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A Quantitative Analysis of the Value-Enhancing Effects of Nicotine, Bupropion, and Varenicline in Male and Female Rats

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A QUANTITATIVE ANALYSIS OF THE VALUE-ENHANCING EFFECTS OF NICOTINE, BUPROPION, AND VARENICLINE IN MALE AND FEMALE RATS

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A QUANTITATIVE ANALYSIS OF THE VALUE-ENHANCING EFFECTS OF NICOTINE, BUPROPION, AND VARENICLINE IN MALE AND FEMALE RATS

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Smoking and tobacco dependence are serious health concerns in the United States and globally. Reward via the pharmacological effects of nicotine are believed to be the principal motivating factor that drive tobacco dependence. Research reveals differences in sensitivity between males and females to the motivational effects of nicotine in tobacco use. Enhancement of reinforcement value of non-nicotine rewards contributes to overall nicotine reward. Similar value-enhancing effects have been observed by the two most commonly prescribed smoking cessation aids, bupropion and varenicline. The present dissertation investigated the value-enhancing effects of nicotine, bupropion and varenicline in both male and female rats using a behavioral economic, reinforcer demand approach. Additionally, the role of dopamine D1 and D2 receptor families and of α4β2* and α7 nicotinic acetylcholine receptors (nAChRs) were investigated in the enhancing effects of nicotine and of bupropion and varenicline, respectively. In two experiments, rats were trained to lever-press maintained by visual stimulus (VS) reinforcement. The response requirement was systematically increased over blocks of 16 sessions according to the following sequence of fixed ratio schedules: 1, 2, 4, 8, 16, 32, 64, 128, 256, 512. Saline, nicotine and bupropion (Experiment 1) or varenicline (Experiment 2) were administered preceding sessions within each session block. Demand for VS reinforcement was analyzed under each drug condition and between the sexes using a

behavioral economic model. The effects of dopamine (D1 or D2 family, Exp1) or nAChR antagonism (α4β2* and α7, Exp2) under each drug condition were also analyzed on responding maintained by progressive ratio VS reinforcement. Nicotine, bupropion and varenicline each enhanced the value of VS reinforcement in male and female rats. Females showed greater sensitivity to the value-enhancing effects of each drug, especially on measures of persistence. Enhancement by bupropion but not nicotine was attenuated by D2 family antagonism in both sexes. Antagonism of α4β2* but not α7 nAChRs attenuated the value-enhancing effects of nicotine and varenicline in females, but only of nicotine in males.

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1: Reward Enhancement and the Nicotine Reinforcement Conundrum

The Significance of Sex in the Social Costs of Smoking

Tobacco use is the single greatest contributor to the global burden of disease and is the leading cause of preventable death and disease in the world (World Health Organization [WHO], 2004). Cigarette smoking, the primary form of tobacco use, accounts for over 480,000 premature deaths annually in the United States alone. For every smoking-related death, an additional 20 persons suffer daily from serious smoking-related illness (United States Department of Health and Human Services [USDHHS], 2014). The estimated annual economic cost of tobacco addiction amounts to $289 billion; over four times the 2013 federal budget of the U.S. Department of Education (USDHHS, 2014). The health and economic benefits of improving our understanding of the factors that drive smoking and nicotine dependence are enormous.

There are significant differences in the prevalence and nature of nicotine-dependence between the sexes. Women, on average, report higher smoking frequency, are less likely to attempt to quit smoking, and are more likely to relapse after quitting than men (Lynch et al., 2001; Roth et al., 2004). Nicotine replacement therapies for smoking cessation (i.e. nicotine gum or patches) are less effective amongst female smokers, and evidence suggests that the sensory and social contexts of smoking are more influential in women than in men (Perkins et al., 1994; 2002; Perkins, 2009). The U.S. Surgeon General published a 675 page report in 2001 entitled *Women and Smoking* that highlights a massive body of research on the prevalence, risk factors, and health consequences of smoking amongst women and girls; a body of research that has only grown in the past 13 years (USDHHS, 2001). Despite the information now available

documenting differences between men and women regarding smoking, we are a long way off from fully understanding the reasons behind those differences. Most research on sex differences in smoking have focused on differential sensitivity to the primary reinforcing properties of nicotine alone. Interestingly, much of that research has revealed that females may be less sensitive than males to the pharmacodynamic effects of nicotine related to reinforcement and reward (for a review see Perkins, 2009). For instance, Perkins and colleagues (2002) found that amongst abstinent smokers, males preferred puffs from nicotine-containing cigarettes over de-nicotinized cigarettes, whereas females did not exhibit a preference. Recently, the LeSage laboratory found that male rats increased their nicotine intake significantly more than females when nicotine infusion doses were progressively decreased across self-administration sessions (Grebenstein et al., 2013).

While evidence suggests that females may be less sensitive than males to the primary rewarding properties of nicotine, increasing evidence suggests that females are more sensitive to the sensory elements of smoking. For instance, Perkins et al. (1994) found that the presentation of a lit cigarette cue shifted preference toward smoking more in females than males in a procedure arranging concurrent availability of cigarette puffs and monetary reward on competing response alternatives. Caggiula and colleagues found that female rats earn more presentations of a sensory reinforcer than males on low fixed-ratio schedules of reinforcement, which corresponded to higher rates of lever pressing for sensory reinforcement coupled with response contingent nicotine infusions on an fixed ratio (FR) 5 schedule of reinforcement (Chaudhri et al., 2005). Despite these recent observations, the majority of research on the behavioral effects of nicotine are still conducted exclusively in males, with little attention to potential differences between the

sexes. One of the primary aims of the work presented in this dissertation was to investigate differences between males and females in the rewarding properties of nicotine and the two most commonly prescribed non-nicotine smoking cessation pharmacotherapies, bupropion (Zyban®) and varenicline (Chantix®).

The Conundrum of Nicotine Reinforcement

The central nervous system effects of nicotine are widely accepted as the primary motivator that drives addiction to tobacco products (USDHHS, 2014). Specifically, the primary reinforcing properties of nicotine are commonly indicated as the principle driving factor that establishes and maintains smoking behavior (USDHHS, 1988). Indeed, great research effort has been made to demonstrate that nicotine directly reinforces behavior upon which its delivery is made contingent (Corrigall and Coen, 1989; Donny et al., 1995; 1998; 1999). The quintessential demonstration of primary reinforcement by nicotine (or any drug, for that matter) is the drug self-administration procedure. In this preparation, subjects (typically rodents or non-human primates) are prepared with an indwelling catheter that can be connected to a drug-syringe pump. Completion of an experimenter-defined operant task, such as lever-pressing, results in drug infusion via brief activation of the drug-pump (Corrigall and Coen, 1989; Donny et al., 1995; 1998; 1999). Significantly higher rates of responding on the active manipulandum than on an inactive manipulandum, or compared to a saline control condition, are interpreted as evidence of a primary reinforcing effect of the drug in question. Generally speaking, most drugs of abuse (cocaine, amphetamines, heroin, alcohol, etc.) show some evidence of primary reinforcing capacity when studied in the self-administration paradigm (see Panlilio and Goldberg, 2007). However, studies investigating the primary reinforcing

properties of nicotine through the drug self-administration procedure have yielded mixed results (Corrigall and Coen, 1989; Donny et al., 1995; 1998; 1999; 2003). Those studies that manage to demonstrate nicotine self-administration typically show relatively low rates of self-administration behavior compared to those engendered by prototypical drugs of abuse, such as cocaine or amphetamine (Panlilio and Goldberg, 2007). Notably, the majority of studies that demonstrate nicotine self-administration employ the use of supporting stimuli to signal drug delivery and/or the availability of drug infusions, such as cue lights, lever insertion, or auditory stimuli (Corrigall and Coen, 1989; Caggiula et al., 2001; 2002; Donny et al., 1995; 1998; 1999; 2003; Grebenstein et al., 2013). In fact, Caggiula and colleagues argue that the inclusion of such stimuli may be requisite for establishing reliable rates of nicotine self-administration (Caggiula et al., 2001; 2002). Given the peculiarities in establishing nicotine self-administration in non-human animals, the degree to which the field asserts that the primary reinforcing properties of nicotine are the principle driving mechanism of smoking behavior is somewhat puzzling. How can the reinforcing properties of nicotine, which appear to be weak at best, establish and maintain smoking behavior at the rates and tenacity that are readily observed in human smoking populations?

Several accounts have been created to help resolve the apparent dichotomy between the tenacity of human smoking behavior and the weak reinforcing capacity of nicotine in self-administration studies. Secondary (i.e. conditioned) reinforcement via nicotine-associated stimuli is commonly invoked as an additional component of the nicotine reinforcement equation. That is, nicotine in tobacco is delivered in the context of many other environmental stimuli that reliably occasion the primary reinforcing effects of

nicotine. The sight, smell and taste of a cigarette, interaction with friends with whom one smokes, the time and place of smoke breaks at work or after a meal, and countless other variables that occasion smoking, may all enter into Pavlovian associations with the primary reinforcing effects of nicotine and thereby acquire some degree of its reinforcing strength. Arguably, the combination of primary reinforcement by nicotine and secondary reinforcement by nicotine-associated stimuli may combine to generate greater overall reinforcement from smoking. However, a significant problem arises with this account when one stops to consider that secondary reinforcers acquire their strength from the primary reinforcement with which they are associated (Kelleher and Gollub, 1962), and in the case of nicotine, that is not a lot. Therefore, although stimuli associated with nicotine delivery undoubtedly acquire some reinforcing capacity by virtue of their association with nicotine (Wilkinson and Bevins, 2008), their reinforcement capacity is also arguably insufficient to explain the high rates and tenacity of smoking seen in human populations.

When considering the role of Pavlovian conditioning and secondary reinforcement in overall nicotine reward, it is important to recognize that nicotine, like other drugs, possesses distinct and perceptible interoceptive stimulus properties. Thus, nicotine is both an unconditioned stimulus with primary reinforcing properties and can become a conditioned stimulus when repeatedly paired with other, non-nicotine unconditioned stimuli. Inasmuch as smokers regularly experience the stimulus properties of nicotine in the context of other reinforcing stimuli (e.g. peer interaction, food, sex, relaxation, alcohol or other drug use, etc.), the reinforcing capacity of these stimuli may be partially acquired by the nicotine stimulus as a secondary reinforcer (for a review, see

Bevins and Besheer, 2014). A study by Besheer et al. (2004) provides a clear demonstration of the ability of nicotine to serve as an interoceptive stimulus that comes to evoke approach behavior by virtue of associations formed with appetitive unconditioned stimuli. In their study, rats were administered 0.4 mg/kg nicotine or saline via subcutaneous injection prior to placement in a conditioning apparatus. On nicotine sessions, sucrose solution was noncontingently delivered periodically within the session, beginning approximately 2 min into the session. On saline sessions, no sucrose was presented. On all sessions, conditioned responding was measured as the rate of approach behavior via head entry into the dipper receptacle prior to the first delivery of sucrose (or an equally matched time-interval on saline sessions). Rats rapidly acquired conditioned approach behavior to the dipper receptacle that was more frequent on nicotine sessions. Furthermore, conditioned responding to the nicotine stimulus was extinguished when nicotine was repeatedly administered across sessions and sucrose deliveries were withheld (Besheer et al., 2004). The finding the nicotine can serve as a conditioned stimulus associated with an appetitive unconditioned stimulus has since be replicated in numerous experiments (see Bevins et al., 2012 for a review). Importantly, these studies demonstrate that nicotine can function as an interoceptive stimulus and can acquire evocative control over behavior via associations with non-nicotine unconditioned stimuli in the environment. However, the ability of conditioned associations between the nicotine stimulus and other reinforcing stimuli to increase the reinforcing capacity of nicotine has not yet been adequately demonstrated.

In the past decade, researchers have begun to investigate a different effect of nicotine in relation to supporting environmental stimuli: reward enhancement. Nicotine,

and other psychomotor stimulants, have been shown to increase rates of operant behavior that produce non-nicotine reinforcing stimuli. In an increasingly classic example, Donny and colleagues (2003) trained rats to lever-press maintained by brief presentations of a compound visual stimulus. This visual stimulus (VS) was comprised of 60-s termination of otherwise constant chamber illumination in compound with 5-s illumination of two 28V DC lamps located above the two response levers. In some of the groups of rats, infusions of 0.03 mg/kg nicotine or saline occasioned delivery of the VS (i.e. prototypical nicotine self-administration). In other groups, infusions of nicotine or saline were also delivered but their timing was controlled by rats in the response-contingent delivery groups. Finally, additional groups received response-contingent infusions of nicotine or saline, but no VS (i.e. unsignaled nicotine self-administration). Donny and colleagues observed that response rates were highest in groups where responding was maintained by presentation of VS and also received nicotine, regardless of whether nicotine delivery was response-contingent or controlled by a yoked partner. Interestingly, responding maintained by nicotine infusions in the absence of VS was not significantly different than responding maintained by saline infusions under similar conditions, and was significantly lower than responding maintained by VS in the absence of nicotine (Donny et al., 2003).

These findings show that nicotine alone does not possess strong primary reinforcing properties, as responding maintained by nicotine in the absence of VS was not significantly different than responding maintained by saline. More importantly, responding maintained by VS showed signs of primary reinforcement by VS presentation, which was enhanced when nicotine was also delivered either contingently or noncontingently. This latter finding, that nicotine enhances the strength of other

reinforcing stimuli has been invoked repeatedly in research over the past decade as a potential contributor to nicotine reinforcement of smoking.

Since 2003, reward enhancement has been increasingly explored as a contributing mechanism in nicotine reinforcement of smoking behavior. Indeed, enhancement of reinforcement value by nicotine appears to hold some promise in helping to elucidate the mechanisms by which nicotine drives tobacco abuse without discarding the contributions of nicotine primary and secondary reinforcement. Rather, the reward enhancing effects of nicotine may support and otherwise strengthen the contributions that nicotine-associated primary and secondary reinforcers give in the equation of overall nicotine reinforcement. This concept is well described by Bevins and Palmatier (2004), wherein they conceptualize overall nicotine reward as being the summation of four critical components: 1) the primary reinforcing properties of nicotine, 2) the secondary reinforcing properties of nicotine-associated stimuli, 3) the acquired, secondary reinforcing properties of the nicotine stimulus by virtue of its association with other primary reinforcers in the smoker's environment, and 4) the incentive amplifying effects of nicotine on each of the three aforementioned components.

Reward Enhancement and Nicotine

The work of Donny et al. (2003) represent the first clear demonstration that nicotine can enhance rates of operant responding maintained by reinforcing, non-nicotine stimuli. In that same study, Donny and colleagues convincingly demonstrate that this enhancement effect of nicotine was not the result of an associative learning process, as enhanced responding disappeared and returned immediately following termination and reintroduction of nicotine administration, respectively. Similarly, continuous infusion of

nicotine across the duration of experimental sessions also enhanced responding maintained by VS, removing the possibility that serendipitous pairings of nicotine infusion and VS presentation resulted in enhanced responding through Pavlovian conditioning (Donny et al., 2003). Additionally, Palmatier et al. (2007a) found that the reward enhancing effects of nicotine increase with repeated nicotine administration and rats who switched from receiving nicotine injections 1 h post-session to 5 min pre-session expressed immediate increases in operant responding that did not differ from those of rats who always received pre-session injections of nicotine. The finding that repeated nicotine administration increased the reward enhancing effects of nicotine in Palmatier et al. (2007a) is corroborated by the finding that repeated nicotine exposure may be requisite to the expression of reward enhancement (Barrett and Odum, 2011). Taken together, the above findings suggest that the reward enhancing effects of nicotine are an inherent result of its pharmacology that develop with repeated exposure as a change in responsiveness to nicotine rather than through acquisition via an associative learning process (Palmatier et al., 2007a).

Although the expression of reward enhancement does not appear dependent upon associative learning between nicotine and non-nicotine unconditioned stimuli, that does not necessarily mean that the reward enhancing effects of nicotine are not affected by associative learning processes. For instance, Barrett and Bevins (2013) found that nicotine increased lever-pressing under extinction conditions only when it had also been administered in the preceding reinforcer-maintenance conditions, and this was not merely an effect of repeated nicotine exposure. That is, nicotine enhanced non-reinforced lever-pressing only when nicotine had occasioned reinforced lever-pressing in the past.

Palmatier, O'Brien and Hall (2012) found that the reward-enhancing effects of nicotine are related to reinforcer salience, and that they are also influenced by conditioning history. In separate experiments, rats were trained to lever-press on a progressive ratio schedule maintained by presentations of liquid sucrose, and the effects of nicotine to enhance progressive ratio breakpoints were assessed across a range of varying sucrose concentrations (0 to 60%, w/v). In the first experiment, rats were exposed to variations in sucrose concentration over several sessions prior to subsequent assessment of the effects of nicotine to enhance responding maintained by sucrose solution of varying concentration. In the second experiment, experience with sucrose reinforcement was restricted to a single concentration until later assessment with nicotine on varying concentrations. Notably, nicotine enhanced progressive ratio breakpoints of responding reinforced by the full range of concentrations of sucrose reward in Experiment 1, but enhancement was concentration dependent in Experiment 2. In other words, differential experience with varying sucrose concentration between the experiments appear to have caused differential sensitivity of nicotine enhancement to sucrose concentration (Palmatier et al., 2012).

As another example of how nicotine enhancement may be affected by associative learning processes, consider the findings of Palmatier et al. (2013). In their study, rats were trained to associate 30-s presentation of an illuminated dipper receptacle (the conditioned stimulus; CS) with delivery of a rewarding sucrose solution (the unconditioned stimulus; US). The effects of nicotine on conditioned approach to the dipper receptacle during CS presentations was then assessed. Interestingly, nicotine did not enhance conditioned approach behavior when the CS and US were delivered in the

same dipper receptacle. However, when the presentations of CS and US were separated into two different receptacles, nicotine enhanced approach to the CS (i.e. sign-tracking) but not to the US (i.e. goal-tracking). The failure to enhance goal-tracking (i.e. US-directed responding) was observed across groups of rats trained with differing concentrations of sucrose (0 to 20%, w/v), reducing the likelihood that the lack of increases in goal-tracking behavior were the result of a ceiling effect of sucrose reinforcement value (Palmatier et al., 2013).

These findings, coupled with those of Palmatier et al. (2012) suggest that the effects of nicotine to enhance behavior may not be as simple as increasing the reinforcement value of non-nicotine rewards, but may result from an enhancement of other motivational properties of incentive stimuli, such as those that evoke conditioned responses, approach behavior, or preparatory responses (Palmatier et al., 2012; Konorski, 1967). While this alternative theoretical framework for the effects of nicotine to enhanced reinforced behavior is intriguing, parsing apart reinforcement value from the other elements of what is commonly referred to as incentive salience may be a futile endeavor, to some degree. Indeed, one could argue that the distinctions between the incentive amplification and value-enhancement accounts may simply be an issue of semantics. Therefore, for the remainder of this dissertation, I will use the terms "value-enhancement" and "incentive amplification" more or less interchangeably, though there are subtle theoretical distinctions between the two terms that are beyond the scope of discussion in this dissertation.

One of the important features of value-enhancement by nicotine is its relationship to the basal reinforcement value of the stimuli upon which it acts. As mentioned above,

Palmatier et al. (2012) demonstrated that the degree to which nicotine enhanced progressive ratio breakpoints of sucrose maintained behavior depended, in part, upon the sucrose concentration. Palmatier et al. (2007) found something similar with responding maintained by sensory reinforcement: nicotine moderately enhanced response rates maintained by a mildly reinforcing VS (adding 28V DC lamp illumination to an already illuminated chamber for 5 s) and greatly enhanced response rates maintained by a more reinforcing VS (turning off all chamber illumination for 5 s). These findings suggest that the greater the basal incentive salience a stimulus exerts, the greater the degree of enhancement that will be observed following nicotine administration. However, Barrett and Bevins (2013) observed that an enhancement effect of nicotine was more difficult to detect on behavior maintained by 26% sucrose solution than on behavior maintained by 4% sucrose solution. Similarly, Raiff and Dallery (2008) observed reliable enhancement of responding maintained by relatively low strength conditioned reinforcers, but failed to observe enhancement of food-pellet maintained behavior in the same sessions. The combined observations of these studies may reflect that nicotine enhances the value of reinforcing stimuli up to a threshold, beyond which observing increases in the value of these stimuli may be difficult to detect because of ceiling effects on either the reinforcement value of the stimuli or on the behavioral performance from which value is inferred.

The value-enhancing and primary reinforcing effects of nicotine operate as two distinct elements that contribute to overall nicotine reward (see Caggiula et al., 2009 for a review of this "dual reinforcement hypothesis"). Evidence of this distinction comes from observations that value-enhancement and primary reinforcement by nicotine are

behaviorally and pharmacologically dissociable. The behavioral dissociation between the value-enhancing and primary reinforcing effects of nicotine was demonstrated in a cleverly designed experiment by Palmatier et al. (2006). In this experiment, rats who had already acquired the lever-press response via training with food pellets, were presented two levers upon which they could response for nicotine infusions, VS presentations, or both. In one group, only nicotine was available (NIC-Only); in another group only VS presentations were available (VS-Only); and in yet a third group, nicotine infusions and VS presentations were both available contingent upon presses on the same lever (NIC+VS). Finally, the critical group of interest received nicotine infusions by responding on one of the levers and VS presentations by responding on the other lever (2-Lever). Thus, rats in the 2-Lever group could choose to respond for deliveries of either nicotine or VS independent of each other. After experience with these conditions on fixed ratio schedules of reinforcement, clear patterns emerged for the different groups. In the NIC-Only group, low response rates on the active lever were only slightly higher than responding on the inactive lever. Active lever-pressing in the VS-Only group was moderately higher than that of the NIC-Only group. Active lever-pressing in the NIC+VS group was synergistically enhanced to levels 2 to 3x that of the VS-Only group, and far greater than would be expected by summation of responding in the NIC-Only and VS-Only groups. Importantly, responding on the nicotine lever of the 2-Lever group did not differ from than of the NIC-Only group, and responding on the VS lever did not differ from that of the NIC+VS group. These differences collapsed when saline was substituted for nicotine in all the groups: VS lever-pressing in the 2-Lever, NIC+VS and VS-Only groups did not differ from each other and nicotine (now saline) lever-pressing in the 2-

lever and NIC-Only groups decreased slightly but did not differ from each other. It is worth noting that nicotine intake was highest in the NIC+VS group, where both the primary and value-enhancing effects of nicotine operated on responding on the same lever. These results demonstrate that while nicotine can simultaneously exert both primary reinforcing and value-enhancing effects on behavior, these effects operate as separate behavioral processes that may combine to drive overall reinforcement by nicotine.

Using the procedure developed by Palmatier et al. (2006), a few studies have been able to show that not only are the primary reinforcing and value-enhancing effects of nicotine behaviorally distinct, they are pharmacologically dissociable as well. For instance, Palmatier et al. (2008) explored the role of metabotropic glutamate 5 (mGlu5) receptors in the primary reinforcing and value-enhancing effects of nicotine in a series of experiments using a similar design to Palmatier et al. (2006) described above. Notably, when lever-pressing across all the groups had been established for 10 days on a fixed ratio (FR) 2 schedule, the mGlu5 receptors antagonists 2-methyl-6-(phenylethynyl)-pyridine (i.e. MPEP) or 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (i.e. MTEP) were tested on all the rats and the effects on responding maintained by nicotine, VS, or both were observed. MPEP and MTEP administration decreased response rates on the nicotine-producing lever in the 2-Lever, NIC-Only, and NIC+VS groups. Lever pressing on the VS-producing lever was also decreased in the 2-Lever groups, but not in the VS-Only groups. Because mGlu5 receptor antagonism decreased nicotine intake in the 2-Lever group, decreases in responding on the VS-producing lever may have been a result of reductions in nicotine intake and not an attenuation of the value-enhancing effects of

nicotine. To investigate this possibility, the effects of MPEP and MTEP administration were explored in combination with nicotine replacement via delivery of non-contingent nicotine infusions. The combination of mGlu5 receptor antagonism and nicotine replacement resulted in decreases in responding only on the nicotine-producing lever of the 2-Lever, NIC-Only and NIC+VS groups, and no effects of the VS-producing lever of the 2-Lever or VS-Only groups. The principal conclusion from these experiments was that mGlu5 receptors were involved in the primary reinforcing effects of nicotine, but not in the value-enhancing properties of nicotine on responding for VS (Palmatier et al., 2008).

In another study, the primary reinforcing and value-enhancing effects of varenicline were investigated using a refined version of the two-lever design described above, with the following groups: concurrently available saline and VS (2L:SAL), concurrently available nicotine and VS (2L:NIC), concurrently available varenicline and VS (2L:VAR), varenicline only (1L:VAR), varenicline infusion coupled with VS on the same operandum (1L:VAR+VS), and response-contingent delivery of VS combined with noncontingent delivery of varenicline controlled by a yoked partner in the 1L:VAR+VS group (Schassburger et al., in press; Palmatier et al., 2006). Varenicline (i.e. Chantix®) is a FDA approved smoking cessation aid developed by Pfizer which has shown relatively good efficacy in helping reduce smoking and risk of relapse (Tonstad, 2006; Koegelenberg et al., 2014). Schassburger et al. (in press) found enhanced response rates of VS-reinforced behavior in the 2L:NIC and 1L:VAR+VS groups compared to the two-lever saline (2L:SAL) group. Additionally, increased responding relative to the saline control group on the infusion-producing lever was observed in the same 2L:NIC and

1L:VAR+VS groups. These findings suggest that nicotine and varenicline produce value-enhancement effects on responding maintained by VS; this effect was observed to be greater with nicotine than with varenicline, corresponding to a similar finding by Levin et al. (2012). Notably, responding on the infusion-lever in the 2L:VAR and 1L:VAR groups was not significantly different from the saline control group, suggesting a lack of primary reinforcing effects of varenicline. Their results imply that somewhere in the shared mechanisms between varenicline and nicotine, we may possibly find the receptor mechanisms of their common value-enhancing effects. In contrast, somewhere in the disparate mechanisms of action between varenicline and nicotine may possibly lie the relevant receptor mechanisms of the primary reinforcing properties of nicotine (Schassburger et al., 2014).

Like nicotine, varenicline acts as an agonist at α4β2-containing nicotinic acetylcholine receptors (nAChRs), though only partially, and acts as a full agonist at α7 and α3β4* nAChRs, where * indicates additional unspecified subunits (Grady et al., 2010; Ortiz et al., 2012). Considerable evidence suggests that β2-containing nAChRs on dopaminergic neurons in the midbrain regions of the ventral tegmental area and nucleus accumbens mediate the primary reinforcing effects of nicotine (Placzek and Dani, 2009; Picciotto et al., 1998; Picciotto and Mineur, 2014; Brunzell and Picciotto, 2009). Varenicline has mixed agonist and antagonist effects on α4β2-containing nAChRs, which may account for the apparent lack of primary reinforcing effects of varenicline (Schassburger et al., in press; Grady et al., 2010; Ortiz et al., 2012).

Regarding the value-enhancing effects of nicotine and varenicline, there is little evidence to suggest that α7 nAChRs as a mechanism (Liu et al., 2007; Guy and Fletcher,

2013), despite the high affinity of varenicline at these receptors (Mihalak et al, 2006). Previous work has shown that the α7-selective antagonist, methyllycaconitine (MLA), does not impair the value-enhancing effects of nicotine on responding for VS (Liu et al., 2007), but no study has investigated the role of α7 receptors in value-enhancement by varenicline. Investigation of the α3β4* receptor may have promise, but little research has been conducted on the role of α3β4* nAChRs in nicotine reward given the lack of highly specific agonist or antagonist compounds for this receptor subtype. The novel compound AT-1001 is a relatively selective antagonist at α3β4* nAChRs, but is presently only synthesized in a few laboratories and is not widely available or well researched (Toll et al., 2012). Dihydro-β-erythoidine (DHβE) acts as an antagonist at β2 and β4-containing nAChRs, but its far greater affinity for β2-containing receptors renders it less ideal than AT-1001 (Harvey and Luetje, 1996; Harvey et al., 1996).

Value-Enhancement and Smoking Cessation Pharmacotherapy

The finding that varenicline, one of the most effective and commonly prescribed smoking cessation pharmacotherapies, possesses value-enhancing properties similar to nicotine should not be surprising. Given the premise that nicotine enhances the reinforcement value of other rewarding stimuli, then smoking cessation results in not only a loss of smoking-related reinforcers, but also a loss in the value of additional sources of reinforcement in the smoker's environment (Raiff and Dallery, 2008). The efficacy of varenicline, therefore, may be partially related to the replacement of the value-enhancing effects of nicotine in tobacco smoke with value-enhancing effects of varenicline. In this sense, varenicline treatment may function in a similar fashion as nicotine replacement therapy. Notably, the other most commonly prescribed

pharmacotherapy for smoking cessation is the anti-depressant, bupropion (Zyban®/Wellbutrin®), which also possesses value-enhancing properties (Palmatier et al., 2009).

The value-enhancing effects of bupropion are interesting from a pharmacological perspective in that the shared mechanisms of bupropion and nicotine on nAChRs are fairly limited. Bupropion acts as a noncompetitive antagonist of α3β4, α3β2, and α4β2-containing nAChRs, as well as a weak reuptake inhibitor of dopamine, norepinephrine and serotonin (Carroll et al., 2014; Dwoskin et al., 2006). In contrast, nicotine functions as an agonist at these same nAChRs, though following initial activation by nicotine, many nAChRs rapidly desensitize and require a period in inactivity before recovering to their resting state (Pitchford et al., 1992; Wang and Sun, 2005). However, both nicotine and bupropion share similar downstream effects in the dopaminergic signaling pathways of the midbrain; that is, increased dopaminergic tone in the ventral tegmental area and nucleus accumbens (Palmatier et al., 2009; Dwoskin et al., 2006). Notably, Palmatier et al. (2009) found that nicotine and bupropion enhanced responding maintained by a mildly reinforcing VS, but observed that antagonism of nAChRs via mecamylamine attenuated the value-enhancing effects of nicotine, but not bupropion. Conversely, antagonism of α1-norepinephrine receptors attenuated the reward-enhancing effects of bupropion, but not of nicotine. Taken together, these findings show that value-enhancement may result from disparate primary targets of different drugs that lead to similar downstream effects in the dopaminergic pathways of the midbrain (Palmatier et al., 2009).

The cases of value-enhancement by varenicline and bupropion demonstrate how replacing the value-enhancing effects of nicotine in tobacco with enhancement effects

from a non-nicotine alternative may be helpful as a smoking cessation aid. Moreover, by furthering our understanding of the pharmacological mechanisms by which nicotine, varenicline, bupropion, and other value-enhancing compounds produce their effects, we may gain a greater understanding of the important variables that drive nicotine reinforcement and tobacco dependence and the neurobiological mechanisms that underlie those variables. The work presented in the later chapters of this dissertation represent part of a larger programmatic effort to elucidate these variables and increase our understanding of their driving mechanisms, and to extend that investigation to include both males and females.

Chapter 2: Quantitatively Representing Reinforcement Value

Issues with Reinforcement Value

Reinforcement value is an ambiguous construct; therefore, the assertion that nicotine enhances the reinforcement value of rewarding environmental stimuli has ambiguous implications. Part of this ambiguity stems from the diverse traditions whereby researchers have attempted to quantify reinforcement value and the methods by which value is inferred from behavior. The fact that reinforcement value is a hypothetical construct inferred from behavior is important to recognize; this fact regulates the utility of the reinforcement value construct to that of an intervening variable and not an ultimate cause of behavior. That is, biological and environmental variables specific to the organism and the situation determine effects on sensitivity of behavior to variables such as reinforcer delay, magnitude, contingency, or response-cost. In turn, these behavioral dispositions specific to the reinforcement situation are observed by changes in rate of response, behavioral persistence, reinforcer consumption, or choice and preference between reinforcement alternatives. The construct of reinforcement value is therefore a theoretical representation of the effects of biological and environmental factors to determine differential sensitivity to reinforcing stimuli on observable behavior.

One of the issues with the construct of reinforcement value is that it is difficult to quantify or represent in any single measure. Given that value is actually a reflection of multiple dimensions of behavioral disposition toward the different variables of reinforcement delivery, this should come as no surprise. For this reason, decades of behavioral research have generated many different metrics that encapsulate diverse facets of the reinforcement value construct. For instance, both Thorndike and Pavlov regularly

employed response latency as a measure of response strength, from which the term reinforcement descends (Thorndike, 1911; Pavlov, 1927). Skinner related response strength to probability of response measured via response rates (Skinner, 1938; Ferster and Skinner, 1957). Herrnstein further built upon Skinner's ideas by developing a model of the effects of relative rates of reinforcement on relative rates of response (Herrnstein, 1961; 1970). Mazur's model of hyperbolic delay discounting is based on the idea that reinforcement value is inversely related to delays in reinforcer presentation (Mazur, 1987). Nevin developed his own model that represents response strength as persistence of behavior in the face of disrupting variables (Nevin, 1974; Nevin and Grace, 2000). Finally, Hursh and Silberburg advocate a behavioral economic model of reinforcer demand that relates value to the relationship between reinforcer consumption and unit response cost (Hursh, 1980; Hursh and Silberburg, 2008). As you can see from this brief overview, the research literature and tradition of developing measurements intended to represent the construct of response strength and/or reinforcement value has a rich and diverse history.

A natural question arises when considering the sundry options for measuring reinforcement value and the effects of drugs to modify that value: what measure is most appropriate? On the surface, this question may appear to have no correct answer. That is, if each measure accurately detects variations in reinforcement value, then any method should be adequate. However, selection of the appropriate measure that best represents the dimensions of reinforcement value where experimental manipulations are hypothesized to exert their effects can make or break the results and interpretation of a particular study. Moreover, some methodologies are arguably more powerful than others

in that they simultaneously generate multiple measures of reinforcement value along distinct behavioral dimensions, or generate measures that represent multiple behavioral dimensions of reinforcement value in a single metric. The reinforcer demand method developed by Hursh and Silberburg is one such approach, yielding indices of value that represent behavioral persistence, maximal sustainable response effort, basal intensity of reinforcer demand, and sensitivity to escalations in unit response cost (Hursh and Silberburg, 2008; Barrett and Bevins, 2012).

Reinforcement Value as Demand

The basic framework of the reinforcer demand approach is rooted in behavioral economic theory, where reinforcement value is conceptualized in terms of reinforcer consumption in relation to its price in units of response cost. As the price of each unit of the reinforcer increases, consumption of the reinforcer decreases, and the rate of these decreases in consumption represents what is termed *elasticity of demand*. Demand curves, generated by plotting rates of consumption as a function of unit response cost, are generally characterized by a portion of inelastic change in consumption followed by a domain of elastic change in reinforcer consumption. Inelastic demand refers to decreases in consumption that are relatively insensitive to increases in reinforcer price; elastic demand is characterized by relatively dramatic decreases in consumption with increases in unit price. The point of unit elasticity, or P_{max}, is the price at which there is a 1:1 relationship between proportional increases in unit cost and proportional decreases in unit consumption of the reinforcer. P_{max} is one of the four principal measures of reinforcement value generated by reinforcer demand analyses, which each represent different dimensions of the value construct. P_{max} reflects the price at which consumption of the

reinforcer shifts from inelastic demand to elastic demand, and thereby represents tolerance toward increases in response cost for reinforcement (i.e. persistence). The concept that increased P_{max} is reflective of greater behavioral persistence is supported by repeated findings that higher estimates of P_{max} correlate with increased breakpoints on progressive ratio schedules maintained by a wide variety of reinforcers (see Bickel, Marsch and Carroll, 2000 for a review).

The second principal metric of reinforcement value obtained in reinforcer demand analyses is the maximum response effort sustainable by the reinforcer, O_{max}. Values of O_{max} are directly related to P_{max}, as maximum responding is always predicted at price P_{max}. However, that is not to suggest that O_{max} is solely determined by P_{max}; differences in basal reinforcer consumption (i.e. the ordinate intercept) and the rate of change in elasticity of demand (i.e. the second derivative) can also lead to differences in O_{max} at values of the same P_{max}. Therefore, O_{max} reflects the interaction between response persistence (P_{max}), intensity of demand (Q_0), and the rate at which sensitivity to increases in unit cost changes from inelastic to elastic. In other words, O_{max} represents three distinct dimensions of reinforcement value within a single metric. Unsurprisingly, O_{max} is often revealed as the demand metric with the greatest predictive utility of outcomes related to a behavior maintained by a specific reinforcer. For instance, higher estimates of O_{max} as assessed by a hypothetical cigarette purchase task are highly correlated with questionnaire-assessed nicotine dependence, increased sensitivity to the value-deflating effects of reinforcer delay on cigarettes, and increased levels of puffs per cigarette during ad libitum cigarette consumption (Chase et al., 2013; MacKillop and Tidey, 2011).

In order to understand the two remaining principal measures of reinforcement value obtained though reinforcer demand analyses, we must turn to the quantitative model of reinforcer demand developed by Hursh and Silberburg (2008). This model relates reinforcer consumption to unit response cost via the following equation:

$$log\ Q\ =\ log\ Q_0\ +\ k(e^{-\alpha * Q_0 * C}\ -\ 1)$$

where *Q* represents units of reinforcer consumption, Q_0 is predicted consumption when the reinforcer costs nothing to obtain (i.e. the ordinate intercept), *k* is a constant reflecting the range of the demand function in log units of consumption, *e* is the base of the natural logarithm, *C* is the response cost per reinforcer delivery, and α represents the rate of change in decline in consumption in standardized price (Q_0 * *C*). Notably, the values of Q_0 and α are adjusted to maximize the fit of the demand model to the data of individuals, and may be conceptualized to represent intensity of demand (Q_0) and the essential value of the reinforcer after accounting for differences in unit consumption in relation to unit cost (α; Hursh and Silberburg, 2008). Importantly, Hursh and Silberburg (2008) propose that the true value of a reinforcer is best represented by the rate of change in elasticity of demand as measured by the α parameter. The significance of α will be discussed momentarily, but first we will turn to consumption at unit cost zero, Q_0.

As noted above, Q_0 represents intensity of demand as consumption freed from constraints of response cost. Therefore, Q_0 represents consumption where the only remaining limiting factor is satiation (i.e. diminishing marginal utility). For this reason, increases in reinforcer magnitude, as in number of food pellets or infusion dose of self-administered drug, have decreasing effects on Q_0. That is, satiation occurs more rapidly with higher magnitudes of food or drug reinforcement, and this leads to decreases in

levels of consumption at Q_0, unless you normalize Q_0 as unitary consumption. As previously mentioned, Q_0 is fundamentally related to O_{max} via interaction with P_{max} and the rate of change in the slope of the inelastic portion of the demand function. As such, increases or decreases in Q_0 often result in similar effects in O_{max}, but these variables can vary independently when differences are observed in one or both of the other factors of O_{max}. Notably, values of Q_0 have been shown to predict preference between reinforcement alternatives when both alternatives are available ad libitum, supporting the idea that Q_0 represents basal intensity of demand for a reinforcer when freed from response cost (Bickel, Marsch and Carroll, 2000).

Finally, essential value as represented by α is the purported measure of the true value of a reinforcer independent of scalar differences in magnitude (Hursh and Silberburg, 2008; Hursh and Winger, 1995). That is, the essential value of one food pellet is equivalent to that of two food pellets as both represent scalar differences of the same reinforcer, all other variables being equal. The ability of the model to account for differences in magnitude of the same reinforcer results from the standardization of price relative to basal consumption in the demand model (i.e. $Q_0 * C$; Hursh and Silberburg, 2008). Essential value reflects the rate of change in demand elasticity (i.e. the second derivative) across the full domain of the demand function. Note that O_{max} is affected by the rate of change in demand elasticity in the inelastic portion of the demand function; this is not the same measure as essential value, which represents rate of change in elasticity across the entire demand function. This distinction may seem trivial, but it is important to recognize that consumption under the elastic and inelastic portions of the demand function represent behavior primarily affected by a different set of variables.

That is, in the inelastic portion of the demand function, consumption is primarily limited by satiation (i.e. diminishing marginal utility); in the elastic portion of the demand function, consumption is primarily limited by increasing price (i.e. constraint). Thus, the essential value parameter reflects the limiting effects of both satiation and price on consumption by representing the rate at which consumption shifts from being limited by satiation to limited by response requirement (Bickel, Marsch and Carroll, 2000; Hursh and Silberburg, 2008; Johnson and Bickel, 2006). For this reason, behavioral economists argue that α is a single parameter estimate of the essential value of a reinforcer, wherein value is well characterized by reinforcer consumption as a function of response cost (Hursh and Silberburg, 2008).

Extension of Reinforcer Demand to Nicotine Value-Enhancement

Demand analyses have been widely applied to characterize the primary reinforcing effects of several drugs of abuse, including alcohol, cocaine, amphetamines, opiates, phencyclidine (PCP), and nicotine (Galuska et al., 2011; Hursh and Winger, 1995; Murphy and MacKillop, 2006; Shahan et al., 1999; 2001; Wade-Galuska, Galuska, and Winger, 2011). However, few studies have used the reinforcer demand methodology to investigate the enhancing effects of nicotine on reinforcement value. The results of these studies so far largely support the notion that the value-enhancing effects play a large part in overall reinforcement derived from nicotine. For instance, Shahan and colleagues (1999) studied cigarette smokers with no immediate plans to quit smoking who were paid to operate a pull-mechanism plunger manipulandum to obtain access to puffs of two types of unlabeled cigarettes: nicotine-containing or de-nicotinized. The number of responses required for opportunity to smoke either kind of cigarette was

systematically increased over sessions. When puffs on either kind of cigarette were available independently, puffs per session and plunger response rates were similar between both types of cigarettes. When both kinds of cigarettes were made available concurrently, puffs per session and plunger response rates were significantly higher for nicotine-containing cigarettes than de-nicotinized cigarettes. Furthermore, subjects rated nicotine-containing cigarettes higher for their "taste", "smoothness", "potency", and "enjoyment". This study shows that current smokers will work equally hard for cigarettes containing or lacking nicotine when those cigarettes are offered independently, responding even thousands of times for a single puff of either type of cigarette. This finding suggests that the sensory elements of cigarette smoking are sufficient to maintain high rates of smoking behavior. However, when offered a choice, a clear preference for nicotine-containing cigarettes was observed and subjects reported greater satisfaction from smoking nicotine-containing cigarettes (Shahan et al., 1999). This finding suggests that nicotine enhanced the reinforcing elements of cigarette smoking sufficiently for participants to develop a preference for one "brand" over another. Interestingly, mean elasticity between types of cigarettes was similar on both the concurrent and independent availability conditions (Shahan et al., 1999). Inspection of the data pattern from the concurrent availability condition suggests potentially higher Q_0 and O_{max} for nicotine-containing cigarettes, but these parameters were not measured or reported in this study.

Pre-clinically, the value-enhancing effects of nicotine have only been investigated using reinforcer demand techniques in three published studies. Barrett and Bevins (2012) trained rats to lever-press maintained by VS in a procedure adapted from Donny et al. (2003). Nicotine (0.4 mg/kg) or saline was injected 5 min preceding experimental

sessions and rats could then lever-press to obtain VS presentations maintained by FR schedules of reinforcement. The FR response requirements were systematically increased over blocks of sessions and VS presentation between drug conditions was observed as a function of unit price. The authors found that nicotine increased VS consumption relative to saline across FR schedules up through FR 32. Furthermore, nicotine enhanced measures of VS reinforcement value relative to saline on Q_0, P_{max}, O_{max} and essential value (α). Finally, enhancement by nicotine of VS reinforcement did not differ in a manner analogous to a scalar increase in the magnitude of VS delivery (Barrett and Bevins, 2012).

Cassidy and Dallery (2012) trained rats to lever-press maintained with food pellets under conditions where all daily food rations were obtained within the experimental sessions (i.e. closed economy) versus when food was made available outside experimental sessions via post-session feeding (i.e. open economy). Additionally, rats could earn either 1 or 2 food pellets under each economy condition in separate experimental phases. The authors found that essential value of food-pellets did not differ between reinforcer magnitude conditions in a closed economy, but did in an open economy, likely reflecting an interaction with economy type on the consumption limiting influence of satiation for food pellets. Rats were then implanted with osmotic mini-pumps that delivered a constant chronic dose of nicotine and demand for 1 or 2 food pellets was again assessed under conditions of closed economy. The authors found that nicotine enhanced essential value of food in the 1-pellet condition, but not in the 2-pellet condition (Cassidy and Dallery, 2012). Effects of nicotine on Q_0, P_{max}, or O_{max} were not reported, but visual inspection of their data suggests that nicotine likely also enhanced Q_0

and possibly O_{max} in the 1-pellet condition, and had no effects on these parameters in the 2-pellet condition (Cassidy and Dallery, 2012).

Finally, Cassidy and Dallery (in press) also investigated the effects of nicotine to enhance primary and conditioned reinforcement using a demand analysis in the observing response procedure. In this procedure, presses to one lever (i.e. the food lever) resulted in either food arranged on a variable interval (VI) 15 s schedule or extinction. Presses to a second lever (i.e. the observing lever) presented stimuli differentially correlated with the schedule in operation on the food lever. Previous work has demonstrated that stimuli associated with food availability in this procedure function as conditioned reinforcers (see Shahan, 2002). Over sessions, the FR requirement to produce the observing stimuli was increased, and baseline demand for conditioned reinforcement was assessed. Nicotine was then administered via osmotic mini-pumps and demand for conditioned reinforcers was reassessed using the same procedure. The authors found evidence of nicotine-enhancement of essential value of conditioned reinforcement in all rats. Measures of Q_0, P_{max} and O_{max} were not reported, but visual inspection of their presented demand curves suggest that nicotine may have enhanced value on all of these measures. The authors also observed nicotine-induced increases in responding on the food lever during intervals of presentation of the food-associated observing stimulus. This latter effect suggests that nicotine may have also enhanced the value of food primary reinforcement, which may account for some of the increases in value of the conditioned reinforcer (Cassidy and Dallery, in press).

Taken together, these three studies demonstrate that nicotine enhances the essential value of other, nonpharmacological reinforcers, though this effect may be

affected by conditions of open or closed economy (Barrett and Bevins, 2012; Cassidy and Dallery, 2012; in press). Importantly, nicotine also appears to enhance value as measured by the demand model. These results support the hypothesis that value-enhancement by nicotine may be an important variable in the overall reward derived from nicotine. Notably, these studies also demonstrate the feasibility of using a reinforcer demand methodology to investigate deeper issues regarding the value enhancing effects of nicotine.

In Chapter 1, we highlighted some of the unknowns regarding the effects of nicotine to alter reinforcement value. The experiments presented in this dissertation were designed to help elucidate the role of value-enhancement by nicotine as a driving motivational mechanism underlying tobacco dependence. As noted previously, females show greater sensitivity to the sensory elements of smoking, and nicotine is known to enhanced sensory reinforcers (Perkins, 2009; Caggiula et al., 2009). The present experiments explored whether female rats differ in sensitivity to reinforcement by VS and/or the enhancement of value of sensory reinforcers. Additionally, bupropion and varenicline engender value-enhancing effects, and these effects have been postulated to be at the heart of their clinical efficacy in smoking cessation (Palmatier et al., 2009; Levin et al., 2013; Schassburger et al., 2014). The present experiments characterize the value-enhancing effects of bupropion and varenicline in comparison to nicotine and attempt to determine whether the effects of each operate by similar or dissimilar behavioral mechanisms. Furthermore, the present experiments investigate the possibility of differences between the sexes in the value-enhancing effects of bupropion or varenicline. Finally, the pharmacological mechanisms of enhancement by bupropion and

varenicline may prove informative to understanding the pharmacological mechanisms of enhancement by nicotine and of the neurobiological mechanisms of perception of reinforcement value in general. The present experiments investigate the roles of dopamine receptors and specific subtypes of nAChRs in the enhancing effects of nicotine alongside those of bupropion and varenicline, respectively.

Chapter 3: Common Procedures

The following sections outline the common procedural details between the two experiments wherein we assessed the effects of nicotine, bupropion, and varenicline on the value of reinforcing sensory stimuli. Each experiment consisted of several phases, including lever-press training, reinforcer demand assessment, progressive ratio schedule training, and receptor antagonism on progressive ratio performance. The procedural specifics unique to each experiment are described further in Chapters 4 and 5 for Experiments 1 and 2, respectively.

Subjects

Forty-eight experimentally-naïve Sprague-Dawley rats (*n*=12 per sex, per experiment; Harlan, Indianapolis, IN), aged 9 weeks upon arrival, were individually housed in clear polycarbonate tubs lined with TEK Fresh® cellulose bedding in a temperature- and humidity-controlled colony. Rats were given two days to acclimate to the colony followed by three additional days of repeated handling prior to initiation of training procedures. Throughout each experiment, water was continuously available and rats were given 12 or 15 grams (for females and males, respectively) of laboratory chow daily (unless otherwise specified). Previous studies from the laboratory have shown that rats maintain healthy rates of growth while still encouraging exploratory behavior under these feeding conditions. Sessions were conducted during the light phase of a 12:12 hour light/dark cycle. Experimental protocols were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee.

Apparatus

Sessions were conducted in sixteen conditioning chambers (ENV-008CT; Med-Associates, Inc., St. Albans, VT; measuring 30.5 x 24.1 x 21.0 cm, l x w x h) enclosed in light- and sound-attenuating cubicles fitted with a fan to mask noise and provide airflow. Sidewalls were aluminum; the ceiling and front and back walls were clear polycarbonate. One sidewall featured a dipper receptacle, occupying a 5.2 x 5.2 x 3.8 cm (l x w x h) recessed space, into which a dipper arm could provide 0.1 ml of sucrose solution when raised. Retractable response levers were featured on either side of the dipper receptacle, approximately 5 cm above the rod floor. White 28 V DC (100 mA) lamps were located 3 cm above each lever, hereafter referred to as the right and left lever lights. Two external 28 V DC (100 mA) lamps were also located above the conditioning chamber, but within the sound attenuating cubicle, hereafter referred to as the house-light. An infrared emitter/detector unit, positioned 4 cm above the rod floor, bisected the chamber 14.5 cm from the sidewall featuring the dipper receptacle and functioned to monitor chamber crosses (locomotor activity) during experimental sessions. Data collection and presentation of experimental events were controlled via personal computer with Med Associates interface and software (MedPC for Windows, IV) located in the same room as the chambers.

Drugs

(-)-Nicotine hydrogen tartrate (0.4 mg/kg, 5 min injection-to-placement interval; IPI), bupropion hydrochloride (10 and 20 mg/kg; 15-min IPI), and varenicline dihydrochloride (0.1 and 1.0 mg/kg; 30-min IPI), SCH-23390 (10 and 30 µg/kg; 45-min IPI), eticlopride hydrochloride (10 and 30 µg/kg; 45-min IPI), dihydro-β-erythoidine

(DHβE; 1.0 and 3.0 mg/kg; 45-min IPI), and methyllycaconitine (MLA; 3.0 and 10 mg/kg; 45-min IPI) (Sigma or NIDA [RTI]) were each mixed in 0.9% saline and injected in volumes of 1 mL/kg of body weight. As is standard in the field, nicotine doses are reported as base form; all other drug doses were salt form. The pH for nicotine was adjusted to 7.0±0.2 with a NaOH solution. All doses and IPIs were based on published research, including previous work from our laboratory on interoceptive conditioning (Reichel et al., 2010; Wilkinson et al., 2010). Please note that bupropion, SCH-23390, and eticlopride were only used in Experiment 1, and varenicline, DHβE, and MLA were only used in Experiment 2. Nicotine was injected subcutaneously, all other drugs were injected intraperitoneally.

Acquisition

Rats were trained to lever press over four "auto-shaping" sessions using 26% (weight/volume) liquid sucrose (Brown and Jenkins, 1968; as described in Charntikov et al., 2013). Auto-shaping sessions began with the random insertion of one of the two response levers. After either a lapse of 15 s or a lever press, the response lever was immediately retracted and the dipper arm was raised for 4 s. Following a variable length time out (average 60 s, range = 30-90 s), the opposite lever was inserted into the chamber initiating a new auto-shaping trial as just described. The lever inserted on odd numbered trials was always randomly determined, and the opposite lever always followed on even numbered trials. Thus, over these 60 trial sessions, each lever was inserted 30 times but was never presented more than 2 times in succession. Each session was conducted in continuous house-light illumination and no other stimuli were presented during these sessions.

Over the following 10 days, rats were trained to lever-press maintained by reinforcement by visual stimuli (VS), consisting of 60 s termination of houselight illumination compounded with 5 s illumination of lever cue lights. Active and inactive lever assignments were pseudo-randomly determined and counterbalanced. Sessions were 60 min, conducted daily during the light phase of a 12:12 light dark cycle. Across all 10 sessions, VS reinforcement was delivered according to a fixed ratio (FR) 1 schedule (1 response:reinforcer) for responding on the active lever; responses on the inactive lever were recorded but produced no programmed consequences. Active and inactive lever assignments were pseudo-randomly determined and counterbalanced across rats (within the sexes). In order to familiarize the rats to injection procedures and to provide sufficient nicotine pre-exposure to minimize the response and locomotor suppressant effects of nicotine, each rat received an injection of saline 5 min preceding placement into the chamber and an injection of nicotine 15 min following termination of each session.

Reinforcer Demand Assessment

Following the tenth day of FR1 training on the VS reinforcement procedure, rats continued to lever-press maintained by VS reinforcement in 60-min sessions as described above, only the response requirement (i.e. fixed ratio (FR) schedule) was systematically increased over blocks of 16 sessions. The sequence of response costs followed an exponential base 2 sequence ranging from FR 1 to FR 512. Over different sessions within each FR block, rats received injections of 0.9 % saline, nicotine, and bupropion (Experiment 1) or varenicline (Experiment 2) prior to placement in the apparatus. Sessions proceeded with the restriction that each drug condition was experienced once before repeating and no drug condition was experienced two days in succession. Each

drug condition was experienced four times within each FR block, but only the last three were included in analyses to capture stable performance on each reinforcement schedule. Demand assessment continued for each rat until the last session of FR 512 or until the last session of a FR block in which a rat the mean number of VS presentations earned is less than 1 across all drug conditions.

Progressive Ratio Performance

This phase began 24 h after the last demand assessment session. Over a single session, lever pressing was reestablished via 1 h auto-shaping sessions using 26% liquid sucrose. Over the next 15 sessions, responding for VS stabilized on a progressive ratio (PR) schedule of reinforcement. The PR sequence followed an exponential base 2 sequence in one-third logarithmic steps, rounded to the nearest whole number (i.e. 2, 3, 4, 5, 6, 8, 10, 13, 16, 20, 25, 32, etc.). This sequence was chosen because it included the ratios experienced in the demand assessment phase and progressed slowly enough so as to minimize ratio strain in the beginning of ratio progression. This progression also afforded the possibility of encountering schedules as high as, or higher than, each rat's breakpoint schedule in the demand assessment phase. Rats received injections of saline, nicotine, bupropion (Experiment 1) or varenicline (Experiment 2) preceding each experimental session as described above.

Antagonist Testing on Progressive Ratio Performance

Over 36 sessions, rats continued to respond on the PR schedule described above. Over these sessions, rats received an injection of the specific antagonist respective to their experiment (SCH-23390, eticlopride, DHβE, or MLA) followed by administration of saline, nicotine, or a single dose of the experiment-specific nAChR agonist (bupropion

or varenicline). Each antagonist compound was assessed at two different doses and against a saline benchmark, and in combination with saline, nicotine, or bupropion/varenicline across two determinations, requiring 18 days of testing for each antagonist. Within each experiment, testing with one antagonist was completed before testing began with the next antagonist. The specific antagonist doses selected were based on previous research indicating differential effects of each compound on goal-directed behavior (Palmatier et al., 2009; Liu et al., 2010; Struthers et al., 2009; Wooters, Bevins and Bardo, 2009).

Dependent Measures and Analyses

The computers recorded number of presses on both the active and inactive levers, general locomotor activity via breaks of the centrally bisecting infrared beam (see Apparatus section), and the number of VS presentations earned within each session. In the demand assessment phases of each experiment, locomotor activity and total lever-presses on the active and inactive levers were averaged over the last three days of each drug condition from each FR schedule block, so as to represent only stable responding on each FR schedule. Each of these measures were analyzed using 3-factor mixed-measures ANOVA with Sex as a between-subjects factor and Drug condition and Unit Cost (i.e. FR schedule) as within-subject factors. Because males tended to exit the demand assessment phase earlier than females by meeting breakpoint criteria on lower FR schedules, only data from those FR schedules representing all the rats in each group were included in analyses (i.e. up to FR 32 for both experiments). *A priori* pairwise comparisons were conducted on the effects of Sex within each condition of Drug, the effects of Drug within each condition of Sex, and the effects of Drug within each condition of Unit Cost.

Additional post-hoc pairwise comparisons were also conducted upon detection of other significant interactions where appropriate. All pairwise comparisons employed Fisher's LSD test with significance set at $p<0.05$. Significant main effects of Drug or Unit Cost, and significant interactions were followed up with appropriate simplified ANOVA and Fisher's LSD contrasts with significance criteria set at $p<0.05$.

The number of VS presentations earned over sessions of the demand assessment phases of each experiment was analyzed using the exponential reinforcer demand model proposed by Hursh and Silberberg (2008) and the values of Q_0, P_{max}, O_{max} and α were calculated from the model fits for each rat individually using nonlinear least squares regression. Analyses on the effects of nicotine and bupropion/varenicline on the parameters of the reinforcer demand model used 2-factor, mixed measures ANOVA with Drug and Sex as independent variables. *A priori* comparisons were then conducted on the effects of Drug within each condition of Sex, and the effects of Sex within each condition of Drug. All planned comparisons used Fisher's LSD tests with significance set at $ps<0.05$.

The primary measures of interest during the antagonist testing phases of each experiment were the total number of active lever-presses and the total number of locomotor beam breaks emitted in test sessions under the PR schedule of reinforcement. The number of VS presentations earned is another potential measure than can be used on PR schedules, but it is redundant with and not as sensitive as active lever-pressing rates. Analyses of active lever-pressing and locomotor activity measures over the antagonist testing sessions were conducted using 3-factor, mixed measures ANOVA with Sex as a between-subjects factor and Antagonist Dose and Drug conditions as within-subjects

factors. The datasets from each antagonist testing phase (SCH-23390, eticlopride, DHβE, or MLA) were analyzed in separate analyses. *A priori* comparisons were conducted on the effects of antagonist dose within each drug condition of each sex; the effects of drug condition within each Sex in the absence of antagonist (i.e. the saline control condition of Antagonist Dose); and the effects of Sex within each drug condition in the absence of antagonist. Additional post-hoc pairwise comparisons were conducted upon detection of additional significant interactions where appropriate. All pairwise comparisons employed Fisher's LSD test with significance set at $ps<0.05$.

Chapter 4: Bupropion and Dopamine Receptor Antagonism

In Experiment 1, we investigated the role of the D1 and D2 families of dopamine receptors in the reward enhancing effects of nicotine and bupropion in male and female rats. Bupropion has been shown to exert a value-enhancement effect pharmacologically distinct from that of nicotine (Palmatier et al., 2009). However, nicotine and bupropion increase dopamine release in nucleus accumbens, an effect frequently implicated as a mechanism of reward (Wise, 1987). The role of dopamine receptors in the effects of nicotine and bupropion to enhance the value of other primary reinforcers has not been investigated (but see Guy and Fletcher, 2014). Moreover, the value-enhancing effects of bupropion have not been investigated using a technique that yields a quantitative assessment of reward along multiple behavioral dimensions of reinforcement value. In Experiment 1, we applied the reinforcer demand model and the PR performance model to characterize the value-enhancing effects of nicotine and bupropion in reference to each other and to saline. We also investigated the role of the D1 and D2 families of dopamine receptors as mechanisms of value-enhancement by both nicotine and bupropion using the PR performance model. Furthermore, we explored the possibility of sex differences in the value-enhancing effects of nicotine and bupropion, or in the involvement of D1- and D2-family dopamine receptors in the expression of value-enhancement by these agents.

There are multiple reasons to suspect a role of D1- or D2-like dopamine receptors in the reward-enhancing effects of nicotine and bupropion. First, both receptor families are found in high densities in the nucleus accumbens, and dopamine transmission in this area is strongly associated with reward (Wise, 1987; Berridge and Robinson, 1998). In nicotine self-administration, blockade of either D1 or D2 receptors decreased nicotine

intake (Corrigall and Coen, 1991); this effect may reflect a blunting of nicotine primary reinforcement or nicotine enhancement of infusion-related cues. Additionally, antagonism of either D1 or D2 receptors also attenuated cue-induced reinstatement of nicotine seeking in rats (Liu et al., 2010). Furthermore, antagonism of D1 or D2-like receptors in the nucleus accumbens did not alter expression of nicotine-induced conditioned place preference, but D1 antagonism in the nucleus accumbens shell impaired acquisition of nicotine-induced conditioned place preference (Spina et al., 2006). Ohmura and colleagues (2011) found that administration of D1-family and D2-family agonist differentially attenuated somatic signs of nicotine withdrawal in rats. Lastly, Guy and Fletcher (2014) found that antagonism of either D1 or D2-like receptors decreased responding for a water-associated conditioned reinforcer and attenuated nicotine-induced increases in responding for conditioned reinforcement. Together, these studies implicate a role of D1- and D2-family dopamine receptors in the motivational properties of nicotine, including value-enhancement. Furthermore, bupropion acts as a partial antagonist of nAChRs, but also inhibits dopamine reuptake (Dwoskin et al., 2006; Carroll et al., 2014). Given that bupropion also enhances operant behavior in a manner analogous to nicotine (Palmatier et al., 2009), these observations suggest that activation of D1- or D2-like dopamine receptors may, in part, underlie the value-enhancing effects of bupropion.

Procedures

Experiment 1 followed the general procedural design described in Chapter 3, with the specifications described hereafter. In the reinforcer demand assessment phase, 0.4 mg/kg nicotine, 10 or 20 mg/kg bupropion, or saline were administered preceding session

initiation at their respective IPIs (see Drugs section in Chapter 3). In the antagonist testing phases, only the 20 mg/kg dose of bupropion was tested; this dose produced robust levels of enhancement in both male and female rats and provided a suitable baseline for assessing the effects of dopamine antagonist to decrease enhanced responding. Additionally, the D1-family receptor antagonist, SCH-23390, and the D2-family receptor antagonist, eticlopride, were used in the antagonist testing phases of Experiment 1.

Results - Lever Pressing and Locomotor Activity Across Escalating FR Schedules

All rats acquired the lever-press response over the 4 auto-shaping sessions and continued to press at moderate rates by the end of the 10-session VS training phase. Administration of nicotine and either dose of bupropion each increased lever-pressing rates relative to saline in both males and females across all FR schedules. Figure 1 displays mean active and inactive lever-pressing between the sexes over the terminal 3 sessions of each drug condition within each FR-schedule session-block. Analysis of active lever-pressing via 3-factor ANOVA (Sex x Drug x Unit Cost) revealed significant main effects of Sex [$F(1,22)=5.200$; $p=0.0326$], Drug [$F(3,66)=44.44$; $p<0.0001$], and Unit Cost [$F(5,110)=19.53$; $p<0.0001$]. The Sex x Drug [$F(3,66)=5.892$; $p=0.0013$], Sex x Unit Cost [$F(5,110)=2.806$; $p=.0200$], and Drug x Unit Cost interactions [$F(15,330)=10.38$; $p<0.0001$] were each significant. Further investigation of the Sex x Drug interaction found equivalently increased active lever-pressing rates under nicotine and both bupropion dose conditions relative to saline in females. In contrast, bupropion enhanced response rates intermediately between saline and nicotine levels in males at either dose [ps<0.05]. Additionally, active lever-pressing by females was significantly

higher than males across all drug conditions, including saline [*p*s<0.05]. Analysis of the Drug x Unit Cost interaction found that nicotine increased active lever-pressing relative to saline on all FR schedules except FR 1; both doses of bupropion likewise increased active-lever pressing relative to saline but did not begin to do so significantly until FR 4 and higher. Active lever-pressing was significantly lower in both bupropion conditions compared to nicotine on FR 32, and 10 mg/kg bupropion was lower than 20 mg/kg bupropion and nicotine on FR 16 [*p*s<0.05]. Finally, the Sex x Unit Cost interaction was characterized by significantly higher active lever-pressing in females than males on FRs 16 and 32, but not any of the lower FR schedules [*p*s<0.05].

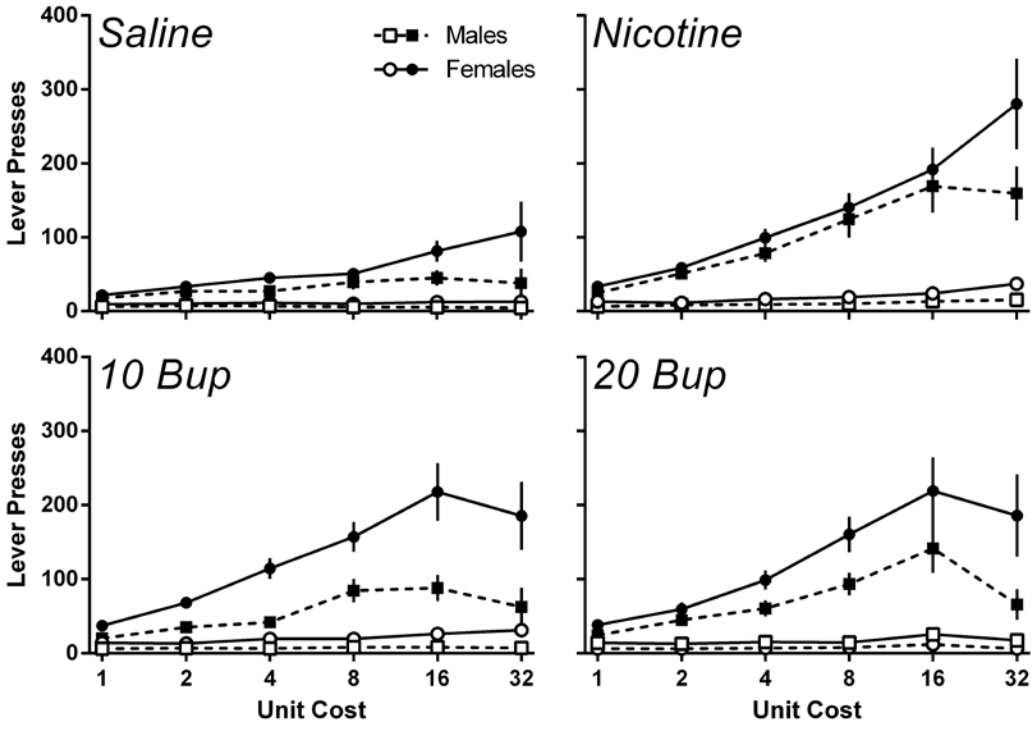

Figure 1. Mean number of presses on the active (filled symbols) and inactive levers (open symbols) averaged over the terminal 3 sessions of each drug condition within blocks of FR schedules. Only data from those FR schedules where all the rats in both sexes remained in the demand assessment phase prior to meeting breakpoint criteria were included. Error bears represent ± 1 SEM.

Nicotine and bupropion also increased inactive lever-pressing, but not nearly to the extent of active lever-pressing, as is shown in Figure 1. Analysis of inactive lever-pressing revealed significant effects of Sex [$F(1,22)=20.80$; $p=0.0002$], Drug [$F(3,66)=13.28$; $p<0.0001$], and Unit Cost [$F(5,110)=4.671$; $p=0.0007$]. The interactions between Sex and Drug [$F(3,66)=4.664$; $p=0.0052$], and between Drug and Unit Cost [$F(15,330)=5.918$; $p<0.0001$] were also statistically significant. Further analysis of the Sex x Drug interaction found that inactive lever-pressing was increased only by nicotine in males, whereas nicotine and both doses of bupropion increased inactive lever-pressing in females [ps<0.05]. In addition, inactive lever pressing was significantly higher in females at each condition of drug, including saline [ps<0.05]. Investigation of the Drug x Unit Cost interaction found that nicotine increased inactive lever-pressing above the saline condition on FR 8 through FR 32, above both bupropion conditions on FR 32, and additionally above 20 mg/kg bupropion on FR 8 [ps<0.05]. Bupropion at 10 mg/kg increased inactive lever-pressing relative to saline on FR 4 through FR 32, and above 20 mg/kg bupropion on FR 32 [ps<0.05]. Lastly, the 20 mg/kg bupropion increased inactive lever-pressing relative to saline only on FR 16 [$p<0.05$].

The effects of nicotine, bupropion, and unit cost progression on locomotor activity are shown in Figure 2. Analysis revealed significant effects of Sex [$F(1,22)=9.527$; $p=0.0054$], Drug [$F(3,66)=65.86$, $p<0.0001$] and Unit Cost [$F(5,110)=3.267$; $p=0.0087$] on locomotor activity, as well as significant Sex x Drug [$F(3,66)=14.27$; $p<0.0001$] and Sex x Unit Cost interactions [$F(5,110)=6.175$; $p<0.0001$]. Follow-up analysis on the Sex x Drug interaction revealed significant differences in locomotor activity between each of the drug conditions in females, with increases above

saline levels in the nicotine condition and further increases above nicotine by bupropion in a dose-dependent manner [*p*s<0.05]. In males, nicotine did not increase locomotor activity above saline levels, but activity was higher than both saline and nicotine in both bupropion conditions [*p*s<0.05]; no differences in activity between bupropion doses were detected [*p*s>0.05]. In addition, higher locomotor activity in females was observed in the 10 and 20 mg/kg bupropion conditions, but no sex differences in locomotor activation by nicotine or saline were detected [*p*s<0.05]. The effects on locomotor activity of drug condition at different levels of unit cost were inconsistent. Briefly, nicotine increased locomotor activity relative to saline on FR 1 and FR 4; 10 mg/kg bupropion relative to saline on FR 1, and 20 mg/kg bupropion relative to saline on FR 1 through FR 16 [*p*s<0.05]. The Sex x Unit Cost interaction was characterized by significant differences in locomotor activity between males and females on all FR schedules that became increasingly large following progressions in Unit Cost [*p*s<0.05]. This effect likely resulted from an trend toward increasing locomotor activity in females rats with increases in Unit Cost in contrast with no apparent effects of Unit Cost on locomotor activity in males [*p*s<0.05].

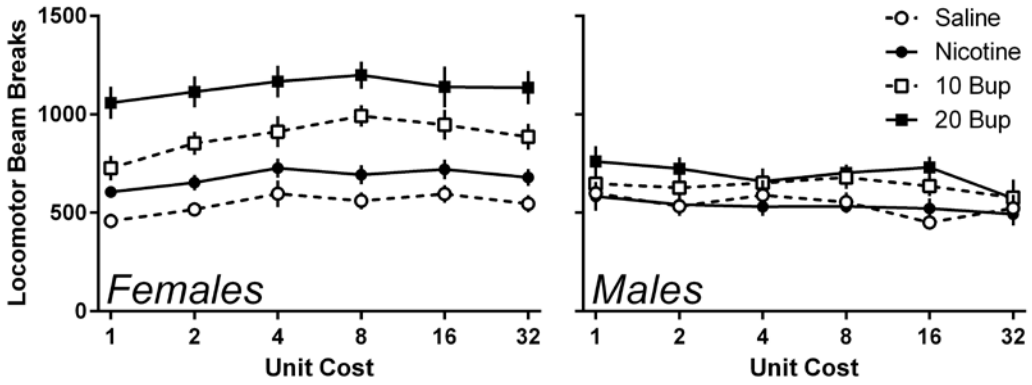

Figure 2. Mean locomotor activity as measured by breaks of the chamber bisecting infrared beam averaged across the terminal 3 sessions of each drug condition within session blocks of each FR schedule. Only data from those FR schedules where all the rats in both sexes remained in the demand assessment phase prior to meeting breakpoint criteria were included. Error bears represent ± 1 SEM.

Results - Analysis of Demand for VS

Figure 3 presents the demand functions between the sexes for VS reinforcement under saline, nicotine and both bupropion dose conditions. Fits of the reinforcer demand model to the male and female group data are also presented as representative data; all analyses exclusively used fits to the data of individual rats. Because individual rats exited the demand assessment phase upon reaching breakpoint criteria at different FR schedules, not all of the data are presented in Figure 3; only data from those FR schedules where at least a quarter of the rats of each sex remained in the demand assessment phase are presented (FR 128 for males; FR 512 for females).

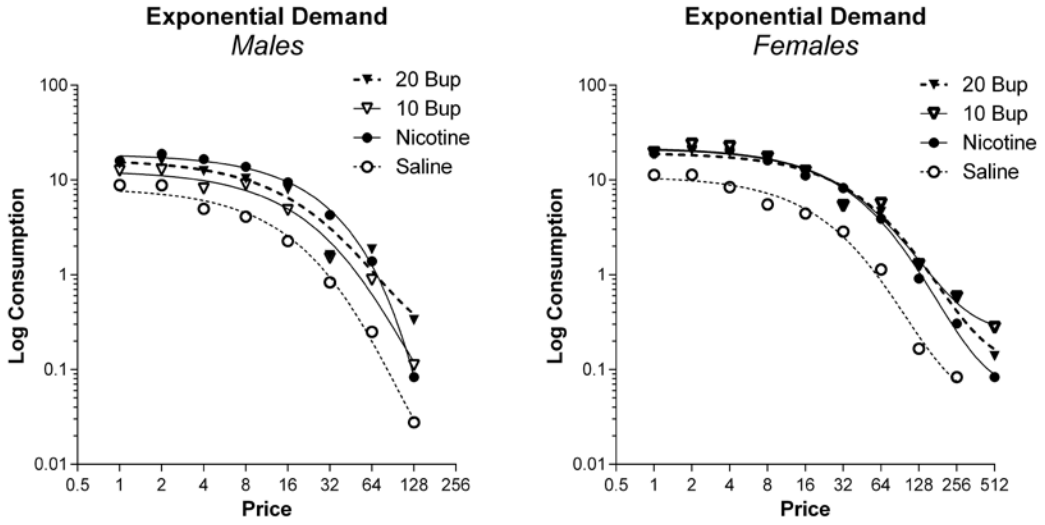

Figure 3. VS consumption as a function of FR schedule between males (left) and females (right), and across the four administration conditions of nicotine (filled circles), saline (open circles), 10 bupropion (open triangles), and 20 bupropion (closed triangles).

In the females, nicotine and both doses of bupropion increased VS consumption to similar levels across all FR schedules except the highest FR schedules, where both doses of bupropion comparably increased consumption to levels above nicotine. Nicotine and both doses of bupropion also increased VS consumption relative to saline in the males, but in contrast to the females, bupropion dose dependently enhanced responding to levels slightly below those of the nicotine condition. In addition, the males completed the demand assessment phase by reaching breakpoint criteria earlier than the females; 50% of the males had reached breakpoint criteria by FR 128, whereas the females reached the same condition at FR 256. Indeed, VS consumption in the females was consistently higher than in the males across all drug conditions throughout the demand assessment phase, as more clearly shown in Figure 4.

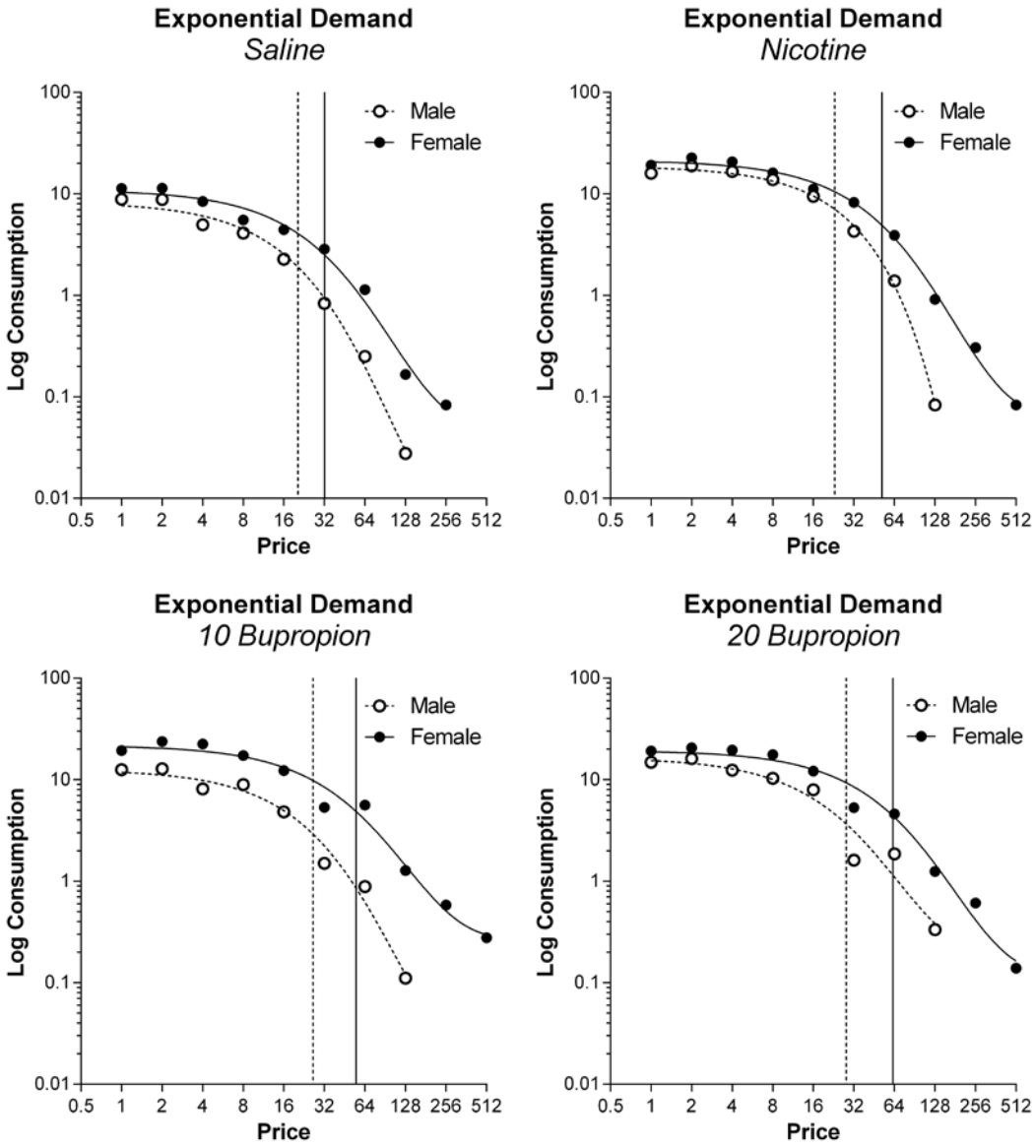

Figure 4. VS consumption between the sexes and across drug conditions as a function of FR schedule. Males are represented by open circles; females by the filled circles. The P_{max} estimates from the group-fitted demand curves are represented by the vertical lines (dashed=males, solid=females).

The values of Q_0, P_{max}, O_{max} and α were obtained via fits of the reinforcer demand model to the data of individual rats, and were each analyzed via two-factor, mixed-measures ANOVA with Sex and Drug as factors with Fisher's LSD used for *a priori* planned pairwise comparisons. The estimates of Q_0 for males and females and across drug conditions are presented in Figure 5. Analysis of the Q_0 parameter revealed a

significant effect of Drug [F(3,66)=25.07; p<0.0001], but not of Sex [F(1,22)=2.633; p=0.1189] or a Sex x Drug interaction [F(3,66)=2.388; p=0.0768]. Pairwise comparisons revealed nicotine and both doses of bupropion increased Q_0 above saline levels in both the sexes [ps<0.05]. In females, neither bupropion dose differed from nicotine; in males 20 mg/kg bupropion did not differ from nicotine, but 10 mg/kg bupropion differed from nicotine and 20 mg/kg bupropion [ps<0.05]. Thus, 10 mg/kg bupropion increased Q_0 to moderate levels that were intermediate the enhancing effects of nicotine and 20 mg/kg bupropion in males, whereas both doses of bupropion and nicotine all increased Q_0 to similar levels in females. Consequently, the only drug condition where Q_0 significantly differed between the sexes was 10 mg/kg bupropion, where enhancement was greater in females than males [p<0.05].

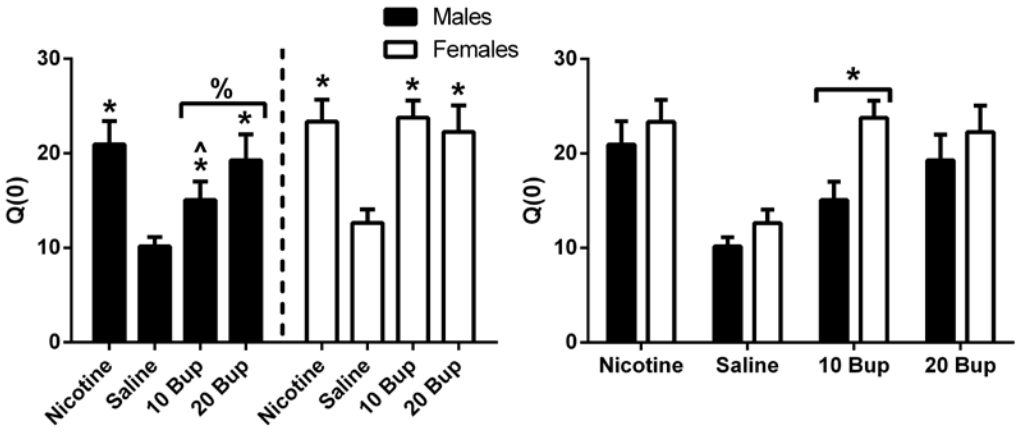

Figure 5. Estimates of Q_0 derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from saline. ^ indicates a significant difference from nicotine. % represents a difference between bupropion conditions. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

Estimates of P_{max} across drug conditions and between the sexes are shown in Figure 6. Analysis revealed significant main effects of Sex [F(1,22)=5.606, p=0.0271] and of Drug [F(3,66)=9.066, p<0.0001], but not a Sex x Drug interaction [F(3,66)=1.992,

p=0.1237]. Further investigation revealed that nicotine and both doses of bupropion increased estimates of P_{max} in females relative to saline; for males, only nicotine increased P_{max} relative to saline [*p*s<0.05]. Comparing between the sexes, estimates of P_{max} were higher in females in the nicotine condition and the 10 and 20 mg/kg bupropion conditions [*p*s<0.05], but no differences were observed between the saline conditions between the sexes [*p*>0.05].

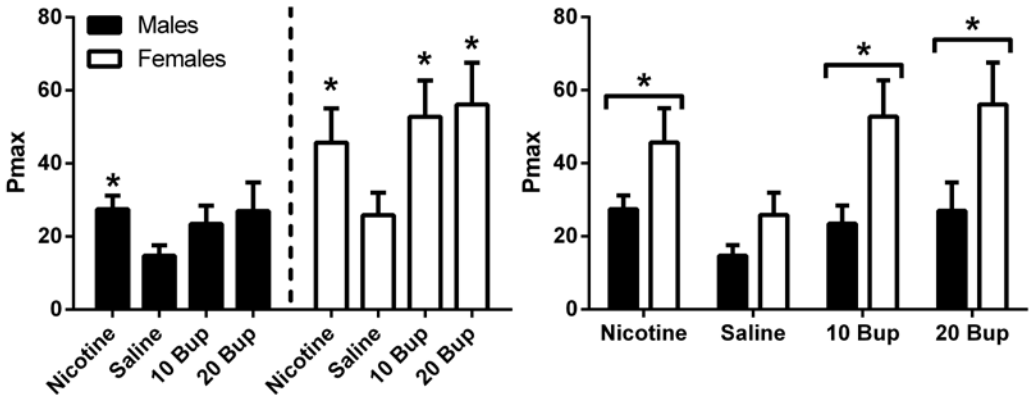

Figure 6. Estimates of P_{max} derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from the saline condition for each sex. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

The model-derived estimates of O_{max} from the demand assessment phase are portrayed in Figure 7. Statistical analysis revealed significant main effects of Sex [F(1,22)=10.30, *p*=0.0104] and of Drug [F(3,66)=18.59, *p*<0.0001], as well as a significant Sex x Drug interaction [F(3,66)=6.344, *p*=0.0008]. Post-hoc comparisons found significant increases in O_{max} under nicotine and both bupropion conditions relative to saline in females; nicotine and 20 mg/kg bupropion increase values of O_{max} relative to saline in males [*p*s<0.05]. Between the sexes, O_{max} values were higher in females under

nicotine and both bupropion conditions, but not following saline administration [*p*s<0.05].

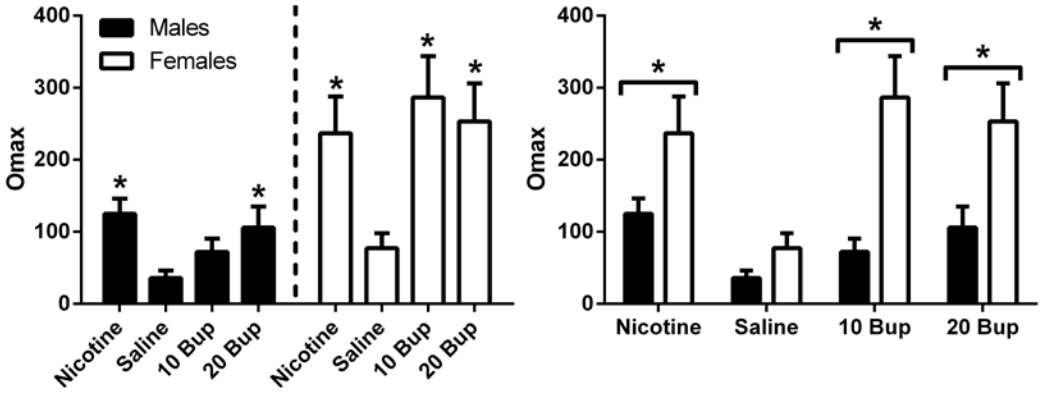

Figure 7. Estimates of O_{max} derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from saline. ^ indicates a significant difference from nicotine. % represents a difference between bupropion conditions. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

Finally, the estimates of the essential value parameter, α, estimated by the reinforcer demand model are displayed in Figure 8. Analysis revealed a significant main effect of Drug [F(3,66)=34.47, *p*<0.0001], but not of Sex [F(1,22)=2.111, *p*=0.1603] and not significant Sex x Drug interaction [F(3,66)=1.663 , *p*=0.1834]. Post-hoc comparisons revealed significant enhancement of essential value relative to saline by nicotine and both bupropion doses in males and females [*p*s<0.05]. There were no differences in essential value between nicotine and either bupropion condition in females. In males, essential value was greater (i.e. lower estimates of α) in the nicotine condition than the 10 mg/kg bupropion condition [*p*s<0.05]. When comparing between the sexes, females showed greater essential value of VS reinforcement than males in both bupropion conditions and in the saline condition [*p*s<0.05].

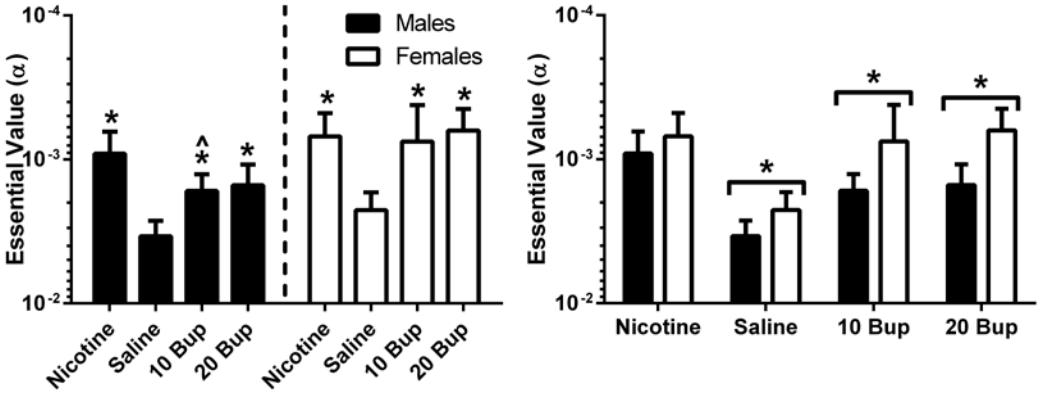

Figure 8. Estimates of essential value parameter (α) derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from saline. ^ indicates a significant difference from nicotine. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

The demand assessment phase revealed that nicotine and bupropion exert value-enhancing effects on responding maintained by VS. This enhancement was observed in both males and females, though some differences between the sexes were evident regarding the enhancing effects of bupropion in particular. Specifically, females showed greater sensitivity to the value-enhancing effects of bupropion than males. Consider the observation that in females, bupropion at either dose, enhanced Q_0, P_{max}, O_{max}, and essential value to similar levels as nicotine. However, in males, enhancement by bupropion showed dose-dependency. For example, 10 mg/kg bupropion enhanced the Q_0 and essential value parameters to levels intermediate to saline and nicotine, whereas the 20 mg/kg dose produced enhancement indistinguishable from nicotine. Notably, females showed greater enhancement by bupropion than males on all model estimates but Q_0 at 20 mg/kg, which failed to reach statistical significance but continued to show a tendency towards greater enhancement in females. Taken together, the results of demand

assessment phase suggest that while nicotine and bupropion produce value-enhancement, females may be more sensitive to the value-enhancing effects of bupropion than males.

Results - D1 Antagonism on Progressive Ratio Performance

Active lever-pressing maintained by the PR schedule of VS reinforcement during SCH-23390 testing phase is shown in Figure 9. Nicotine and bupropion both increased active lever-pressing relative to saline in each sex, and systemic antagonism of the D1 receptor family with SCH-23390 attenuated this effect. Analysis revealed significant main effects of Sex [$F(1,22)=5.994$; $p=0.0228$], Drug [$F(2,44)=38.35$; $p<0.0001$], and Antagonist Dose [$F(2,44)=17.53$; $p<0.0001$], as well as significant Sex x Drug interaction [$F(2,44)=7.985$; $p=0.0011$]. Planned comparisons found that 30 µg/kg SCH-23390 decreased active lever-pressing in both sexes under nicotine and bupropion treatment conditions, and also under the saline condition in females [ps<0.05] (Figure 9, left panel). Active presses were also reduced in males following antagonism with 10 µg/kg SCH-23390 under nicotine and bupropion treatment conditions [ps<0.05]. Additionally, SCH-23390 dose-dependency was observed only in males on the nicotine condition [ps<0.05]. Further analysis revealed that nicotine and bupropion enhanced lever-pressing to similar levels in the absence of SCH-23390 [ps<0.05]. Finally, response rates on PR were significantly higher in females at each drug condition in the absence of SCH-23390 [ps<0.05] (Figure 9, right panel).

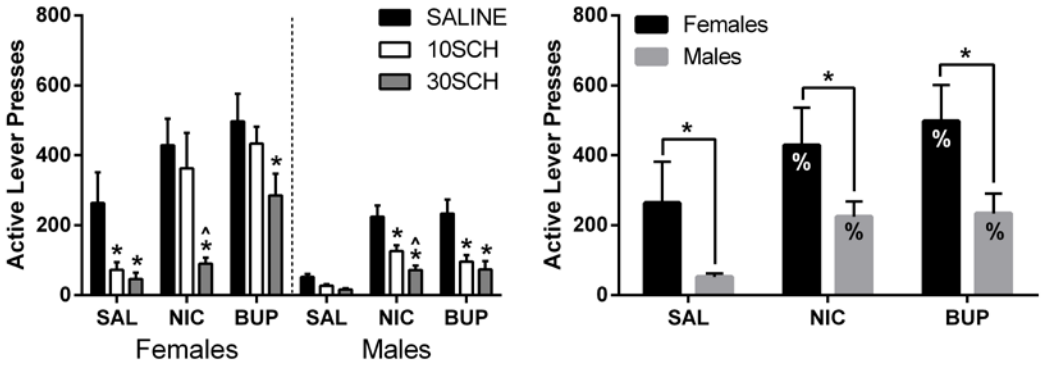

Figure 9. Active lever-pressing across the sessions of the D1 antagonism testing phase using SCH-23390. Left: Active lever-pressing between the sexes, averaged across the 2 determinations of each of the SCH-23390 test conditions. * represents a significant difference from the saline control condition of antagonist dose within a drug condition. ^ represents a significant difference between doses of SCH-23390 within a drug condition. Right: The effects nicotine and bupropion on active lever-pressing between the sexes in the absence of SCH-23390. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. ^ represents a significant difference from the nicotine condition within each sex. Error bears represent ± 1 SEM.

Figure 10 shows measures of locomotor activity across sessions of the SCH-23390 testing phase. Analysis uncovered significant effects of Sex [$F(1,22)=13.57$; $p=0.0013$], Drug [$F(2,44)=21.33$; $p<0.0001$], and Antagonist Dose [$F(2,44)=30.75$; $p<0.0001$]. The Sex x Drug [$F(2,44)=8.927$; $p=0.0006$] and Sex x Antagonist Dose interactions [$F(2,44)=4.875$; $p=0.0122$] were also significant. Planned comparisons found decreases in locomotor activity by 30 µg/kg SCH-23390 under the nicotine and bupropion conditions in both sexes, and under the saline condition in females [$ps<0.05$] (Figure 10, left panel). Further investigation of the effects of drug condition in the absence of SCH-23390 uncovered significant increases in locomotor activity following bupropion administration in females. There were no effects of nicotine or bupropion on locomotor activity relative to saline in males, but significantly higher activity in the bupropion condition than the nicotine condition in males [$ps<0.05$]. Locomotor activity was significantly higher in females compared to males in the nicotine and bupropion

conditions in the absence of SCH-23390 [*p*s<0.05] (Figure 10, right panel). Finally, analysis of the Sex x Antagonist Dose interaction found significantly depressed locomotor activity by 30 µg/kg SCH-23390 relative to saline in both sexes, but locomotor depression was greater in females than in males [*p*s<0.05].

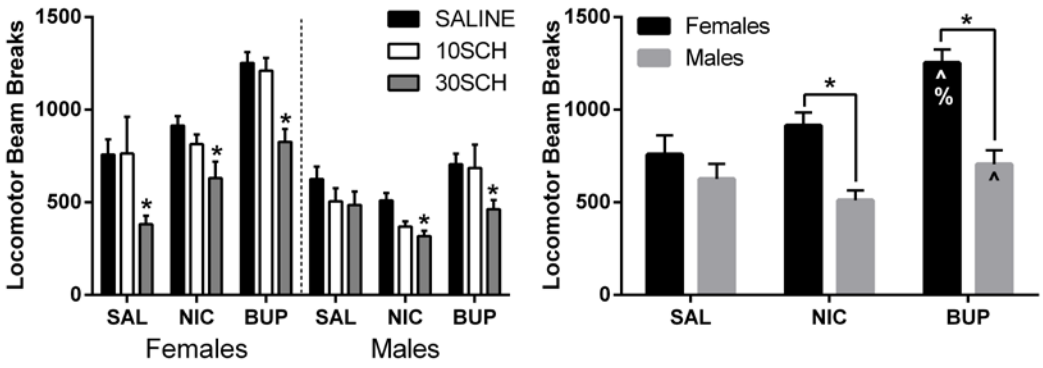

Figure 10. Locomotor activity across the sessions of the D1 antagonism testing phase using SCH-23390. Left: Locomotor activity between the sexes, averaged across the 2 determinations of each of the SCH-23390 test conditions. * represents a significant difference from the saline control condition of antagonist dose within a drug condition. ^ represents a significant difference between doses of SCH-23390 within a drug condition. Right: The effects nicotine and bupropion on locomotor activity between the sexes in the absence of SCH-23390. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. ^ represents a significant difference from the nicotine condition within each sex. Error bears represent ± 1 SEM.

Results - D2 Antagonism on Progressive Ratio Performance

Active lever-pressing across the sessions of antagonist testing with eticlopride is shown in Figure 11. Mixed-measures ANOVA revealed significant effects of Sex [F(1,22)=7.845; *p*=0.0104], Drug [F(2,44)=29.22; *p*<0.0001], and Antagonist Dose [F(2,44)=18.35; *p*<0.0001]. The Sex x Drug [F(2,44)=6.779; *p*=0.0027] and Drug x Antagonist Dose interactions [F(4,88)=3.443; *p*=0.0116] were also significant. Planned pairwise comparisons revealed significant decreases in active lever-pressing by 30 µg/kg eticlopride in the bupropion condition of both sexes; 10 µg/kg eticlopride also decreased bupropion-enhanced responding in the males, but not in females [*p*s<0.05] (Figure 11,

left panel). Statistical investigation of the effects of nicotine and bupropion in the absence of eticlopride (Figure 11, right panel) found significantly higher responding in females across all drug conditions, as in the preceding D1 antagonist testing phase [*p*s<0.05]. Additionally, nicotine and bupropion increased lever-pressing to similar levels in males, but levels of bupropion-enhanced responding were higher than those of nicotine in females [*p*s<0.05]. Investigation of the Drug x Antagonist Dose interaction found significantly decreased responding by 30 µg/kg eticlopride only under bupropion conditions [*p*<0.05]; eticlopride at either dose did not significantly reduce responding under the nicotine or saline conditions [*p*s>0.05].

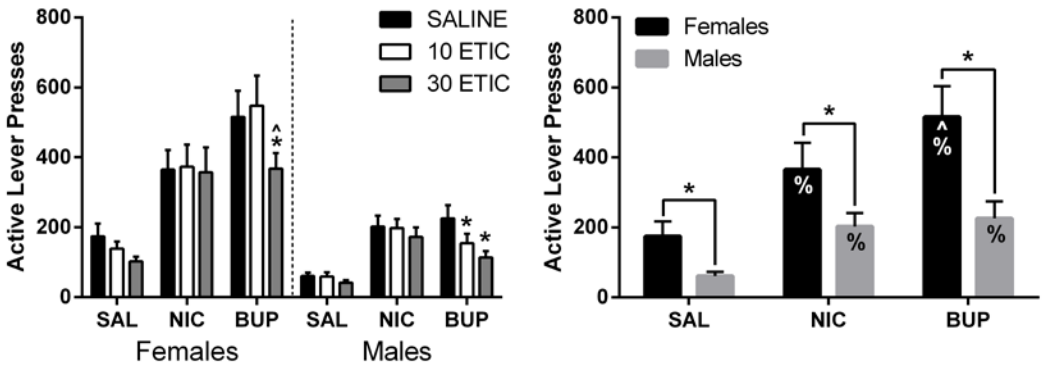

Figure 11. Active lever-pressing across the sessions of the D2 antagonism testing phase using eticlopride. Left: Active lever-pressing between the sexes, averaged across the 2 determinations of each of the eticlopride test conditions. * represents a significant difference from the saline control condition of antagonist dose within a drug condition. ^ represents a significant difference between doses of eticlopride within a drug condition. Right: The effects nicotine and bupropion on active lever-pressing between the sexes in the absence of eticlopride. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. ^ represents a significant difference from the nicotine condition within each sex. Error bears represent ± 1 SEM.

Locomotor activity over the sessions of D2 receptor antagonism testing with eticlopride is presented in Figure 12. Statistical analysis found significant effects of Sex [F(1,22)=20.13; *p*=.0002], Drug [F(2,44)=31.25; *p*<0.0001], and Antagonist Dose [F(2,44)=3.533], and a significant Sex x Drug interaction [F(2,44)=13.03; *p*<0.0001].

Eticlopride, at either dose, had no significant effects on locomotor activity within any of the drug conditions in either sex [*p*s>0.05] (Figure 12, left panel). However, the main effect of eticlopride did reveal that 30 µg/kg eticlopride decreased locomotor activity relative to saline when collapsing across the conditions of Sex and Drug [*p*<0.05]. Analysis of the effects of nicotine and bupropion on locomotor activity in the absence of eticlopride (Figure 12, right panel) discovered significantly higher activity under bupropion conditions in the females and differences between bupropion and nicotine in both the sexes [*p*s<0.05]. Locomotor activity in the females was greater than in the males at each drug condition in the absence of eticlopride [*p*s<0.05].

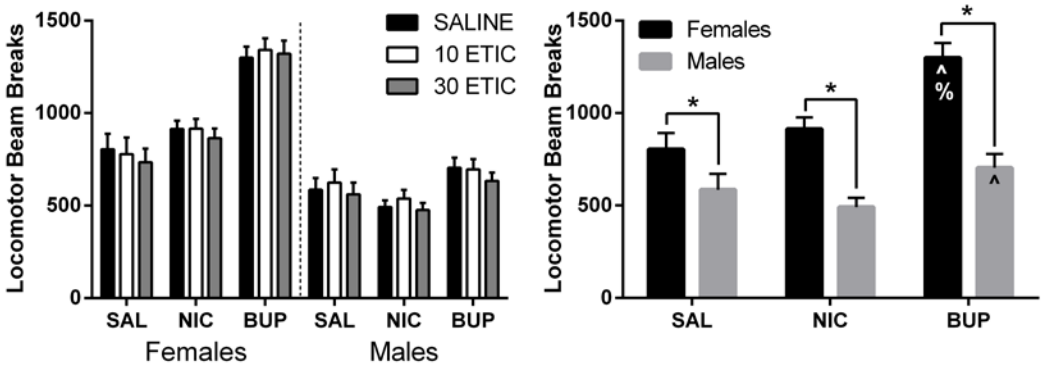

Figure 11. Locomotor activity across the sessions of the D2 antagonism testing phase using eticlopride. Left: Locomotor activity between the sexes, averaged across the 2 determinations of each of the eticlopride test conditions. * represents a significant difference from the saline control condition of antagonist dose within a drug condition. ^ represents a significant difference between doses of eticlopride within a drug condition. Right: The effects nicotine and bupropion on locomotor activity between the sexes in the absence of eticlopride. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. ^ represents a significant difference from the nicotine condition within each sex. Error bears represent ± 1 SEM.

The PR performance data reproduce the observations from the demand assessment phase that nicotine and bupropion enhance responding maintained by VS reinforcement, and that enhanced levels of responding were higher in females. Interestingly, lever-pressing was higher than females under saline conditions in the

absence of either antagonist, suggesting a tendency for females to exhibit higher lever-pressing under baseline conditions (cf. Chaudhri et al., 2005; Grebenstein et al., 2013). Additionally, the greatest sex differences in lever-pressing were reliably observed under bupropion conditions. Nicotine and bupropion also increased locomotor activity in females, but not in males. Notably, D1 antagonism with SCH-23390 decreased active lever-pressing as well as locomotor activity in both sexes and under saline conditions. In contrast, D2 receptor family antagonism via eticlopride decreased lever-pressing in the bupropion conditions in each sex, and had no effects under saline or nicotine conditions. Additionally, no effects of eticlopride on locomotor activity were detected. Together, these effects suggest a role of D2-family dopamine receptors in the value-enhancing effects of bupropion, but not of nicotine. D1-family dopamine receptors may also be involved in the reward-enhancing effects of nicotine or bupropion, but locomotor suppression by SCH-23390 makes this finding difficult to interpret.

Chapter 5: Varenicline and Nicotinic Acetylcholine Receptor Antagonism

In Experiment 2, we investigated the roles of α4β2-containing and homomeric α7 nAChRs in the reward enhancing effects of nicotine and varenicline in male and female rats. Varenicline has been shown to enhance rates of operant responding for mildly reinforcing visual stimuli in a fashion similar to nicotine, albeit to lower levels (Levin et al., 2012). Previous work has shown that antagonism of α4β2-containing receptors, but not of α7 receptors, partially attenuates the value-enhancing effects of intravenous nicotine on a FR5 schedule of VS reinforcement (Liu et al., 2007). As mentioned in Chapter 1, varenicline is a partial agonist at α4β2-containing nAChRs and a full agonist at α7 receptors. We hypothesized that the moderate value-enhancing effects of varenicline result from its action on α4β2-containing receptors, and not its α7 receptor activity, similar to nicotine. Experiment 2 was partially designed to test this hypothesis. We also designed the present experiment as a method for comparing the value-enhancing effects of varenicline and nicotine using the reinforcer demand model, which represents value along multiple behavioral dimensions (see Chapter 2). Finally, the present experiment also investigated whether the value-enhancing effects of varenicline would differ between the sexes, while simultaneously replicating our investigation of nicotine from Experiment 1.

Regarding the nAChR subtypes investigated here, there are a few important reasons for the selection of α4β2* and α7 receptors. First, α4β2* and α7 receptors are believed to be the two most common forms of the nAChR in the dopaminergic reward pathways of the midbrain (see Placzek and Dani, 2009; Brunzell and Picciotto, 2009). Previous work has shown that presentation of a DHβE-paired conditioned visual stimulus

increased current-intensity thresholds of reinforcing electrical stimulation of the medial forebrain bundle in rats (Kenny and Markou, 2006). Nicotine decreases reward thresholds in this preparation, which is congruent with the interpretation of reward-enhancement (Kenny and Markou, 2006). Additionally, administration of DHβE into the ventral tegmental area decreased nicotine self-administration in rats (Corrigall et al., 1994). Furthermore, antagonism of ventral tegmental area α7 receptors via MLA microinjection blocked nicotine-conditioned place preference (Laviolette and van der Kooy, 2003). Furthermore, high doses of MLA attenuated nicotine self-administration in rats (Markou and Paterson, 2001). Importantly, Liu et al. (2007) found that antagonism of α4β2* but not of α7 receptors attenuated enhancement of VS-maintained responding by intravenous nicotine. Similarly, antagonism of α4β2* but not of α7 receptors attenuated nicotine-enhanced responding for a water-associated conditioned reinforcer (Guy and Fletcher, 2013). Finally, administration of MLA, but not of DHβE, reduced the attenuated cue-induced reinstatement of nicotine-seeking behavior in rats, but produced no effects on nicotine self-administration or cue-induced food-seeking behavior (Liu, 2014). Together, these findings suggest a role of α4β2-containing and α7 nAChRs in the motivational effects of nicotine, but suggest that α4β2* receptors may be more directly involved in the reward-enhancing effects of nicotine. Varenicline also exerts reward-enhancing effects on responding and acts as a full agonist at α7 nAChRs and as a partial agonist at α4β2*, α3β4*, and α6β2* nAChRs (Coe et al., 2005; Foulds, 2006; Grady et al., 2010; Mihalak et al, 2006; Rollema et al., 2007). However, no previous study has investigated the receptor mechanisms of value-enhancement in varenicline.

Procedures

Experiment 2 followed the general procedures described in Chapter 3, with the following distinctions. In the reinforcer demand assessment phase, 0.4 mg/kg nicotine, 0.1 or 1.0 mg/kg varenicline, or saline were administered preceding experimental sessions at their respective IPIs (refer to Drugs section in Chapter 3). In the antagonist testing phase, only the 1.0 mg/kg dose of varenicline was tested, as were nicotine and saline. Antagonism of α4β2-containing and α7 receptors was tested using DHβE and MLA, respectively. One female rat was excluded from testing in both the antagonist testing phases because of health concerns regarding the development of tumors.

Results - Lever Pressing and Locomotor Activity Across Escalating FR Schedules

All rats acquired the lever-press response over the 4 auto-shaping sessions and continued to press at moderate rates by the end of the 10-session VS training phase. Both nicotine and varenicline increased frequency of lever-pressing behavior relative to saline in male and female rats. Figure 13 shows mean active and inactive lever-pressing, in either sex, averaged over the terminal 3 sessions of each drug condition within each FR schedule session-block. Statistical analysis of active lever-pressing revealed significant effects of Drug [$F(3,66)=72.80$; $p<0.0001$] and Unit Cost [$F(5,110)=19.62$; $p<0.0001$], but not of Sex [$F(1,22)=1.990$; $p=0.1724$]. The Drug x Unit Cost interaction was also significant [$F(15,330)=17.84$; $p<0.0001$], and further analysis found that nicotine enhanced active lever-pressing across all FR schedules relative to saline, while varenicline dose-dependently increased active pressing relative to saline on all schedules but FR1 [ps<0.05]. Nicotine-enhanced rates of active lever-pressing exceeded those of both varenicline doses on schedules FR 2 though FR 32 [ps<0.05]. Planned comparisons

on the effects of sex under each drug condition found significantly higher rates of active lever-pressing in females than in males under all but the saline condition [*p*s<0.05]. Additionally, nicotine increased lever-pressing to the highest levels in females, followed by a dose-dependent enhancement of responding by varenicline [*p*s<0.05]. Similarly, nicotine increased active lever response rates above all other conditions in males; however, varenicline enhanced responding relative to saline but did not differ show dose dependency in males [*p*s<0.05].

Nicotine and varenicline administration also had effects on inactive lever-pressing that varied with changes in FR schedule (Figure 13). Analysis revealed significant main effects of Drug [F(3,66)=11.82; *p*<0.0001] and of Unit Cost [F(5,110)=3.760; *p*=0.0035], but not of Sex [F<1]. There was also a significant Drug x Unit Cost interaction [F(15,330)=4.899; *p*<0.0001] and Sex x Drug x Unit Cost interaction [F(15,330)=2.181; *p*=0.0068]. In females, nicotine increased inactive presses relative to saline across all FR schedules, 0.1 mg/kg varenicline relative to saline on FR 4 and FR 32, 1.0 mg/kg varenicline relative to saline on all schedules but FR 4, and nicotine relative to 1.0 mg/kg varenicline on FR 16 and FR 32 [*p*s<0.05]. In males, inactive lever-pressing was increased by nicotine and 1.0 varenicline relative to saline only on schedules FR 8 through FR 32, 0.1 mg/kg varenicline compared to saline on FR 8, and nicotine relative to 1.0 varenicline on FR 16 [*p*s<0.05]. Further comparisons found that females pressed the inactive lever more than males under the nicotine and 1.0 mg/kg varenicline conditions, except on FR 4 and FR 8. [*p*s<0.05]. Inactive pressing was also higher in females following 0.1 mg/kg varenicline and saline administration on FR 2 [*p*s<0.05].

Analysis of the Drug x Unit Cost interaction found that nicotine increased inactive lever-pressing relative to saline across all FR schedules, and was higher than varenicline at the 0.1 and 1.0 mg/kg doses on schedules FR 8 through 32, and FR 16 through 32, respectively [*p*s<0.05]. Varenicline increased inactive lever-pressing at the 0.1 mg/kg dose on FR 8 and FR 32 schedules; the 1.0 mg/kg dose increased inactive lever-pressing on schedules FR 8 through 32 [*p*s<0.05]. Finally, increases in inactive lever-pressing by the 1.0 mg/kg dose of varenicline were greater than those of the 0.1 mg/kg dose on FR 16 and FR 32 [*p*s<0.05].

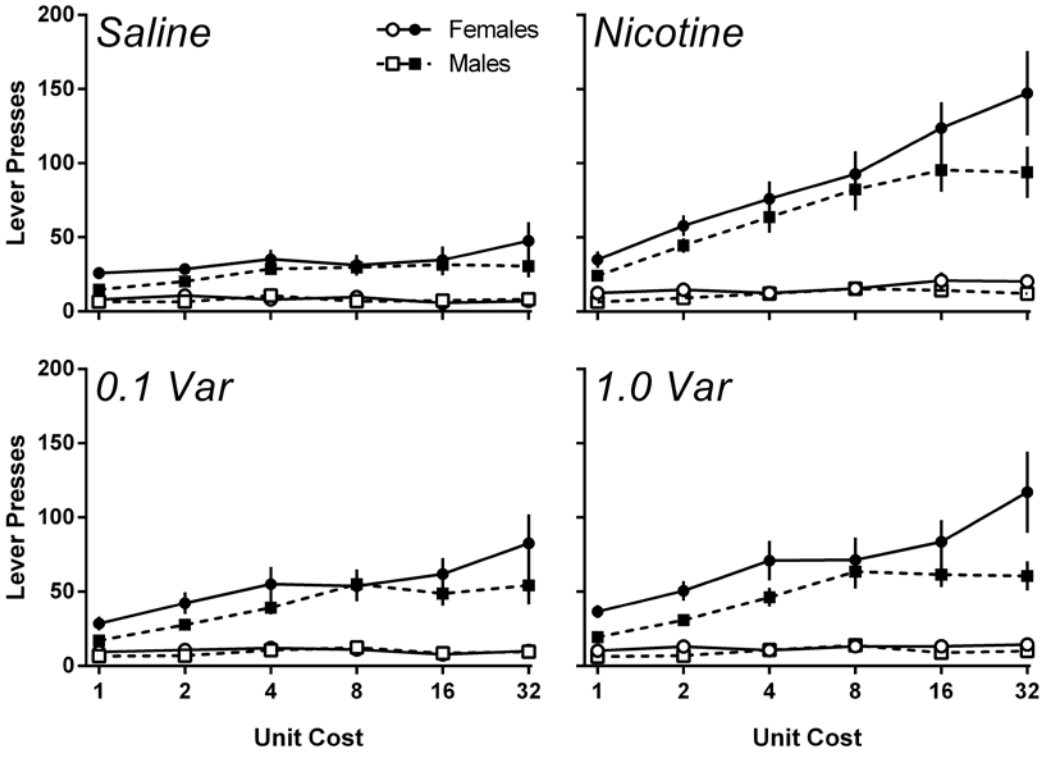

Figure 13. Mean number of presses on the active (filled symbols) and inactive levers (open symbols) averaged over the terminal 3 sessions of each drug condition within blocks of FR schedules. Only data from those FR schedules where all the rats in both sexes remained in the demand assessment phase prior to meeting breakpoint criteria are included. Error bears represent ± 1 SEM.

The effects of nicotine and varenicline of locomotor activity across FR schedules and between the sexes is shown in Figure 14. Three-factor ANOVA revealed main effects of Sex [$F(1,22)=7.539$; $p=0.0118$], Drug [$F(3,66)=11.35$; $p<0.0001$], and Unit Cost [$F(5,110)=4.661$; $p=0.0007$], as well as significant Sex x Drug [$F(3,66)=18.27$; $p<0.0001$] and Sex x Unit Cost interactions [$F(5,110)=3.802$; $p=0.0032$]. The three-factor interaction approached conventional levels of significant [$F(15,330)=1.695$; $p=0.0503$]. The Sex x Drug interaction was characterized by differences in locomotor activity between each of the drug conditions in females (i.e. nicotine > 1.0 varenicline > 0.1 varenicline > saline), but no differences in activity between drug conditions in the males [ps<0.05]. Additionally, locomotor activity was higher in females than males under each drug condition but saline [ps<0.05]. Further analysis of the interaction between Sex and Unit Cost found that locomotor activity was greater in females than in males on schedules FR 4 through FR 32, driven largely by a trend toward increased activity with later FR schedules in females and the absence of such a trend in males [ps<0.05].

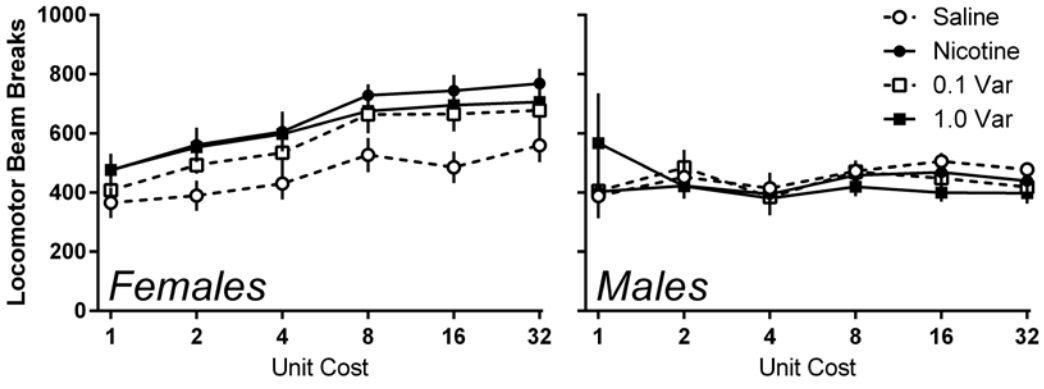

Figure 14. Mean locomotor activity as measured by breaks of the chamber bisecting infrared beam averaged across the terminal 3 sessions of each drug condition within session blocks of each FR schedule. Only data from those FR schedules where all the rats in both sexes remained in the demand assessment phase prior to meeting breakpoint criteria are included. Error bears represent ± 1 SEM.

Results - Demand Assessment

Both nicotine and varenicline administration increased lever-pressing rates compared to saline conditions in male and female rats. Enhanced response rates were observed across FR schedules in the demand assessment phase. Figure 15 portrays the demand curves as fit to the grouped data of males and females between each of the drug conditions throughout the demand assessment phase. Note that the curves presented here are merely for representative purposes and that all comparative analyses were conducted using the results of fitting the reinforcer demand model to the data of individual rats. Because individual rats completed the demand assessment phase after meeting breakpoint criteria at different FR schedules, not all the data are represented in Figure 15. Only data up to those FR schedules where at least a quarter of the rats of each sex remained in the demand assessment phase are presented (FR 128 for males; FR 256 for females).

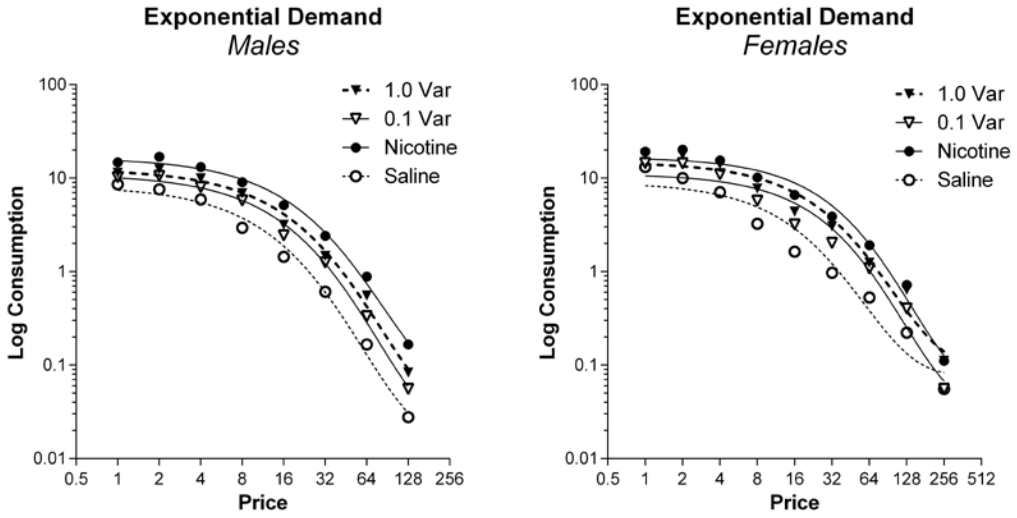

Figure 15. VS consumption as a function of FR schedule between males (left) and females (right), and across the four administration conditions of nicotine (filled circles), saline (open circles), 0.1 varenicline (open triangles) and 1.0 varenicline (closed triangles).

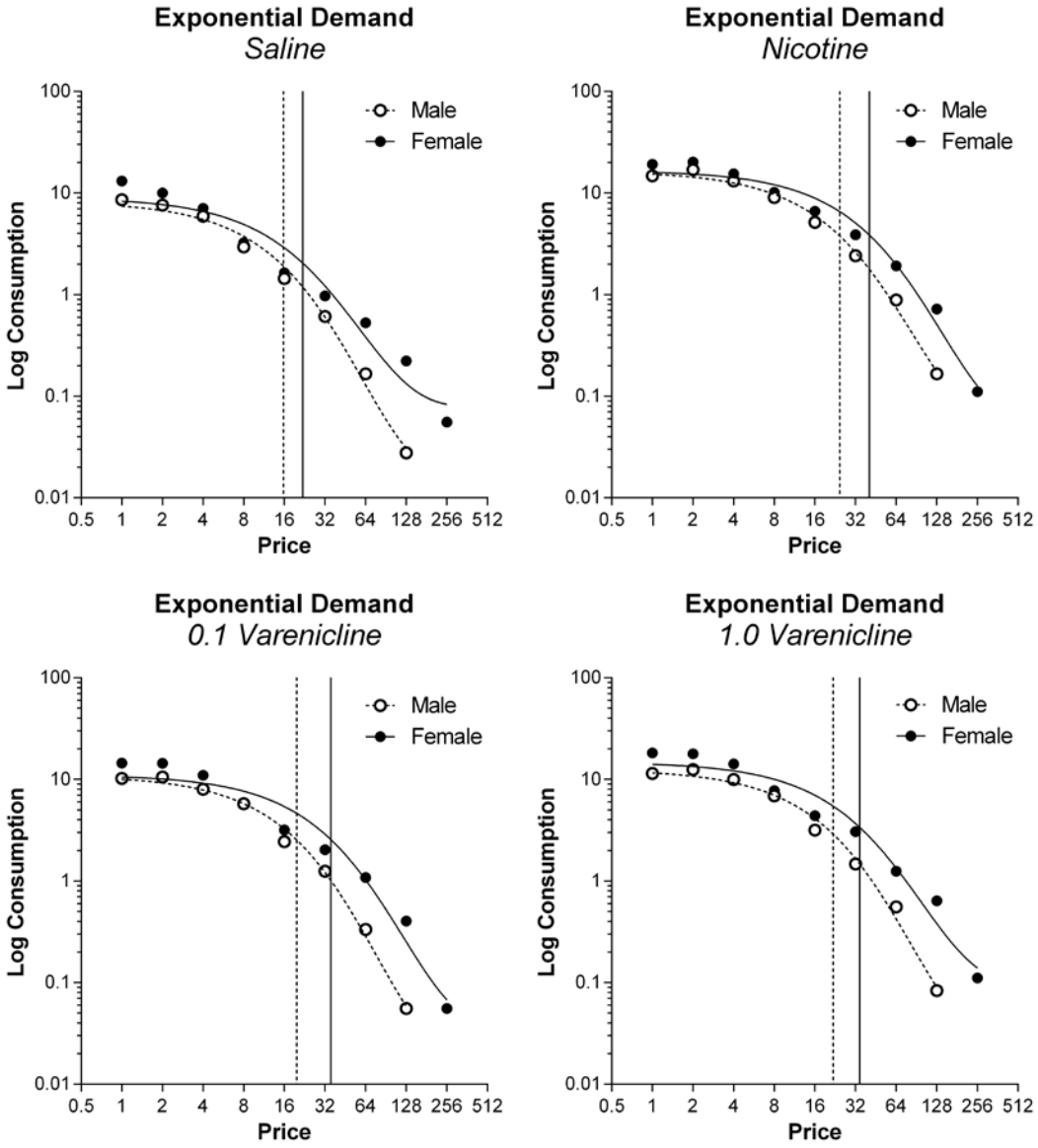

Figure 16. VS consumption between the sexes and across drug conditions as a function of FR schedule. Males are represented by open circles; females by the filled circles. The P_{max} estimates from the group-fitted demand curves are represented by the vertical lines (dashed=males; solid=females).

Inspection of the VS demand curves revealed that in both sexes, nicotine enhanced VS consumption to the highest rates of all conditions across each of the FR schedules. Varenicline also increased VS consumption relative to saline in dose-dependent manner. The point by which 50% of the rats of each sex had reached breakpoint criteria was FR 64 for males and FR 128 for females. Figure 16 shows that

within each drug condition, the demand curves for females were consistently above the curves for males, particularly at the higher FR schedules.

Values for Q_0, P_{max}, O_{max} and α of the reinforcer demand model were obtained by fitting the model to the data of individuals rats, and were compared using two-factor mixed measures ANOVA with Sex and Drug as factors. The model estimates of Q_0 for males and females and across drug conditions are shown in Figure 17. Statistical analysis revealed a significant main effect of Drug [$F(3,66)=3.951$; $p=0.0118$], but not of Sex [$F(1,22)=2.243$; $p=0.1484$], nor a significant Sex x Drug interaction [$F<1$]. Further analysis found that nicotine induced higher Q_0 values than any of the other drug conditions in males; no significant effects of Drug were detected in females [ps<0.05]. Additionally, values of Q_0 were higher in females in the 1.0 mg/kg varenicline condition [$p<0.05$], but no other effects of sex were detected across the other drug conditions [ps>0.05].

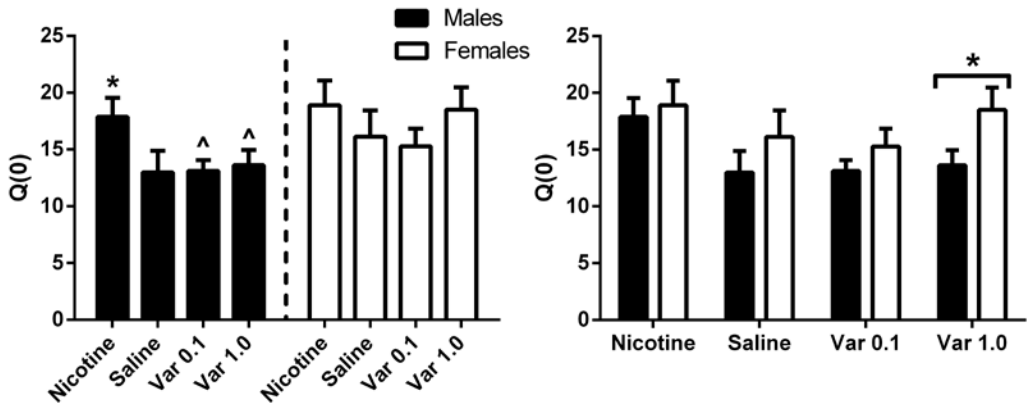

Figure 17. Estimates of Q_0 derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from saline. ^ indicates a significant difference from nicotine. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

The calculated values of P_{max} across drug conditions and between the sexes are shown in Figure 18. Analyses revealed a significant main effect of Drug [$F(3,66)=14.78$, $p<0.0001$], but not of Sex [$F(1,22)=1.068$, $p=0.3126$], or a Sex x Drug interaction [$F(3,66)=1.618$, $p=0.1935$]. Planned comparisons on the effects of Drug within the sexes found that nicotine and 1.0 mg/kg varenicline significantly elevated P_{max} values relative to saline in males [ps<0.05]. In females, nicotine and both varenicline doses differed from saline, and both varenicline dose conditions were significantly lower on P_{max} than nicotine [ps<0.05]. Statistical inspection of the effects of sex at the different drug conditions found higher values of P_{max} in females in the nicotine and 0.1 mg/kg varenicline conditions [ps<0.05].

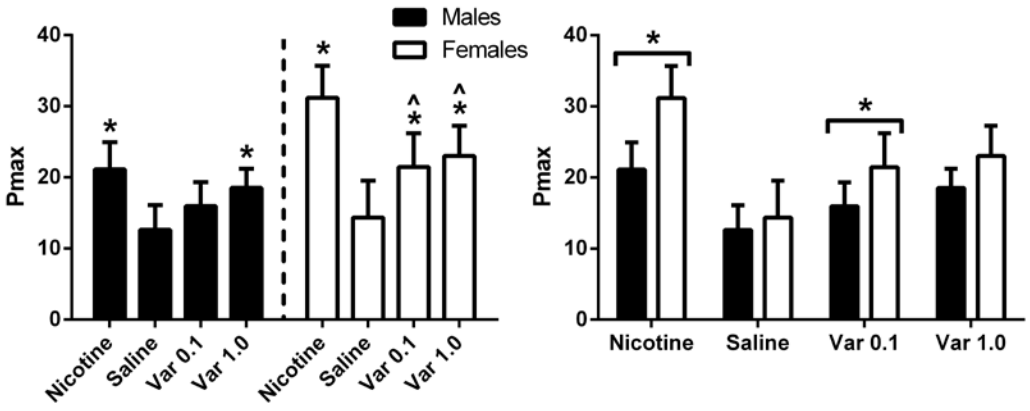

Figure 18. Estimates of P_{max} derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from the saline condition for each sex. ^ indicates a significant difference from nicotine. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

Estimates of O_{max} derived from the reinforcer demand model are displayed in Figure 19. Analyses revealed a significant main effect of Drug [$F(3,66)=43.77$, $p<0.0001$], but not of Sex [$F(1,22)=2.125$, $p=0.1590$] or a Sex x Drug interaction [$F(3,66)=2.280$, $p=0.0874$]. Analysis of the effects of nicotine and varenicline

administration within the sexes found that nicotine and 1.0 mg/kg varenicline increased O_{max} estimates relative to saline in males and females; 0.1 mg/kg varenicline also increased O_{max} relative to saline in females [*p*s<0.05]. Both varenicline dose conditions also differed from nicotine in each of the sexes [*p*s<0.05]. Additionally, significant differences in O_{max} were observed between varenicline conditions in the females, with higher O_{max} associated with higher varenicline dose [*p*<0.05]. Additional analysis on the effects of sex at each drug condition found significantly higher estimates of O_{max} in females under drug all conditions but saline [*p*s<0.05].

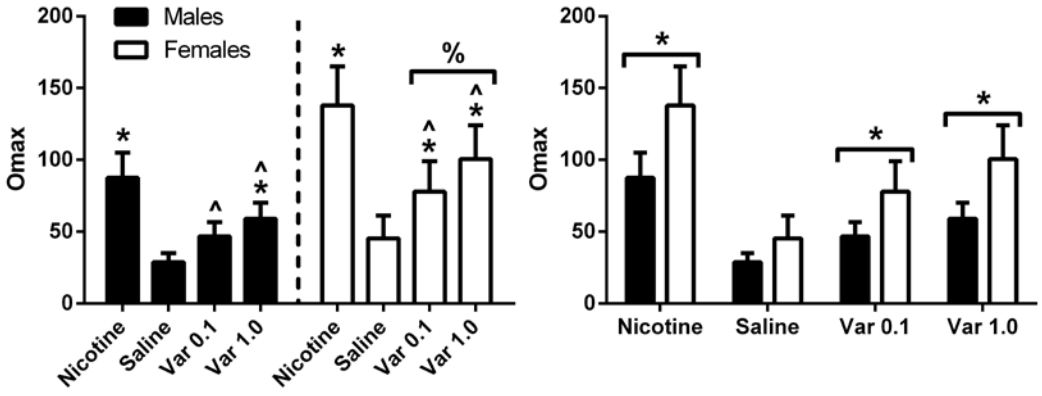

Figure 19. Estimates of O_{max} derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from saline. ^ indicates a significant difference from nicotine. % indicates a significant difference between varenicline doses. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

The estimates of essential value of VS reinforcement across drug conditions and between the sexes are shown in Figure 20. Analyses revealed a significant main effect of Drug [$F(3,66)=31.24$, $p<0.0001$], but not of Sex [$F<1$] or a Sex x Drug interaction [$F<1$]. *A priori* comparisons of the effects of Drug within the sexes revealed identical patterns in males and females: nicotine and both doses of varenicline significantly enhanced essential value relative to saline, and enhancement by 0.1 mg/kg varenicline was

intermediate to that of nicotine [*p*s<0.05]. Additionally, essential value was significantly greater (i.e. lower values of α) in 1.0 mg/kg varenicline than 0.1 mg/kg varenicline [*p*<0.05]. However, greater essential value was observed in females than in males in the nicotine condition, but not at any of the other drug conditions [*p*s>0.05].

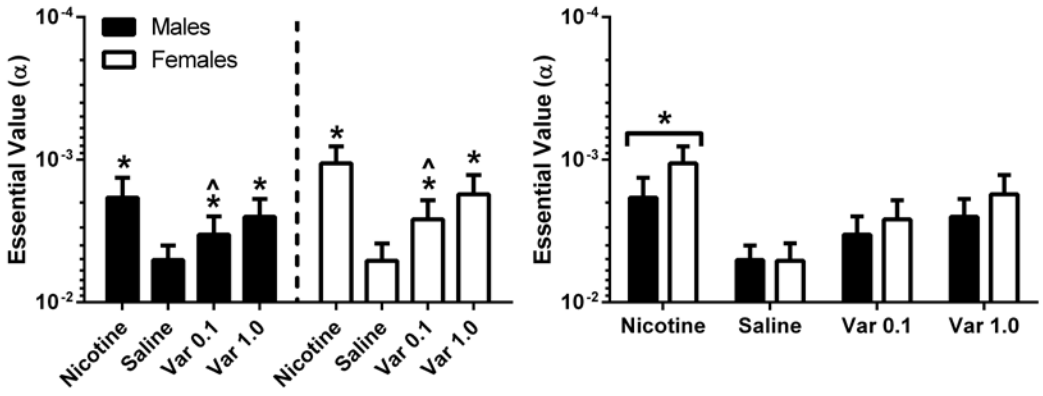

Figure 20. Estimates of essential value parameter (α) derived from the reinforcer demand model. Female rats are represented by the open bars, and male rats by the filled bars. Left: the effects of drug within each sex. * indicates significant differences from saline. ^ indicates a significant difference from nicotine. Right: the effects of sex under each drug condition. Asterisks indicate significant differences between the sexes. Error bears represent ± 1 SEM.

Overall, the results of the demand assessment phase indicate that nicotine and varenicline enhanced the reinforcement value of VS relative to saline conditions. Enhancement was observed in males and females, with some notable differences between the sexes observed on different metrics of the reinforcer demand model. Namely, nicotine-enhanced measures of VS reinforcement value were greater in females with exception of the Q_0 parameter. Additionally, females showed higher Q_0, P_{max}, and O_{max} values than males in the varenicline conditions, though the effects on Q_0 and P_{max} differed by varenicline dose. In combination, the findings of the demand assessment phase suggest that nicotine and varenicline enhance the value of reinforcing sensory stimuli in

males and females, but that females may be more sensitive than males to the enhancing effects of varenicline and nicotine on sensory reinforcement.

Results - α4β2 Antagonism on Progressive Ratio Performance

Active-lever pressing on the PR schedule of VS reinforcement across sessions of the DHβE testing phase is displayed in Figure 21. Nicotine and varenicline increased active lever-pressing on the PR schedule relative to saline in males and females. Analysis revealed significant effects of Sex [$F(1,21)=8.769$; $p=0.0075$], Drug [$F(2,42)=50.15$; $p<0.0001$] and Antagonist Dose [$F(2,42)=11.65$; $p<0.0001$]. The Sex x Drug [$F(2,42)=6.587$; $p=.0032$], Sex x Antagonist Dose [$F(2,42)=4.630$; $p=0.0152$], and Drug x Antagonist Dose interactions [$F(4,84)=7.313$; $p<0.0001$] were significant as well. Finally, the Sex x Drug x Antagonist Dose interaction was also significant [$F(4,84)=2.860$; $p=0.0286$]. Planned pairwise comparisons found that 3.0 mg/kg DHβE decreased active lever-pressing in all drug conditions in females, and in the nicotine condition in males [ps<0.05] (Figure 21, left panel). The lower dose of 1.0 mg/kg DHβE also reduced active lever-pressing in females in the saline and varenicline conditions [ps<0.05]. Analysis of the effects of nicotine and varenicline within the sexes in the absence of DHβE found that both drugs increased active lever-pressing relative to saline; levels of enhanced responding were similar between nicotine and varenicline in the females, but nicotine-enhanced responding was greater than that of varenicline in males [ps<0.05]. Investigation of differences between the sexes in responding under each drug condition in the absence of DHβE revealed consistently higher responding in females than in males across drug conditions [ps<0.05] (Figure 21, right panel). Further analysis of the Sex x Drug x Antagonist Dose interaction revealed that antagonism with DHβE at

either dose attenuated the differences in nicotine and varenicline-enhanced responding in males, but both remained significantly higher than saline [*p*s>0.05]. In contrast, decreases in responding by 3.0 mg/kg DHβE were greater in the nicotine condition than the varenicline condition in females, such that nicotine levels of responding were intermediate to saline and varenicline [*p*<0.05].

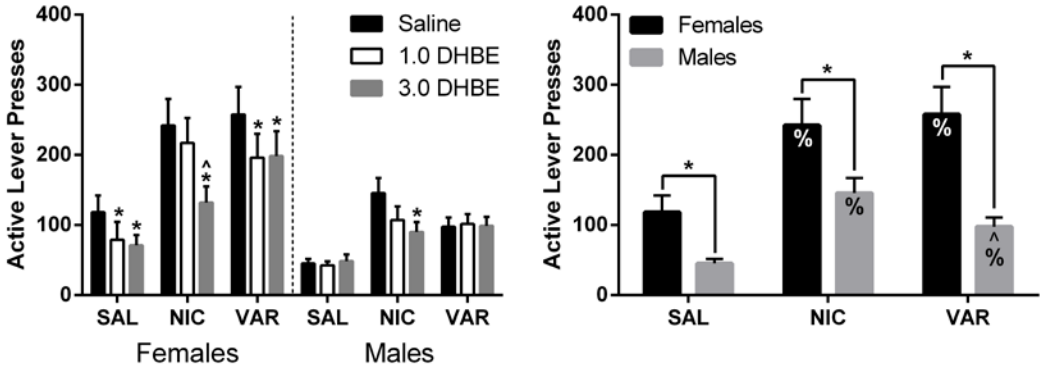

Figure 21. Active lever-pressing across the sessions of the α4β2 antagonism testing phase using DHβE. Left: Active lever-pressing between the sexes, averaged across the 2 determinations of each of the DHβE test conditions. * represents a significant difference from the saline control condition of antagonist dose within a drug condition. ^ represents a significant difference between doses of DHβE within a drug condition. Right: The effects nicotine and varenicline on active lever-pressing between the sexes in the absence of DHβE. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. Error bears represent ± 1 SEM.

Figure 22 shows the locomotor activity across sessions of the α4β2 antagonism testing phase. Multi-factorial ANOVA revealed significant effects of Sex [$F(1,21)=26.51$; $p<0.0001$], of Drug [$F(2,42)=10.08$; $p=0.0003$], and a significant Sex x Drug interaction [$F(2,42)=41.07$; $p<0.0001$], but no main effect of Antagonist Dose [$F(2,42)=1.397$; $p=0.2585$]. Planned pairwise comparisons discovered a significant decrease in locomotor activity by 1.0 mg/kg DHβE under the saline condition in females [$p<0.05$], but no other effects of DHβE administration on locomotor activity within any of the drug conditions in either of the sexes [$p<0.05$] (Figure 22, left panel). Although a decrease was detected at the 1.0 mg/kg DHβE dose in females, the small effect size and lack of an effect at the

3.0 mg/kg DHβE dose suggest that this finding likely represents a Type I detection error. Further analysis on the effects of drug on locomotor activity within the sexes in the absence of DHβE (Figure 22, right panel) found that nicotine and varenicline increased locomotor behavior in females to similar levels [*p*s<0.05]. In contrast, both drugs produced significant decreases in locomotor activity in males [*p*s<0.05]. Additionally, locomotor activity in the absence of DHβE was significantly higher in females than in males under all drug conditions, including saline [*p*s<0.05].

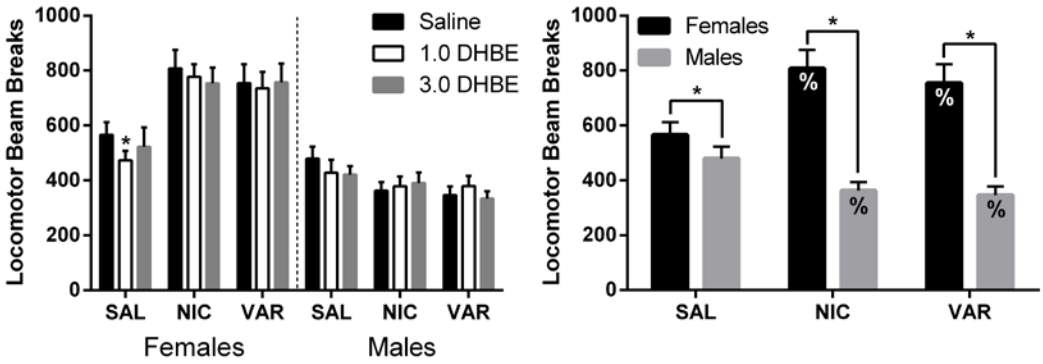

Figure 12. Locomotor activity across the sessions of the α4β2 antagonism testing phase using DHβE. Left: Locomotor activity between the sexes, averaged across the 2 determinations of each of the DHβE test conditions. * represents a significant difference from the saline control condition of antagonist dose within a drug condition. Right: The effects nicotine and varenicline on locomotor activity between the sexes in the absence of DHβE. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. Error bears represent ± 1 SEM.

Results - α7 Antagonism on Progressive Ratio Performance

Active lever-pressing across the sessions of the antagonist testing phase with MLA is presented in Figure 23. Multi-factorial statistical analysis revealed significant effects of Sex [F(1,21)=7.831; *p*=0.0108] and Drug [F(2,42)=53.29; *p*<0.0001], but not of Antagonist Dose [F(2,42)=1.060; *p*=0.3555] and no significant interactions. Planned pairwise comparisons on the effects of MLA dose within drug conditions of each sex (Figure 23, left panel) found no effects of MLA antagonism of α7 nAChRs on active

lever-pressing in any drug condition [*p*s>0.05]. In the absence of MLA, nicotine and varenicline enhanced lever-pressing to similar levels in females, but enhancement by nicotine was greater than enhancement by varenicline in males [*p*s<0.05] (Figure 23, right panel). Furthermore, females lever-pressed more than males in each drug condition [*p*s<0.05].

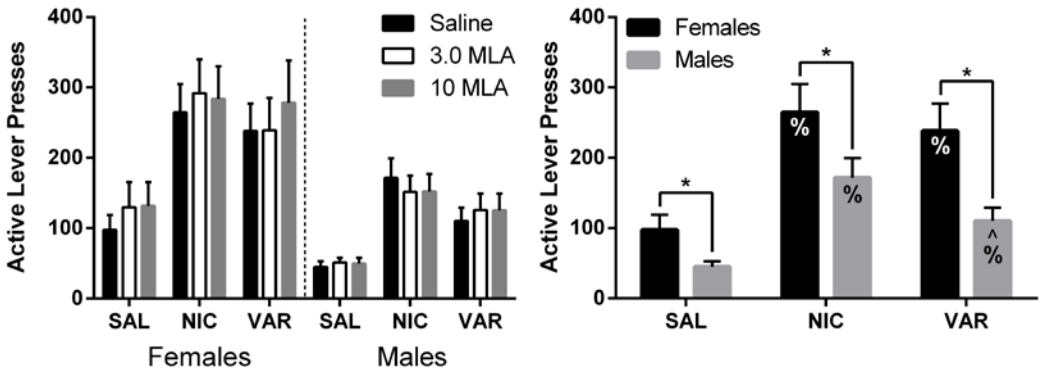

Figure 23. Active lever-pressing across the sessions of the α7 antagonism testing phase using MLA. Left: Active lever-pressing between the sexes, averaged across the 2 determinations of each of the MLA test conditions. Right: The effects nicotine and varenicline on active lever-pressing between the sexes in the absence of MLA. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. Error bears represent ± 1 SEM.

Locomotor activity over the sessions of the MLA testing phase is presented in Figure 24. Analyses revealed significant effects of Sex [$F(1,21)=28.21$; $p<0.0001$], and of Drug [$F(2,42)=5.854$; $p=0.0059$], and a Sex x Drug interaction [$F(2,42)=17.52$; $p<0.0001$]. *A priori* pairwise comparisons discovered no effects of MLA administered at either dose on locomotor activity within drug conditions of either sex [*p*s>0.05] (Figure 24, left panel). Further analysis on the effects of nicotine and varenicline on locomotor activity in the absence of MLA (Figure 24, right panel) revealed significant locomotor activation by both drugs in females relative to saline, but no effects of either drug on locomotor activity in males [*p*s<0.05]. Additionally, in the absence of MLA, females

expressed greater locomotor activity than males under the nicotine and varenicline conditions, but not following injection of saline [*p*s<0.05]. However, differences in locomotor activity following saline injection were significantly higher in females in the 3.0 and 10 mg/kg MLA conditions [*p*s<0.05].

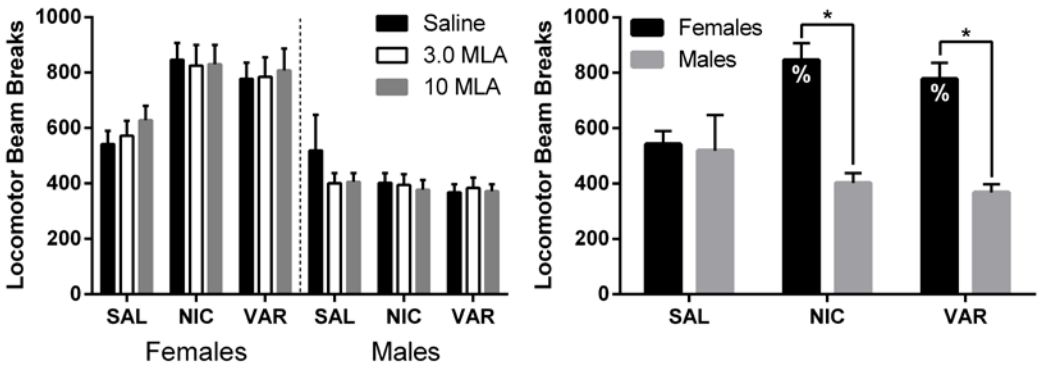

Figure 24. Locomotor activity across the sessions of the α7 antagonism testing phase using MLA. Left: Locomotor activity between the sexes, averaged across the 2 determinations of each of the MLA test conditions. Right: The effects nicotine and varenicline on locomotor activity between the sexes in the absence of MLA. * represents significant differences between the sexes within a drug condition. % represents a difference from the saline condition within each sex. Error bears represent ± 1 SEM.

The PR performance data corroborate the previous observation that nicotine and varenicline enhance responding maintained by VS reinforcement, and that enhanced levels of responding were higher in females. Additionally, the observation that females lever-pressed more than males under saline baseline conditions was also replicated. Interestingly, nicotine and varenicline induced hyper-locomotion in females, but neither drug increased locomotor behavior in males. In fact, nicotine and varenicline induced locomotor suppression in males while simultaneously enhancing active lever-pressing. This latter observation strongly implies that the value-enhancing effects of nicotine and varenicline are not an artifact of parallel effects of locomotor activation. Notably, α4β2-containing nAChR antagonism via DHβE decreased active lever-pressing under the nicotine condition in both sexes without reliable effects of locomotor behavior. A similar

effect was observed in the varenicline condition of females. Additionally, α7 nAChR antagonism with MLA yielded no effects on lever-pressing or locomotion. Together, these findings suggest a critical role of α4β2-containing nAChRs, but not of α7 nAChRs, in the reward-enhancing effects of nicotine and varenicline.

Chapter 6: Summary and Discussion of Main Findings

Chapters 3, 4 and 5 outlined the methods and results of two experiments designed and conducted in an effort to improve our understanding of the value-enhancing effects of nicotine and the smoking cessation aids, bupropion and varenicline. Each experiment represents an independent investigation of value-enhancement with special attention to biological sex and the role of dopamine or nicotinic acetylcholine receptors as neuropharmacological mechanisms of enhancement. Despite the procedural differences between the present experiments, which are relatively few, the combined results of each help to construct a larger picture of value-enhancement and overall reinforcement by nicotine. In this chapter, I summarize the main findings of interest from the vast amount of data generated in the multiple phases of each experiment. Thereafter, I will consider alternative interpretations of what the present findings reflect and why they are meaningful in the context of each experiment. Finally, we will consider the implications of the present findings in the wider context of nicotine reward and the driving motivational mechanisms of smoking behavior.

Summary of Experiment 1

Experiment 1 was designed to investigate the value-enhancing effects of nicotine and bupropion using the quantitative methods of the reinforcer demand model. As highlighted in Chapter 2, the reinforcer demand model provides a unique method for characterizing the performance of behavior maintained by reinforcing stimuli that may arguably represent multiple facets of the reinforcement value construct (Barrett and Bevins, 2012; Hursh and Silberburg, 2008). A strength of Experiment 1 is that the value-enhancing effects of nicotine and bupropion were characterized side-by-side in a within-

subjects design, using the reinforcer demand model and more traditional measures of response strength. Such an approach generates the possibility for direct comparison between the effects of each compound on the behavior of individuals as they respond under schedules of VS reinforcement.

In Experiment 1, we found that nicotine and bupropion increased lever-press response rates and this effect was more pronounced on the active lever. This effect was observed across a range of FR schedules and was observed in both sexes. Interestingly, bupropion engendered far greater enhancement of response rates in females than in males, which is consistent with other findings that females are more sensitive to the response activating effects of many psychomotor stimulants (Van Swearinggen, Walker, and Kuhn, 2013; Reichel et al., 2012; Eubig et al., in press). Indeed, females also showed pronounced activation of locomotor behavior by both nicotine and bupropion, whereas males showed only moderate and inconsistent locomotor activation by bupropion, and no activation by nicotine. However, the increases in active lever-pressing by nicotine and bupropion observed in either sex are probably not caused principally by locomotor activation since enhancement by nicotine was observed in males in the absence of locomotor activation, and 10 mg/kg bupropion increased locomotor behavior in females to greater levels than nicotine, yet both drugs enhanced active lever-pressing to similar levels. A more thorough treatment of the relationship between locomotor activation and value-enhancement will be provided later when we consider the findings of both experiments together.

Demand for VS reinforcement was also enhanced by both nicotine and bupropion in Experiment 1. One of the most notable findings made evident by the reinforcer

demand analysis is that males and females differed in their consumption of VS reward under bupropion conditions. VS consumption in either bupropion condition was indistinguishable from the nicotine condition in females, whereas bupropion enhanced VS consumption in a dose-dependent manner in males, but not to the extent of enhancement by nicotine. Responding maintained by VS reinforcement was consistently greater in females across all drug conditions, and especially so at higher unit costs. From these observations, it would appear that females are generally more responsive to sensory reinforcement by VS than males, and the estimates of the components of the reinforcer demand model seem to corroborate this hypothesis. Closer inspection of potential sex differences on the four measures generated by the reinforcer demand model revealed that females and males consistently differed under bupropion conditions across all the indices of the demand model. Importantly, on the essential value parameter (α), the purported single-parameter encapsulation of reinforcement value (Hursh and Silberburg, 2008), sex differences were also observed in the saline condition. Finally, bupropion enhancement of VS reinforcer demand in males showed dose-dependency that was not observed in females, and with intermediate or no enhancement being reliably observed at the 10 mg/kg dose condition. These findings combine to suggest that males and females may differ in basal value derived from the VS reinforcer used in these studies, and that they also differ in their sensitivity to the value-enhancing effects of bupropion. Notably, females showed greater sensitivity to the effects of nicotine, and the greatest sensitivity to bupropion on demand indices related to response persistence and maximal sustainable effort (P_{max} and O_{max}).

Over the sessions of the antagonist testing phases, nicotine and bupropion enhanced active lever-pressing to a greater degree in females than in males. Performance on PR schedules is often used as an assay of response persistence, of which PR breakpoints are a traditional measure (Bickel et al., 2000). The purpose of the PR in the present experiments was not to obtain breakpoints; nevertheless, the utility of testing on a PR schedule was partly to observe the effects of nicotine and bupropion treatment on behavior maintained under conditions that challenge persistence. In this way, the PR schedule served as a single session proxy for the demand assessment methodology with the benefit of allowing for quick and efficient testing of the antagonist effects, but at the drawback of not permitting an analysis of reinforcer consumption as a function of reinforcer price. In this fashion, the methods of the demand assessment phase and of the PR schedule in the antagonist testing phases should be viewed as complimentary, allowing for investigation of the effects of increasing response cost as an assay of behavioral persistence across and within sessions. The findings between the demand analysis and PR schedule converge to suggest that females may be more sensitive to enhancement of behavioral persistence by nicotine and bupropion.

Antagonism of the D1 receptor family by SCH-23390 decreased lever-pressing and locomotor activity in both males and females. Unfortunately, this makes interpreting the role that D1-family receptors play in the value-enhancing effects of nicotine and bupropion somewhat difficult. On the one hand, decreases in responding may actually reflect a decrease in basal reinforcement value of the VS that further decrease levels of value-enhancement by nicotine and bupropion. On the other hand, decreases in locomotor activity, particularly under saline conditions suggests a likely impairment of motor

behavior which may have interfered with lever-pressing for reasons not related to change in reinforcement value. Given the significant role that dopamine plays in locomotor behavior generally (Beninger, 1983; Hoffman and Beninger, 1985), a motor impairment account seems more parsimonious.

In contrast, the results from the antagonist testing phase with eticlopride are considerably less ambiguous. Eticlopride decreased lever pressing only in the bupropion conditions of both sexes, and these decreases were not accompanied by locomotor suppression. Although 30 µg/kg eticlopride did decrease responding when evaluating the main effect of antagonist dose, the size of this effect was considerably small and should not be interpreted as significant motor impairment. Rather, D2-receptor family antagonism appears to have partially attenuated the value-enhancing effects of bupropion without affecting the enhancing effects of nicotine or the basal reinforcement value of VS. Taken together, the results from both antagonist testing phases suggest that activation of D2-like receptors is a critical component of the enhancing effects of bupropion but not of nicotine, highlighting a pharmacological distinction in the mechanisms of value-enhancement between these two drugs. Activation of D1-like receptors may also be involved in the value-enhancing effects of nicotine and bupropion, but this possibility will require further investigation using techniques that can parse apart the role of these receptors in locomotor behavior versus processing reinforcement value.

Summary of Experiment 2

Experiment 2 evaluated the reward-enhancing effects of nicotine and varenicline using a side-by-side comparison within individual rats, in addition to comparison between the sexes. Nicotine and varenicline increased active lever-pressing rates relative

to saline, and did so over a wide range of FR schedules. Increases in inactive-lever pressing were also observed, but the size of these changes were small and nowhere near in proportion to the increases observed on the active lever. In both sexes, enhancement of lever-pressing by nicotine surpassed levels of enhancement by varenicline, corroborating the findings of previous studies that the value-enhancing effects of varenicline are weaker than those of nicotine (Levin et al., 2012; Schassburger et al., in press). Locomotor activity was also enhanced by nicotine and varenicline in females, but not in males. The apparent sex differences in the locomotor stimulating effects of nicotine in Experiment 2 are a direct replication of a similar effect in the preceding experiment; together, these findings confirm observations from previous studies finding little or no locomotor activation by nicotine using similar procedures with male rats (Barrett and Bevins 2012; 2013). Again, the response-elevating effects of nicotine and varenicline are not sufficiently explained by a locomotor activation account, since enhancement of response rates by both drugs was observed in males in the absence of locomotor activation. Indeed, enhancement of responding in males was observed in conditions where locomotor behavior was, in fact, decreased by nicotine and varenicline.

Evaluation of demand to consume VS reinforcers as a function of price in units of FR response cost revealed similar patterns of enhancement by nicotine and varenicline between the sexes. Nicotine robustly increased consumption of VS across values of unit cost, whereas varenicline dose-dependently enhanced VS consumption to levels generally below those of nicotine. As in Experiment 1, females showed greater levels of VS consumption than males under each of the drug conditions, but this may not actually reflect an increased sensitivity to VS sensory reinforcement in females. Notably, females

did not differ from males in any of the parameters or predictions of the reinforcer demand model under any saline conditions in Experiment 2. However, females did show greater enhancement by nicotine and varenicline largely on indices related to behavioral persistence (P_{max} and O_{max}), though it should be noted that nicotine-enhanced levels of essential value were also greater in females than in males.

Enhancement of value by nicotine and varenicline was detected by each of the indices of the reinforcer demand model assessed within the sexes, with the exception of Q_0; only nicotine increased estimates of Q_0 and only in males. In fact, the effects of nicotine on Q_0 appear somewhat blunted in both the sexes in Experiment 2 when compared to previous work (Barrett and Bevins, 2012; Cassidy and Dallery, 2012, in press), including Experiment 1. This observation might result from repeated activation and prolonged desensitization of α4β2 nAChRs by repeated administration of nicotine and varenicline that may thereby cause a weakening of the value-enhancing effects of both compounds; or it could reflect a sample of rats with decreased sensitivity to sensory reinforcement or the value-enhancing effects of nicotine. Without confirmatory research, either account remains speculative. Nonetheless, the apparent lack of effect of nicotine or varenicline on Q_0 in the female rats represents a point of departure in Experiment 2 from previous work that may warrant further investigation. It should be noted that although values of Q_0 were higher in females than males at the 1.0 mg/kg varenicline dose, no enhancement of Q_0 relative to saline was observed in females at this or any other condition. Therefore, the differences in Q_0 between the sexes wrought by varenicline may not reflect a difference in enhancement by varenicline. Q_0 aside, the effects of nicotine and varenicline on each of the other model estimates suggest that both compounds

enhanced the value of VS reinforcement within the sexes, and that enhancement by nicotine was by some degree stronger than enhancement by 1.0 mg/kg varenicline.

Nicotine and varenicline also enhanced lever-pressing performance on the PR schedule across the antagonist testing phases. Enhancement of PR performance by 1.0 mg/kg varenicline was essentially equivalent to nicotine in females, but decreased relative to nicotine in males. Differences in lever-pressing were observed between the sexes, echoing the findings of Experiment 1 that females may have a tendency toward greater response persistence and enhancement of persistence by nicotine. Antagonism of α4β2-containing nAChRs decreased active lever-pressing maintained by VS reinforcement in each of the drug conditions in females, but only in the nicotine condition in males. The reason for this discrepancy in unclear. These findings may reflect a role on α4β2-containing receptors in the perception of reinforcement value and enhancement of value by nicotine and varenicline that is more pronounced in females than in males. Alternatively, these findings may result from a floor-effect that prohibited detection of parallel effects in the males. A floor-effect account is somewhat undermined by the observation that lever-pressing rates in the saline control condition in females were roughly equivalent to varenicline-enhanced rates in males, yet DHβE decreased responding in the saline conditions of females but not in the varenicline conditions in males. That is, decreases in response rates should have been detectable in the varenicline conditions of males given that such decreases were readily observed from similar rates in females under saline conditions. Nonetheless, DHβE decreased lever-pressing in the nicotine conditions of both sexes, suggesting that α4β2-containing nAChRs mediate the value-enhancing effects of nicotine, and possibly varenicline. The finding that DHβE had

no reliable effects on locomotor activation under any of the drug conditions for either of the sexes, suggests attenuation of value-enhancement by DHβE was not the result of locomotor suppression.

Antagonism of α7 nAChRs by MLA had no effects on lever-pressing or locomotor activity under any of the drug conditions in either of the sexes. These findings confirm previous work with nicotine that found no role of α7 receptors in the value-enhancing effects of nicotine (Liu et al., 2007), and extends this finding to varenicline. The null effect of α7 antagonism in the varenicline conditions is notable because varenicline is a full agonist at, and binds with great affinity to α7 nAChRs (Grady et al., 2010; Ortiz et al., 2012). In combination with previous work, the present results reinforce the conclusion that α7 nAChRs are not involved in the reward-enhancing effects of nicotine or the expression of reward-enhancement generally.

Discussion of Main Findings

The combined findings of Experiments 1 and 2 provide a number of interesting observations regarding the reward-enhancing effects of nicotine and those of varenicline and bupropion. Foremost, nicotine potently enhances the reinforcement value of VS sensory reinforcement and this effect is not adequately explained by locomotor activation. Each experiment here represents a replication of this effect also observed in previous work, including our own work using the reinforcer demand model to quantify the value-enhancing effects of nicotine in male rats (Barrett and Bevins, 2012).

An interesting observation from the present experiments is that lever-pressing occurred at higher rates and persisted onto higher values of unit cost in females compared to males. Previous studies have shown a similar tendency for females to exhibit higher

baseline response rates (Chaudhri et al., 2005; Grebenstein et al., 2013). As noted above, this observation may reflect a tendency toward increased sensitivity to sensory reinforcement and/or to the enhancing effects of nicotine, bupropion and varenicline in females. However, the findings of the reinforcer demand model do not provide adequate support for this conclusion. In Experiment 1, the model estimated greater essential value in females compared to males under saline conditions, but did not find similar effects with any of the other model indices. Furthermore, the model estimates yielded no differences between the sexes under saline conditions in Experiment 2. Undeniably, a trend towards increased value sensitivity in females is observable across each of the model estimates in the saline conditions of both experiments. However, in neither experiment did the main effect of Sex reach significance in the analyses of the essential value parameter. Whether the difference between the sexes in essential value under saline conditions in Experiment 1 is an accurate representation or a Type I detection error remains unclear; further experimentation that includes a replication of the present demand assessment methodology between the sexes will be required to confirm or reject this observation. Saline conditions aside, sex differences in the effects of nicotine, bupropion, and varenicline were observed across experiments. Notably, the measures that most reliably detected differences between the sexes were those that provide some representation of persistence of responding (i.e. P_{max}, O_{max}, and PR response rates). This observation may suggest that observed differences in response rates between the sexes is caused by another behavioral mechanism other than differential sensitivity to basal reinforcement value or to value-enhancing effects.

One possibility is that females may be more sensitive to the associative links in the response-reinforcer or context-reinforcer relationships inherent in the present designs. Indeed, the observation that sex differences were most readily detected by measures reflecting persistence of responding may suggest that responding by females had acquired more behavioral momentum than responding by males. Behavioral momentum is a theoretical framework that relates strength of response (i.e. behavioral mass) to persistence of responding in the face of disrupting variables, such as changes in motivation, context, or reinforcement contingencies (Nevin, 1974; Nevin and Grace 2000). Importantly, momentum is determined by the strength of conditioned associations formed between the response, reinforcing stimulus, and context of reinforcement; manipulations that strengthen or weaken these associations likewise affect persistence of responding. Thus, increased persistence of responding in the females in the present experiments could possibly result from heightened sensitivity to the stimulus elements of the context, or from stronger connections in the context-reinforcer or response-reinforcer associations. Such effects would strengthen persistence of responding without necessarily enhancing reinforcement-value as measured by reinforcer demand. While examination of these possibilities is beyond the scope of the present work, future research should investigate the possibility of sex differences in behavioral momentum acquired through sensory reinforcement, and the effects of nicotine within the behavioral momentum framework.

The present experiments also extend our understanding of the nature of value-enhancement by bupropion and varenicline, and represent the first attempt to characterize reward-enhancement by either of these compounds in females. The original findings that

bupropion and varenicline possess value-enhancing properties that may contribute to their clinical efficacy in smoking cessation was replicated in the present experiments (Palmatier et al., 2009; Levin et al., 2012; Schassburger et al., in press). Importantly, there were differences in the behavioral characteristics of enhancement by bupropion and varenicline that may be informative to understanding the mechanisms of their clinical efficacy. Specifically, we observed that bupropion produced robust reward-enhancement that was more pronounced in females. Levels of bupropion-enhanced responding approximated those of nicotine, or even exceed nicotine-enhanced levels in some instances. Likewise, bupropion produced pronounced locomotor activation that was greater than activation by nicotine, and this effect was also greater in females. In contrast, varenicline enhanced lever-pressing to levels some degree lower than enhancement by nicotine, and this pattern was generally consistent between the sexes. Females did show greater enhancement by varenicline on measures of persistence, but showed no effects of varenicline of basal intensity of demand (Q_0), and did not differ from males in varenicline-enhanced levels of essential value. Additionally, varenicline only increased locomotor activity in females; in males it had no effect or even decreased locomotor activity. Combined, these findings suggest that bupropion and varenicline may exert their enhancing effects on reinforced behavior via different mechanisms. This possibility should not come as a surprise, because we already knew that bupropion and nicotine enhance value via different pharmacological mechanisms (Palmatier et al., 2009), and the pharmacological mechanisms of enhancement between nicotine and varenicline are likely shared (Levin et al., 2012; Schassburger et al., in press). The differential effects of D2-receptor family antagonism on nicotine and bupropion-enhanced responding in

Experiment 1, and the common effects of α4β2-containing nAChR antagonism on nicotine and varenicline-enhanced responding in Experiment 2 support the transitive extension that the pharmacological mechanisms of enhancement differ between bupropion and varenicline.

Previous work found that bupropion enhanced operant responding for VS via activation of α1-norepinephrine receptors and not via action at nAChRs (Palmatier et al., 2009). The present findings extend previous findings by implicating a role of D2-family dopamine receptor activation in the enhancing effects of bupropion as well. Recall that the primary effects of bupropion on neurotransmission are inhibition of norepinephrine, dopamine, and serotonin reuptake, increasing vesicular monoamine transport, and noncompetitive antagonism of nAChRs (for a review, see Dwoskin et al., 2006). It seems likely that the value-enhancing effects of bupropion result from its ability to indirectly increase dopamine activity in the nucleus accumbens, either via reuptake inhibition or via activation of dopaminergic projections from the pre-frontal cortex into the nucleus accumbens through activation by norepinephrine (cf. Palmatier et al., 2009). The latter mechanism has been proposed based on the similarities in structure and function between bupropion and amphetamine, and the observation that activation in the α-norepinephrine receptor system of the prefrontal cortex is involved in the motivational effects of amphetamines (Darracq et al., 1998; Palmatier et al., 2009). An interesting question for consideration in future studies is whether it is the activation of pre- or post-synaptic D2-family dopamine receptors that is primarily responsible for the dopaminergic mechanisms of enhancement by bupropion. However, such studies will have to be carefully designed

to account for the significant involvement of dopamine receptor function in motor behavior.

As mentioned in Chapter 1, varenicline has a complex pharmacological profile on nAChRs, but its clinical efficacy as a smoking cessation aid is believed to result from its partial agonist/antagonist activity at α4β2-containing receptors, both mimicking and antagonizing the effects of nicotine at these receptors. Varenicline acts as a full agonist at α7 receptors with high binding affinity, and also activates α6β2* and α3β4* receptors (Bordia et al., 2012; Grady et al., 2010; Ortiz et al., 2012; Rollema et al., 2007; Mihalak et al., 2006). The present work suggests that activation of α4β2-containing receptors, but not α7 receptors, plays a critical role in the value-enhancing effects of varenicline. Notably, this is a feature that it shares with nicotine, in which activation of α4β2-containing receptors has been implicated in both the primary reinforcing and value-enhancing effects of nicotine (Palmatier et al., 2009). However, recent findings suggest that varenicline does not appear to have primary reinforcing effects, despite sharing a mechanism of action with nicotine at α4β2-containing receptors (Schassburger et al., in press). Additionally, DHβE also works as an antagonist at receptors containing the α6β2 and α3β4 subunits (Harvey and Luetje, 1996; Harvey et al., 1996), and the role of α4β2-containing nAChRs in the value-enhancing effects of nicotine has only been investigated via antagonism with DHβE (Liu et al., 2007). Together, these findings suggest that the α4β2 subunit may not be solely responsible for the value-enhancing effects of nicotine and varenicline, but that some other receptor subtype also antagonized by DHβE may be involved, such as α6β2* or α3β4* nAChRs (Schassburger et al., in press). Further investigation using more specific receptor antagonists, such as AT-1001 (Toll et al.,

2012), or knockout gene expression may be critical in further elucidating the mechanisms by which nicotine and varenicline enhance reinforcement value.

Nicotine, bupropion, and varenicline each produce locomotor activating effects that were observed primarily in females in the present experiments. Increases in inactive lever-pressing by nicotine, bupropion, and varenicline were also observed in the present experiments. Inactive lever-pressing is an oft employed measure of nonspecific behavioral activation and has served as a proxy for more direct locomotor activity measures (e.g. Barrett and Odum, 2011; Donny et al., 2003) . In the present work, increases in inactive lever-pressing were greater in females than in males, though these increases in either sex were relatively small in comparison to the effects of drug administration on active lever-pressing. However, the observation that locomotor activity and inactive lever-pressing were greater in females than in males should not be interpreted as the primary causal mechanism of sex differences in the value-enhancing effects observed in the present experiments. As briefly mentioned earlier, there are important distinctions between the effects of nicotine, bupropion, and varenicline on behavioral activation and their value-enhancing effects on active lever-pressing maintained by VS. First, the enhancing effects of drug administration on active lever-pressing were sensitive to reinforcement schedule, with greater proportional enhancement occurring on arrangements of higher unit response cost. Locomotor activity and inactive lever-pressing were also affected to some degree by progression of response requirement, but these effects were inconsistent between the sexes and nowhere near the degree of enhancement of active lever-pressing.

Second, locomotor activity was increased by nicotine, bupropion, and varenicline principally in the females, with little or no activation by these drugs in males. However, enhancement was observed in both the sexes, albeit levels of enhancement were generally greater in females than in males. This observation was especially true of responding on the PR schedule for VS reinforcement. It is worth noting that locomotor activation by bupropion was greater than that of nicotine on the PR schedule of Experiment 1, yet enhancement of lever-pressing did not reliably reveal the same pattern. Finally, enhancement of active lever-pressing was observed in the same conditions as significant decreases in locomotor activity, as was the case with nicotine and varenicline in males during the DHβE antagonist testing phase of Experiment 2. These findings substantiate those of previous studies that demonstrate that the locomotor activating and value-enhancing effects of nicotine may vary independently of each other, and extends those findings to varenicline and bupropion (Barrett and Bevins, 2012; 2013; Palmatier et al., 2006; 2009; Schassburger et al., in press).

The present work adds to a now extensive body of literature demonstrating the robust effect of nicotine to enhance the value of other reinforcing environmental stimuli (see Caggiula et al., 2009 for a review). The additional observation that this enhancement effect is greater in females than males is congruent with observations of increased tenacity of tobacco dependence and increased sensitivity to non-nicotine reinforcement factors of smoking in women (see Perkins 2009 for a review). By bridging these two bodies of research, the present work serves to enrich our understanding of the variables that differentially drive motivation for smoking in men and women. An important direction for future research will be to replicate the present finding that females are more

sensitive the value-enhancing effects of nicotine, bupropion, and varenicline in preclinical and clinical populations. Additionally, future studies should investigate the mechanisms that underlie the differences in sensitivity to value-enhancement between the sexes. While conjecture on the specific mechanisms from the present results is certainly speculative, a few areas for future investigation suggest themselves as likely candidates. For instance, future studies should investigate whether males and females differ only in their sensitivity to value-enhancement, or whether this applies to sensitivity to sensory reinforcement in the absence of nicotine. The present experiments do not provide conclusive evidence for or against differential sensitivity to basal reinforcement value, but there is a clear tendency towards higher value in females. Carefully designed experiments that use a range of value metrics and parametrically investigate sensitivity to reward along a continuum of basal reinforcement value may be illuminating. If, for instance, females are indeed more sensitive to reward by sensory reinforcers, then some of the differential sensitivity to value-enhancement may stem from differential amplification of basal reinforcement value (Palmatier et al., 2007).

Future experiments may also investigate whether differences in responsiveness to the value-enhancing effects of nicotine, bupropion, and varenicline stem from differences in pharmacokinetics or pharmacodynamics. That is, are the sex differences in reward-enhancement the result of differences in absorption, distribution, metabolization or elimination of these drugs, or the result of differences in the receptor activity of these drugs in critical brain regions? Previous work has shown that blood-plasma levels of nicotine are higher in female rats than in males shortly after repeated intravenous administration (Harrod et al., 2007), suggesting sex differences in the pharmacokinetics

of nicotine may play a role in its behavioral effects. Interestingly, these sex differences were attenuated by gonadectomy, suggesting a role of gonadal hormones as a mechanism for pharmacokinetic differences between the sexes (Harrod et al., 2008). Additionally, clearance rates (i.e. elimination) of nicotine and cotinine, a prominent metabolite of nicotine, are higher in women than in men, and use of oral contraceptives further hasten these clearance rates in women (Benowitz et al., 2006). Together, these findings suggest that differences in pharmacokinetics may play a role in determining sex differences in the behavioral effects of nicotine, including reward-enhancement. However, there are two reasons to suppose that pharmacokinetic differences between the sexes does not exclusively account for observed differences in the reward-enhancing effects of nicotine (Perkins, 2009). First, the elimination half-life of nicotine is about 2 hours, but differences in the rewarding and reward-enhancing effects of nicotine have been observed in far briefer intervals following administration. Second, differences in nicotine pharmacokinetics should also yield differences in the dose-response relations on all measures of nicotine, including heart rate, mood, and locomotor behavior, but such effects have not be observed in studies where nicotine administration levels were controlled between the sexes (see Perkins, 2009). Indeed, faster nicotine clearance should result in decreased locomotor activation in females, but we observed the opposite effect in the present experiments.

As mentioned above, gonadal hormones have been implicated as an important factor in the sex differences in the pharmacokinetics of nicotine (Harrod et al., 2008; Benowitz et al., 2006; Benowitz et al., 2009). Additionally, some evidence suggests that gonadal hormones may cause differences in nicotine pharmacodynamics as well. That is,

ovarian steroid hormones have been shown to have regulatory effects on nAChR density and function (see Pauly, 2008 for a review). Ovariectomized rats show decreases in the density of α7 nAChRs in the hypothalamus, amygdala, raphe nucleus, and cerebellum; and estrogen replacement attenuates this effect (Morley et al., 1983; Miller et al., 1982, 1984; Miller and Billiar, 1986; Arimatsu et al., 1985; Koylu et al., 1997; Centeno et al., 2006). However, gonadal hormones do not appear to regulate the density of non-α7 nAChRs, and the number, synaptic location, subtype distribution, and nicotine-induced up-regulation of nAChRs do not appear to differ reliably between male and female rats (cf. Pauly, 2008). However, given the findings that antagonism of α7 receptors does not attenuate the reward-enhancing effects of nicotine (Experiment 2 and Liu et al., 2007), the relative influence of gonadal hormones on the reward-enhancing effects of nicotine is uncertain. The effects of hormone levels or estrous cycling are beyond the scope of this dissertation; the present studies did not monitor hormone levels or estrous cycling. However, future research should investigate the role of estrous or other sex hormones in the reward-enhancing effects of nicotine and other drugs.

In the present studies, the greatest differences between the sexes were observed under bupropion conditions. Given that the mechanisms of bupropion enhancement are particularly mediated by dopamine and norepinephrine receptors (Experiment 1 and Palmatier et al., 2009), sex differences in the dopaminergic or adrenergic responses to nicotine and bupropion may be informative in the context of reward-enhancement. Indeed, an increasingly large body of literature implicates the heightened dopaminergic response of females in striatal cells as a mechanism for sex differences in rewarding effects of a variety of drugs of abuse (cf. Roth et al., 2004; Carroll and Anker, 2010).

There is some evidence that gonadal hormones, specifically estrogen, regulate dopaminergic response in striatal tissue (Becker, 1999; cf. Roth et al., 2004; Carroll and Anker, 2010). For instance, nicotine-evoked dopamine release has been shown to be greater in estrogen-treated ovariectomized rats (Dluzen and Anderson, 1997), and density of dopamine uptake sites varies with estrogen level through phases of estrous cycling (Morissette and Di Paolo, 1993). Differences in dopaminergic function between the sexes have been implicated as a mechanism for sex differences in the primary reinforcing effects of psychomotor stimulants, including nicotine (cf. Roth et al., 2004; Carroll and Anker, 2010). These pharmacokinetic differences may also underlie differences in the reward-enhancing effects of nicotine and other psychomotor stimulants. Future research should investigate the relationship between estrogen-regulated differences in dopaminergic tone in the midbrain and sensitivity to the reward-enhancing effects of nicotine, bupropion or other psychomotor stimulants.

Finally, future research should investigate the role that value-enhancement replacement by bupropion and varenicline may play in the efficacy of both agents as smoking cessation aids. If value-enhancement by these agents is critical to their efficacy as pharmacotherapies for smoking cessation, then perhaps bupropion and varenicline may be differentially effective treatments for men and women trying to quit smoking. Alternatively, differences in sensitivity to the value-enhancing effects of bupropion and varenicline may also be accompanied by differences in sensitivity to the side-effects of both these agents for tobacco-abstaining patients. Regardless, an extension of the present findings to human populations of smokers with special attention to the behavioral and biological mechanisms of value-enhancement promises to be a fruitful endeavor and may

provide great insights into the best practices toward treating tobacco dependence between the sexes.

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