To my parents, for their unending love and support, and to my advisor, Dr. Neil E. Rowland, for his guidance
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The purpose of this study was to examine how a flavor cue compares to a compound visual stimulus (VS) in a nicotine self-administration (NSA) paradigm in rats. Because people who smoke cigarettes not only receive nicotine, but the flavor and other stimuli associated with the delivery of the drug, it is important to understand how these stimuli contribute to nicotine addiction and abuse. It has been suggested that nicotine not only makes conditioned reinforcers out of associated stimuli but may also enhance the rewarding properties of already reinforcing stimuli. In the present study, rats were initially trained to lever-press for food, and then underwent intraoral (IO) and/or intravenous (IV) catheter implantations. During the experimental phase, all rats received brief IV infusions of 0.017 mg free base nicotine contingent on lever pressing and were trained to self-administer nicotine paired either with a light cue or a one second simultaneous IO infusion of a flavor (.05% cherry flavored Kool-Aid in a .1% saccharin solution). Separate rats were used to examine the rates of responding for the flavor without any nicotine exposure. The rats who received simultaneous infusions of the flavor were unable to maintain responding at an equal rate to those who acquired the behavior when infusions were paired with a VS, despite the finding the rats will
responding at consistent rates for the flavor by itself. After two weeks of NSA, nicotine was withheld and extinction of the operant behavior was recorded. When the nicotine was removed and replaced with saline, responding for the VS alone was higher than for the flavor alone. Responding for simultaneous infusions of nicotine of a flavor concentration of .2% as well as a .05% flavor concentration at an infusion rate of 10 seconds were also looked at. More rats were able to achieve stable rates of self administration with the .2% flavor concentration. Lastly, rates of responding for a .2% flavor during the presence of non-contingent nicotine were recorded as well, but this concentration was not responded for in the presence of non-contingent nicotine. Then these rats were given a two-bottle preference test between the previously paired cherry flavor and a grape flavor for three days and the number of licks was recorded. The cherry flavor that had been associated with nicotine was then avoided in a two-bottle preference test as compared to a novel grape flavor.
CHAPTER 1
INTRODUCTION

Cigarette smoking is the leading cause of preventable death among the 45 million adult smokers in the United States. Harming nearly every organ in the body, 1 in every 5 deaths is smoking related, with around 440,000 people prematurely dying every year (CDC, 2002). Though the number of adults who smoke has decreased over the past 2-3 decades in the United States, the number of teens who engage in smoking has not. Likewise, smoking is becoming a much bigger problem elsewhere, with over 40% of people smoking in developing countries, particularly males (Benowitz, 2008a). It is estimated that over 5 million deaths occur worldwide every year and that this number will increase to over 10 million annually in 30-40 years time (Benowitz, 2008a; Peto et al., 1996). Smoking in psychiatric populations has increased as well, particularly in people with schizophrenia of whom around 90% use tobacco (Dalack et al., 1998).

Despite the health risks associated with smoking cigarettes, people continue to engage in the behavior. Although over 70% of people who smoke express a desire to quit, only 3% of them are successful over a long-term period (Shiffman et al., 1998). Similarly, 80% of people who attempt to quit on their own, using various methods available, relapse within the first month (Hughes et al., 1992). Around half of smokers attempt to cut down on daily intake (West, 2001). The success rates of the different smoking interventions available are anywhere from 10-30% for the first 6 months to a year (Hughes et al., 2009; Silagy et al., 2007; West, 2001), with the average success rate for different nicotine replacement therapies at around 17% compared with 10% in control groups (Silagy et al., 2007). Combining therapies leads to a modest increase in
success rates, whereas additional counseling does not give much added benefit (Silagy et al., 2007).

In order to comprehend why it is so difficult to quit smoking despite available therapies and public knowledge about the health risks, it is important to understand the neurobiology of nicotine dependence. Though cigarettes contain over 4,000 chemicals, at least 43 of which are known carcinogens (Shiffman et al., 2008), it is the ingredient nicotine whose pharmacological action fosters addiction (USDHHS, 1988). Like all drugs of abuse, nicotine dependence is characterized by drug liking, trouble quitting, symptoms of tolerance and withdrawal, and problems with relapse (Palmatier et al., 2007b). The neurobiological mechanisms underlying these psychological and physiological characteristics of addiction have been extensively studied. A large number of neurotransmitters are engaged during nicotine use, including but not limited to, dopamine, norepinephrine, acetylcholine, glutamate, serotonin, β-endorphins, and γ-aminobutyric acid (GABA) (Benowitz, 2008b), along with neuropeptides such as hypocretin/orexin (Corrigall et al., 2009).

Nicotine is an agonist at nicotinic acetylcholine receptors (nAChRs), which are ligand gated ion channels located peripherally and centrally. They are activated by the endogenous neurotransmitter acetylcholine and they modulate neurotransmitter release. This is why nicotine modulates the release of so many different neurotransmitters (Markou, 2009). The primary reinforcing properties of nicotine depend on activation of nAChRs, as seen in studies which use the nAChR antagonists to eliminate nicotine self-administration (NSA) (Liu et al., 2006; Palmatier et al., 2007c). There are nine homologous subunits of these receptors, α4β2 and α7 being the most
important in nicotine addiction. The α7 subunits are important in sensory gating, the activation of which may underlie the reason behind why so many people with schizophrenia smoke (Benowitz, 2008b). The α4β2 subunits possess a high affinity for nicotine which is absent in β2 knock-out (KO) mice and mostly gone in α4 KOs. It seems the β2 receptors mediate some of the rewarding effects of nicotine (Cordero-Erausquin et al., 2000; Picciotto et al., 1998; Picciotto et al., 2002). Mutant mice indicate that the α4β2 subunits are necessary and sufficient for tolerance and sensitization (Brody et al., 2006). β2 KOs no longer show increases in striatal dopamine release when administered nicotine and likewise will not self-administer the drug (Cordero-Erausquin et al., 2000; Picciotto et al., 1998).

The most extensively studied neurotransmitter in relation to nicotine is dopamine, most likely because of its known role in other drugs of abuse. The mesolimbic dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is important in the reinforcing aspect of drug addiction (Carelli et al., 2000; Grimm et al., 2000; Sellings et al., 2008). Many drugs of abuse including nicotine have the ability to acutely decrease brain reward thresholds, a change mediated by increases in the transmission of dopamine and serotonin in the NAc (Koob et al., 2006). Nicotine binds to nAChRs in many brain areas including the substantia nigra, the VTA and NAc and in particular, causing an increase in extracellular dopamine levels (Epping-Jordan et al., 1998; Marubio et al., 2000; Picciotto et al., 2002). Injections of nicotine into the NAc increase DA, and lesions of DA projections to this area result in decreased nicotine self-administration in rats, similar to results seen in other drugs of abuse (Balfour et al., 2000). Dopamine also seems to mediate the change from “drug liking” to active “drug
seeking” (Balfour et al., 2000; Le Foll et al., 2003). Specific blockade of Dopamine D₃ receptors through receptor-specific antagonists reduce this drug seeking behavior (Le Foll et al., 2003).

Mesocortical dopamine release is inhibited by GABA, which projects to the VTA from the tegmental pendunculopontine (TPP) nucleus and NAc (Laviolette et al., 2002). Increased GABA transmission decreases the nicotine-induced increases in dopamine release that accompany the rewarding affects of nicotine. Thus, inhibition of enzymes involved in GABA metabolism decreases nicotine self-administration by increasing GABA levels (Markou et al., 2008). GABA agonists infused into the TPP reduce nicotine self administration, but not cocaine self administration (Piccotto et al., 2002). GABAₐ receptors in particular seem important to nicotine addiction, and administration of GABAₐ receptor agonists into the VTA, NAc shell, and TPP, reduce the reinforcing affects of nicotine. These receptors also reduce responding for food, although the doses of pharmacological agents needed to produce this effect are higher than those that reduce NSA (Markou, 2008).

In addition to the NAc, VTA dopaminergic neurons also project to the amygdala and the frontal cortex. Regulation of dopamine release in these areas is through the release of glutamate. Nicotine increases glutamate release at presynaptic nAChRs in the VTA, prefrontal cortex, hippocampus, and amygdala. There are also glutamate projections from some of those areas to other brain regions with dopaminergic cells. Administering nicotine to the VTA increases glutamate release and subsequently increases the firing rate of dopamine neurons in the area. Blocking mGlu5 glutamate receptors and NMDA receptors with antagonists decreases self administration in
rodents, an effect caused by a reduction in glutamate-mediated dopamine release. mGlu2/3 receptor agonists on the other hand also decrease nicotine self administration, as these receptors negatively modulate dopamine release. Decreases in glutamate transmission result in increases in withdrawal symptoms. Therefore, increased glutamate occurs during nicotine seeking and administration and decreased glutamate is seen during withdrawal (Markou, 2008). Nicotine activates dopamine secreting neurons via NMDA receptors which change the firing pattern of the neurons and increase dopamine overflow (Balfour et al., 2000). The bed nucleus of the stria terminalis (BNST) has glutamate projecting neurons to VTA dopamine neurons, and research seems to indicate that only self-administration of nicotine, as opposed to passive administration of the drug, results in the potentiation of VTA dopamine activity. This indicates a role for this system in behavioral conditioning and reinforcement learning via changes in glutamate activity at NMDA receptors (Caille et al., 2009).

The transition from acute smoking to chronic nicotine use is accompanied by tolerance and withdrawal symptoms between cigarettes (Benowitz, 2008a). The avoidance of these withdrawal symptoms, along with the reinforcing effects of nicotine itself, are the principal factors that seem to promote smoking (Markou, 2008). People who smoke every day have almost complete saturation of α4β2 nAChRs throughout the day, with desensitization occurring in over half of the receptors. High occupancy of these receptors correlates with decreased craving in smokers. It has been estimated that one cigarette produces enough nicotine to occupy up to 95% of the receptors, at least in the short term. It is therefore important to understand why smoking persists throughout the day when these nAChRs are almost fully occupied. Smoking might occur
sustain a high level of occupancy and so alleviate symptoms of craving (Brody et al., 2006). If smoking a cigarette results in high occupancy of all α4β2, then periods of abstinence, for example while asleep, will be associated with falls in plasma nicotine levels and reduced occupancy of relevant receptors. This may be why increased craving is experienced in the morning and people report that the most satisfying cigarette of the day is the first one. Smokers then continue to smoke through the day to maintain desensitization and high receptor occupancy (Benowitz, 2008a).

Smoking to avoid withdrawal symptoms is only one of the reasons people smoke. Nicotine itself may serve as a primary reinforcer which sets in motion the release of many neurotransmitters that result in long term changes to brain-reward regions. However, nicotine appears to be a much weaker reinforcer than other drugs of abuse such as cocaine. Given the near complete nAChR occupancy from the nicotine and the resulting loss of a biologic response to nicotine as well as a loss of dopamine secretion, something else must be helping to maintain the behavior (Balfour et al., 2000; Benowitz, 2008b). The initial dopamine release is thought to influence communication between areas of the brain involved in encoding the rewarding properties of a stimulus (Di Chiara, 2000).

Nicotine addiction seems to be heavily dependent on contextual cues. When people smoke cigarettes they not only take in the nicotine, but they perceive smells, tastes, and other stimuli in their environment that they may associate with smoking. There seems to be an increase in a person’s desire to smoke when they are presented with these stimuli (Caggiula et al., 2002). People who are presented with environmental stimuli that they associate with smoking are more likely to relapse into the behavior or
feel a craving for the drug (Conklin et al., 2002). When people smoke at a high enough level that the relevant nAChRs are desensitized, it is most likely the conditioned reinforcers within the cigarette smoke that maintain the behavior. Several studies have looked at human smoking behavior in the presence of smoking cues without nicotine, as well as in the reverse condition. The presentation of cues to smokers while they are not allowed to smoke results in increased craving (Caggiula et al., 2001). Blocking cues on the other hand reduces satisfaction. For example anesthetizing the respiratory airway but allowing people to smoke, leads to reduced liking as does the blockade of olfactory cues (Baldinger et al., 1995). Similarly the effect of denicotinized cigarettes on craving and satisfaction has been examined, and these cigarettes are rated as more pleasurable and produce a large reduction in craving. This effect seems to be due to the fact that the denicotinized cigarettes produce the same amount of smoke intake as regular cigarettes, a crucial cue involved in smoking despite lower levels of nicotine. This finding helps to illustrate the importance of associated cues in maintaining dependence (Butschky et al., 1995). The importance of cues in nicotine dependence may partially account for why nicotine cessation programs have such poor success because they do nothing to account for the effect that cues have on intake, with addicts seeming to smoke both for the nicotine and for the associated stimuli that come with smoking cigarettes (Caggiula et al., 2001).

The neurobiology of the interaction between nicotine and cues and their importance on human smoking and relapse has also been extensively studied. For example, when presented with smoking cues (ashtrays, cigarettes, pictures of people smoking etc.) people take longer puffs and smoke their first cigarette sooner when given
the opportunity (Payne et al., 1991). The effect of cues on craving and relapse seem to be more important for women than for men, particularly for olfactory and taste stimuli which seem to be more reinforcing for women. They are also more poorly able to identify the changes in nicotine levels in cigarettes and place more emphasis on cues when rating smoking satisfaction (Perkins et al., 2001). Brain imaging studies have been used to evaluate the effect of cues on brain activation in smokers, and have seen increased activity in areas like the amygdala, ventral striatum, thalamus, hippocampus, orbitofrontal cortex, and dorsolateral prefrontal cortex, indicating a role for the emotional significance of the cues presented. Increased activity was positively correlated with self-reported craving (Franklin et al., 2007). Cues seem to have the greatest effect on brain activation when associated with an expectancy to smoke and drug availability (McBride et al., 2006).

Rats are more able to acquire the self-administration behavior and move up in reinforcement schedules (from FR1 to FR5) when the NSA is paired with some type of stimulus such as a light cue, without which they self-administer nicotine at much lower levels (Cohen et al., 2005). These cues are then able to maintain responding when nicotine is removed and high levels of responding can be reinstated after periods of withdrawal and extinction for a long period of time (Cohen et al., 2005; Liu et al., 2007). These effects have been specifically blocked with drugs such as the cannabinoid receptor antagonist rimonabant (Cohen et al., 2005), the nonselective nAChR antagonist mecamylamine (Liu et al., 2007), the GABA_B receptor antagonist CGP44532 (Paterson et al., 2005), the D_3 receptor antagonist BP 897 (Le Foll et al., 2003), the mGlu5 receptor antagonist MEP and mGlu2/3 receptor agonists (Markou, 2009).
Most research on nicotine and its relationship with different stimuli has been done looking at pairing infusions of nicotine with a compound visual stimulus (CVS) and its effect on the acquisition and maintenance of nicotine self-administration. The compound visual stimulus usually consists of the house light turning off and a dim cue light above the response device turning on, although studies have examined these stimuli individually and alongside auditory cues as well. A study examining the difference between turning the houselight on versus turning it off as stimuli associated with nicotine found no discernable difference between the two in their ability to maintain lever pressing and work as primary reinforcers, with nicotine increasing responding for both types of stimuli (Raiff et al., 2009). On the contrary, another study found that nicotine only increases responding for turning the houselight off, though this was compared with turning the stimulus light on, so this effect could have been due to differences in stimulus intensity (Palmatier et al., 2007b). Though tones and light cues have been paired together, few if any studies have examined a tone by itself in conjunction with nicotine (Chaudhri et al., 2006a). Two competing theories have been proposed concerning how stimuli combine with nicotine to foster self-administration and addiction. The traditional view is that this occurs through the typical Pavlovian method of conditioning. It is possible that the light itself is relatively neutral and when it combines with the nicotine, the reinforcing properties of the nicotine transfer onto the stimulus leading to an increase in responding. Rats will lever press for the light cue and the nicotine independently of each other, however, indicating the compound visual stimulus (CVS) may not be neutral and when the two cues are presented together, lever-pressing increases dramatically in a synergistic manner (Caggiula et al., 2002). An
alternate theory more recently proposed suggests that nicotine is important in two ways. As in the first theory, nicotine is considered a weak primary reinforcer. Lever-pressing for the administration of nicotine is then reinforced by the neurobiological effects and pharmacological properties of the nicotine itself, so that drug using behavior is reinforced by the effects of the drug (Chaudhri et al., 2006b; Raiff et al., 2006). Additionally, it is proposed that nicotine has a reinforcement-enhancing effect on other stimuli present at the time. It has been shown that when nicotine is randomly infused, rats will press at much higher levels for the CVS. Here the compound visual stimulus is not contingent on nicotine delivery, yet rats will press more for the stimulus in the presence of nicotine. This indicates that the visual stimulus can act as a primary reinforcer while nicotine increases responding for the nonpharmacological stimulus. However, the pairing in these studies between the nicotine and compound visual stimulus was weakened due to the noncontingent delivery of the visual stimulus, which suggests that responding for the visual stimulus was not due to a Pavlovian association between the two, but was a direct result of the nicotine (Donny et al., 2003).

Evidence seems to indicate that the strength of a stimulus and the degree that it is reinforcing in and of itself helps to determine which property of nicotine dominates in the facilitation of self-administration. Thus, responding for nonpharmacological stimuli which are moderately to strongly reinforcing will be acquired at greater rates in the presence of nicotine because of the reinforcement-enhancing properties of nicotine. On the other hand, a nonpharmacological stimulus which is in utility a weak primary reinforcer and serves as an unconditioned stimulus will be potentiated by the primary reinforcing actions of nicotine, enabling the weak stimulus to subsequently serve as a
conditioned stimulus. Under these assumptions, contingent nicotine therefore increases responding for weaker non-pharmacological stimuli, whereas non-contingent nicotine can be seen to elevate responding for already reinforcing stimuli (Chaudhri et al., 2006b; Chaudhri et al., 2007; Palmatier et al., 2007a). It has also been shown that by increasing the reinforcing property of a stimulus (i.e. by previously pairing it with sucrose) the greater the effect that nicotine will have on responding for that stimulus (Chaudhri et al., 2006b). Clinical observations seen in women have been corroborated with sex differences seen in rats (Perkins et al., 2001). Female rats were found to show a greater rate of responding when nicotine and a visual stimulus were paired together, indicating the possibility that either female rats show greater sensitivity to nicotine’s reinforcing capabilities or they are more sensitive to the combined effects of the nicotine plus visual stimulus (Chaudhri et al., 2005).

If the idea that nicotine enhances responding for other stimuli is correct, it stands to reason that there should not be anything about the CVS in particular that is special in terms of the acquisition and maintenance of nicotine self-administration. Rather, it is the properties of the nicotine which make this pairing and synergy possible in the first place. Therefore, any stimulus should be able to substitute for the light cue and facilitate administration in the same way. It is also possible that because the light cue is reinforcing by itself, another reinforcing stimulus should be able to take the place of the light cue and promote NSA. The focus however, on pairing a stimulus with nicotine has been almost entirely placed on a light cue, leaving a lot of opportunity for the exploration of other types of cues. It is important, when trying to understand whether the CVS works through a Pavlovian method of conditioning or by acting as a primary reinforcer
combined with special properties of nicotine, to examine the generality of the CVS to other modalities of stimuli.

The goal of this study therefore is to more completely replicate the way drugs of abuse are experienced by humans, and examine nicotine’s effects on another relevant stimulus. It is well-known that rodents have a high propensity to associated changes in interoceptive state with tastes or flavors. In this study, the stimulus chosen was a saccharin solution flavored with Cherry Kool-Aid which was available upon response completion simultaneously with either contingent or non-contingent nicotine. Subsequent tests then evaluated differences in NSA with varying concentrations and infusions lengths of the flavor; responding for the flavor itself was also examined.
CHAPTER 2
METHODS

Subjects

Adult male Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN) and were allowed 5-7 days to acclimate to the vivarium before the start of the experiment. Rats were housed in polycarbonate cages with wood chips as bedding whenever they were not in the operant chambers. They were given ad libitum access to Purina (No. 5001) pelleted chow until a week after surgery. After that, they were fed a daily ration of about 6-18g in order to maintain their body weights between 85-90% of pre-surgery weights. Ad libitum access to water was provided for the entirety of the experiment, except for the evening before their first exposure to a saccharin solution, when water was removed in order to provide additional motivation to consume the new, sweetened water.

The Psychology Department vivarium is staffed and managed by Animal Care Services (ACS) at the University of Florida and has Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accreditation. Animal care and use was consistent with the principles in the NRC Guide for the Care and Use of Laboratory Animals and was reviewed and approved by the University of Florida (Institutional Animal Care and Use Committee). Behavioral test chambers were located in the same room as animal housing and were maintained by the investigator. The vivarium was kept at a constant 22-26 °C with 50-70% humidity.

Surgery

Rats were anesthetized in preparation for surgery using inhalation of 2.5% isoflurane in oxygen. Verification that the rats were fully anesthetized was through
ensuring areflexia to hind-paw pinching from time to time throughout the surgery. Once a rat was anesthetized, an area of about 3 x 3 cm was shaved on the dorsal scapular area. For rats additionally receiving an intraoral catheter, the top of the skull was also shaved. Additionally, for implanting the jugular vein catheter, an area of 1 x 3 cm was shaved on the ventral side, directly above the right jugular vein. The shaved areas were sterilized using a Betadine solution (10% Povidone-Iodine Topical Solution: Henry Schein Animal Health; Melville, NY) and Paralube was placed on the corneas to prevent them from drying out.

The first surgical incision, 1.5cm in length, was through the skin and connective tissue overlying the right jugular vein. The area was then blunt dissected until the jugular vein was located, stripped of connective tissue and secured with a suture. Jugular vein catheters were made from 9 cm lengths of MicroRenathane tubing (.58mm ID x .94mm OD, MRE-037, Braintree Scientific, Inc, Braintree, MA), with a collar 3.4 cm from one end and another at the opposite end, and were sterilized with ethylene oxide beforehand. Catheters were filled with sterile heparinized saline immediately prior to implantation. A catheter was then inserted through a small incision in the exposed jugular vein, was advanced to the 3.4 cm collar, and then was tied and secured in place with sterile silk suture. Next, a 2 cm incision was made in the scapular region. Curved hemostats were then used to tunnel subcutaneously from the dorsal incision in front of the shoulder to the incision on the ventral side; the catheter was grasped and pulled to the dorsal site where it was attached to 23 gauge stainless steel tubing bent 90 degrees in a prefabricated port with a nylon threaded hub and a small disc of surgical mesh (313-000BM-15, Plastics One Inc., Roanoke, VA). A piece of polyvinyl tubing about 2
cm in length (.51mm ID x 1.52mm OD; Norton Performance Plastics, Akron, OH) was fitted to the exterior or distal end of the 23 gauge tubing and this was closed with a stainless steel pin. The mesh disc was fitted in the subcutaneous space and both incisions were then closed using interrupted sutures (Braunamid white, Aesculap Inc., Center Valley, PA).

Some rats additionally received intraoral catheters immediately after closing the jugular catheter incisions. Intraoral catheters were made from a single piece of sterilized polyethylene tubing (PE60), 75mm in length. This tubing was flared at one end and a small (~3 mm diameter), flat piece of silicon sheet was slid down to the flared end; the purpose of the flare and silicon washer was to hold the catheter in the oral cavity. An incision was made on top of the skull about 1cm in length. The skull was then cleaned of connective tissue and was dried using 70% isopropyl alcohol on a sterile cotton swab. The rat’s mouth was then propped open and a 21 gauge needle was attached to the non-flared end of the catheter. The needle was pushed through the left of the oral cavity just lateral to the first molar, and was tunneled subcutaneously through the jaw muscles and to the skull incision, taking care to pass rostrally to the eye orbit. The catheter was pulled so that the flared end was flush up against the inside of the cheek, the needle was removed, and a port attached. Three small holes were then drilled 1mm into the skull and three screws were inserted into the skull in order to secure the port and provide an anchor for the dental cement cap. The port was made from 23 gauge stainless steel tubing (made from a hypodermic needle, filing the ends blunt) bent at a ~80 degree angle with the bend about 2/3 down the length of the needle. A small (~1cm) piece of plastic tubing was placed on the longer end and closed
with a small pin. This port was then secured to the top of the skull first by inserting 3 stainless steel jeweler’s screws into the exposed skull, then securing the port with a small amount of dental acrylic (Lang Dental: Henry Shein Animal Health; Melville, NY) placed around the screws and port and allowed to dry. The incision was then closed to overlay the cement mound using non-absorbable suture (Braunamid white, Aesculap Inc., Center Valley, PA). This allows the skin to heal over the cement and provides a more secure attachment to the skull.

Once the surgery was completed, rats were given subcutaneous injection of 5 mg/kg of Ketorolac tromethamine (Sigma Chemicals) and 10 mg/kg of enrofloxacin (Bayer HealthCare LLC, Shawnee Mission, KS). Subcutaneous injections of warm saline (2ml) were also administered to those rats that had both the IO and IV surgeries done. Rats were placed in a recovery chamber, warmed from underneath with a heating pad, until they were fully ambulatory, before returning to their individual home cages.

**Catheter Maintenance**

Jugular vein catheters were flushed once a day with a heparinized saline solution (0.17 mg/ml) for the entirety of the experiment, beginning two days after surgery. Subcutaneous injections of enrofloxacin were given for 7 days after the surgery. Once a week rats were given streptokinase (Sigma Chemicals Co., 400 units) to prevent fibrin formation at the tip of the catheter.

Intraoral catheters were flushed once a day by injecting 10ml autoclaved water into the IO port; this serves to remove saliva and any small particles of food in the catheter.
Behavioral Test Chambers

Experiments were conducted in eight Operant test chambers (30.5 cm x 24.1 cm x 21.0 cm, ENV-008CT, Med Associates, St. Albans, VT). The chambers themselves were located in ventilated, sound-attenuating boxes. Each chamber had one retractable lever and a cue light situated about 5 cm above the lever. There was also a house 28V light about 2 cm below the top of the chamber. In the roof of the chamber was a hole about 5 cm in diameter to allow passage of one or two infusion lines. These lines were made of polyethylene tubing (PE60). The distal end of each line was attached to a 30 ml syringe driven by an infusion pump (PHM-100 set at 3.33 RPM, Med Associates). This line led to one input arm of a double lumen swivel (Instech Laboratories, Inc. Plymouth Meeting, PA) that was suspended above the cage with a counterbalance mechanism (Med Associates). The output from the swivel was a ~30 cm PE60 line, protected from biting with spring wire, was attached to the ports on the rat when in use. This configuration allowed the rats to have unlimited movement within the chamber. For rats receiving nicotine only, one line was used. Nicotine was passed from the syringe through a filter (0.22 µm; Cameo #25ES; Osmonics) before entering the lines and being infused into the rat. For rats receiving IO infusions also, the second lumen of the swivel was used for these solutions.

Before rats were allowed to self-administer nicotine they were trained to press a lever for pellets (45 mg, Purified Rodent Tablet, 5TUL, TestDiet, Richmond, IN; composition expressed as a % kilocalories was fat 12.7%, carbohydrate 66.8%, protein 20.5%) in the chambers. The pellet receptacle for the pellet dispenser was located next to the lever, and one lever press resulted in delivery of a single pellet (FR 1 schedule). All devices were interfaced to a computer through an input/output module (DIG-716,
Med Associates) and all responses, food rewards and infusions were monitored and recorded by a software package (Med-PC Version IV). Chambers were cleaned daily and pans filled with Sani-Chips were refreshed daily and chambers were wiped down with a 70% ethanol solution every day between sessions.

Nicotine

Nicotine hydrogen tartrate (Sigma Chemicals) was dissolved in a sodium-phosphate buffer (pH~7.35) at a concentration of 0.4mg/ml. Nicotine infusions were passed through a nitrocellulose filter (0.22μm; Cameo #25ES; Osmonics) before injection. The syringes were 30 ml and with the pump motor yielded an infusion rate of 2.385 ml/min (0.04ml/sec). With 1-sec infusions, this gave 25 injections per ml. The parameters of the unit nicotine infusion remained constant throughout the experiment.

Saccharin Acclimation

After the period of acclimation, all rats were water deprived overnight and then given access to bottles filled with a solution made up of autoclaved water and .1% saccharin for two days in place of their normal water. This was to ensure that the saccharin solution would not be novel when the rats in the flavor group received infusions of the nicotine-paired flavor. The amount of saccharin water consumed was recorded each day, although rats typically consumed all of the solution.

Training Protocol

For at least two days, or until the task was completed, rats were placed in the chambers for 30 minute sessions to learn to lever-press for food. During this time, the chamber house light remained on and at no point did the house light turn off. In contrast to typical training protocols, there was no cue light presented to indicate that a pellet had been dispensed contingent upon a lever press. The reason training was completed
this manner was to maintain an identical training protocol between groups subsequently assigned to flavor or light cues, with no possibility of differential carry-over to the cues in the next phase of the experiment. Completion of this training task occurred comparably fast to previous studies in this lab using a cue light, so did not seem to be affected by omission of the light cue. Rats were trained using an FR1 schedule, where a single lever press resulted in one pellet being dispensed into the pellet receptacle adjacent to the lever. Completion of the task was declared when the rat pressed the lever 75 times in 30 minutes on two consecutive days and consumed the majority of the pellets dispensed. Subsequent pellet “reminder” sessions were given if self-administration of nicotine became very low (usually once or twice through the remainder of the study).

**Compound Visual Stimulus (CVS) Group**

We started with \( n = 17 \) rats but some data were lost due to sick rats and catheter problems and the actual Ns are shown in the results. The day after a rat reached the criterion for pellet training it was hooked up to the infusion lead(s) in the chamber. The rat was then placed in the behavioral operant chamber for one 50 minute session (for which stable responding throughout the session has been previously seen: see Rowland et al., 2008) every day for approximately two weeks. During these sessions, a lever press resulted in a one-second infusion of nicotine through the IV port and into the jugular vein. Once the lever had been pressed, the house light then turned off and the cue light turned on for a 30 second period. During these 30 seconds, the lever retracted and the rat was no longer allowed to self-administer until the lever was protruded, the cue light turned off, and the house light turned back on. The FR1 schedule used here was also used for all subsequent groups. Though the intention was to move rats up to
an FR5 schedule, this was not realistic based on the level of responding seen throughout the studies.

**Flavor Group**

We started with $n = 12$ rats, but some data were lost due to sick rats and catheter problems and the actual Ns are shown in the results. As for the CVS, a rat was then placed in the behavioral operant chamber for one 50 minute session every day for approximately two weeks. During these sessions, a lever press resulted in a one-second infusion of nicotine through the IV port and into the jugular vein. Simultaneously, a one-second infusion of flavored saccharin was infused into the IO port, directly into the mouth of the rat. There was a 30 second timeout in this condition, during which the lever retracted and the rat was unable to press for more nicotine and favored solution. The house light remained on and there was no change in the cue light. The flavor solution was made up of autoclaved water containing .1% saccharin and .05% Cherry Kool-Aid (Kraft Foods Global Inc.; Northfield, IL) powder. The flavor was added to a saccharin solution, rather than to plain water, because previous protocols have shown this combination is reinforcing, preferred to plain water, and works well in preference testing and in combination with self administration of intragastric glucose (Myers et al., 2003).

**Parametric Tests**

The parameters described above are known to work for a light cue associated nicotine self-administration, but may not be optimal for the taste delivery. Previous studies from our lab have shown that the chosen concentration of flavor can work as an adequate pairing when licked from a spout and followed by glucose reinforcement, but may not hold true for nicotine as a reinforcer or when the flavor is directly infused in the
mouth. Thus, we performed a series of parametric tests varying the concentration of the tastant and the infusion characteristics.

**Flavor Concentration**

In addition to the .05% concentration of Cherry Kool-Aid, there was a higher concentration of the Kool-Aid that was examined within the above protocol at .2% instead. Nicotine self-administration was then recorded under the two new flavor concentration groups and compared with the results of the initial flavor study. We started with \( n = 12 \) rats but some data were lost due to sick rats and catheter problems and the actual Ns are shown in the results.

**Duration of Infusion**

The above flavor protocol from the first part of the experiment was then conducted with a different flavor infusion duration. One way to vary the volume of the infusion is to change the size of the syringe that is placed into the pump to administer the flavor. This was not chosen however, because that would cause an increase in the volume of the flavor dispensed in the mouth during the one second infusion to rates that might exceed the rats’ ability to swallow. Instead, we chose to vary the duration of the infusion. A longer infusion length of 10 seconds (thus, 10 times the volume delivered) was examined. Nicotine self-administration was recorded under the new flavor infusion length and compared with the results of the initial flavor study. We started with \( n = 10 \) rats but some data were lost due to sick rats and catheter problems and the actual Ns are shown in the results.

**Self-Administration of Flavor Alone**

Since some reports indicate that the CVS by itself is reinforcing, it was important to examine whether the flavor itself is reinforcing. Therefore, the cherry flavor study was
conducted in the same manner, except no infusion of nicotine accompanied the flavor infusion contingent upon the lever-press (n = 8). Lever-pressing was then recorded to see if there was a difference between random lever-pressing and pressing for the flavor, in the absence of all light cues and nicotine. To help understand any increases or decreases in self-administration in the flavor protocol as compared to the light group lever pressing for the flavor alone was compared to lever pressing for a flavor and nicotine simultaneously.

**Non-contingent Nicotine**

Previous studies which examined the relationship between the CVS and nicotine have examined the rate of responding for the CVS in the presence of non-contingent administrations of nicotine. Typically, non-contingent nicotine administration uses a yoked control design, in which the self-administration pattern for a ‘contingent’ rat is imposed on another who thus receives non-contingent injections of nicotine. This was not feasible in this study however, because self-administration was hard to acquire in the previous nicotine-flavor studies. Instead, a program was written to administer 25 programmed infusions of nicotine throughout the 50 minutes session randomly, with infusions being no closer than 30 seconds apart to account for the 30 second time outs that would have been seen had a yoked design been employed. Therefore, rats were given access to a lever on an FR1 schedule, where a single lever press resulted in an infusion of flavor in the presence of non-contingent infusions of nicotine. We started with n = 17 rats but some data were lost due to sick rats and catheter problems and the actual Ns are shown in the results. The flavor was made up of .1% Saccharin and .2% Kool-Aid (instead of the .05% Kool-Aid concentration used in the initial flavor studies, because of subsequent results indicating more rats might achieve self-administration
with increased concentrations of the flavor). Self-administration of the flavor was recorded.

**Preference Testing**

Rats from the non-contingent nicotine/flavor study (n = 10) were then given a two bottle preference test with Cherry and Grape Kool-Aid to see if there would be a preference or avoidance of the Cherry flavor that was self-administered in the presence of nicotine. One bottle was filled with .2% Cherry Kool-Aid and the other was filled with .2% Grape Kool-Aid and were positioned in the chambers on retractable sipper devices (Med Associates) and made accessible from the chamber when the program was turned on. The flavors were fully counterbalanced on each side of the chamber over the course of three days. The sessions were 50 minutes long, and licks on each bottle were recorded via a contact lickometer (Med Associates). Preferences were classified as the number of licks for one flavor being 70% or higher of the total licks, and likewise aversions were defined by number of licks on one flavor being 30% or less of total licks.

**Data Analysis**

All measures were analyzed with SPSS statistical software (SPSS, Inc., Chicago, IL). The initial comparisons between the flavor and light groups were analyzed with a one-way analysis of variance (ANOVA) with the type of stimulus (either flavor or light) as the independent variable and the number of infusions for each day as the dependent variable. Extinction was also examined in the same way. The parametric tests were analyzed using a one-way ANOVA with the parametric change as the independent variable and the number of responses as the dependent variable. Tukey’s post-hoc test for multiple comparisons was done for each day to determine where the differences between the groups lie. A one-way ANOVA was then used to determine if there was an
effect of parametric changes on the percent of rats able to attain stable nicotine self-administration, with the parametric change as the independent variable and the percentage of rats over 6 or 10 responses as the dependent variables. Tukey’s post-hoc tests for multiple comparisons were then looked at for all days to determine where the differences were occurring. A one-way ANOVA was also used to determine if there was an effect of flavor in the 2-bottle preference test, and a paired-samples t-test was then subsequently used for each day to determine if the effect persisted over all three days. In all cases, $p < .05$ was considered significant, though significances between $p < .05$ and $p < .1$ were sometimes mentioned. Graphs were generated using SPSS statistical software as well.
CHAPTER 3
RESULTS

Cherry Kool-Aid Flavor and Visual Stimulus

In general, rats in the flavor condition pressed less for nicotine than those in the visual stimulus condition (Figure 3-1). This group difference was significant on days 2 [F (1, 29) = 5.073, \( p < .05 \)], 5 [F (1, 26) = 13.999, \( p < .01 \)], 6 [F (1, 24) = 20.841, \( p < .01 \)], 7 [F (1, 23) = 15.218, \( p < .01 \)], 8 [F (1, 26) = 34.669, \( p < .01 \)], 10 [F (1, 20) = 12.211, \( p < .01 \)], 11 [F (1, 20) = 20.429, \( p < .01 \)], and 12 [F (1, 19) = 14.848, \( p < .01 \)], and approached significance on days 1 [F (1, 29) = 3.104, \( p = .089 \)], 4 [F (1, 27) = 3.642, \( p = .06 \)], and 9 [F (1, 22) = 4.008, \( p = .058 \)].

During extinction, when nicotine was replaced with saline, rats in the flavor group responded less than those in the CVS group (Figure 3-2), with significant differences between groups responding for the flavor and visual stimulus on days 1 [F (1, 23) = 12.508, \( p < .01 \)] and 3 [F (1, 22) = 10.601, \( p < .01 \)], and near significance on day 2 [F (1, 21) = 3.863, \( p = .063 \)].

Responding for flavor alone without any prior responding for nicotine and flavor together was also recorded (Figure 3-3). There was no difference over the course of 8 days for responses made for the flavor only [F (7, 48) = 1.226, \( ns \)].

Parametric Tests: Flavor Concentration

There was no clear distinction between the different flavor concentrations. Rats in the .2% flavor group responded more than rats in the .05% flavor group over several days (Figure 3-4). A one-way ANOVA followed by a Tukey’s post-hoc between the two concentration manipulations revealed significant differences on days 4 [F (2, 30) = 7.228, \( p < .01 \)], 11 [F (2, 20) = 3.572, \( p < .05 \)], and 12 [F (2, 19) = 3.902, \( p < .05 \)].
Contrary to that however, rats in the .05% flavor condition responded more than rats in the .2% flavor group on day 6 [$F(2, 25) = 4.265, p < .05$].

Compared with rats in the .05% flavor concentration group, there was a higher percentage of rats in the .2% flavor concentration who were able to reach stable levels of responding using a criterion of 6 or more responses (Figure 3-5) with a main effect of parametric change on responding [$F(2, 347) = 3.421, p < .05$]. A one-way ANOVA followed by Tukey’s post-hoc tests revealed this difference was on days 4 [$F(2, 30) = 5.428, p < .01$], and 11 [$F(2, 30) = 6.334, p < .01$]. There was no significant difference in the percentage of rats in the two flavor concentrations reaching stable levels of responding when the criterion was 10 or more responses (Figure 3-7). However, there was a significant difference between the two flavor concentration groups in the percentage of rats with responding over 10 for day 6 [$F(2, 25) = 3.796, p < .05$], with Tukey’s post-hoc revealing that rats in the .05% flavor concentration responded more than those in the .2% group. Despite lack of statistical difference between these groups, there is a clear trend for more rats in the .2% flavor group being able to maintain responding than those in the .05%, particularly after week one (Figures 3-5–3-8).

**Parametric Tests: Amount and Duration of Taste Infusion**

There did not appear to be any clear-cut difference between groups varying in flavor infusion length (Figure 3-6). A one-way ANOVA and subsequent Tukey’s post-hoc tests showed that rats in the .05% flavor condition (1 second infusion) responded more than the 10 second infusion group on day 5 [$F(2, 27) = 3.527, p < .05$], and those in the .2% concentration group (1 second infusion) responded more than those in the 10 second group on day 12 [$F(2, 19) = 3.90, p < .05$]. There was however, a significantly greater percentage of rats in the 10 second flavor concentration who were able to reach
stable levels of responding using a criterion of 6 or more responses than those in the .05% concentration group on day 4, \[F (2, 20) = 6.334, p < .01\]. No significant difference was seen between the two infusion length groups (Figure 3-8) for the percentage of rats able to attain stable self administration using the criterion of 10 or more infusions.

**Non-contingent Nicotine**

Responses for the flavor concentration in the presence of non-contingent infusions of nicotine were recorded (Figure 3-9), with responding decreasing dramatically over time.

**Two-bottle Preference Test**

The .2% cherry flavor that had been previously paired with nicotine was licked less than the novel .2% grape flavor when rats were given simultaneous access to both (Figure 3-10), \[F (1, 62) = 11.499, p < .001\]. A paired samples t-test indicated that the grape flavor was licked more than the cherry flavor on days 1 \[t (11) = 2.617, p < .05\] and 2 \[t (9) = 2.257, p < .05\], but failed to find a significant difference between the grape and cherry flavors on day 3.

The criterion for avoidance of the cherry flavor was defined as 70% or more of the total licks on the grape flavor, and this occurred in 9 out of 12 rats (75%) on the first day, in 6 out of 10 rats (60%) on the second day, and in 4 out of 10 rats (40%) on the last day.
Figure 3-1. Mean nicotine infusions over the course of twelve days in groups, for which nicotine was paired with a flavor or a light cue.

Shown are the M +/- SE for ns = 9-14 (flavor) and 12-17 (light). *P < 0.05, **P < 0.01 flavor v. light group difference.
Figure 3-2. Mean responses made over the course of three days for saline and a flavor or light cue in groups for which nicotine was previously paired with the cue.

Shown are the M +/- SE for ns = 10-11 (flavor) and 13-14 (light) **P < 0.01 flavor v. light group difference.
Figure 3-3. Mean responses for the .05% flavor only over the course of seven days. Shown are the M +/- SE for n = 8.
Figure 3-4. Mean number of nicotine infusions over the course of twelve days in groups for which nicotine was paired with different concentrations of the flavor and different infusion lengths of the flavor.

Shown are the M +/- SE for ns = 6-12 (.2% flavor: a), 7-10 (10 second infusion: b), 9-14 (.05% flavor: c). *P < 0.05, **P < 0.01, with ab = difference between .2% flavor and 10 seconds, ac = difference between .2% flavor and .05% flavor; and bc = difference between 10 seconds and .05% flavor.
Figure 3-5. The percent of rats in groups who responded more than 5 times per session over the course of twelve days.

Shown are the M +/- SE for ns = 6-12 (.2% cherry flavor) and 9-14 (.05% cherry flavor).

**P < 0.01 flavor v. light group difference.
Figure 3-6. The percent of rats in different infusion length groups who responded more than 5 times per session over the course of twelve days.

Shown is the percent for rats for ns = 7-10 (10 second infusion) and 9-14 (one second infusion ns).
Figure 3-7. The percent of rats in different flavor concentration groups who responded more than 9 times per session over the course of twelve days.

Shown is the percent for rats for ns = 6-12 (.2% cherry flavor) and 9-14 (.05% cherry flavor). *P < 0.05 flavor v. light group difference.
Figure 3-8. The percent of rats in different flavor infusion length groups who responded more than 9 times per session over the course of twelve days.

Shown is the percent for rats for ns = 7-10 (10 second infusion) and 9-14 (one second infusion).
Figure 3-9. Mean responses for .2% cherry flavor in the presence of noncontingent nicotine over the course of ten days.

Shown are the M +/- SE responses for ns = 12-17.
Figure 3-10. Mean responses over the course of three days for .2% cherry and .2% grape flavors after ten days of responding for the .2% cherry flavor in the presence of noncontingent nicotine.

Shown is the M +/- SE licks for the two flavors over three days (day 1 ns = 12; day 2 ns = 10; day 3 ns = 10) *P < 0.05, **P < 0.01 .2% cherry flavor v. .2% grape difference.
CHAPTER 4
DISCUSSION

The goal of this project was to test the hypothesis that flavor may be a more effective conditional stimulus than a visual (light) cue when associated with the weak primary reinforcer, nicotine. The principal finding was that a flavor solution (Kool-Aid flavored saccharin) does not improve the acquisition of nicotine self administration in rats, or produce higher levels of drug taking, compared to a light cue. In extinction, when saline was substituted for nicotine, rats trained to receive the flavor stimulus simultaneously with nicotine exhibited a much greater decrease in lever pressing compared with rats trained to receive a light cue with nicotine. Although responding for the flavor alone was not able to be maintained, light cues sustained stable levels of responding across several days of extinction, a result which suggests that the flavor was unable to serve as a conditioned reinforcer (Caggiula et al., 2001; Mathier-Kia et al., 2002). Further, despite relatively stable levels of responding for the flavor solution itself, rats did not press at high rates for the flavor in the presence of noncontingent nicotine infusions. Because responding on a lever in the absence of pellets was not examined, we cannot be sure that the responding seen for the flavor reflects its ability to serve as a reinforcer under these conditions and is not simply an extinction curve from the lever training experience with pellets.

Though largely not statistically significant, there did appear to be a slightly higher percentage of rats that were able to achieve more stable levels of responding over the course of 12 days if they were receiving the higher concentration of the flavor compared with the lower concentration and the longer infusion length. These changes in the salience of the flavor, by changing the concentration of the Kool-Aid, seemed to
increase the number of rats able to acquire stable nicotine self-administration, but did not markedly affect responding in the presence of noncontingent nicotine. When rats were given a two bottle preference test between two flavors, one previously associated with noncontingent infusions of nicotine and the other a novel flavor, there was a significant avoidance for the flavor that had been paired with nicotine during the first two days, and persisted in some rats even on the third day. An additional confound is that the non-nicotine flavor was novel, which could have influenced the rats’ motivation to try the new flavor over one with which they already had experience.

There are several stimuli that are paired with nicotine in human smoking, many of which are inherent to the cigarettes and cigarette smoke and are independent of the smoking environment. The flavor of cigarettes is one such cue that is always associated with smoking.

Several previous studies have examined the aversive properties of nicotine using a taste or flavor association task. In these protocols, a flavor (the S+) is consumed before acute administration of nicotine. In a discriminative version of this protocol, the S+ is delivered prior to nicotine injection while another flavor (the S-) is delivered prior to saline injection; this is usually performed on alternating days for up to 3 cycles, then on the 7th day both flavors are presented and preference for one flavor over another is measured (Fenu et al., 2001; Shafe et al., 1998). Tests like this show either a lack of preference for the nicotine-paired flavor or often, taste avoidance (Parker, 1995). Some research seems to suggest that this avoidance is due not to aversion but to anticipatory contrast, the concept that when a flavor which is favorable is presented before a drug, the flavor is avoided in anticipation of the more rewarding effects of the drug. (Grigson
et al., 2002; Risinger et al., 2002). Thus, a lower preference in this type of protocol may not necessarily indicate an aversive effect.

However, no study prior to the present work has examined the notion that nicotine might be able to increase responding for a flavor when that flavor is repeatedly paired simultaneously with the nicotine. The results of the current two-bottle preference study support the idea that a nicotine-paired flavor is avoided since the preference for the non-nicotine paired flavor was preferred. In contrast to typical flavor aversions that are much more resistant to extinction however (Lamprecht, et al., 1997), the avoidance in the present work lasted for only the first two days of the preference testing before there was no longer significant responding for one flavor over the other. When a Saccharin solution is given to rats directly before self-administering a drug like morphine, rats will subsequently avoid Saccharin solutions in the future, though they will continue to administer the drug and show a preference for the environment in which the drug is administered. It is possible that drugs which lend themselves to conditioned taste aversions, rather than avoidance, produce nausea as a side effect. However, the palatability of a particular flavor or solution is not affected when paired with drugs that are reinforcing using other paradigms like conditioned place preference, indicating that any avoidance of a flavor is not a consequence of sickness from an aversion (Parker, 2003; Reicher, et al., 1977). Latent inhibition refers to a phenomenon in which prior exposures to an unconditional stimulus (drug, in this case) before conditioning trials can help to overcome any taste avoidance in flavors paired with drugs (Parker, 1995). This was not used in this study because pretreatment with nicotine may affect the association of a cue with nicotine (Olausson et al., 2004) and it was important to see the
pairing of the two without additional confounds; however, prior injections of nicotine do not seem to affect (in either direction) acquisition of NSA (Shoaib et al 1997).

It has been shown that adolescent rats consume more of a nicotine paired flavor than do adult rats in studies which pair the two together, showing that the appearance of taste aversions in the presence of nicotine demonstrate a decline in younger rats, for whom it seems the rewarding properties of nicotine are stronger (Wilmouth et al., 2004). Rats in the present study were past adolescence because of the need for more fully grown animals to maintain stable IV and IO cannulas, and possibly better recovery from surgery.

Because flavor is paired with nicotine in human smoking, it was important to understand whether this pairing contributes to nicotine seeking behavior. The most efficient way to pair nicotine and a flavor in this way is by simultaneous administration of the two, with nicotine being administered intravenously and the flavor being infused directly into the mouth by way of an intraoral catheter. Though this double surgery is difficult and the recovery process is more involved than just implanting an IV catheter, we showed that the two surgeries were possible. There were postsurgical problems with recovery and infection in some double catheter rats however, so it is possible that even asymptomatic rats could have had subclinical infections that compromised their behavioral responding in the chambers. One way to have examined if this affected responding would have been to perform the double surgery on all rats, including those in the cue light group, and compared levels of responding between the two groups. This was decided against however, because of the higher incidence of premature death and
infection in the IV/IO rats. Similarly, because of this problem, it was not possible to look at reinstatement following extinction.

One concern was with varying the amount of flavor the rats were receiving was that increasing the syringe size and therefore increasing the size of the bolus injected into the mouth might be too overwhelming for the rat because it may not be able to swallow as fast as the delivery. Instead, we chose to vary the infusion duration. The infusion length examined in the parametric portion of the study was 10 seconds long. Since the IO catheter opens up into the mouth it was possible that the flavor might get displaced with mouth movement or saliva, so it was important to ensure that the rats were actually getting enough of the flavor to make an association. Likewise, the light stimulus itself was quite a bit longer than 1 sec, with the houselight turning off and the cue light turning on for the duration of the 30 second time out, so it was important to see if making the flavor stimulus longer as well would have an effect.

The method of simultaneous administration was extremely beneficial despite some surgical issues however, because it allowed us to directly manipulate the size and length of both the nicotine and flavor infusions for maximal pairing. It seems that in order for any stimulus to be paired with nicotine and result in assisting in the acquisition and maintenance of the behavior, that stimulus needs to be presented in a way that is contingent upon the lever-pressing for the nicotine infusion (Caggiula et al., 2001; Grimm et al., 2000). This does not occur when nicotine delivery is contingent upon the flavor, as is the case in two bottle tests or when nicotine is placed within a flavored solution. The reverse is true as well, as it has been seen that when the nondrug is delivered noncontingently, the synergistic effect of drug and nondrug disappears. In that
case, the synergistic effect in responding seen by pairing nicotine and the cue light together disappeared when rats were given access to nicotine in the presence of noncontingent visual stimulus presentations (Chaudhri et al., 2006b).

Understanding which aspects of cigarette smoking contribute to nicotine addiction is crucial for devising novel smoking cessation treatments. As the importance of environmental stimuli is becoming more realized, so in turn is the notion that providing smokers with an alternative means for receiving nicotine is not going to be sufficient to help people quit. Not only does the addiction to nicotine need to be treated, so do the stimuli that have previously been associated with nicotine delivery (Liu et al., 2008). Studies have shown that reducing olfactory and taste cues in smoking attenuates the pleasure and behavioral reinforcement derived from smoking, indicating that the presence of the flavor experience does in fact play a role in nicotine addiction (Rose, 2006). It is possible however, that smokers initially smoke in spite of the flavor that is at first found to be either inconsequential or more likely even aversive (Palmatier et al., 2007a), and then over time the valance of the flavor is switched. One study gave direct injections of both nicotine and a dopamine receptor antagonist into the VTA, and found that the antagonist was able to switch the valance of a previously aversive dose of nicotine to make it rewarding (Laviolette et al., 2003). Broad spectrum rather than receptor-specific dopamine antagonists (Di Chiara et al., 2004) are also able to abolish conditioned taste aversions in other paradigms (Fenu et al., 2001), so it is possible then that the mesolimbic dopamine system, particularly in the VTA, works to establish positive associations between nicotine and previously aversive (or avoided) stimuli, like a flavor. More research would need to be done here to examine if this is correct,
possibly by administering noncontingent infusions of nicotine in the presence of forced exposure to a particular flavor over days. It is important to elucidate how this mechanism works in order to more effectively treat nicotine dependence.
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BIOGRAPHICAL SKETCH

Patricia E. Grebenstein graduated cum laude from Wittenberg University in Springfield, OH in 2007 with a Bachelor of Arts in psychology with a minor in English. Her interest in behavioral neuroscience began after taking several classes on physiological psychology, sensation and perception, and psychophysiology. An interest in drug addiction arose during a summer internship, where she assisted in writing a literature review on the neurobiology and neuropsychological effects of MDMA (Ecstasy). In the fall of 2007, she was accepted to the University of Florida's Behavioral Neuroscience PhD program in the Department of Psychology under the mentorship of Dr. Neil E. Rowland whose research interest included ingestive behavior and nicotine addiction. Patricia was interested in the relationship between drug and food reward as well as her work for her master's thesis. Her master's thesis was presented in spring 2010 and focused on gaining a better understanding of the role of sensory reinforcers in maintaining and sustaining nicotine self-administration, particularly a flavor stimulus. She received her Master of Science from the University of Florida in the spring of 2010, and continued on in the program with the hopes of being awarded a PhD.