Aversive control of behavior: punishing effects of intravenous nicotine in rats

by
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To

Bà Nội

Vang

Nhan, Loan

Sang, Phuong, John

&

Brian

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List of Abbreviations

CNS: central nervous system

DHβE: dihydro-beta-erythroidine

FR: fixed ratio

Hr: hour

IM: intramuscular

INJ: injection

IP: intraperitoneal

ITI: intertrial interval

IV: intravenous

KO: knock-out

LEDs: light-emitting diodes

Min: minute

nAChR: nicotinic acetylcholine receptor

S: second

S.C.: subcutaneous

VTA: ventral tegmental area

Abstract

The behavioral effects of nicotine vary widely, yet investigation of the aversive effects of nicotine has been limited. A careful examination of how the aversive effects of nicotine results in punishment of behavior may contribute to the understanding of the control of behavior by nicotine. The overall goal of these studies was to develop punishment procedures to evaluate the aversive effects of intravenous nicotine in rats. Using punishment procedures, rats were trained to respond on a lever that delivered sucrose pellets, then the effects of response-independent and -dependent nicotine injections on sucrose-reinforced lever responding were examined. Manipulation of behavioral and pharmacological parameters included the dose of nicotine delivered per injection (0.01-0.18 mg/kg), the behavioral alternatives to punished responding (reinforced or non-reinforced), and the delay of scheduled nicotine delivery. Antagonist studies were also conducted to elucidate the role of nicotinic acetylcholine receptor(s) in mediating the punishing effects of nicotine. In general, sucrose was a reinforcer of lever responding, whereas nicotine dose-dependently decreased sucrose-reinforced lever responding, such that higher doses of nicotine were required to decrease lever responding when it was administered response-independently compared to its response-dependent delivery. Punishment by nicotine was attenuated if scheduled delivery of nicotine was delayed after a response, or if the behavioral alternative to punished responding did not deliver a positive reinforcer. While the punishing effects of nicotine are mediated by nicotinic acetylcholine receptors, the $\alpha 4\beta 2^*$ subtype is not involved (as assessed by dihydro-beta-erythroidine administration). Punishment procedures can be used to study the aversive effects of nicotine in rats. Consideration of both the pharmacological and environmental conditions in which nicotine is self-administered may be important in determining the more general behavioral effects of nicotine.

Chapter 1

General Introduction

Tobacco Epidemic

Tobacco smoking is the leading cause of preventable disease and premature death in the United States (US Department of Health and Human Services 2014). It is estimated that more than 16 million Americans suffer from a disease related to smoking such as cancer, cardiovascular disease, and diabetes, which contributes to over 400,000 premature tobacco-related deaths a year in the United States. Despite the health consequences associated with tobacco smoking, over 40 million adults continue to smoke (Centers for Disease Control and Prevention 2014). While the determinants of tobacco addiction are multifaceted, nicotine, a constituent of tobacco, is considered "the drug in tobacco that causes addiction" (US Department of Health and Human Services 1988).

Reinforcing Effects of Nicotine

Although it is recognized that the effects of nicotine that contribute to addiction are diverse, such as neuroadaptations that may occur with persistent use (e.g., tolerance and dependence) and withdrawal symptoms that may be experienced with cessation (see US Department of Health and Human Services 2010), the ability of nicotine to serve as a reinforcer (i.e., a stimulus that increases the probability of behavior that leads to its delivery) is widely regarded as a crucial property of nicotine

that supports the development and maintenance of the abuse of nicotine-containing products such as tobacco (Young and Herling 1986, Ator and Griffths 2003). A significant part of nicotine abuse research has therefore been devoted to examining the reinforcing effects of nicotine.

Preclinical intravenous (IV) drug self-administration procedures in which animals perform an operant response to obtain nicotine have been the standard by which the reinforcing effects of nicotine have been examined. The use and development of IV self-administration procedures that clearly demonstrate the reinforcing effects of nicotine have been critical in the identification of pharmacological, genetic, and environmental determinants that may contribute to the development of nicotine abuse (see Perkins 1999; Laviolette and van der Kooy 2004). However, while the reinforcing effects of IV nicotine have been demonstrated in rodents (e.g., Corrigall and Coen 1989; Donny et al. 1995; Watkins et al. 1999), non-human primates (e.g., Goldberg et al. 1981; Le Foll et al. 2007), and humans (e.g., Harvey et al. 2004; Sofuoglu et al. 2008), establishing the reinforcing effects of nicotine in controlled studies remains difficult (see Le Foll and Goldberg 2009).

Procedures that facilitate reliable establishment and maintenance of nicotine self-administration require precise control of experimental parameters that include, but are not limited to, drug infusion durations that are rapid (Wakasa et al. 1995); delivering nicotine with drug paired stimuli (Goldberg et al. 1981; Chaudhri et al. 2005; 2006); and limiting access to nicotine through schedule control (Henningfield and Goldberg 1983b; Corrigall and Coen 1989), which seems to be most critical. For example, Goldberg and colleagues (1981) found that under second-order schedules of reinforcement, which both explicitly pair drug delivery with a specific stimulus and space drug availability through time, nicotine was a highly effective reinforcer and maintained rates and

patterns of behavior comparable to those maintained by cocaine in squirrel monkeys. In contrast, studies in which simple or continuous schedules of reinforcement were used either failed to establish self-administration of nicotine or maintained low response rates (see Griffiths et al. 1979). It was suggested by Goldberg and colleagues (1981) that using a second-order schedule ensured that injections were spaced at least 5 min apart, which was believed to mitigate the aversive/and or direct suppressant effects of nicotine when too frequent injections and/or large doses of nicotine were delivered.

Therefore, while self-administration procedures have been developed to demonstrate the reinforcing effects of IV nicotine, very specific conditions are often required to obtain such effects. Consequently, the reinforcing *strength* of nicotine (i.e., the likelihood that nicotine will function as a reinforcer under varying experimental conditions) has been considered weak relative to other drugs of abuse (Griffiths et al. 1979; Dougherty et al. 1981; Henningfield and Goldberg 1983b; Collins 1990). For example, studies using IV self-administration procedures to compare the relative reinforcing strength of cocaine to nicotine found that nicotine either failed to establish, or maintained lower rates of self-administration behavior than cocaine (Pickens and Thompson 1968; Ator and Griffiths 1983; Risner and Goldberg 1983), and under concurrent (and mutually exclusive) schedules of drug self-administration, cocaine is preferred over nicotine (Manzardo et al. 2001).

Aversive Effects of Nicotine

The limited conditions in which nicotine serves as a reinforcer may be related to the aversive effects of nicotine, that is, the stimulus effects of nicotine that cause an organism to behave so as to minimize exposure to nicotine (e.g., Benowitz 1990; Le Foll and Goldberg 2009). Studies using operant procedures to assess the aversive effects of

self-administered nicotine have been documented (Goldberg and Spealman 1982; 1983, Spealman 1983; Henningfield and Goldberg 1983a). In these investigations of IV nicotine self-adminstration, it was noted that doses of nicotine that were reported to be reinforcing in squirrel monkeys could also function to punish food-reinforced lever responding (Goldberg and Spealman 1982; 1983) and to maintain lever responding to avoid its scheduled injection (Spealman 1983). Similarly, it has been reported that humans will also maintain responding to avoid IV injections of nicotine (Henningfield and Goldberg 1983a). These findings suggest that nicotine may have aversive effects that can control behavior. However, investigation of the aversive effects of nicotine using operant procedures beyond these early studies has been limited. Therefore, development of operant procedures to determine both the pharmacological and environmental factors that mediate the aversive effects of nicotine may contribute to a general understanding of how nicotine can control behavior.

Neuronal Nicotinic Receptors and Behavioral Effects of Nicotine

It has been well documented that effects of nicotine are mediated through its agonist actions at nicotinic acetylcholine receptors (nAChRs) that are subdivided into muscle and neuronal subtypes (which is based on their major site of expression). And while actions at muscle nAChRs may mediate effects that contribute to the abuse of nicotine, it is the neuronal subtype that are found in both the peripheral and central nervous system (CNS) that are believed to be predominately involved in mediating the psychoactive effects of nicotine (e.g., Gotti and Clementi 2004; Albuquerque et al. 2009). The neuronal nAChRs are a functionally diverse group of ligand-gated ion channels that exist as pentamers made up of a combination of β (β2-β4) and/or α (α2-α10) subunits. The possible combinations of subunits that can make up a receptor are

responsible for the different subtypes that exist, which differ in their pharmacological and kinetic properties (see Taly et al. 2009). Therefore, considerable efforts have been undertaken to determine the possible role of receptor subtype(s) in mediating specific each of behavioral effects of nicotine.

There is substantial evidence from behavioral studies using pharmacological and genetic techniques that demonstrate that the $\alpha4\beta2^*$ subtype of nAChR are involved in mediating the reinforcing effects of nicotine (see in Picciotto and Kenny 2013). In behavioral pharmacological studies, treatment with dihydro-beta-erythroidine (DHβE), a competitive, $\alpha4\beta2^*$ selective nAChR antagonists (Williams and Robinson, 1984; Sabey et al. 1999; Shoaib et al. 2000), has been shown to attenuate self-administration of nicotine in rats (Corrigall et al. 1994; Watkins et al. 1999; Mansbach et al. 2000; Liu et al. 2007) and squirrel monkeys (Le Foll et al. 2009). In genetic studies, it has been shown that $\beta2$ knock-out (KO) mice that readily self-administer cocaine will not self-administer nicotine (Picciotto et al. 1998), and that self-administration of nicotine could be established with targeted expression of $\beta2$ subunit gene into the ventral tegmental area (VTA) (Maskos et al. 2005), suggesting that $\beta2^*$ receptors are necessary for the maintenance of nicotine self-administration. Similar observations have been reported in $\alpha4$ KO mice, such that nicotine self-administration is attenuated in $\alpha4$ KO mice as compared to wild type mice, and increases in self-administration in $\alpha4$ KO could be obtained with targeted re-expression of $\alpha4$ subunit in the VTA (Pons et al. 2008). It should be noted that a limitation of genetic studies is the possibility of adaptations in neural circuitry that may affect the interpretation of the behavioral effects of nicotine. Therefore, these studies alone are not sufficient to indicate that the $\alpha4\beta2^*$ nAChRs are involved in the reinforcing effects of nicotine, but in combination with studies using pharmacological techniques they do provide strong evidence to support the hypothesis

the $\alpha4\beta2^*$ subtype of nAChR are involved in mediating the reinforcing effects of nicotine.

Interestingly, it has been suggested that $\alpha4\beta2^*$ nAChRs may be involved in mediating the effects of nicotine-induced conditioned taste aversion (Shoaib and Stolerman 1995; Shoaib et al. 2000; 2002; Gommans et al. 2000) and nicotine-induced conditioned place aversion (Laviolette and van der Kooy 2003). However, it should be noted that a limitation of these findings is that these procedures provide an indirect measurement of the aversive effects of nicotine because they do not measure drug-taking behaviors. Pharmacological studies using operant procedures have demonstrated that nAChRs mediate the aversive effects of nicotine such that treatment with mecamylamine, a non-selective nAChR antagonist, is able to dose-dependently block the punishing effects of nicotine (Goldberg and Spealman 1982). Additionally it has been suggested that the aversive effects of nicotine are likely being mediated by nAChRs in the CNS since hexamethonium, a nicotinic antagonist with primarily peripheral effects, was not able to attenuate the aversive effects of nicotine that maintained responding to postpone delivery of IV nicotine in squirrel monkeys. Mecamylamine, on the other hand, which has access to the brain, was able to attenuate these aversive effects of nicotine (Spealman 1983). However, it is unclear whether the $\alpha4\beta2^*$ nAChRs are involved in these effects.

Recent findings from a genetic study using IV nicotine self-administration procedures in mice suggest that the aversive effects of nicotine maybe mediated through nAChRs containing the $\alpha5$ subunit (Fowler et al. 2011). In this study, it was shown that both $\alpha5$ KO mice and wild-type mice showed similar patterns of IV nicotine self-administration, since inverted U-shaped dose-response curves were obtained with both groups at doses of nicotine (0.03-1.0 mg/kg base formulation) that have been

shown to be reinforcing in other nicotine self-administration procedures in mice (see Shannon et al. 2007). However, α5 KO mice showed increased self-administration behavior at high unit doses of nicotine that are typically found on the descending (0.4-0.6 mg/kg), but not the ascending limb of the inverted U-shaped dose-response curve of nicotine self-administration in wild-type mice (Fowler et al. 2011). While it was suggested that the nAChRs containing the α5 subunit may be involved in mediating the aversive effects of high doses of nicotine that are thought to limit rates of responding (e.g., Katz 1989; Rose and Corrigall 1997), pharmacological studies have yet to verify the role of the α5 subunit in self-administration behaviors due to limited availability of antagonists that are selective for nAChRs containing the α5 subunit (see Daly 2005). Therefore, further investigation of the nAChR subtype(s) that mediate the aversive effects might be useful in understanding how the actions of nicotine on specific nAChRs control behavior.

Choice Procedures

Choice procedures, an approach that has not been used previously to examine the aversive effects of nicotine may be useful in this regard. Choice procedures are operant procedures in which subjects can respond under one of two or more alternative schedules of reinforcement (typically available for responding on different manipulanda) that are presented concurrently. It has been suggested that an advantage of using choice procedures over procedures that use single operant situations is that it may provide a more sensitive measure of the aversive effects of non-drug (Azrin and Holz 1966) and drug stimuli (Woolverton 2003; Negus 2005; Podlesnik 2010; Woolverton 2012). For instance, Azrin and Holz (1966) reported that while decreases in rates of responding on a manipulandum that delivered food paired with electric shock was

inversely related to the intensity of the shock delivered in a single operant situation, the punishing effects of electric shock could be increased if pigeons were presented with a concurrently available manipulandum that delivered food without shock. That is, under a choice situation, the intensities of electric shock that were effective at suppressing rates of responding on the manipulandum that delivered food + electric shock were lower than in a single operant situation. The authors concluded that having an unpunished outcome as an alternative consequence enhanced the punishing effects of electric shock. Similar conclusions have also been made in a study examining the punishing effects of IV histamine on food-reinforced responding in rhesus monkeys (Woolverton 2003). In this study, rhesus monkeys were given the choice to respond on levers that delivered either food or food paired with an IV histamine injection. It was reported that monkeys decreased their responding on the lever that delivered food + histamine and reallocated responses to the lever that delivered food alone. Decreases in responding on the lever that delivered the food + histamine were dependent on the dose of histamine, with greater decreases observed with larger doses. While the study did not examine the effects of histamine in a non-choice situation, it was noted by the author that the doses of histamine that were found to be punishing in the study were approximately 10 fold lower than the ones used in published studies that used single operant situations (e.g., Goldberg 1980; Katz and Goldberg 1986).

Another possible advantage of using choice procedures is that it allows for a direct comparison between the relative strength of reinforcers and punishers by measuring the allocation of responding between manipulanda that deliver either a positive reinforcer paired with an aversive stimulus (i.e., punished consequence), or a positive reinforcer alone (i.e., unpunished consequence). Furthermore, examining the allocation of responding in choice situations can be used as a control to determine

whether decreases in positively reinforced behaviors is a result of a punishing effect or a direct effect (i.e., unconditioned rate suppressant effects) of the aversive stimulus being evaluated. For instance, if decreases in responding on a manipulandum that delivered a punished consequence also resulted in the reallocation of responses (i.e., increases in responding) on a different manipulandum that delivered a unpunished consequence, this demonstrates that decreases in responding were not a result of the direct suppressant effects of the punishing consequence (e.g., Woolverton 2003; Negus 2005). Accordingly, choice procedures may provide a more sensitive measure of the aversive effects of nicotine than single operant situations, because a viable behavioral alternative is offered, which may offer advantages in terms of differentiating the punishing effects of a stimulus from its direct effects.

Aims

The goal of these studies is to contribute to a general understanding of how operant behavior can be controlled by the aversive effects of IV drug administration. The specific goal of this research was to develop IV drug punishment procedures to study the aversive effect of nicotine, and to identify pharmacological and environmental variables that contribute to punishment by nicotine. The rationale for studying the punishing effects of nicotine is that it may provide insight on how the aversive effect of nicotine may contribute to the control of nicotine-taking behavior.

Specific Aim 1

The first set of experiments (presented in Chapter 2) was designed to examine independent variables that may affect the punishing effects of IV drug administration in the rat. Using a choice procedure, rats were presented with two concurrently available

levers, in which responding on either lever resulted in the delivery of a sucrose pellet with or without an IV injection of histamine (a putative aversive stimulus). This was used to examine whether manipulating the schedule of drug delivery or a response-contingent drug history would affect the punishing effects of IV histamine. These studies were conducted to provide guidance in developing an operant procedure to study the aversive effects of IV nicotine and interpretation of data.

Specific Aim 2

The second set of experiments (presented in Chapter 3) was designed to evaluate the aversive effects of IV nicotine by examining its punishing effects. In these experiments, rats were trained to respond on a lever that delivered sucrose pellets, and then the effects of response-independent and -dependent nicotine injections on sucrose-reinforced lever responding were examined. In addition, the dose of nicotine delivered per injection (0.01-0.18 mg/kg), the behavioral alternative to punished responding (i.e., choice vs. non-choice situation), and the delay between the response and the delivery of nicotine were manipulated to examine whether nicotine-induced decreases in rates of responding were due to a punishing effect or to a direct suppressant effect of the drug, and to determine whether nicotine is characteristically similar to other punishers.

Specific Aim 3

The final set of experiments (presented in Chapter 4) was designed to elucidate the receptors involved in mediating the punishing effects of IV nicotine on sucrose-reinforced lever responding in rats observed in Aim 2. Using the same punishment procedure developed in Aim 2, antagonist studies evaluating the effects of

mecamylamine and dihydro-beta-erythroidine pretreatments on the punishing effects of IV nicotine were conducted.

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Chapter 2

Effects of drug history and intertrial interval schedules on punished responding by IV histamine

Introduction

The development of intravenous (IV) self-administration procedures to study a drug's capacity to function as a positive reinforcer (i.e., a stimulus that increases the frequency of a behavior, which leads to the drug's delivery) has been extremely useful in studying drugs of abuse (Johanson and Schuster 1981; Woolverton and Nader 1991; Jones and Comer 2013). However, the reinforcing effects of drugs alone may not describe all aspects of drug-taking behavior. For example, rates of responding tend to decrease with larger doses of drugs of abuse, as illustrated by the descending limb of the typical inverted U-shaped dose-response function of IV drug self-administration (Katz 1989). These decreases in behavior may be due in part to the aversive properties of drugs (Stolerman and D'Mello 1981; Katz 1989). Developing procedures to examine the aversive effects of drugs may be useful in understanding the general control of behavior by drugs of abuse.

One approach to studying the aversive effects of IV drugs is the development of punishment procedures, in which the ability of a drug to function as a punisher (i.e., a stimulus that decreases the frequency of behavior that leads to its delivery) is measured. Because punishment procedures measure a decrement in behavior, it can

be studied only if there is reasonable level of response behavior occurring. Such a level can be maintained with positive reinforcement, and therefore punishment studies must assess the effects of punishment in relation to some baseline of positively reinforced responding. Accordingly, studying the punishing effects of drugs is not without its difficulties. If an IV drug injection is delivered simultaneously with a positive reinforcer such as food, and if the behavior that was positively reinforced with food delivery decreases, then these decreases can be attributed to the effects of the drug (Goldberg 1980; Katz and Goldberg 1986). However, controls are necessary to establish that the observed decreases in positively reinforced behavior are a result of punishment and not due to something else entirely, such as a direct suppressant effect of the drug upon reinforced responding. Additionally, since the punishing drug is studied in relation to its effects on the rates of a positively reinforced behavior, the nature of the reinforcer and its scheduled delivery must also be evaluated. For example, the magnitude (or amount) of a positive reinforcer has been reported to be a determinant of punishment: behaviors maintained by lower-magnitude reinforcers are more readily punished than higher-magnitude reinforcers (Johanson 1977; Poling and Thompson 1977).

Choice procedures have been developed to study the punishing effects of IV drugs by examining the allocation of responding between two concurrently available manipulanda, which response-contingently deliver either the positive reinforcer with the IV drug injection, or the positive reinforcer alone (Woolverton 2003; Negus 2005; Podlesnik et al 2010). In these procedures, if responding is allocated toward the manipulandum that delivers the positive reinforcer alone, then this suggests that the drug is a punisher and does not have direct suppressant effects. Although choice procedures have been developed to evaluate the punishing effects of drugs (Goldberg 1980; Katz and Goldberg 1986 Woolverton 2003; Negus 2005; Podlesnik et al. 2010),

less is known about behavioral determinants of punishment as compared to reinforcement.

The purpose of the present study was to further evaluate the punishing effects of IV histamine injections (Woolverton 2003; Negus 2005; Podlesnik et al 2010) by using a choice procedure to examine whether schedule control of punisher delivery (through manipulation of intertrial interval length), or subjects' drug histories affect punishment of sucrose-reinforced lever pressing in rats. In self-administration studies examining the reinforcing effects of IV cocaine, altering time-out (i.e., the time following reinforcement during which response manipulanda are available but responding has no scheduled consequences) has been shown to affect rates of responding (Winger 1993; Woolverton 1995; Rowlett et al. 1996; Nader and Morgan 2001; Martelle et al. 2008). However, it remains unclear whether varying the amount of programmed time between response-dependent delivery of a punisher can affect punishment. Furthermore, researchers have noted that a drug's ability to act as a reinforcer and maintain later drug self-administration in a subject can be influenced by a previous history with that drug (see Young et al. 1981). For instance, rhesus monkeys increase their response rates for smaller doses of cocaine that were not initially self-administered if they had established a self-administration history with a larger dose of cocaine (Goldberg 1973; Downs and Woods 1974). Although a drug history can influence the effects of positively reinforcing compounds, it is not known what kind of drug history might affect the punishing effects of drugs. The ability of drug histories to subsequently alter a drug's ability to punish positively reinforced behaviors has not been investigated and may be important in predicting behavioral effects.

Methods

All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals and performed in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health.

Subjects

Male Sprague-Dawley rats (300-375 g) were obtained from Harlan (Indianapolis, IN) and housed in a temperature- (21-23 °C) and humidity-controlled environment on a 12-h dark/light cycle, with lights on at 7:00 A.M. Rats were housed three per cage during non-drug behavioral training procedures, and then individually housed after surgery was completed. Except during experimental sessions, rats had free access to tap water and were on food-restricted diets of Purina rodent chow that maintained at least 80% of their pre-restriction body weights. All experiments were conducted 5-7 days/week between 9:00 A.M and 5:00 P.M.

Apparatus

Med Associates (St. Albans, VT) operant conditioning chambers and manipulanda were used for all experiments. Each operant conditioning chamber was approximately 30 cm long, 24 cm wide, and 21 cm high, and each chamber was contained in a sound-attenuating cubicle. The front panel of each operant conditioning chamber was equipped with two retractable levers, 6.8 cm above the grid floor, and 1.3 cm from the side walls, with an array of red, yellow, and green light-emitting diodes (LEDs) above each lever. A 2.8V white incandescent house light was located at the top center of the rear panel. Located between the two levers was a magazine in which a 45 mg sucrose pellet (Bio-Serv, Frenchtown, NJ) could be delivered. IV drug injections were delivered by a motorized syringe driver (PHM-107; Med Associates) through Tygon tubing (S-54-

HL, Norton Performance Plastics, Akron, OH) connected to a swivel that was held in place by a counterbalanced arm. Syringe drivers were located outside the sound-attenuating chamber. Injection durations were determined by the weight of each rat divided by the drug delivery pump flow rate (0.072 ml/s). Data were collected with Med Associates software.

Non-Drug Behavioral Training Procedures

Rats first received one 60 min session in which they were trained to eat sucrose pellets from the magazine located inside the operant chamber. No levers were available during this time, and sucrose pellets were delivered on a random-time 60 s schedule. Rats then received two 60 min daily sessions in which they were trained to respond on both levers. In these sessions, each lever was presented individually in alternating sequence. Rats were given 8 s to press the lever each time it was presented. If a response was made (or no response was made after 8 s), then a sucrose pellet was delivered, the lever retracted, and the LEDs were turned off until the lever was presented again. Intertrial intervals (ITI), which are timed intervals between trials when no lever is available and, therefore, no responses could be made, were each 60 s long. Each ITI began when the lever retracted.

Behavioral training finished with a single 90 min “choice” training session. In this session, a response made on either lever, delivered a sucrose pellet. The choice training session began with two “lever trials” followed by a series of “choice trials.” Lever trials were initiated when one of the two levers was randomly extended into the chamber with the three LEDs above that lever turned on. Rats were given 60 s to press the lever after it was presented. If a response was made, sucrose was delivered, the lever retracted, and the LEDs were turned off. If no response was made within the 60 s, then the lever retracted, the LEDs were turned off, and no sucrose was delivered. Lever

trials were presented to allow rats to sample the consequences of responding on each lever before choice trials began. After the two lever trials were presented, choice trials were initiated with the simultaneous extension of both levers and the LEDs above both of the levers turned on. Under a concurrent (and mutually exclusive) fixed ratio 1- fixed ratio 1 (FR1-FR1) schedule, rats were presented with the opportunity to respond on one of the two available levers. If a response was made on either lever, sucrose was delivered, both of the levers retracted, and all of the LEDs were turned off. If no response occurred within 60 s, both levers retracted, all of the LEDs were turned off and no sucrose was delivered. Each ITI was 120 s long and began when the lever(s) retracted.

Surgery

After non-drug behavioral training was completed, rats were surgically implanted with chronic indwelling IV catheters. Rats were anesthetized with ketamine (100 mg/kg; IP) and xylazine (10 mg/kg; IP) before a longitudinal incision was made to expose the femoral vein into which a catheter constructed from Micro-Renathane (Braintree Scientific, Inc., Braintree, MA) was inserted. The catheter was passed subcutaneously to an incision made between the scapulae and was then connected to a metal cannula that exited the skin. Catheters were flushed daily with 0.5 ml (100 U/ml) of heparinized saline to maintain patency. Rats were allowed at least 5 days to recover from surgery; during this time rats had unrestricted access to food and water.

Testing Procedures

All testing procedures began after rats recovered from surgery. For each experiment there were two phases (I and II) that were conducted sequentially (see Table 2.1 for schematic overview of experiments). Each phase consisted of 10 daily experimental sessions, and the choice procedure used in all sessions was the same

one as described for the choice training session with the exception of the consequences of responding on the levers and length of ITIs.

Experiment 1. Evaluating the punishing effects of histamine on lever responding, which delivered either sucrose+histamine, or sucrose alone, under different ITI schedules (5, 60, or 120 s). In phase I, the lever on which a rat made fewer responses during choice training was designated lever 1, and responding on this lever resulted in the delivery of a sucrose pellet. The lever on which a rat made more responses during choice training was designated lever 2, and the consequence of responding on lever 2 was the delivery of a sucrose pellet paired with an IV injection of 1.0 mg/kg histamine (sucrose+histamine). In phase II, the consequences of responding on the levers were reversed, such that responding on lever 1 now delivered sucrose+histamine, and responding on lever 2 now delivered a sucrose pellet alone. The consequences of responding on the levers were reversed to determine whether a reinforcer preference (i.e., responding controlled by consequences) or a position preference (i.e., responding controlled by location of the physical lever) was controlling response behavior. Three separate groups were used to evaluate lever responding with programmed ITI times of 5, 60, or 120 s (n=6 per group). For each 90 min session, the maximum number of choice trials that could be completed was 48 with a 5 or 60 s ITI and 43 with a 120 s ITI.

Experiment 2. Evaluating the punishing effects of histamine on lever responding, which delivered either sucrose+histamine, or sucrose alone, in rats with different drug histories. In phase I, the lever on which a rat made fewer responses during choice training was designated lever 1, and the assigned consequence of responding on this lever was the delivery of a sucrose pellet. The lever on which a rat made more responses during choice training was designated lever 2,

and consequence of responding on lever 2 was the delivery of an IV injection of saline or histamine (0.3 or 1.0 mg/kg per injection). In phase II, the consequences of the levers were changed, such that responding on lever 1 now delivered a sucrose pellet paired with an IV injection of 1.0 mg/kg histamine, and responding on lever 2 now delivered sucrose alone. Therefore in phase II, all rats were presented with the choice to respond on lever 1, which delivered sucrose+histamine, or lever 2, which delivered sucrose alone. Three separate groups were used to evaluate lever responding in rats with different drug histories (saline, 0.3, or 1.0 mg/kg histamine history) (n=6 per group). For each 90 min session, the maximum number of choice trials that could be completed was 43 with each ITI 120 s long.

Drugs

Histamine dihydrochloride was obtained from Sigma-Aldrich (St.Louis, MO) and dissolved in 0.9% saline solution. Histamine doses were calculated using the salt form of the drug and chosen according to reported behavioral activity in rats (Podlesnik et al. 2010).

Data Analyses

For all experiments, the dependent variable measured in each session was the number of responses made on each lever. In general, ANOVAs were used to determine changes in lever responding across sessions and differences in lever responding among groups. Significant ANOVAs were followed by Bonferroni post hoc tests. All statistical tests used an alpha of 0.05, two-tailed. Analyses were performed using Prism 6.0 (GraphPad Software, La Jolla, CA, USA).

Experiment 1. To examine the punishing effects of histamine under each ITI schedule, two-way repeated measures ANOVAs with the within-subjects factors of lever and sessions were used to analyze lever responding in each phase. To determine

if there was a difference in punishment among groups in each phase, punished-choice responding defined as [(number of ratios completed on the sucrose+histamine lever during choice trials ÷ total number of ratios completed for all choice trials)×100] were analyzed using two-way repeated measures ANOVAs with length of ITI (5 s vs. 60 s vs. 120 s), and sessions as the two factors. Punished-choice responding was used for analyses because of differences in the maximum number of responses that could have been made among the different ITI schedules.

Experiment 2. In each group, to determine if pairing a 1.0 mg/kg histamine injection with sucrose delivery in phase II punished responding on lever 1, a one-way repeated measures ANOVA was used to analyze responses made on lever 1 across sessions 10-20. One-way repeated measures ANOVAs were also used to determine if changing the consequence of responding on lever 2 in phase II affected lever responding on lever 2. To determine if drug histories affected punishment, responses made on lever 1 were averaged across sessions in phase I, and phase II, and the mean responses made in each phase by each group was compared using a two-way ANOVA with the within-subjects factor of consequence of lever 1 in each phase (sucrose vs. sucrose+histamine) and the between-subjects factor of drug history (saline vs. 0.3 vs. 1.0 mg/kg histamine).

Results

Effects of ITI schedule on the punishing effects of histamine on lever responding, which delivered either sucrose+histamine (1.0 mg/kg per injection), or sucrose alone. In general, all groups' responding on lever 1 (sucrose) increased and responding on lever 2 (sucrose+histamine) decreased across sessions in phase I. When the consequences of the levers were reversed in phase II, responding on lever 1

(sucrose+histamine) decreased as responding on lever 2 (sucrose) increased across sessions (Fig. 2.1a-c). In the 5 s ITI group (Fig. 2.1a), differences in responding between levers in phase I were affected by sessions [main effect of lever: F (1,10)=20.68, p=0.001; session x lever interaction: F (9,90)=10.51, p<0.0001]. Rats made more responses on lever 1 than on lever 2 in each session from 4 to 10. Additionally, the average number of total lever responses made in each session differed across sessions in phase I [main effect of session: F (9,90)=5.93, p=<0.0001], such that the number of total responses made in each session from 6 to 10 was greater than in session 1. In phase II, there was no significant difference in responding between levers, however responding on lever 2 differed across sessions [main effect of session: F (9,90)=4.12, p=0.0002; session x lever interaction: F (9,90)=5.56, p<0.0001], such that responses were significantly greater in session 19 and 20 compared to session 11.

In the 60 s ITI group (Fig. 2.1b), differences in responding between levers in phase I were affected by sessions [main effect of lever: F (1,10)=25.47, p=0.005; session x lever interaction: F (9,90)=15.86, p<0.0001]. Rats made more responses on lever 1 than on lever 2 in each session from 4 to 10. When the consequences of the levers were reversed in phase II, responding on lever 1 decreased while responding on lever 2 increased [session x lever interaction: F (9,90)=2.78, p=0.006], however there was no significant difference in lever responding between levers or changes in total number of responses across sessions.

In the 120 s ITI group (Fig. 2.1c), differences in responding among levers in phase I were different [main effect of lever: F (1,10)=17.20, p=0.002] with more responses made on lever 1 than lever 2. However, differences in lever responding were not affected by sessions. When the consequences of the levers were reversed in phase II of the experiment, responding on lever 1 decreased while responding on lever 2

increased [session x lever interaction: F (9,90)=7.94, p<0.0001], however there was not significant difference in responding between levers across sessions.

Punished-choice responding (i.e., percent of total responding allocated on the lever that delivered sucrose+histamine) was not different among ITI groups (Fig 2a and b). However, the punished-choice responding differed across sessions in phase I [main effect of session: F (9,126)=9.07, p<0.0001] (Fig. 2.2c) and across sessions in phase II [main effect of session: F (9,126)=8.02, p<0.0001] (Fig. 2.2d). In phase I, punished-choice responding in each session from 6 to 10 decreased as compared to session 1 and decreases were also observed in each session from 18 to 20 compared to session 11.

Effects of drug history on responding on levers, which delivered either sucrose+histamine (1.0 mg/kg per injection), or sucrose alone. In phase I, each group readily acquired responding on lever 1 (sucrose), and made little or no responses on lever 2 (injection). By session 10 each group made all of their responses on lever 1 (sucrose) (Fig. 2.3a-c). In the initial sessions, in which rats did make responses on lever 2 (injection), the 1.0 mg/kg histamine history group received, on average, the greatest total daily histamine intake among the groups, such that this group received approximately 4 times more histamine (Table 2.2). For instance, in session 1, the 1.0 mg/kg histamine and 0.3 mg/kg groups received 11.2±1.7 and 2.8±0.8 mg/kg histamine respectively, whereas the saline history group received no histamine, because the only injections the rats could receive was saline.

When the consequences of responding on the levers were changed in phase II of the experiment, responding on lever 1 (sucrose+histamine) generally decreased across sessions in each group, with some increase in responding on lever 2 (sucrose) (Fig. 2.3a-c). In the saline history group, responding differed across sessions on lever 1 [main

effect of session: F (10, 50)=13.20, p<0.0001] and lever 2 [main effect of session: F (10, 50)=3.61, p=0.001] (Fig. 2.3a). Rats in the saline history group made fewer responses on lever 1 in each session from 11 to 20 as compared to session 10 and made more responses on lever 2 in each session from 18-20 compared to session 10.

In the 0.3 mg/kg histamine history group, responding differed across sessions on lever 1 [main effect of session: F (10, 40)=5.16, p<0.0001] and lever 2 [main effect of session: F (10, 40)=2.99, p=0.006] (Fig. 2.3b). Rats in the 0.3 mg/kg histamine history group made fewer responses on lever 1 in each session from 11 to 20 compared to session 10, and increased responding on lever 2 in each session from 18 to 20 compared to session 10.

In the 1.0 mg/kg histamine history group, responding differed across sessions on lever 1 [main effect of session: F (10, 50)=40.06, p=0.0004] and lever 2 [main effect of session: F (10, 50)=2.49, p=0.01] (Fig. 2.3c). Rats in the 1.0 mg/kg histamine history group made fewer responses on lever 1 in session in 11, and each session from 15 to 20 compared to session 10. However, while responding on lever 2 differed across sessions, none of the individual sessions significantly differed from responding in session 10.

Between-subject comparisons revealed that the punishing effects of 1.0 mg/kg histamine injections in phase II were dependent on the unit dose of histamine injections available in phase I (Fig. 2.4). Group-averaged responding on lever 1 decreased in phase II [main effect of consequence: F (1, 28)=51.75, p<0.0001], and the decreases were dependent on the unit dose of histamine available in phase I, in which the greatest decrease was observed in the saline history group, followed by the 0.3 mg/kg histamine history group. Averaged responding on lever 1 by the 1.0 mg/kg histamine did not

significantly decrease in phase II, with rats making significantly more responses on lever 1 compared to the saline history group.

Discussion

The purpose of the present study was to examine whether manipulation of ITI length and/or drug history affected the punishing effects of IV histamine injections on sucrose-reinforced lever responding in rats. In general, sucrose served as a reinforcer of lever responding, whereas IV histamine injections served as a punisher of sucrose-reinforced lever responding. When rats were presented with levers that delivered either sucrose or sucrose+histamine, rats generally decreased their responding on the sucrose+histamine lever while increasing their responding on the sucrose-only lever. These findings are consistent with published studies in which IV histamine injections punished both food- (Woolverton 2003; Podlesnik et al 2010) and cocaine-reinforced behaviors (Negus 2005; Woolverton et al. 2012), while increasing "unpunished" responding (i.e., the positively reinforced behavioral alternative) in choice situations. Although histamine injection served as a punisher of sucrose-reinforced lever responding under the present conditions studied, attenuation of the punishing effects of histamine was observed in rats that had a drug history involving response-contingent histamine when compared to rats with no drug history. In contrast, the punishing effects of histamine may have been increased with a shorter ITI (e.g., 5 s).

Effects of ITI on punishment by histamine

When rats were presented with the choice to respond on levers that delivered either sucrose only or sucrose+histamine, punished-choice responding decreased across sessions in both phase I and phase II (after lever consequences were reversed). This suggests that responding was controlled by consequences, and not position

preference. The decreases in punished-choice responding indicates that pairing histamine with sucrose delivery resulted in rats allocating fewer of their total responses on the sucrose+histamine lever, and/or increased their responses on the sucrose lever across sessions. These changes across sessions demonstrate that histamine served as a punisher of sucrose-reinforced responding, and in some instances, punishment coincided with rats increasing their responding on the alternative lever, which delivered sucrose alone.

Although there was no difference in punished-choice responding among the different ITI groups, the total number of responses made within each session differed. In the 5 s ITI group, the total number of responses made in each session differed across sessions in both phase I and phase II, such that the total number of responses made in the initial sessions of both phases was lower (because of missed trials) than responses made in sessions at the end of each phase. Rats in the 60 and 120 s ITI groups did not significantly miss any trials, and the total number of responses made in each session did not differ across sessions. These findings suggest that a 5 s ITI may have resulted in some direct suppressant effects on behavior.

However, the reduction in responses made on the sucrose+histamine lever was more complete in the 5 s ITI group, such that responding was reduced to zero in phase I, and remained low in phase II compared to the 60 and 120 s ITI groups. Therefore, while a shorter ITI may have resulted in some direct suppressant effects, it may have also increased the punishing effects of histamine. For instance, responding on the sucrose lever by rats in the 5 s ITI group remained completely suppressed for several sessions in phase II despite the histamine consequence being removed. This may have occurred because the punishing effects established in phase I were long-lasting. Other studies have noted that the effects of punished behavior may remain even after the

punishment contingency has been removed (see Azrin and Holz 1966).

One explanation as to how a shorter ITI could have increased the punishing effects of histamine is that rats in the 5 s ITI can be punished with greater frequency compared to the 60 or 120 s ITI. It has been reported that punishment schedules that are analogous to a continuous schedule of reinforcement (i.e., FR1 schedule of reinforcement without scheduled timeouts) result in greater punishment of behavior (Azrin et al. 1963). In rhesus monkeys, Negus (2005) found that punishment of cocaine-reinforced behavior by intravenous histamine injections was directly related to the "probability" (or schedule) of histamine being delivered with cocaine. In this study, response-contingent histamine significantly decreased cocaine-reinforced responding when the probability of histamine delivery with cocaine was 100% (an injection followed every reinforced response). In contrast, when the probability of delivery was decreased to 33% (an injection followed every third reinforced response), responding was similar to that of subjects that had 0% probability of receiving histamine injections. These findings suggest that the frequency of punisher delivery is important in punishment, just as it is with reinforcement (see Spealman and Goldberg 1978). Therefore, decreasing the length of ITI in the present study may have actually resulted in greater punishment (albeit with the possibility of some direct suppressant effect), which is not apparent in the analysis of punished-choice responding.

Effect of drug histories on punishment by histamine

When the consequence of responding on lever 1 was changed from the delivery of sucrose alone to the delivery of sucrose paired with an injection of histamine (1.0 mg/kg), responding on lever 1 decreased. However, the punishing effect of histamine on sucrose-reinforced lever responding was attenuated in groups with a history of histamine compared to groups that had a saline history (i.e., no drug history). The

extent to which histamine punished sucrose-reinforced responding on lever 1 was related to the unit dose of histamine previously delivered as a consequence of responding on lever 2. Accordingly, attenuation of punishment was greater in the 1.0 mg/kg histamine history group compared to the saline history group.

It is possible that a drug history of response-contingent IV histamine may have resulted in tolerance to the punishing effects of histamine, such that rats that received histamine in phase I were less sensitive to the punishing effects of 1.0 mg/kg histamine in phase II. Tolerance to the punishing effects of electric shock, for instance, has been reported to occur with repeated exposure in rhesus monkeys (Bergman and Johanson 1981). However, development of tolerance to histamine cannot account for the fact that the punishing effects of histamine persist across sessions in phase II (in which rats are repeatedly exposed to histamine). Alternatively, a history of IV histamine or saline self-administration established on lever 2 could have attenuated the punishing effects of histamine by affecting reallocation of punished responding in phase II. For instance, although the consequence of the levers were changed in phase II (so that responding on lever 2 delivered sucrose alone, and responding on lever 1 delivered sucrose paired with an injection of 1.0 mg/kg histamine), responding on lever 2 was low in all groups, with responding completely suppressed for several sessions in the 1.0 mg/kg histamine history group. The saline history group, while not statistically significant, made, on average, 10.2±5.5 responses on the lever 2 (sucrose), which was more than the responses made by 0.3 or 1.0 histamine history group, which averaged 9.3±5.8 and 3.6±2.0 respectively. The low number of responses made on lever 2, and differences in responding among the groups may reflect a long-lasting effect of the self-administration history established on the respective lever when it had previously delivered an injection of saline or histamine (0.3 or 1.0 mg/kg). While it is possible that histamine had direct

suppressant effects that prevented reallocation of behavior, the 1.0 mg/kg histamine history group received more injections than either the saline or 0.3 mg/kg histamine history group, and yet the total amount of responses made in each session did not differ among groups.

Other studies using choice procedures have demonstrated that providing an equivalent reinforcer that is unpunished as an alternative response consequence may facilitate a more sensitive measurement of punishment, such that the intensity of electric shock (Azrin and Holz 1966) or the dose of IV histamine (Woolverton 2003) needed to punish behavior is less than when no alternative unpunished reinforcer is available. This suggests that the saline history group was not more sensitive to the punishing effects of 1.0 mg/kg histamine (i.e., less tolerant of histamine), but, rather, that the measurement of punishment was affected by whether rats reallocated punished responding. A future study in which no alternative unpunished reinforcer consequence is provided in phase II may help determine whether the attenuation of the punishing effect of histamine was a result of drug history that affected reallocation of behavior, or a tolerance that developed to histamine.

Conclusions

The results of this study demonstrate that while histamine functioned to punish sucrose-reinforced lever responding, drug history and schedule of punisher delivery may be important variables to consider when examining the punishing effects of IV drugs. Therefore, examining conditions under which drugs are studied may have practical implications in understanding the general effects of drugs. The findings in this study agree with the general conception that the behavioral effects of drugs can be altered by a number of conditions including the ongoing rate of responding, behavioral and drug history, and the current behavioral conditions (see Morse and Kelleher 1977).

Table 2.1 Consequences of responding on lever 1 and lever 2 in phases I and phase II for each histamine punishment experiment conducted.

	Consequences of responding on levers			
	Phase I: Sessions 1-10		**Phase II: Sessions 11-20**	
	Lever 1	**Lever 2**	**Lever 1**	**Lever 2**
Experiment 1 Evaluating the punishing effects of intravenous histamine injections under different ITI schedules (5 vs. 60 vs. 120 s)	sucrose	sucrose+ histamine (1.0 mg/kg histamine per injection)	sucrose+ histamine (1.0 mg/kg histamine per injection)	sucrose
Experiment 2 Evaluating the punishing effects of intravenous histamine in rats with drug histories (saline vs. 0.3 vs. 1.0 mg/kg histamine history)	sucrose	saline injection **or** 0.3 mg/kg/inj histamine **or** 1.0 mg/kg/inj histamine	sucrose+ histamine (1.0 mg/kg histamine per injection)	sucrose

Table 2.2 Values for total injections delivered and total histamine intake (mg/kg) across sessions 1-10.

	Saline injection		Histamine (0.3 mg/kg/inj)		Histamine (1.0 mg/kg/inj)	
	Total injections	Total intake mg/kg	Total injections	Total intake mg/kg	Total injections	Total intake mg/kg
Session 1	14.8±4.0	n/a	9.6±2.7	2.8±0.8	11.2±1.7	11.2±1.7
Session 2	3.6±0.9	n/a	3.2±1.3	0.9±0.3	4.2±1.8	4.2±1.8
Session 3	0.8±0.5	n/a	2.2±1.2	0.6±0.3	0.8±0.3	0.8±0.3
Session 4	1.6±0.9	n/a	.06±0.4	0.01±0.01	0.6±0.4	0.6±0.4
Session 5	0.6±0.4	n/a	.04±0.4	0.01±0.01	0	0
Session 6	0	n/a	00	0	0.4±0.4	0.4±0.4
Session 7	0	n/a	00	0	0	0
Session 8	0	n/a	0.2±0.2	0.06±0.06	0	0
Session 9	0.8±0.8	n/a	0	0	0.4±0.2	0.4±0.2
Session 10	0.2±0.2	n/a	0	0	0.2±0.2	0.2±0.2

Figure 2.1

	Phase I: sessions 1-10	Phase II: sessions 11-20
lever 1	sucrose	sucrose+1.0 mg/kg histamine
lever 2	sucrose+1.0 mg/kg histamine	sucrose

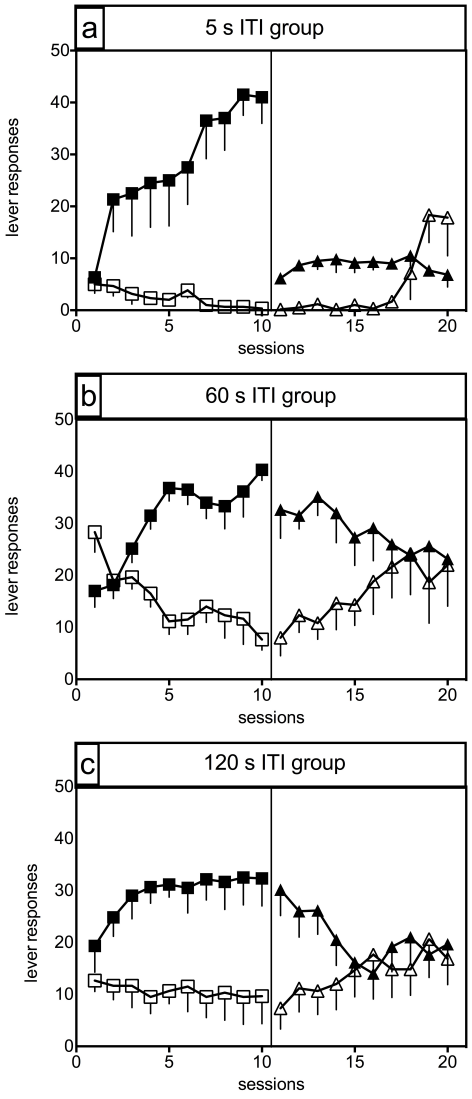

Figure 2.1 Responding on levers that delivered either sucrose+histamine (1.0 mg/kg) or sucrose under different ITI schedules. Each graph displays data from individual groups that differ by their ITI schedule: (a) 5 s ITI, (b) 60 s ITI, and (c) 120 s ITI (*n*=6 per group). Closed and open symbols represent the mean (±SEM) responses made on levers 1 and 2 respectively. Legends next to the symbols listed in the table indicate the consequences of responding on lever 1 and lever 2 across sessions 1-20.

Figure 2.2

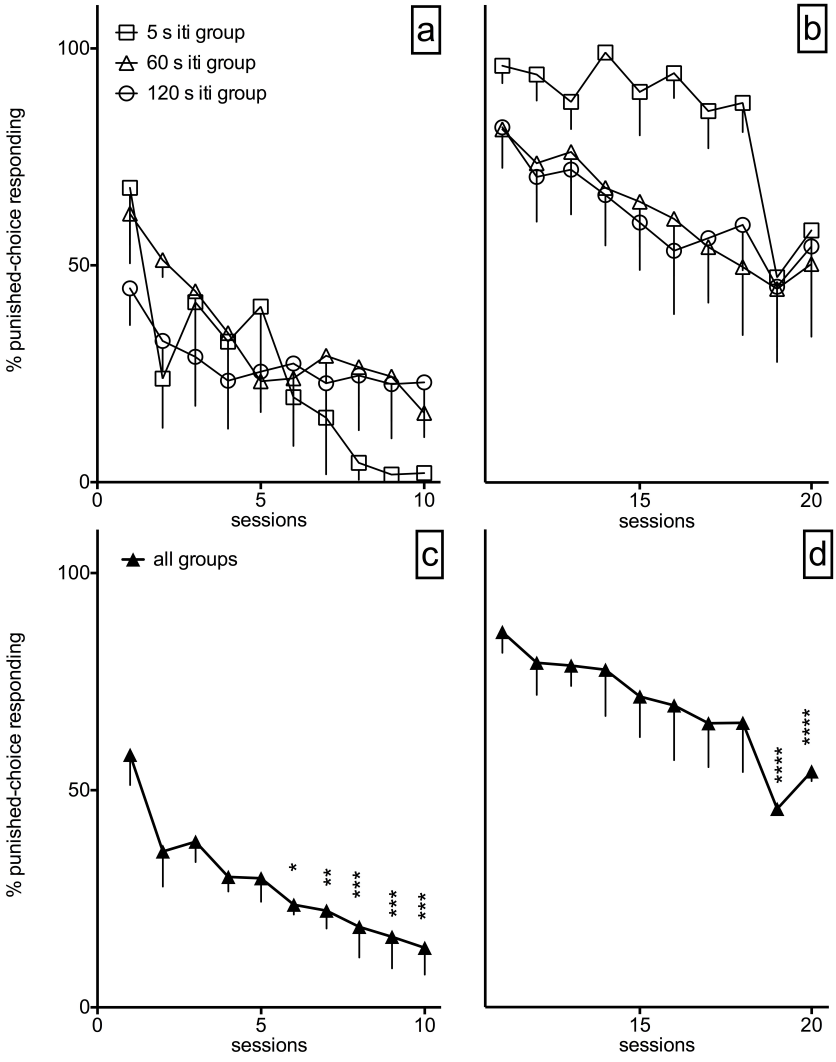

Figure 2.2 Punished-choice responding under different ITI schedules. Open and closed symbols represent the mean (±SEM) percent of punished-choice responding by individual groups (a and b) (*n*=6 per group) and of all groups respectively (c and d). *, p<. 05, **, p<0. 01***, p<0. 001, ****, p<0. 0001. Significant decrease in punished-choice responding across sessions compared to the first session within each phase.

Figure 2.3

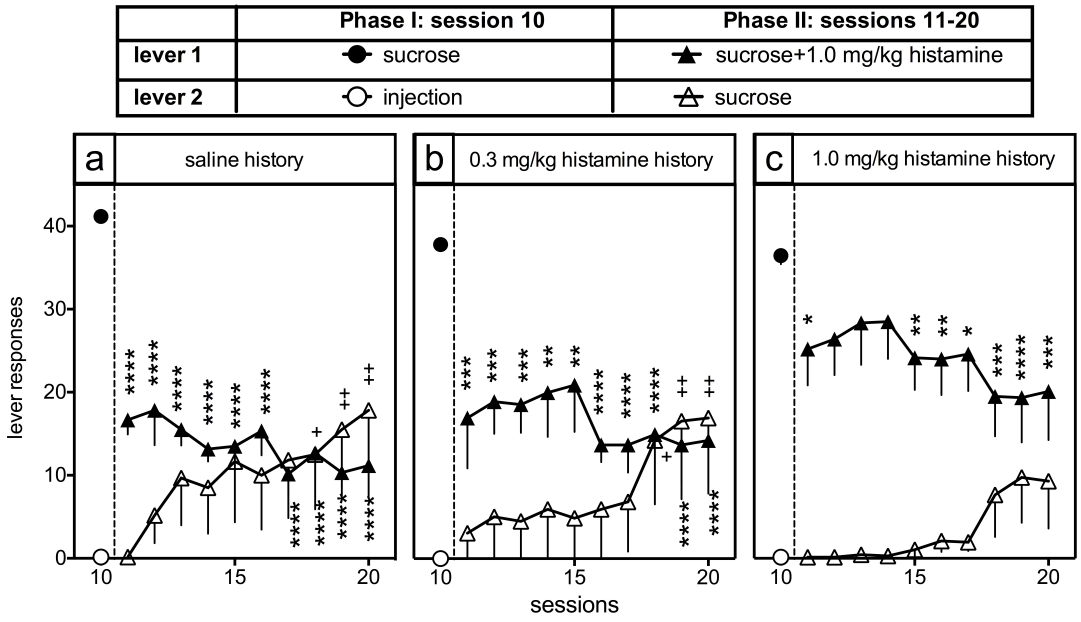

Figure 2.3 Effects of drug history on lever responding that delivered either sucrose+histamine (1.0 mg/kg) or sucrose. Each graph displays data from individual groups that differ by their drug history, which corresponds to the dose of histamine or saline "injection" available on lever 2 in phase I: (a) saline history, (b) 0.3 mg/kg histamine history and (c) 1.0 mg/kg histamine history (*n*=6 per group). Closed and open symbols represent the mean (±SEM) responses made on levers 1 and 2 respectively. Legends next to the symbols listed in the table indicate the consequences of responding on lever 1 and lever 2 across sessions 10-20. *, p<. 05, **, p<0. 01***, p<0. 001, ****, p<0. 0001. Significant difference in responding on lever 1 compared to session 10. +, p<. 05, ++, p<0. 01. Significant difference in responding on lever 2 compared to session 10.

Figure 2.4

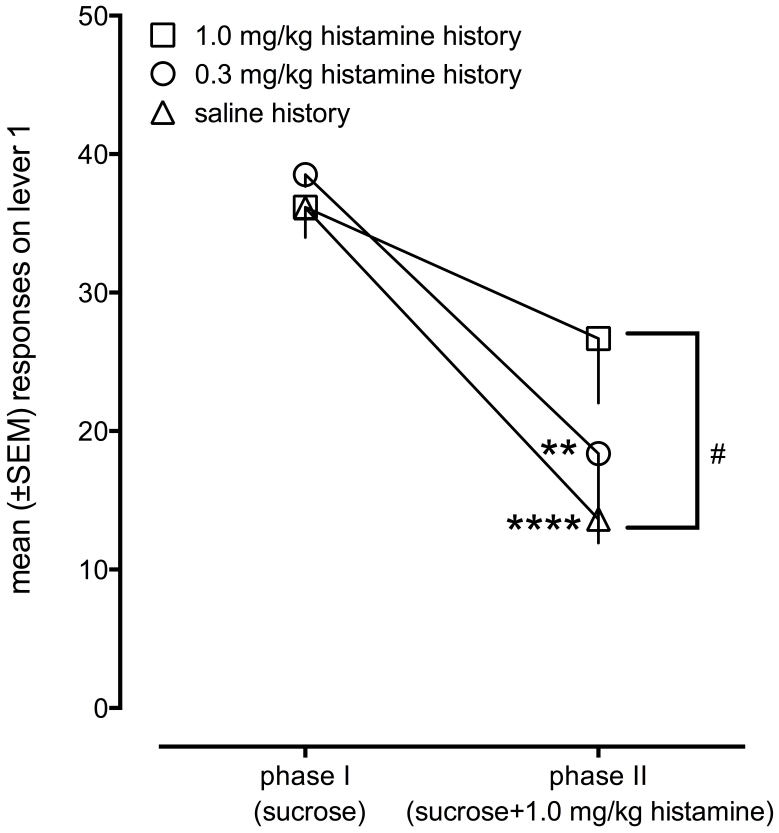

Figure 2.4. Responding on lever 1 in phase I and phase II. Symbols represent data from individual groups that differ in their drug history as indicated by legends to the right of the symbols (*n*=6 per group). Responses made on lever 1 in phase I (sucrose consequence) and phase II (sucrose+1.0 mg/kg histamine consequence) by each group are presented as the mean (±SEM). **, p<0. 01***, p<0. 001. Significant difference in responding on lever 1 in phase I compared to phase II. #, p<0. 05. Significant difference in responding on lever 1 in phase II between saline history and 1.0 mg/kg histamine history group.

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Chapter 3

Punishing effects of intravenous nicotine in rats

Introduction

Intravenous (IV) nicotine self-administration studies have demonstrated that nicotine can function as a positive reinforcer (i.e., a stimulus that increases the frequency of behavior that leads to its delivery) in rodents (e.g., Corrigall and Coen 1989; Donny et al. 1995; Watkins et al. 1999), non-human primates (e.g., Goldberg et al. 1981; Le Foll et al. 2007), and humans (e.g., Harvey et al. 2004; Sofuoglu et al. 2008). However, compared to other drugs of abuse, establishing the reinforcing effects of nicotine in controlled studies remains difficult (see Le Foll and Goldberg 2009). For instance, it has been reported that control of experimental parameters such as drug infusion duration (Wakasa et al. 1995), non-drug paired stimuli (Goldberg et al. 1981; Chaudhri et al. 2006), and schedule control of nicotine availability (Henningfield and Goldberg 1983) are all important in establishing and maintaining self-administration behavior. Therefore, while self-administration procedures have been developed to demonstrate the reinforcing effects of IV nicotine, very specific conditions are often required to obtain such effects.

These difficulties in demonstrating the reinforcing effect of nicotine may be due in part to the aversive properties of nicotine (Benowitz 1990). It is hypothesized that higher reinforcing doses of nicotine found on the descending limb of the inverted U-shaped dose-response function of nicotine self-administration may also

have aversive properties that limit rates of responding and total drug intake (e.g., Katz 1989; Rose and Corrigall 1997). However, it is unclear whether decreases in rates of self-administration are a result of direct suppression of behavior and/or if they are due to the aversive effects of nicotine. Early investigations of IV nicotine self-administration noted that unit doses of nicotine that were reinforcing in squirrel monkeys (Goldberg et al. 1981) could also function to punish food-reinforced lever responding (Goldberg and Spealman 1982; 1983), and maintain lever responding to avoid its scheduled injection (Spealman 1983). Similarly, it has been reported that humans will also maintain responding to avoid IV injections of nicotine (Henningfield and Goldberg 1983). These findings indicate that the behavioral effects of nicotine can be divergent. However, investigation of the aversive effects of nicotine beyond these early studies described has been limited. A better understanding of the aversive effects of nicotine may be useful in understanding control of behavior by nicotine in more general circumstances.

The purpose of this study is to extend the evaluation of the aversive effects of IV nicotine injections by determining variables that may influence the punishing effects of IV nicotine on sucrose-reinforced responding in the rat. Using punishment procedures, dose of nicotine delivered per injection, the behavioral alternative to punished responding (i.e., reinforced vs. non-reinforced), and the delay of nicotine delivery were manipulated to determine whether punishment by nicotine is characteristically similar to other functional punishers. Additionally, the effects of response-independent nicotine were examined to determine what the rate-limiting cumulative dose of nicotine is. These experimental parameters were chosen because they have been shown to affect suppression of response behavior by punishers such as electric shock (Azrin and Holz 1966; Grove and Schuster 1974) and IV histamine (Woolverton 2003; Woolverton et al. 2012).

Methods

All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals and performed in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health.

Subjects

Male Sprague-Dawley rats (300-375 g) were obtained from Harlan (Indianapolis, IN) and housed in a temperature- (21-23 °C) and humidity-controlled environment on a 12-h dark/light cycle, with lights on at 7:00 A.M. Rats were housed three per cage during non-drug behavioral training procedures, and then individually housed after surgery was completed. Except during experimental sessions, rats had free access to tap water and were on food-restricted diets of Purina rodent chow that maintained at least 80% of their pre-restriction body weights. All experiments were conducted 5-7 days/week between 9:00 A.M and 5:00 P.M.

Apparatus

Med Associates (St. Albans, VT) operant conditioning chambers and manipulanda were used for all experiments. Each operant conditioning chamber was approximately 30 cm long, 24 cm wide, and 21 cm high, and each chamber was contained in a sound-attenuating cubicle. The front panel of each operant conditioning chamber was equipped with two retractable levers, 6.8 cm above the grid floor, and 1.3 cm from the side walls, with an array of red, yellow, and green light-emitting diodes (LEDs) above each lever. A 2.8V white incandescent house light was located at the top center of the rear panel. Located between the two levers was a magazine in which a 45 mg sucrose pellet (Bio-Serv, Frenchtown, NJ) could be delivered. IV drug injections were delivered

by a motorized syringe driver (PHM-107; Med Associates) through Tygon tubing (S-54-HL, Norton Performance Plastics, Akron, OH) connected to a swivel that was held in place by a counterbalanced arm. Syringe drivers were located outside the sound-attenuating chamber. Injection durations were determined by the weight of each rat divided by the drug delivery pump flow rate (0.072 ml/s). Data were collected with Med Associates software.

Non-Drug Behavioral Training Procedures and Surgery

Rats first received one 60 min session in which they were trained to eat sucrose pellets from the magazine located inside the operant chamber. No levers were available during this time, and sucrose pellets were delivered on a random-time 60 s schedule. Rats then received two 60 min daily sessions in which they were trained to respond on both levers. In these sessions, each lever was presented individually in alternating sequence. Rats were given 8 s to press the lever each time it was presented. If a response was made (or no response was made after 8 s), then a sucrose pellet was delivered, the lever retracted, and the LEDs were turned off until the lever was presented again. Intertrial intervals (ITI), which are timed intervals between trials when no lever is available and, therefore, no responses could be made, were each 60 s long. Each ITI began when the lever retracted.

Behavioral training finished with a single 90 min “choice” training session. In this session, a response made on either lever, delivered a sucrose pellet. The choice training session began with two “lever trials” followed by a series of “choice trials.” Lever trials were initiated when one of the two levers was randomly extended into the chamber with the three LEDs above that lever turned on. Rats were given 60 s to press the lever after it was presented. If a response was made, sucrose was delivered, the lever retracted, and the LEDs were turned off. If no response was made within the 60 s,

then the lever retracted, the LEDs were turned off, and no sucrose was delivered. Lever trials were presented to allow rats to sample the consequences of responding on each lever before choice trials began. After the two lever trials were presented, choice trials were initiated with the simultaneous extension of both levers and the LEDs above both of the levers turned on. Under a concurrent fixed ratio 1- fixed ratio 1 (FR1-FR1) schedule, rats were presented with the opportunity to respond on one of the two available levers. If a response was made on either lever, sucrose was delivered, both of the levers retracted, all of the LEDs were turned off, and no sucrose was delivered. If no response occurred within 60 s, both levers retracted and all of the LEDs were turned off. Each ITI was 120 s long and began when the lever(s) retracted.

After non-drug behavioral training was completed, rats were surgically implanted with chronic indwelling IV catheters. Rats were anesthetized with ketamine (100 mg/kg; IP) and xylazine (10 mg/kg; IP) before a longitudinal incision was made to expose the femoral vein into which a catheter constructed from Micro-Renathane (Braintree Scientific, Inc., Braintree, MA) was inserted. The catheter was passed subcutaneously to an incision made between the scapulae and was then connected to a metal cannula that exited the skin. Catheters were flushed daily with 0.5 ml (100 U/ml) of heparinized saline to maintain patency. Rats were allowed at least 5 days to recover from surgery; during this time rats had unrestricted access to food and water.

Testing Procedures

There were two phases (I and II) within each of the three separate experiments conducted (see Table 3.1 for schematic overview). Phase I consisted of 5 daily sessions and was followed by phase II, which consisted of 10 daily sessions. The punishment procedures used in all sessions was the same one described for the choice training session, with the exception of the consequences of responding on each lever. In each

90 min session, the maximum number of choice trials that could be completed was 43, and each ITI was 120 s long.

Phase I

Experiments 1-4: Acquisition of responding on a lever that delivered sucrose. After recovery from surgery, rats received 5 daily sessions to acquire responding on a lever that delivered a sucrose pellet. The lever a rat made fewer responses on during the choice training session was designated lever 1, and the consequence of responding on lever 1 was the delivery of a sucrose pellet. The lever a rat made more responses on during the choice training session was designated lever 2, and responding on lever 2 resulted in the delivery of a saline injection.

Phase II

Experiment 1. Evaluating the effects of pairing nicotine (0.01, 0.03, and 0.1 mg/kg per injection) and saline injection with sucrose delivery on lever responding, which delivered either sucrose+injection, or sucrose alone. In phase II the consequence of responding on lever 1 was changed from the delivery of a sucrose pellet to the delivery of a sucrose pellet paired with a simultaneous injection (i.e., sucrose+injection) of nicotine (0.01, 0.03, or 0.1 mg/kg per injection) or saline, and the consequence of responding on lever 2 was changed from the delivery of a saline injection to the delivery of a sucrose pellet (n=6-7/group).

Experiment 2. Evaluating the effects of pairing nicotine injection (0.03, 0.1, and 0.18 mg/kg per injection) with sucrose delivery on lever, which delivered either sucrose+injection, or nothing. In phase II the consequence of responding on lever 1 was changed from the delivery of a sucrose pellet to the delivery of sucrose+injection of nicotine (0.03, 0.1 or 0.18 mg/kg per injection), and the

consequence of responding on lever 2 was changed from delivery of a saline injection to the delivery of nothing (i.e., null consequence) (*n*=6/group).

Experiment 3. Evaluating the effect of delaying nicotine (0.1 and 0.18 mg/kg per injection) and saline injections paired with sucrose delivery on lever responding, which delivered either sucrose injection, or nothing. In phase II, the consequence of responding on lever 1 was changed from delivery of a sucrose pellet to the delivery of sucrose+injection of nicotine (0.1 or 0.18 mg/kg per injection) or saline, and the consequence of responding on lever 2 was changed from delivery of a saline injection to the delivery of nothing. For every response made on lever 1, the injection was either administered simultaneously with sucrose delivery (no delay), or 60 s after sucrose delivery (delay). Each injection delivery condition was conducted for 5 consecutive sessions, and the order of the conditions conducted was counterbalanced within groups (*n=5*-6/group).

Experiment 4. Evaluating response-independent delivery of IV nicotine injection on lever responding. In phase II, the consequence of responding on lever 1 continued to deliver sucrose, while the consequence of responding on lever 2 was changed from the delivery of saline to the delivery of nothing. Within each session conducted, rats could respond on lever 1 or lever 2 while being administered a continuous 90 min infusion of response-independent nicotine totaling 1.0 or 1.8 mg (or saline) in a volume of 4.8 ml. Three independent groups were used to test each dose of nicotine or saline administered (*n*=6/group).

Drugs

(-)-Nicotine hydrogen tartrate salt was obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in 0.9% saline solution. Response-dependent and –independent nicotine or

saline injections were administered intravenously under all conditions studied, and drug doses were calculated on the basis of the salt form of the drug.

Data Analysis

For all experiments, the dependent variable measured in each session was the number of responses made on each lever. One-way repeated measures ANOVAs were used to determine if changing the consequence of responding on each lever affected lever responding across sessions. To determine if lever responding was different among groups that received different injections (either by drug or dose), responses made on each lever across all sessions in phase II were averaged by group, and the mean responses were compared using two-way ANOVAs. Significant ANOVAs were followed by Bonferroni post hoc tests. All statistical tests used an alpha of 0.05, two-tailed. Analyses were performed using Prism 6.0 (GraphPad Software, La Jolla, CA, USA).

Results

Experiments 1-4: Acquisition of responding on lever 1 (sucrose). All rats readily acquired lever responding, with all responses made almost exclusively on lever 1 (sucrose) compared to lever 2 (saline injection) (data not shown). By session 5, group-averaged responses made on lever 1 in Experiments 1, 2, 3, and 4 was 39.9±0.3, 38.2±1.0, 38.3±0.5, and 39.2±1.1, respectively. Group-averaged responses made on lever 2 in Experiments 1, 2, 3, and 4 was 1.8±0.3, 3.5±1.3, 4.5±0.4, and 2.6±1.1, respectively. This pattern of responding indicates that responding on lever 1 was maintained by the reinforcing effect of sucrose.

Experiment 1. Effects of pairing nicotine or saline injection with sucrose delivery on lever responding, which either delivered sucrose+injection, or sucrose alone. When an injection was delivered with sucrose in phase II, responding on lever 1

generally decreased as responding on lever 2 increased (Fig. 3.1a-c), and the extent to which responding decreased on lever 1 and increased on lever 2 was dependent on the dose of nicotine delivered with sucrose (Fig 3.2). In the saline group, changing the consequences of responding on lever 1 and lever 2 changed responding on both lever 1 [$F(10, 50)=2.30$, $p=0.02$] and lever 2 [$F(10, 50)=4.61$, $p=0.02$]. Rats made fewer responses on lever 1 in session 12 and 14 compared to session 5, and made more responses on lever 2 in session 12 and 14 compared to session 5 (Fig. 3.1a). In the 0.01 mg/kg nicotine group, changing the consequences of responding on lever 1 and lever 2 in phase II changed responding on both lever 1 [$F(10, 60)=4.70$, $p<0.0001$] and lever 2 [$F(10, 60)=4.61$, $p<0.0001$]. Rats made fewer responses on lever 1 in each session from 12 to 15 compared to session 5, and made more responses on lever 2 in each session from 13 to 15 compared to session 5 (Fig. 3.1b). In the 0.03 mg/kg nicotine group, changing the consequences of responding on lever 1 and lever 2 in phase II changed responding on both lever 1 [$F(10, 60)=6.41$, $p<0.0001$] and lever 2 [$F(10, 60)=6.86$, $p<0.0001$]. Rats made fewer responses on lever 1 in each session from 10 to 15 compared to session 5 and made more responses on lever 2 in each session from 10 to 15 compared to session 5 (Fig. 3.1c). In the 0.1 mg/kg nicotine group, changing the consequences of responding on lever 1 and lever 2 in phase II changed responding on both lever 1 [$F(10, 60)=4.81$, $p<0.0001$] and lever 2 [$F(10, 60)=4.81$, $p<0.0001$]. Rats made fewer responses on lever 1 across each session from 6 to 15 compared to session 5 and made more responses on lever 2 in each session from 6 to 15 compared to session 5 (Fig. 3.1d). Between-subjects analysis revealed that the average number of responses made across sessions 6-15 in phase II was not different among groups. However, there was a difference in responding on levers such that as the unit dose of nicotine injection increased, responses for lever 1 decreased, while responding on lever

2 increased [main effect of lever: $F(1, 46)=24.75$ $p<0.0001$; lever x dose interaction: $F(3,46)=6.15$, $p=0.001$] (Fig. 3.2). The 0.1 mg/kg nicotine group made the fewest responses on lever 1 (sucrose+injection) and the most responses on lever 2 (sucrose) compared to the saline group, which made the most responses on lever 1 and the fewest responses on lever 2.

Experiment 2. Effects of pairing nicotine injection with sucrose delivery on lever responding, which either delivered sucrose+injection, or nothing. Each group responded almost exclusively on lever 1, with few or no responses made on lever 2 across sessions 5-15 (Fig. 3.3a-c). In the 0.03 mg/kg nicotine group, pairing an injection of 0.03 mg/kg nicotine with sucrose delivery did not change responding on lever 1 in phase II (Fig. 3.3a). However, in the 0.1 mg/kg nicotine group, pairing an injection of 0.1 mg/kg nicotine with sucrose delivery changed responding on lever 1 [$F(10, 50)=13.57$, $p<0.0001$] (Fig. 3.3b). When the consequence of responding on lever 1 was changed from sucrose to sucrose+injection, rats made fewer responses on lever 1 across each session from 7 to 15 as compared to session 5 (Fig. 3.3b). In the 0.18 mg/kg nicotine group, pairing an injection of 0.18 mg/kg nicotine with sucrose delivery changed responding on lever 1 [$F(10, 50)=14.08$, $p<0.0001$]. When the consequence of responding on lever 1 was changed from sucrose to sucrose+injection, rats made fewer responses on lever 1 across each session from 6 to 15 as compared to session 5 (Fig. 3.3c). Additionally, while there was a change in responding on lever 2 in session 9 [$F(10, 40)=2.39$, $p=0.02$], responding in session 9 was not significantly different from responding in session 5. Between-subjects analysis revealed that the average number of responses made across sessions 6-15 in phase II was different among groups [main effect of dose: $F(2, 28)=35.95$, $p<0.0001$]. The number of responses made on lever 1 was affected by the dose of nicotine delivered [main effect of lever: $F(2, 28)=35.95$,

$p<0.0001$; lever x dose interaction: $F(2, 28)=29.64$, $p<0.0001$] (Fig. 3.4). Responding on lever 1 was dependent on dose with rats that received the highest unit dose of nicotine making the least amount of responses (0.18<0.1<0.03 mg/kg).

Experiment 3. Effect of delaying nicotine injection on lever responding, which either delivered sucrose injection, or nothing. Rats responded almost exclusively on lever 1 (sucrose+injection) and made few or no responses on lever 2 (nothing) in phase II. However, responding rates on lever 1 (sucrose+injection) were different among groups [main effect of dose: $F(2, 30)=63.31$, $p<0.0001$], with responding affected by the unit dose of nicotine delivered and the immediacy of the injection delivery [main effect of delay: $F(1, 30)=12.45$, $p=0.001$; delay x dose: $F(2, 30)=3.920$, $p=0.03$] (Fig. 3.5). Under the no-delay condition, both the 0.1 and 0.18 mg/kg nicotine groups made fewer responses on lever 1 compared to the saline group, and the 0.18 mg/kg nicotine group made fewer responses than the 0.1 mg/kg nicotine group. Under the delay condition, the 0.18 mg/kg nicotine group made fewer responses on lever 1 compared to both the saline and the 0.1 mg/kg nicotine groups. Both the 0.1 and 0.18 mg/kg nicotine groups made more lever responses when the injection was delayed than when there was no delay. However, in the saline injection group, lever responding did not differ between delay and no-delay injection conditions.

Experiment 4. Effect of response-independent delivery of IV nicotine injection on lever responding. The delivery of response-independent saline or nicotine totaling 1.0 mg per session did not change lever responding across sessions 6-15 as compared to when no infusion was delivered in session 5 (Fig. 3.6a and 3.6b). However, response-independent delivery of 1.8 mg nicotine per session changed responding on lever 1 across sessions [$F(10, 50)=17.90$, $p<0.0001$] (Fig. 3. 6c). Rats made fewer

responses on lever 1 on each session from 8 to 15 as compared to responses made on session 5 when no infusion was administered.

Fig. 3.7 displays average responses (across session 6-15) that were made by each group on lever 1 that delivered sucrose, and lever 2, which delivered nothing. The number of responses made on lever 1 vs. lever 2 differed [main effect of lever: $F(1, 30)=1677$, $p<0.0001$] with the number of responses made affected by the dose of response-independent nicotine administered per session [main effect of dose: $F(2, 30)=204.0$, $p<0.0001$; lever x dose: $F(2, 30)=208.7$, $p<0.0001$]. Rats made more responses on lever 1 than on lever 2. Responding on lever 2 did not differ among groups, but rats that received a dose of 1.8 mg nicotine made fewer responses on lever 1 compared to rats that received a dose of 1.0 mg nicotine or saline. Responses made on lever 1 did not differ between the saline and 1.0 mg nicotine group.

Discussion

The purpose of the present study was to examine the punishing effects of IV nicotine injection on sucrose-reinforced lever responding. In general, sucrose served as a reinforcer of lever responding, whereas nicotine served as a punisher of lever responding. Punishment by nicotine was dependent on the unit dose of injection delivered, the immediacy of injection delivery upon a lever response, and the behavioral alternative to punished responding. Additionally, the cumulative doses of nicotine delivered in punishment situations were not likely due to direct suppressant effects, since comparable doses given response-independently did not affect sucrose-reinforced lever responding.

Punishing effects of nicotine

When rats were presented with levers that delivered either sucrose+injection or sucrose only, all rats decreased their responding on the sucrose+injection lever whether the injection delivered was nicotine or saline. However, the degree to which initial and overall responding on the sucrose+injection lever was reduced was directly related to the dose of nicotine delivered per response, with greater reduction observed as the dose of nicotine increased. These findings have also been observed in squirrel monkeys trained to respond on a lever that delivered food (Goldberg and Spealman 1983). The decreases in responding on the sucrose+injection lever were also accompanied by increases in responding on the alternative lever that delivered sucrose alone. The amount of responding that increased on the sucrose lever reflected the decrease in responding on the sucrose+injection lever (Fig. 3. 2). As a result of the reallocation of responses, rats always made the maximum number of responses per session. Therefore, the nicotine dose-dependent decreases in responding on the sucrose+injection lever could not be attributed to direct suppressant effects. This is the first study that we are aware of to demonstrate that nicotine not only functioned to punish responding leading to its delivery, but also resulted in an increase in responding on the lever that delivered an "unpunished" positive reinforcer. Reallocation of responding resulting from punishment has been reported in studies that used choice procedures to examine punishment of cocaine-reinforced (Johanson 1977) and food-reinforced (Azrin et al. 1965) behavior by electric shock, and punishment of food-reinforced behavior by IV histamine injections (Woolverton 2003; Podlesnik et al 2010). But while punishment resulted in reallocation of behavior in these studies, behavioral histories on the levers were not considered, even though they may be important in determining the effects of punishment. For instance, the increases in responding on the sucrose lever in phase II may have been due in part to the fact that the consequence of

responding on the lever was changed from saline injection to sucrose, which may explain why responding increased on the respective lever among the saline injection group.

Attenuation of the punishing effect of nicotine

Nicotine dose-dependent decreases in responding on the sucrose+nicotine lever were attenuated when the alternative response consequence was null, as compared to when it was sucrose only. For instance, pairing an injection of 0.03 mg/kg nicotine with sucrose delivery did not punish responding on the sucrose+nicotine lever when the consequence of responding on the alternative lever was null (Fig. 3. 3a). While it could be argued that an injection of 0.03 mg/kg nicotine is not a behaviorally active dose under these conditions, the same unit dose of nicotine punished responding on the sucrose+nicotine lever when responding on the alternative lever delivered sucrose (Fig. 3.1c). Furthermore, while unit doses of 0.1 and 0.18 mg/kg nicotine decreased responding on the sucrose+nicotine lever, the degree to which responding decreased with each dose was similar to what was observed with unit doses of 0.03 and 0.1 mg/kg nicotine, respectively, when responding on the alternative lever delivered sucrose only. The degree to which attenuation of punished responding occurs indicates that not having a positively reinforced behavioral alternative decreased the dose-dependent effects of nicotine by a quarter log. Other studies using choice procedures have demonstrated that having an equivalent reinforcer that is unpunished as the consequence of the behavioral alternative may provide a more sensitive measurement of punishment, such that a lower intensity of electric shock (Azrin and Holz 1966) or a smaller dose of IV histamine (Woolverton 2003) was needed to punish behavior when no positively reinforced behavioral alternative was available.

The punishing effects of 0.1 and 0.18 mg/kg per injection nicotine observed when the alternative response consequence is null could be further attenuated if the nicotine injection delivered with sucrose was delayed by 60 s after a response was made. The finding that delaying the delivery of nicotine results in an attenuation of punishment agrees with findings that the punishing effects of a stimulus decreases as the time interval after a response made for the punishing stimulus increases (see Azrin and Holz 1966). For example, Woolverton et al. (2012) found that delaying the delivery of response-contingent histamine injections decreased punishment of cocaine-reinforced lever responding in rhesus monkeys. These findings suggest that a punisher is most effective when it is administered immediately upon a response-contingent behavior.

Control for direct-suppressant effects

The degree to which nicotine punishes lever responding in this study has been seemingly related to the dose of nicotine. However, results from delaying nicotine injections suggests otherwise. When the delivery of the nicotine injection paired with sucrose was delayed for 60 s after a response was made, rats increased their responding on the lever that delivered nicotine with sucrose as compared to when there was no delay. Total daily nicotine intake was therefore greater under the delayed injection condition. While delaying the injection could have interfered with the spacing of nicotine injections (e.g., time between sequential nicotine injections would be shorter), programming the 120 s ITI to begin after a response was made ensured that response-dependent injections were delivered at least 120 s between responses regardless of whether the injection was administered immediately or 60 s after a response was made. Therefore, the finding that more lever responses are made when larger doses of total nicotine intake occurs under the delayed nicotine injection

condition suggests that the behavioral effects of nicotine are neither invariable nor predictable solely on the basis of the drug's inherent pharmacological qualities. Consideration of factors such as the contiguity (i.e., temporal proximity) between a response and consequence of the response being delivered may have practical implications in examining the behavioral effects of drugs. Examining the effects of delay on response-dependent drug delivery may therefore be useful in determining if a decrease in behavior is due to punishment (i.e., decreased responding due to the learned contingency) vs. direct suppression of responding.

Punishing strength of nicotine

While nicotine served as a punisher in this study, responding on the sucrose+injection lever was not completely abolished under any of the described conditions. It could be argued that nicotine injections did not abolish responding on the sucrose+injection lever because nicotine had some reinforcing effect. The unit doses of nicotine used in this study have been found to be reinforcing in IV self-administration studies. For example, using limited-access schedules (e.g., fixed ratio schedules with time outs), rats pre-trained to bar press for nicotine injections will self-administer doses of 0.03- 0.46 mg/kg/inj of nicotine, with a total daily nicotine intake ranging from 0.46- to 4.6 mg/kg (Corrigall and Coen 1989; Chaudhri et al. 2005; Shoaib et al. 1997; Watkins et al. 1999). The decreases in responding on the sucrose+injection lever may therefore represent changes in behavior that would allow rats to attain a total dose of nicotine that is reinforcing (e.g., Corrigall and Coen 1989; Lynch and Carroll 1999). However, this rationalization does not explain why nicotine intake differs among groups that received different unit doses of nicotine (e.g., when choice was sucrose+injection or sucrose, the average daily nicotine intake for the 0.01, 0.03 and 0.1 mg/kg/inj nicotine groups were 0.30, 0.79, and 1.89 mg/kg respectively).

An alternative explanation as to why some responding persists on the sucrose+injection lever may be due to the reinforcing effect (value) of sucrose. The magnitude of the reinforcer that maintains a behavior has been reported to be a determinant of punishment, in which behavior maintained by lower magnitude reinforcers is more strongly punished (Johanson 1977; Poling and Thompson 1977). It is possible that the unit doses of nicotine studied were mildly punishing compared to the highly reinforcing effect of sucrose. Therefore, in principle, responding on the sucrose+injection lever could have been abolished if larger doses of nicotine and/or smaller amounts of sucrose were used to maintain behavior.

Interestingly, Goldberg and Spealman (1982) reported that IV nicotine injections that were reinforcing in squirrel monkeys suppressed food-maintained behavior, such that suppression by nicotine was equivalent to suppression by electric shock, and, in some instances, response behavior was completely abolished. In their study, squirrel monkeys were able to respond on a single lever under a two-component, multiple FR schedule (unpunished and punished signaled components). In both components, every FR 30 resulted in the presentation of food, whereas in the punished component, only the first response in each of FR 30 produced an injection of nicotine. The deliveries of nicotine injections were never given concurrently with food presentation, whereas in the present study, nicotine injections were either given concurrently with sucrose or 60 s after. Methodological differences between the two studies may account for the degree to which nicotine punishes behavior. Using multiple schedules with different FRs for the delivery of sucrose and nicotine injection may be useful in determining if schedule control influences the punishing effects of nicotine in “choice” studies.

Effects of response-independent IV nicotine injections

Delivery of a continuous 90 min infusion of response-independent nicotine totaling 1.0 mg did not change sucrose-reinforced lever responding as compared to when no nicotine was delivered, and responding rates did not differ from those of rats that received saline. In contrast, sucrose-reinforced lever responding did decrease in rats that received a cumulative dose of 1.8 mg nicotine compared to when no nicotine was delivered and responding was lower than responding by rats that received 1.0 mg nicotine or saline. While these findings agree with the findings in which higher cumulative doses of drug result in decreases in self-administration behavior (see Katz 1989), it is unclear whether the observed decrease in lever responding with the delivery of 1.8 mg nicotine resulted from conditioned and/or unconditioned suppressant effects. For instance, when rats were administered 1.8 mg nicotine for the first time in session 6, lever responding decreased by approximately 35% as compared to when no nicotine was delivered, and continued to decrease with repeated response-independent nicotine administration. By the 10^{th} and final administration of 1.8 mg nicotine, responding was almost completely suppressed. Since rats demonstrated that they were capable of making lever responses in session 6, this suggests that administration of 1.8 mg of response-independent nicotine may have resulted in both conditioned and unconditioned behavioral suppressant effects. Although it is unclear what is the exact underlying mechanisms that resulted in the observed decreases in lever responding, these findings are still useful because they provide a rate-limiting cumulative dose of nicotine in which the direct suppressant effects cannot be differentiated from the punishing effects. Accordingly, decreases in behavior observed with cumulative doses of nicotine that are equal to or lower than 1.0 mg of nicotine delivered response-dependently are not likely due to a direct suppressant effect of nicotine. Whereas cumulative doses of nicotine that are greater than 1.0 mg (as observed with the

response-independent delivery of 1.8 mg) may have direct suppressant effects that limit the overall rate of responding on both levers.

Although a cumulative dose of 1.0 mg of nicotine was delivered in both the response-dependent and -independent conditions, the effects of nicotine on rates of lever responding were only observed in the response-dependent condition. 1.0 mg nicotine delivered under the response-independent condition might be behaviorally inactive due to the delivery method of nicotine. Under the response-independent condition, the cumulative 1.0 mg of nicotine was delivered as a continuous infusion, such that rats effectively received one injection in which the infusion rate was 1.0 mg nicotine per 90 min. Under the response-dependent condition, however, the cumulative dose of 1.0 mg nicotine was delivered in pulsatile increments, through individual nicotine injections of 0.1 or 0.18 mg/kg, at an infusion rate of 4-6 s. The maximum frequency in which the individual injections could be delivered was every 2 minutes, and was controlled by the rats' responding. Differences in the delivery of nicotine could have altered the effects of nicotine, such that doses of nicotine and/or drug levels obtained under the response-dependent condition were aversive, which lead to the decreases in lever responding (see Katz 1989; Rose and Corrigall 1997). It has been suggested that the decreases in response behavior observed in preliminary investigations of IV nicotine self-administration studies was a result of the continuous reinforcement schedule that permitted high doses of nicotine to quickly accumulate with continuous successions of injections (see Goldberg and Spealman 1982; Corrigall and Coen 1989). Self-administration studies have also shown that the infusion rate at which IV nicotine is delivered affects the reinforcing effects of nicotine (Wakasa et al. 1995; Sorge and Clarke 2009; Wing and Shoaib 2013). For example, in a concurrent FR1-FR1 choice study in which rats could respond on two concurrently available levers

that delivered either a dose of 15μg/kg nicotine (base) at an infusion rate of 3 s or 30 s, rats made significantly more responses on the lever that delivered nicotine at an infusion rate of 30 s (Sorger and Clarke 2009).

While the effects of response-dependent and independent nicotine have been discussed and compared in terms of the rate of nicotine delivery, it is important to note that the difference in the contingency of nicotine delivery may also account for differences in the effects of nicotine. Studies have reported significant differences in the effects of drugs associated with response-dependent versus response-independent administration (e.g., Mello and Mendelson 1970; Ator and Griffiths 1992). For instance, Dworkin et al. (1995) found that while response-dependent cocaine self-administration was reinforcing in rats, the response-independent administration of the same or similar dosage pattern was lethal. Therefore, while the cumulative dose of nicotine delivered may be related to some of the behavioral effects of nicotine that have been reported (e.g., Corrigall and Coen 1989), the conditions under which nicotine is delivered can alter the effects of the nicotine.

Conclusions

This is the first study of which we are aware that demonstrates that the same doses of IV nicotine injections that are reported to be reinforcing in rats (Corrigall and Coen 1989; Chaudhri et al. 2005; Shoaib et al. 1997; Watkins et al. 1999), could also function to punish response behavior in the present studies. While the pharmacological properties of nicotine (e.g., dose-dependent effects) are important determinants of punishment, environmental conditions in which nicotine is self-administered (e.g., alternative response consequences) are also important. Therefore, consideration of the environmental context in which nicotine is self-administered may have practical implications in understanding the effects of nicotine on behavior.

Table 3.1. Consequences of responding on lever 1 and lever 2 in phase I and II for each nicotine punishment experiment.

	Consequences of responding on levers			
	Phase I: Sessions 1-5		Phase II: Sessions 6-15	
	Lever 1	Lever 2	Lever 1	Lever 2
Experiment 1 Evaluating the effect of pairing nicotine (saline, 0.01, 0.03, or 0.1 mg/kg) injection with sucrose delivery	sucrose	saline injection	sucrose+injection	sucrose
Experiment 2 Evaluating the effect of pairing nicotine (0.03, 0.1, or 0.18 mg/kg) injection with sucrose delivery	sucrose	saline injection	sucrose+injection	nothing
Experiment 3 Evaluating the effect of delaying (saline, or 0.1, 0.18 mg/kg nicotine) injection paired with sucrose delivery	sucrose	saline injection	sucrose+injection **or** sucrose +delayed injection	nothing
Experiment 4 Evaluating the effect of response-independent nicotine (saline, 1.0 or 1.8 mg total) delivery on lever responding	sucrose	saline injection	sucrose	nothing

Figure 3.1

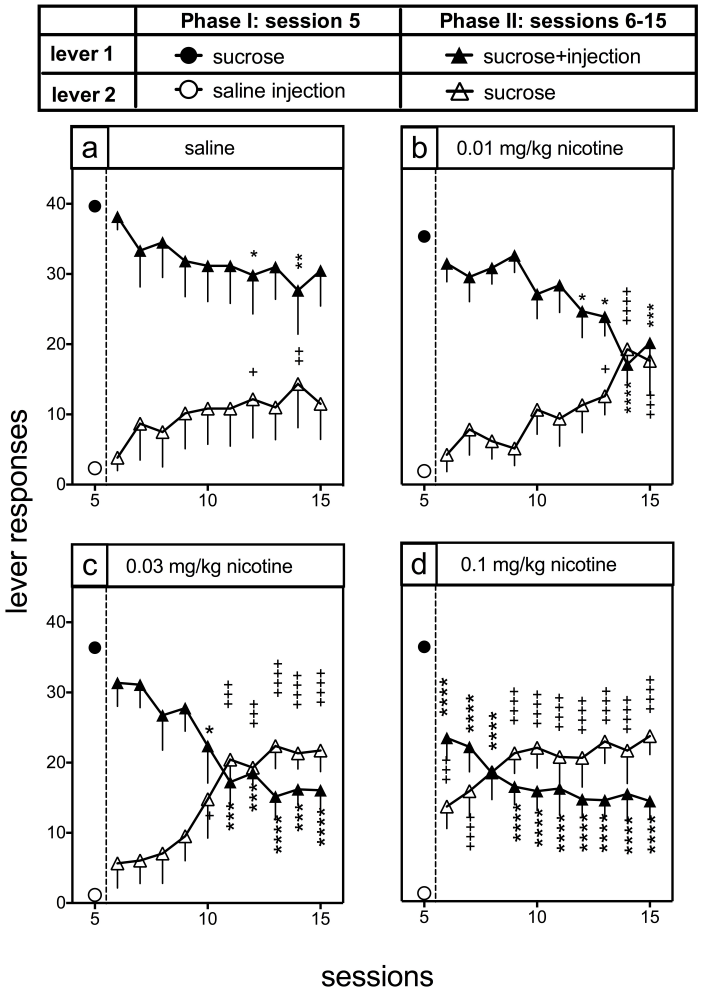

Figure 3.1 Effects of pairing nicotine injection with sucrose delivery on lever responding that delivered either sucrose+injection or sucrose. Each graph displays data from individual groups that differ by the dose of nicotine or saline "injection" paired with sucrose delivery in phase II (a) saline, (b) 0.01 mg/kg nicotine, (c) 0.03 mg/kg nicotine, and (d) 0.1 mg/kg nicotine (n=6 per group). Closed and open symbols represent the mean (±SEM) responses made on lever 1 and lever 2, respectively. Legends next to the symbols listed in the table indicate the consequences of responding on lever 1 and lever 2 in phases I and II. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$. Significant decrease in responses made on lever 1 as compared to responses made in session 5. +, $p<0.05$, ++, $p<0.01$, +++, $p<0.001$, ++++, $p<0.0001$. Significant increase in responses made on lever 2 as compared to responses made in session 5.

Figure 3.2

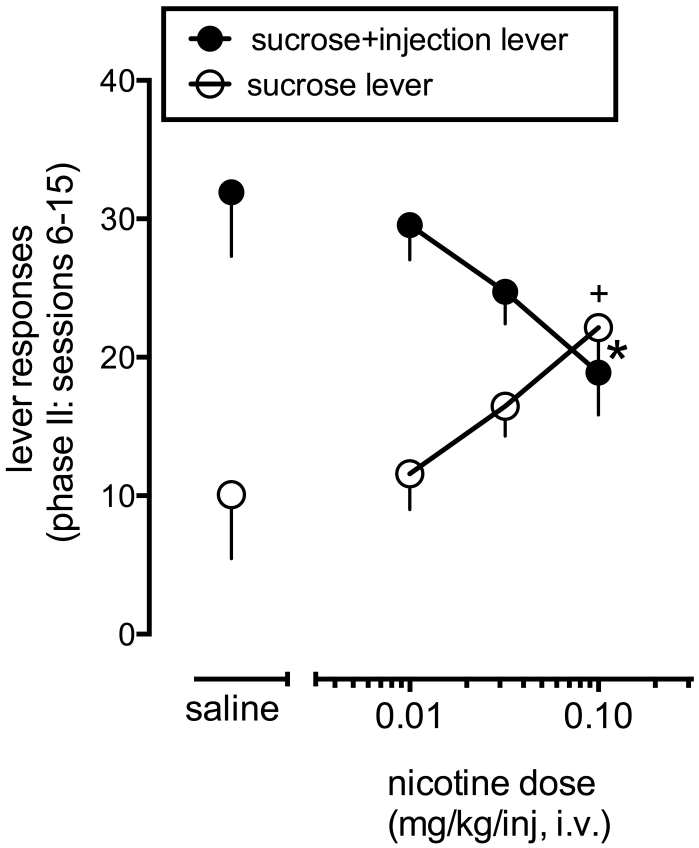

Figure 3.2 Nicotine dose-dependent decreased responding on the sucrose+injection lever, and increased responding on the sucrose lever. Values on the x-axis indicate dose of nicotine or saline "injection" delivered for responses made on the sucrose+injection lever. Closed and open symbols represent the mean (±SEM) responses made on the sucrose+injection and sucrose levers, respectively, in phase II (n=6). *, $p<0.05$. Significant increase in responses made on the sucrose lever as compared to saline. +, $p<0.05$. Significant decrease in responses made on the sucrose+injection as compared to saline.

Figure 3.3

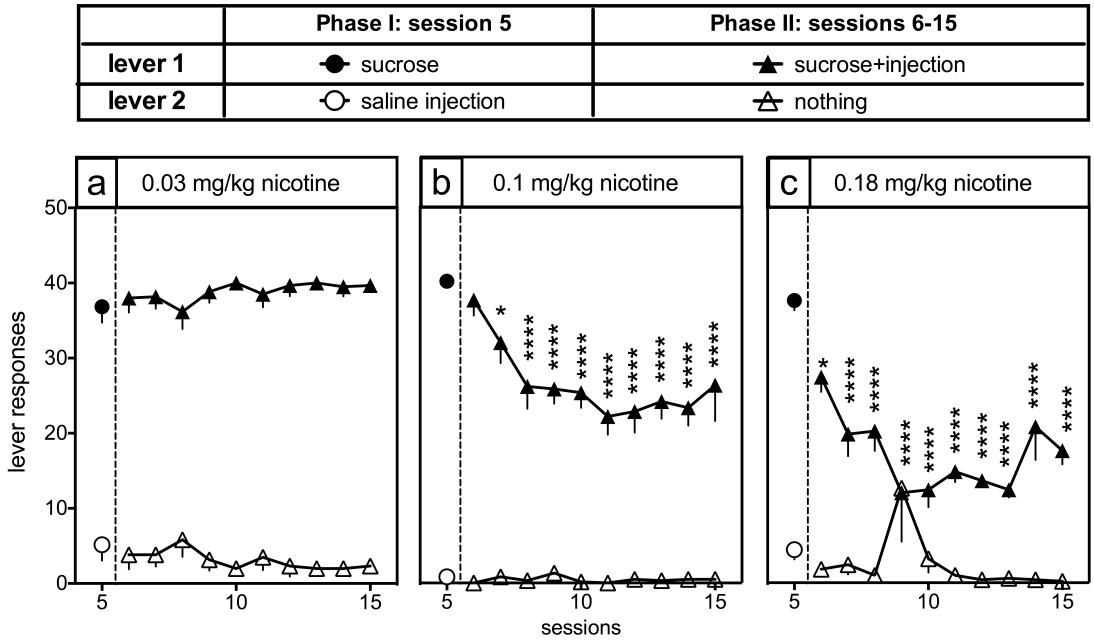

Figure 3.3 Effects of pairing nicotine injection with sucrose delivery on lever responding that delivered either sucrose+injection, or nothing. Each graph displays data from individual groups that differ by the dose of nicotine "injection" paired with sucrose delivery in phase II: (a) 0.03 mg/kg nicotine, (b) 0.1 mg/kg nicotine and (c) 0.18 mg/kg nicotine (*n*=6 per group). Closed and open symbols represent the mean (±SEM) responses made on lever 1 and lever 2 respectively. Legends next to the symbols listed in the table indicate the consequence of responding on lever 1 and lever 2 in phases I and II. *, $p<0.05$, ****, $p<0.0001$. Significant decrease in responses made on lever 1 as compared to responses made in session 5.

Figure 3.4

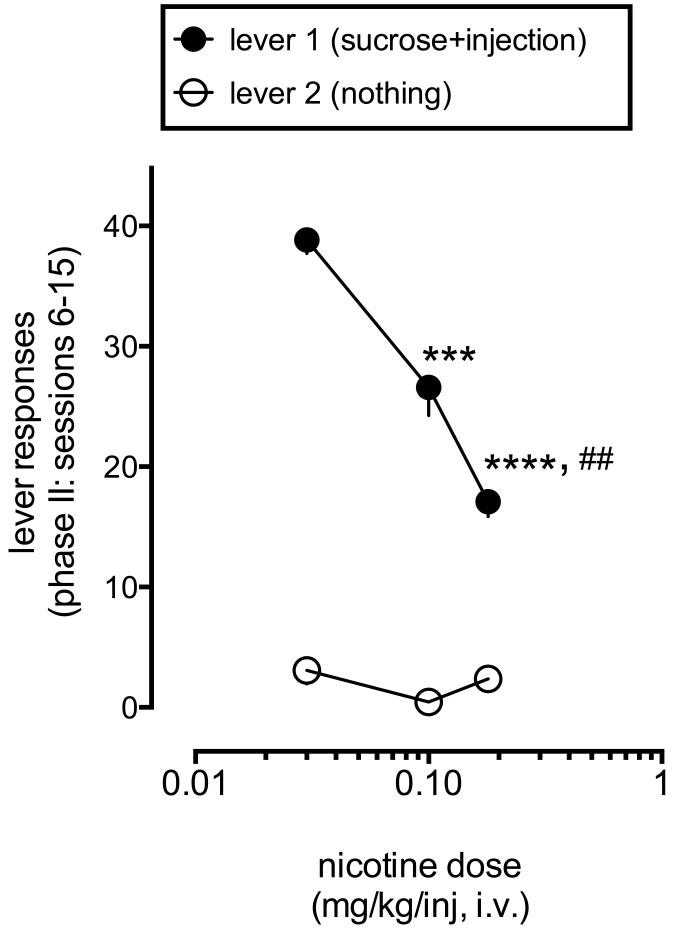

Figure 3.4 Dose-dependent effects of nicotine injection on lever responding that delivered either sucrose injection, or nothing. Values on the x-axis indicate the dose of nicotine "injection" delivered with sucrose for responses made on lever 1. Closed and open symbols represent the mean (±SEM) responses made on lever 1 and lever 2, respectively, in phase II, (*n*=6). ***, p<0.001, ****, p<0.0001. Significant decrease in responding on lever 1 compared to 0.03 mg/kg nicotine injection condition. ##, p<0.01. Significant difference in responding on lever 1 between the 0.1 mg/kg nicotine and 0.18 mg/kg nicotine conditions.

Figure 3.5

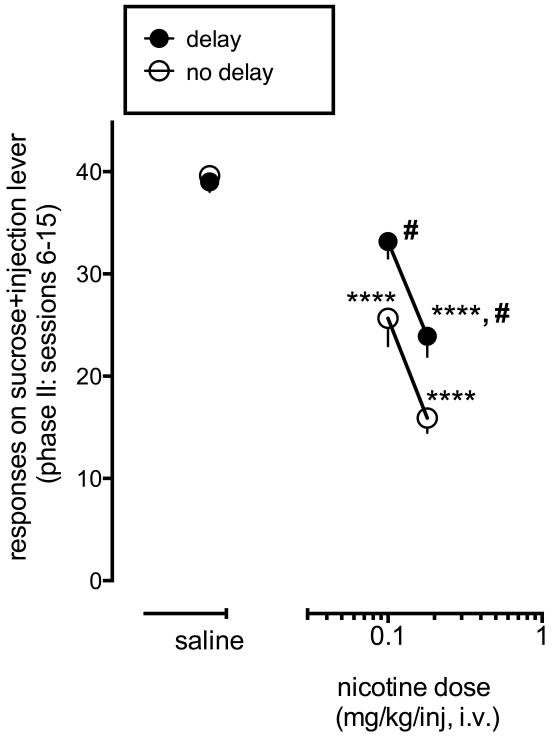

Figure 3.5 Attenuation of the punishing effects of nicotine by delaying injection delivery paired with sucrose. Values on the x-axis indicate the dose of nicotine or saline "injection" delivered with sucrose for responses made on the sucrose+injection lever. Closed and open symbols represent the mean (±SEM) responses made on the sucrose+injection lever when injections were delayed or not delayed, respectively, in phase II (*n*=6). ****, p<0.0001. Significant decrease in responding on sucrose+injection lever compared to saline. #, p<0.05. Significant decrease in responding on sucrose+injection lever compared delayed.

Figure 3.6

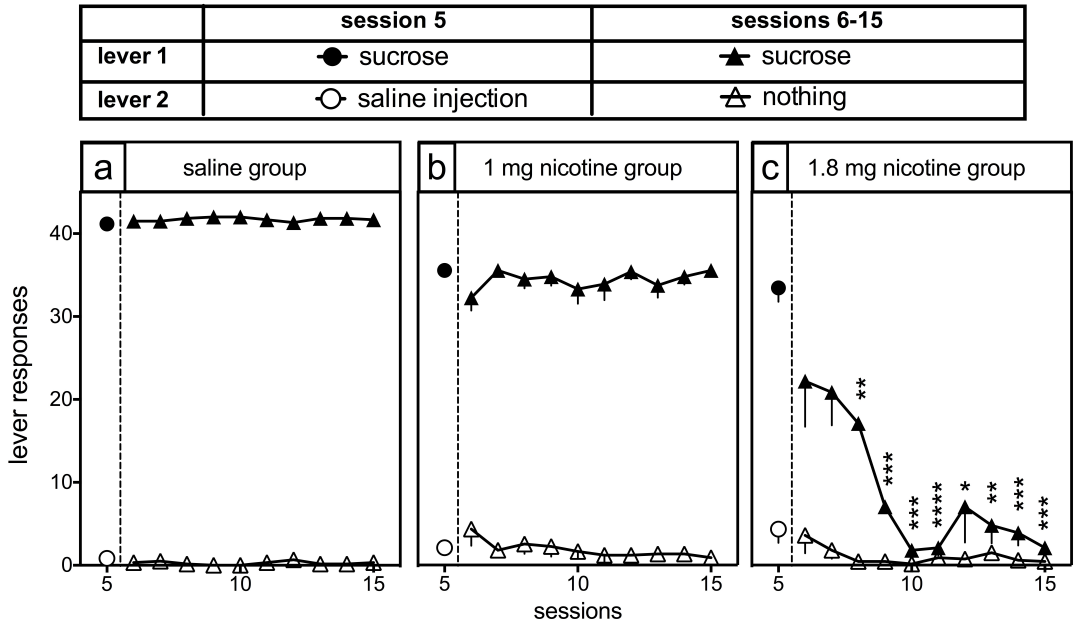

Figure 3.6 Effect of response-independent nicotine on lever responding. Each graph displays data from individual groups that differ by the dose of nicotine or saline delivered per session in phase II: (a) 0.03 mg/kg nicotine, (b) 0.1 mg/kg nicotine and (c) 0.18 mg/kg nicotine (*n*=6 per group). Closed and open symbols represent the mean (±SEM) responses made on lever 1 and lever 2 respectively. Legends next to the symbols listed in the table indicate the consequences of responding on lever 1 and lever 2 by sessions. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$. Significant decrease in responses made on lever 1 as compared to responses made in session 5.

Figure 3.7

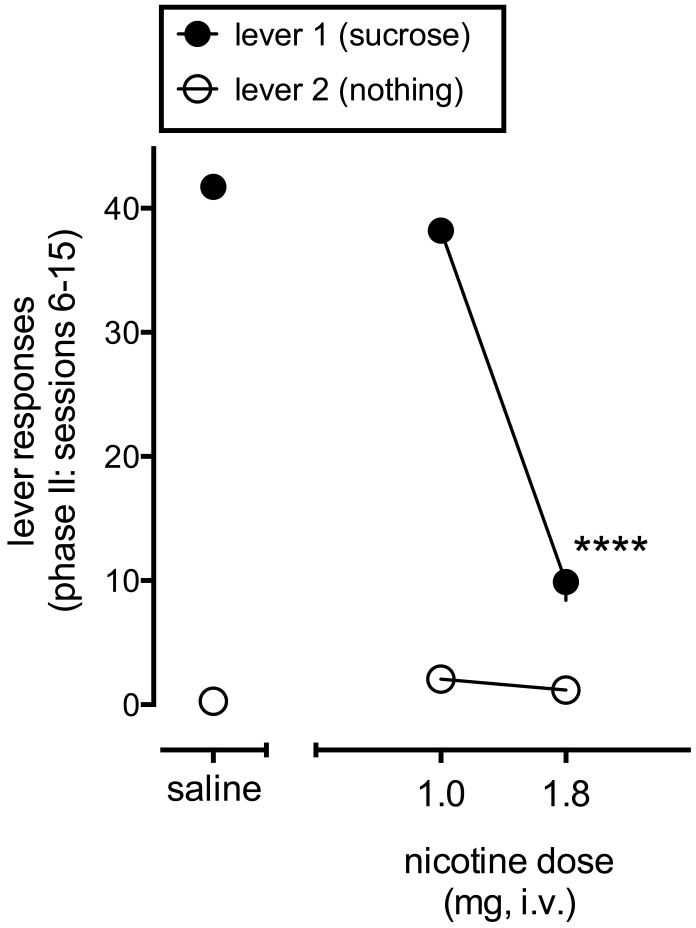

Figure 3.7 Dose-dependent effect of response-independent nicotine on lever responding. Values on the x-axis indicate the dose of nicotine or saline delivered as a response-independent continuous infusion during each session in phase II. Closed and open symbols represent the mean (±SEM) responses made on the lever 1 and lever 2, respectively, in phase II (n=6). ****, $p<0.0001$. Significant decrease in responding on sucrose+injection lever compared to saline.

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Chapter 4

Receptor mediation of the punishing effects of nicotine in rats

Introduction

Tobacco smoking is the leading preventable cause of disease in the United States, resulting in over 400,000 premature deaths a year (US Department of Health and Human Services 2014). Despite the health consequences that result from smoking, over 40 million Americans continue to smoke. Among current smokers, it is reported that a majority want to quit, and among those who have tried to quit, most are unsuccessful (Center for Disease and Control and Prevention 2009). While the determinants of tobacco addiction are multifaceted, a large body of evidence has led to the widely accepted conclusion that nicotine, a primary constituent of tobacco, is "the drug in tobacco that causes addiction" (US Department of Health and Human Services 1988).

It has been well documented that the effects of nicotine are mediated through its agonist actions on neuronal nicotinic acetylcholine receptors (nAChRs) that are found throughout the central (CNS) and peripheral nervous systems. The nAChRs are a functionally diverse group of ligand-gated ion channels that exist as pentamers. To date, there have been twelve different neuronal subunits that have been identified in mammalian tissues: α2-α10 and β2-β4. Combinations of these neuronal subunits make up the different nAChR subtypes (see Gotti and Clementi. 2004). Substantial research efforts has therefore been devoted to elucidating the possible roles of different

nAChR subtypes in mediating the effects of nicotine that regulate nicotine-taking behavior (see Picciotto and Kenny 2013).

Evidence from pharmacological studies suggests that the reinforcing effects of nicotine, which are thought to be important in the abuse of tobacco products (reviewed in US Department of Health and Human Services 2010) are mediated through the $\alpha4\beta2^*$ subtype of nAChR (e.g., Corrigall et al. 1994; Watkins et al. 1999; Mansbach et al. 2000; Liu et al. 2007; Le Foll et al. 2009). Accordingly, the $\alpha4\beta2^*$ nAChRs have been a primary target for smoking cessation agents (see Benowitz 2008; Taly et al. 2009). Although the reinforcing effects of nicotine are important in establishing and maintaining abuse of tobacco, it has been suggested that self-administration of drugs of abuse may be a function of the relative balance between the reinforcing and aversive effects (Stolerman and D'Mello 1981; Katz 1989; Lynch and Carroll 2001; Riley 2011). As such, it has been hypothesized that along with the reinforcing effects of nicotine, aversive effects may also be important in determining nicotine-taking behaviors (see Benowitz 1990; Le Foll and Goldberg 2009). Therefore, the investigation of the nAChR subtype(s) that mediate the aversive effects of nicotine may also be important targets for the development of novel therapeutics for smoking cessation.

Nicotine can produce aversive effects at doses that have been found to be reinforcing in non-human primates (Goldberg and Spealman 1982; 1983; Spealman 1983), rodents (Truong and Woods; unpublished data), and humans (Henningfield and Goldberg 1983). Interestingly, it has been suggested that the $\alpha4\beta2^*$ nAChRs may be involved in mediating the effects of nicotine-induced conditioned taste aversion (Shoaib and Stolerman 1995; Shoaib et al. 2000; 2002; Gommans et al. 2000) and nicotine-induced conditioned place aversion (Laviolette and Kooy 2003). However, recent findings from a molecular genetic study using self-administration procedures suggest

that the aversive effects of nicotine are mediated through different nAChRs that contain the α5* subunit (Fowler et al. 2011). While pharmacological studies using operant procedures have demonstrated that nAChRs mediate the aversive effects of nicotine (Goldberg and Spealman 1982), and that these effects are likely being mediated by nAChRs in the CNS (Spealman 1983), it is unclear whether the $\alpha4\beta2$* nAChRs are involved in these effects.

Using a punishment procedure, the purpose of this study was to examine the antagonism of the aversive effects of IV nicotine in rats. Examining antagonism of the aversive effects of IV nicotine may lead to a more general understanding of the receptor mediated effects of nicotine that control behavior. The antagonists used in this study were mecamylamine (Stone et al. 1956; Martin et al. 1989), a nicotine receptor ion channel blocker that is uncompetitive in its actions, and dihydro-beta-erythroidine (DHβE) (Williams and Robinson, 1984; Sabey et al. 1999; Shoaib et al. 2000), a competitive, $\alpha4\beta2$* selective nAChR antagonist.

Methods

All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals and performed in accordance with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health.

Subjects

Male Sprague-Dawley rats (300-375 g) were obtained from Harlan (Indianapolis, IN) and housed in a temperature- (21-23 °C) and humidity-controlled environment on a 12-h dark/light cycle, with lights on at 7:00 A.M. Rats were housed three per cage during non-drug behavioral training procedures, and then individually housed after surgery was completed. Except during experimental sessions, rats had free access to

tap water and were on food-restricted diets of Purina rodent chow that maintained at least 80% of their pre-restriction body weights. All experiments were conducted 5-7 days/week between 9:00 A.M and 5:00 P.M.

Apparatus

Med Associates (St. Albans, VT) operant conditioning chambers and manipulanda were used for all experiments. Each operant conditioning chamber was approximately 30 cm long, 24 cm wide, and 21 cm high, and was contained in a sound-attenuating cubicle. The front panel of each operant conditioning chamber was equipped with two retractable levers, 6.8 cm above the grid floor, and 1.3 cm from the side walls, with an array of red, yellow, and green light-emitting diodes (LEDs) above each lever. A 2.8V white incandescent house light was located at the top center of the rear panel. Located between the two levers was a magazine in which a 45 mg sucrose pellet (Bio-Serv, Frenchtown, NJ) could be delivered. IV drug injections were delivered by a motorized syringe driver (PHM-107; Med Associates) through Tygon tubing (S-54-HL, Norton Performance Plastics, Akron, OH) connected to a swivel that was held in place by a counterbalanced arm. Syringe drivers were located outside the sound-attenuating chamber. The duration of response-dependent injections were determined by the weight of each rat divided by the drug delivery pump flow rate (0.072 ml/per/s). Data were collected with Med Associates software.

Non-Drug Behavioral Training Procedures

Rats first received one 60 min session in which they were trained to eat sucrose pellets from the magazine located inside the operant chamber. No levers were available during this time, and sucrose pellets were delivered on a random-time 60 s schedule. Rats then received two 60 min daily sessions in which they were trained to respond on both levers. In these sessions, each lever was presented individually in alternating

sequence. Rats were given 8 s to press the lever each time it was presented. If a response was made (or no response was made after 8 s), then a sucrose pellet was delivered, the lever retracted, and the LEDs were turned off until the lever was presented again. Intertrial intervals (ITIs), which are timed intervals between trials when no lever is available and, therefore, no responses could be made, were each 60 s long. Each ITI began when the lever retracted.

Non-drug behavioral training finished with a single 90 min "choice" training session. In this session, a response made on either lever delivered a sucrose pellet. The choice training session began with two "lever trials" followed by a series of "choice trials." Lever trials were initiated when one of the two levers was randomly extended into the chamber with the three LEDs above the lever turned on. Rats were given 60 s to press the lever after it was presented. If a response was made, sucrose was delivered, the lever retracted, and the LEDs were turned off. If no response was made within the 60 s, then the lever retracted, the LEDs were turned off, and no sucrose was delivered. Lever trials were presented to allow rats to sample the consequence of responding on each lever before choice trials began. After the two lever trials were presented, choice trials were initiated with the simultaneous extension of both levers and the LEDs above both of the levers turned on. Under a concurrent fixed ratio 1- fixed ratio 1 (FR1-FR1) schedule, rats were presented with the opportunity to respond on one of the two available levers. If a response was made on either lever, sucrose was delivered, both of the levers retracted, all of the LEDs were turned off, and sucrose was not delivered. If no response occurred within 60 s, both levers retracted and all of the LEDs were turned off. Each ITI was 120 s long and began when the lever(s) retracted.

Surgery

After non-drug behavioral training was completed, rats were surgically implanted with chronic indwelling IV catheters. Rats were anesthetized with ketamine (100 mg/kg; IP) and xylazine (10 mg/kg; IP) before a longitudinal incision was made to expose the femoral vein into which a catheter constructed from Micro-Renathane (Braintree Scientific, Inc., Braintree, MA) was inserted. The catheter was passed subcutaneously to an incision made between the scapulae and was then connected to a metal cannula that exited the skin. Catheters were flushed daily with 0.5 ml (100 U/ml) of heparinized saline to maintain patency. Rats were allowed at least 5 days to recover from surgery; during this time rats had unrestricted access to food and water.

Evaluating DHβE and mecamylamine pretreatment on punished lever responding

All testing procedures began after rats recovered from surgery. The choice procedure used in all sessions was the same one described for the choice training session, with the exception of the consequences of responding on each lever. In each 90 min session, the maximum number of choice trials that could be completed was 43, and each ITI was 120 s long.

Rats received 5 daily sessions to acquire responding on a lever that delivered a sucrose pellet. The lever on which a rat made fewer responses during the choice training session was designated lever 1, and the consequence of responding on lever 1 was the delivery of a sucrose pellet. The lever on which a rat made more responses during the choice training session was designated lever 2, and responding on lever 2 resulted in the delivery of nothing.

After the 5 sessions were conducted, the consequence of responding on lever 1 was changed from delivery of sucrose to the delivery of sucrose paired with an intravenous injection of nicotine (0.1 or 0.18 mg/kg per injection) or saline, while the consequence of responding on lever 2 remained the delivery of nothing. To evaluate

DHβE and mecamylamine pretreatment on punished lever responding, the pairing of a nicotine or saline injection with sucrose delivery was alternated sequentially across sessions, with examination of 0.1 mg/kg nicotine completed first before 0.18 mg/kg nicotine was evaluated. DHβE (vehicle, 1.0, 3.2, and 5.6 mg/kg; s.c.) and mecamylamine (vehicle, 0.1, 0.3, 1.0 mg/kg; s.c.) pretreatments were administered in the home cage 15 min before the start of the test session. Each antagonist treatment was separated by at least 2 days. The order of DHβE and mecamylamine pretreatments, and administration of each antagonist dose were counterbalanced (*n=7*).

Drugs

(-) -Nicotine hydrogen tartrate salt, DHβE and mecamylamine were obtained from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in 0.9% saline solution and doses were calculated on the basis of the salt form of the drug. Doses of DHβE and mecamylamine were chosen based on reported behavioral activity in rats (Jutkiewicz et al. 2011).

Data Analysis

The dependent variable measured in each session was the number of responses made on each lever. Two-way ordinary ANOVAs were used to determine if antagonist pretreatments (mecamylamine or DHβE) affected responding on lever 1 with the within-subjects factors of antagonist dose and dose of nicotine delivered with sucrose (saline vs. 0.1 vs. 0.18 mg/kg nicotine). Significant ANOVAs were followed by Bonferroni post hoc tests. All statistical tests used an alpha of 0.05, two-tailed. Analyses were performed using Prism 6.0 (GraphPad Software, La Jolla, CA, USA).

Results

When rats were given 5 sessions to respond on levers that delivered sucrose or nothing, rats readily increased their responses on lever 1 (sucrose) while decreasing their response on lever 2 (nothing). By session 5, averaged responses made on lever 1 was 38.7±0.9, whereas averaged responses made on lever 2 was 3.2±0.9. This pattern of responding indicated that responding on lever 1 was maintained by the reinforcing effect of sucrose. When the consequence of responding on lever 1 changed to include the delivery of an injection of nicotine or saline, responses continued to be made exclusively on lever 1 as compared to lever 2. However, the number of responses made on lever 1 was affected by the dose of nicotine delivered with sucrose [$F(2,18)=38.39$, $p<0.0001$]. Post hoc tests revealed that responding on lever 1 was dependent on dose of nicotine delivered with sucrose, such that rats that received the highest unit dose of nicotine made the fewest responses (saline>0.1>0.18 mg/kg) (data not shown).

When mecamylamine pretreatments were administered, responding on lever 1 differed by the dose of nicotine injection paired with sucrose delivery [main effect of nicotine dose: $F(2, 72)=119.5$, $p<0.0001$], which was affected by dose of mecamylamine treatment [main effect of mecamylamine: $F(3,72)=10.68$, $p<0.001$; nicotine dose x mecamylamine: $F(6, 72)=4.463$, $p=0.0007$] (Fig. 4.1). Post hoc tests revealed that pretreatments with mecamylamine compared to vehicle did not affect responding on lever 1 when it delivered a saline injection with sucrose. Pretreatment with all doses of mecamylamine attenuated the punishing effect of 0.1 mg/kg nicotine, such that responding on lever 1 did not differ from trials in which the injection delivered was saline. Mecamylamine pretreatment also decreased the punishing effect of 0.18 mg/kg nicotine, such that rats treated with 0.3 or 1.0 mg/kg mecamylamine made more responses on lever 1 compared to vehicle treated rats, but responding on lever 1 was lower than when the injection delivered was saline.

Pretreatments with DHβE (1.0, 3.2, and 5.6 mg/kg) did not affect responding on lever 1 when the injection delivered with sucrose was saline or 0.1 mg/kg nicotine compared to vehicle treatment (Fig. 4.2). Additionally, pretreatment with 5.6 mg/kg DHβE did not affect responding on lever 1 when the injection delivered with sucrose was 0.18 mg/kg nicotine compared to vehicle treatment (data not shown). However, the dose of nicotine injection delivered with sucrose affected responding on lever 1 [main effect of injection type: $F(1, 47)=92.49$, $p<0.0001$] with fewer responses made on lever 1 when 0.1 mg/kg nicotine was delivered with sucrose compared to when saline was delivered with sucrose.

Discussion

The purpose of the present study was to determine whether pretreatments with nAChR antagonists altered the punishing effects of IV nicotine injections. The degree to which IV nicotine injections functioned to decrease sucrose-reinforced lever responding was related to the dose of nicotine, with greater decreases in responding observed with larger doses of nicotine. The finding that rats increased their responding on a lever that delivered the same unit doses of nicotine with sucrose when the delivery of nicotine was delayed for 60 s (see Chapter 3) suggests that the decrease in lever responding observed under the present conditions is not a result of direct suppressant effects, but due to a punishing effect of nicotine. Pretreatments with the non-selective antagonist, mecamylamine, resulted in the attenuation of the punishing effects of nicotine on sucrose-reinforced lever responding, whereas the selective α4β2* nAChR antagonist, DhβE, had no effects on responding. While this is not the first study to indicate that the punishing effects of nicotine are mediated by nAChRs (Goldberg and Spealman 1982), it is the first to demonstrate that the punishing effects of IV nicotine are not mediated by

the $\alpha 4\beta 2^*$ nAChRs subtype, which are believed to mediate the reinforcing effects of self-administered nicotine (Corrigall et al. 1994; Watkins et al. 1999; Mansbach et al. 2000; Liu et al. 2007; Le Foll et al. 2009).

Mecamylamine pretreatments on punished lever responding

Pretreatment with doses of 0.1-1.0 mg/kg mecamylamine had no effect on lever responding when saline injections were delivered with sucrose, but pretreatment with these doses of mecamylamine completely blocked the punishing effects of 0.1 mg/kg nicotine equivalently such that responding on the sucrose+injection lever did not differ from responding when the injection delivered with sucrose was saline. However, pretreatments with the same doses of mecamylamine only partially blocked the punishing effects of 0.18 mg/kg nicotine. The degree to which the punishing effects of 0.18 mg/kg nicotine were attenuated depended on the dose of mecamylamine administered, with attenuation increasing with dose of mecamylamine. Pretreatment with doses of 0.1-1.0 mg/kg mecamylamine therefore resulted in a rightward shift in the dose response function of nicotine, suggesting that the effects of mecamylamine were surmountable. This indicates that the punishing effects of nicotine in rats are mediated by nAChRs, which is consistent with other studies that reported that mecamylamine increased food-reinforced lever responding that had been punished by IV nicotine injections (Goldberg and Spealman 1982), and decreased responding that was maintained by the postponement of IV nicotine injections (Spealman 1983).

DHβE pretreatments on punished lever responding

While DHβE has been reported to block the effects of nicotine-induced conditioned taste aversion (Shoaib et al. 2000; 2002; Gommans et al. 2000) and unbiased conditioned place aversion (Laviolette and Kooy 2003), pretreatments with DHβE in the present study did not alter aversive effects of nicotine as measured by

punishment. It is possible that differences in the cumulative dose of nicotine (0.2-2.0 mg/kg) administered in the conditioned taste aversion studies (Shoaib et al. 2000; Gommans et al. 2000) and the present study (≥2.8 mg/kg) may account for the differences in the effects of DHβE, such that the higher doses of nicotine self-administered could not be antagonized. However, the doses of DHβE used in the present study were comparable to the ones used in the conditioned taste aversion studies (Shoaib et al. 2000, Gommans et al. 2000). This suggests that while the α4β2* nAChRs are involved in mediating the effects of nicotine-induced conditioned taste aversion and nicotine-induced conditioned place aversion, they are not involved in mediating the punishing effects of nicotine. The negative results obtained with DHβE treatment also provides additional evidence that demonstrates that lever responding is not being maintained by a reinforcing effect of nicotine, since it is thought that the reinforcing effects of self-administered nicotine are mediated through the α4β2* nAChR subtype (Corrigall et al. 1994; Watkins et al. 1999; Mansbach et al. 2000; Liu et al. 2007). ***Implications for the development of pharmacotherapies in treatment of tobacco abuse***

Findings from pretreatments with mecamylamine and DHβE suggest that the punishing effects of nicotine are mediated by a population of nAChR subtype(s) that do not include the α4β2* nAChRs receptors. While the identification of the nAChR subtype(s) that mediate the aversive effects of response-contingent nicotine has yet to be established, it has been proposed that nAChRs that contain the α5 subunit may be involved in mediating the aversive effects of nicotine (Fowler et al. 2011). In this study, α5 knockout mice showed increased nicotine self-administration at the high unit doses found on the descending limb of the dose-response curve of nicotine self-administration, which are believed to have aversive properties that may serve to limit

intake (e.g., Katz 1989; Rose and Corrigall 1997). Development of antagonists that are selective at the α5 subunit may be useful in elucidating the nAChR(s) that may be involved in the aversive effects of nicotine that may limit its intake. However, in principle, DhβE or a pharmalogical equivalent that antagonizes the reinforcing effects of nicotine, but not the aversive effects of self-administered nicotine, may be a useful pharmacotherapy in treatment of tobacco abuse. While it is not known whether selective nicotinic antagonists would be a useful aid in smoking cessation treatments in humans, receptor selective antagonists have been useful pharmacotherapies for opioid abuse (Comer et al. 2002, 2006; Sullivan et al. 2006; Lobmaier et al. 2008).

Figure 4.1

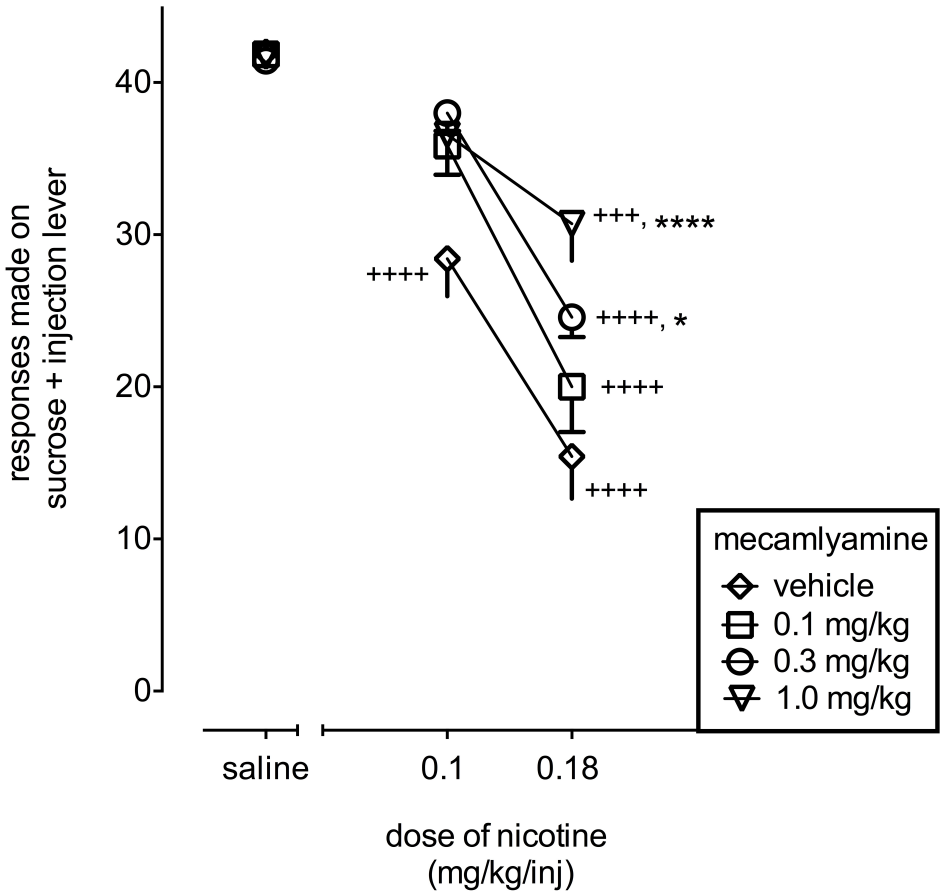

Figure 4.1 The effects of mecamylamine pretreatment on nicotine dose-dependent punished lever responding. Legends to the right of the open symbols indicate the pretreatment dose of mecamylamine. Dose of nicotine or saline injection delivered with sucrose is indicated on the x-axis. ++, $p<0.01$. ++++, $p<0.0001$. Significant decrease in lever responding compared to vehicle pretreatment when injection of saline is delivered with sucrose. *, $p<0.05$, ****, $p<0.0001$. Significant increase in lever responding compared to vehicle treatment when dose of nicotine delivered with sucrose is 0.18 mg/kg.

Figure 4.2

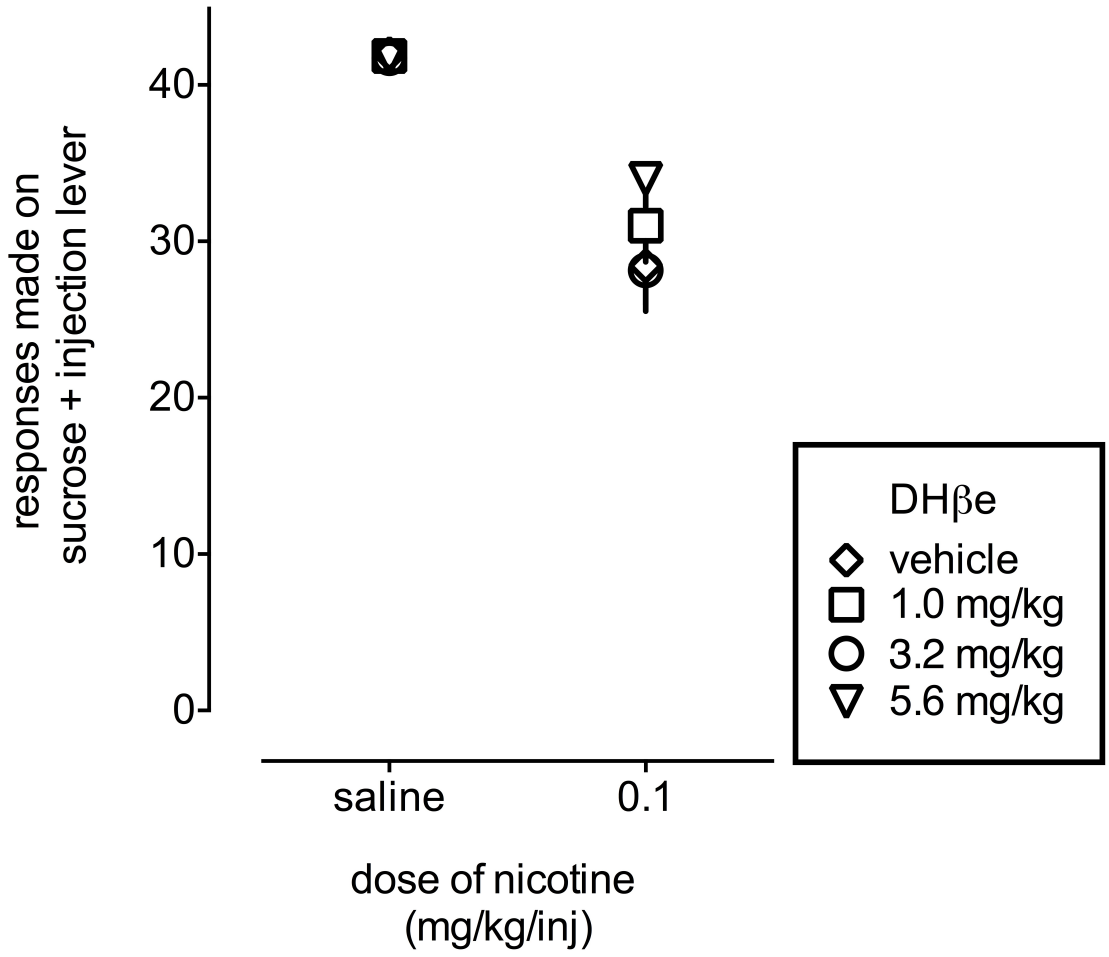

Figure 4.2 Responding on the lever that delivered sucrose+ injection of saline or 0.1 mg/kg nicotine with DHβE pretreatment. Legends to the right of the open symbols indicate the pretreatment dose of DhβE. Dose of nicotine or saline injection delivered with sucrose is indicated on the x-axis.

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Chapter 5

General discussion

Tobacco products contain thousands of chemicals, but nicotine, a constituent of tobacco, is regarded as the psychoactive drug responsible for tobacco addiction (see US Department of Health and Human Services 2010). For instance, IV self-administration studies have clearly demonstrated that nicotine functions as a reinforcer in both non-human (e.g., Henningfield and Goldberg 1983b; Corrigall and Coen 1989; Donny et al. 1995; Le Foll and Goldberg 2007) and human subjects (e.g., Henningfield and Goldberg 1983b; Ator and Griffiths 1983; Harvey et al. 2004). In addition to IV nicotine, humans will also self-administer intranasal nicotine (Perkins et al. 1996) and use medicinal nicotine gum (Hughes et al. 1990). And while it is recognized that the effects of nicotine that contribute to tobacco addiction are diverse, it is widely accepted that the reinforcing effects of nicotine contribute greatly to the development and maintenance of its abuse in humans (Young and Herling 1986; Ator and Griffths 2003). Nevertheless, it is unclear why certain people become heavy smokers (i.e., people who smoke ≥ 2 packs of cigarettes per day), while others smoke intermittently, and still others try tobacco, but never develop a "habit" despite repeated exposure to nicotine. These observations may indicate that the reinforcing effects of nicotine may not account for all nicotine-taking behaviors.

It has been proposed that the rates at which drugs of abuse such as nicotine are self-administered may be influenced by aversive properties of the drug (see Cappell and Le Blanc 1979; Goudie AJ 1979; Russell 1979; Stolerman and D'Mello 1981; Katz

1989; Rose and Corrigall 1997; Le Foll and Goldberg 2009). For example, research with squirrel monkeys have demonstrated that the same doses of nicotine which will maintain self-administration (Goldberg et al. 1981; Goldberg and Spealman 1982; Spealman and Goldberg 1982) could also function to dose-dependently punish food-reinforced lever responding such that the punishing effects of nicotine were effectively equivalent to electric shocks in which both stimuli functioned to decrease food-reinforced responding by over 70% (Goldberg and Spealman 1982; 1983). It has also been reported that IV nicotine injections can also maintain lever responding to avoid its scheduled injection in both squirrel monkeys (Spealman 1983) and smokers (Henningfield and Goldberg 1983a).

However, it is unclear whether the aversive effects of nicotine described in these studies were unique to the conditions under which they were studied, or whether nicotine functioned more generally as an aversive stimulus. For instance, in the punishment procedures used by Goldberg and Spealman (1982; 1983), the first response in each FR30 produced an injection of nicotine and the rate in which nicotine injections could be delivered was limited only by the monkey's responding. Whereas in the self-administration procedures, nicotine was delivered either under a fixed-interval or second order schedule in which the rate of nicotine delivery was limited by either the schedule of nicotine delivery and/or programmed time-outs (Goldberg et al. 1981; Goldberg and Spealman 1982; Spealman and Goldberg 1982). Accordingly, Goldberg and Spealman (1982; 1983) suggested that the same doses (10-30 μg/kg) of nicotine that functioned to punish lever responding could also reinforce lever responding because of differences in the schedules in which nicotine was delivered (i.e., the arrangement of contingencies between responding and delivery of drug) (Goldberg et al. 1981; Goldberg and Spealman 1982; Spealman and Goldberg 1982). These studies

indicated that nicotine can control behavior in divergent ways, and suggested that the nature of the behavioral effects of nicotine may depend on both the pharmacological and environmental conditions in which nicotine is administered.

Prior to the work presented herein, our understanding of the aversive effects of nicotine on self-administration behavior were limited to the studies described above, in which determinants of nicotine punishment had only begun to be examined. The present studies provide strong evidence to support the hypothesis that nicotine has aversive effects that may serve to limit its self-administration, such that nicotine functions similarly to other functional punishers, and that the $\alpha 4\beta 2^*$ nAChR receptors, which are believed to be involved in mediating the reinforcing effects of nicotine (Corrigall et al. 1994; Watkins et al. 1999; Mansbach et al. 2000; Liu et al. 2007; Le Foll et al. 2009) are not involved in mediating the punishing effects of nicotine. In addition, the IV drug punishment procedures developed for these studies may be useful in examining aversive effects of other drugs.

Aversive Stimulus Effects of Nicotine

The aversive effects of nicotine that limit rates of self-administration are generally believed to occur with high doses and/or accumulated drug levels (see Katz 1989; Collins 1990; Rose and Corrigall 1997). Accordingly, it has been suggested that in early nicotine self-administration studies that used continuous schedules of reinforcement fail to maintain stable rates of responding because the relatively high frequency in which injections can be delivered made nicotine aversive compared to the reinforcing effects observed with second-order schedules with programmed time-outs Goldberg et al. 1981; Corrigall and Coen 1989). For example, Corrigall and Coen (1989) were the first to clearly demonstrate that IV nicotine was reinforcing in rats by using a

FR5, with a 60 s time-out schedule, in daily 60-min sessions. It was suggested that limiting scheduled access to nicotine led to higher and more stable rates of nicotine self-administration because it mitigated the direct and/or aversive effects of nicotine that are believed to limit rates of self-administration. However, the results presented in Chapter 3 and Chapter 4 provide strong evidence that while the conditions under which nicotine is studied may influence the behavioral effects of nicotine; nicotine may function more broadly as an aversive stimulus than as a reinforcer.

For instance, doses of nicotine that fall on both the ascending and descending limb of the typical inverted U-shaped dose-response curve of nicotine self-administration obtained from rats (Corrigall and Coen 1989; Shoaib et al. 1997; Watkins et al. 1999; Chaudhri et al. 2005) was able to punish sucrose-reinforced responding in the studies reported in Chapter 3 and Chapter 4. Additionally, nicotine was punishing despite using a schedule of nicotine delivery (FR1, with a 120s ITI, in daily-90 min sessions) that limits the frequency of nicotine injections similarly to self-administration procedures (e.g., Corrigall and Coen 1989; Donny et al 1995; Shoaib et al. 1997; Valentine et al. 1997). Furthermore, the punishing effects of nicotine were sensitive to manipulation of experimental parameters that included dose of nicotine, choice vs. non-choice situations, and the delay of scheduled response-dependent nicotine delivery, which demonstrates that the punishing effects of nicotine are equivalent to punishers such as electric shock or IV histamine injections.

Punishment procedures to examine the aversive effects of IV drugs

Although punishment procedures have been developed to examine the aversive effects of nicotine (Goldberg and Spealman 1982; 1983), the procedures described herein may also be useful in further examining the aversive effects of IV nicotine and

other drugs of abuse, such that these procedures may provide a more sensitive measure of the punishing effects of drugs than single operant conditions alone. For example, in the choice condition, it was found that the doses of nicotine that were needed to punish sucrose-reinforced responding were lower than if no positively reinforced behavioral alternative was available. Furthermore, because rats were able to allocate responding between punished and unpunished responses, this contingency provided a control within the experiment to determine whether decreases in rates of punished responding were due to punishment or direct suppressant effects that would have been expected to affect unpunished responding.

In addition, the study in which the punishing effects of nicotine were attenuated by delaying the delivery of response-dependent nicotine such that rates of nicotine self-administered increased suggest that a punisher is most effective when it is delivered immediately upon a response-contingent behavior. Therefore manipulation of the contiguity between a response and its consequence may be used as a control in single operant conditions to determine whether decreases in behavior are due to a drug's punishing and/or direct suppressant effects. In theory, since punishment is a decrease in responding due to a learned contingency, then manipulation of the relationship of the response and its consequence should affect punishment. Whereas manipulation of variables such as delay should not affect the direct suppressant effects since decreases in responding are unconditioned. Lastly, the study in which response-independent nicotine was delivered may be useful in determining a rate-limiting dose of drugs in which operant behavior can be evaluated without concerns regarding unconditioned effects. Therefore, the procedures described herein contribute to the study of the behavioral effects of drugs by providing procedures in which the punishing effects of drugs may be evaluated thoroughly.

Differentiating the aversive and reinforcing effects of IV nicotine pharmacologically

The psychoactive effects of nicotine are believed to be mediated predominately through its agonist actions on the various neuronal nicotinic acetylcholine receptors (nAChRs), which differ in subunit structure, kinetic and pharmacological properties, and distribution within the nervous system (see Gotti and Clementi 2004; Taly et al. 2009). Accordingly, it has been proposed that each of these nAChR subtypes may also have unique roles in mediating the behavioral effects of nicotine that contribute to tobacco addiction (see Picciotto and Kenny 2013). Although the roles for several of the individual nicotinic receptor subtype(s) in mediating the behavioral effects of nicotine have yet to be elucidated, substantial evidence from behavioral studies using both pharmacological and genetic techniques indicate the $\alpha4\beta2^*$ nAChR subtype is involved in mediating the reinforcing effects of nicotine (see Benowitz 2008; Picciotto and Kenny 2013). Interestingly, antagonist studies with DHβE, a selective $\alpha4\beta2^*$ nAChR subtype antagonist (Williams and Robinson, 1984; Sabey et al. 1999; Shoaib et al. 2000) found that DHβE was able to attenuate both nicotine-induced conditioned taste aversion (Shoaib et al. 2000; Gommans et al. 2000) and nicotine-induced conditioned place aversion (Laviolette and van der Kooy 2003) in rats. While a drawback of both conditioned taste aversion and conditioned place aversion is that the experimenter administers the nicotine, both have been argued to provide useful information on the aversive effects of nicotine. With conditioned place aversion procedures, animals are tested for the development of conditioned avoidance for environments distinctly paired with nicotine. It has been argued that avoidance behavior and decreased time spent in a nicotine-paired environment is a measure of the aversive effects of nicotine. Similarly,

it is argued that conditioned taste aversion is a measure the nicotine's capability to serve as an aversive conditioned stimulus that result in avoidance of tastes paired with its administration. Accordingly, these findings suggest that the α4β2* nAChR subtype may also be involved in mediating the punishing effects of nicotine. However, the antagonist studies with mecamylamine and DHβE presented in Chapter 4 provide evidence to suggest that while the punishing effects of IV nicotine are mediated by nAChRs, they are not mediated by the α4β2* nAChR subtype, as demonstrated by the failure of DHβE to block the punishing effects of nicotine. These findings are important because while it is unclear which nAChR subtype(s) are involved in mediating the punishing effects of IV nicotine, they indicate that the aversive and reinforcing effects of nicotine may be differentiated pharmacologically.

In addition to the proposed role of the α4β2* nAChR subtype in mediating the reinforcing effects of nicotine (Corrigall et al. 1994; Watkins et al. 1999; Mansbach et al. 2000; Liu et al. 2007; Le Foll et al. 2009) it has been reported that the α4β2* nAChR subtype is involved in mediating the discriminative stimulus effects of nicotine, which are believed to promote nicotine self-administration in both humans and non-human subjects because the ability to perceive and identify characteristic interoceptive effects of nicotine encourages the development of the such behaviors (see Stolerman 1992; Smith and Stolerman 2009). However, it is unclear whether the discriminative stimulus effects of nicotine mediated by the α4β2* nAChR subtype are involved in the punishing effects of nicotine. Interestingly, Jutkiewicz et al. (2011), found that while DHβE antagonized the discriminative effects of nicotine in rats, it was less effective at antagonizing the high dose of nicotine (1.78 mg/kg; s.c.) compared to the low dose (0.32 mg/kg; s.c.). Schild analyses of DHβE suggested that different nAChRs subtypes might be mediating the stimulus effects of large and small doses of nicotine.

Accordingly, while the α4β2* nAChR subtype is involved in mediating the discriminative stimulus effects of nicotine that are presumed to be related to the reinforcing effects of nicotine, it is possible that the other nAChR subtype(s), while unknown, are involved in mediating the discriminative stimulus effects that are related to the aversive effects of nicotine.

While the identification of the nAChR subtype (s) that mediates the punishing effects of nicotine remains to be established, there is evidence to suggest that the nAChRs that mediate the aversive effects of nicotine are located in the CNS. For example, Spealman (1983) found that treatment with hexamethonium, a nicotinic antagonist with primarily peripheral effects, was not able to attenuate the aversive effects of nicotine that maintained responding to postpone delivery of IV nicotine in squirrel monkeys, whereas mecamylamine could. In agreement, studies using genetic techniques found that mice with deletion of the α5 nAChR subunit, [which is predominately expressed in brain regions that include the habenulo-interpeduncular pathways, VTA and substantia nigra (Marks et al. 1992)] increased nicotine self-administration at unit doses of nicotine found on the descending limb, but not the ascending limb, of the typical inverted U-shaped dose-response curve of nicotine self-administration (Fowler et al. 2011). It has been proposed that doses of nicotine found on the descending limb of a typical inverted U-shaped dose-response curve have aversive properties that limit rates of responding (e.g., Katz 1989; Rose and Corrigall 1997). This suggests that the nAChRs that are composed of the α5 subunit may be involved in mediating the aversive effects of nicotine. While it is recognized that a limitation of genetic studies is the possibility of adaptations in neural circuitry that may affect the interpretation of the behavioral effects of nicotine, they are useful when pharmacological agents to study individual nAChR subtype are limited. Interestingly, an

increased vulnerability to tobacco addiction has been found in humans with polymorphisms in the α5 nAChR subunit gene, which results in decreased function of the subunit (Bierut et al. 2008; Kuryatov et al. 2011). Polymorphisms in the α5 nAChR subunit has been associated with an increased risk of developing lung cancer and chronic obstructive pulmonary pathway in smokers (Wang et al. 2010; Amos et al. 2008; Le Marchand et al. 2008), which may reflect greater exposure to carcinogens from greater smoking (Macqueen et al. 2014).

Limitation of findings

While studies have shown that nicotine can produce reinforcing effects (e.g., Corrigall and Coen 1989; Shoaib et al. 1997; Watkins et al. 1999; Chaudhri et al. 2005), the findings reported herein show that nicotine can also produce aversive effects, demonstrating that the behavioral effects of nicotine are complex. Presumably it is the interaction/balance of these independent effects that ultimately determine rates of nicotine self-administration in both humans and non-humans. Accordingly, a procedure in which both the reinforcing and aversive effects of nicotine can be detected within the same subjects would help determine the relative contributions of each of these effects in drug self-administration. However, since the effects of individual nicotine injections were not evaluated independent of sucrose delivery in the studies reported in Chapter 3 or Chapter 4, it is unclear whether the reinforcing effects of sucrose alone, or a combination of the reinforcing effects of sucrose and nicotine maintained responding on levers that delivered nicotine with sucrose. Therefore, a limitation of the findings reported in Chapter 3 and 4 is that it is unclear whether punishment by nicotine is a result of the aversive effects of nicotine alone, and/or the reinforcing effects of nicotine being relatively weak compared to its aversive effects, or whether lever responding that

results in nicotine injections is being maintained by the sucrose delivery that is paired with its delivery. However, a separate experiment using the same schedules of reinforcement (FR 1, with 120 s ITI, daily-90 min sessions) found that nicotine injections alone could not maintain lever responding above responding on levers that had no schedule consequence (Figure A1.1), which suggest that punishment is more likely a result of the aversive effects of nicotine alone, and that responding on the lever that results in nicotine injections is being maintained by the sucrose that is delivered with it concurrently.

Alternatively, it could also be argued that the procedures used herein may not provide a sensitive measure of the reinforcing effects of drugs. However these procedures have been used to demonstrate the reinforcing effects of the fast- and short-acting mu opioid receptor agonist remifentanil (Figure A1.2). In studies examining the effects of remifentanil, it was established that response-contingent IV remifentanil paired with sucrose delivery was more reinforcing than delivery of sucrose alone, and it was shown that injections of 10.0 μg/kg remifentanil were more reinforcing than sucrose. This demonstrates that the operant procedures used in Chapter 3 and Chapter 4 can detect the reinforcing effects of drugs such as remifentanil, but suggest that these procedures do not provide a sensitive measure of the reinforcing effects of nicotine. This is not surprising since it has been noted that the reinforcing *strength* of nicotine is considered weak relative to other drugs of abuse (Griffiths et al. 1979; Dougherty et al. 1981; Henningfield and Goldberg 1983b; Collins 1990), and that establishing the reinforcing effects of nicotine remains difficult in controlled studies (see Le Foll and Goldberg 2009).

Since drugs such as nicotine can produce both reinforcing and aversive effects, and it is proposed that the relative contribution of these effects determine drug-taking

behaviors, then it would be advantageous to examine both effects in the same group of subjects. For instance, it would be predicted that subjects that were more sensitive to the aversive effects would be less sensitive to the reinforcing effects and vice versa. Since there are currently no procedures in which both the reinforcing and aversive effects of nicotine can be measured, it may be necessary to use multiple procedures to assess the effects of nicotine. Therefore, while the operant procedures used to study the punishing effects of nicotine reported herein may not provide a sensitive measure of the reinforcing effects of nicotine; it may be used in combination with self-administration procedures that are known to be sensitive to the reinforcing effects to provide a better understanding of drug-taking behaviors.

Consideration of other effects of nicotine that may affect self-administration

While discussion of the effects of nicotine that may affect rates of self-administration have been largely focused on the reinforcing and aversive effects of nicotine, consideration of other effects could also contribute to the understanding of nicotine-taking behaviors. For instance, administration of nicotine in humans through cigarettes and IV injections has been known to increase heart rate and blood pressure in a dose-dependent manner (e.g., Koch et al 1980; Soria et al 1996; Rose et al. 2000), and in some instances these cardiovascular effects coincide with dose-dependent increases in subjective ratings of "drug liking" in participants with histories of drug abuse and cigarette smoking (Henningfrield et al. 1985; Soria et al. 1996; Garrett and Griffths 1997). It has also been noted that although smokers and nonsmokers displayed significant increases in blood pressure and heart rate after nicotine administration, a difference between heart rate measured at later times was greater in nonsmokers that also reported they were less likely to "use [nicotine] again" (Soria et al. 1996).

Cardiovascular effects may therefore contribute to punishment by nicotine.

Other effects of nicotine worth considering are its commonly reported stimulant and anxiolytic effects that may induce pleasure and reduce stress and anxiety in humans (see Picciotto et al. 2002, Benowitz 2010). For instance, high rates of smoking have been observed among patients with affective disorders (Breslau 1995) such that the rate of smoking in individuals with affective disorders is more than double the rate in the general population (Kalman et al. 2005). Interestingly it has been reported that nicotine can produce antidepressant effects in both smokers and non-smokers (Salin-Pascual et al. 1996). Whereas cessation of smoking has been known to induce withdrawal symptoms that include irritability, depressed mood, and anxiety (Hughes and Hatsukami 1986). Behavioral models of anxiety and depression in rodents suggest that nicotine can have both anxiolytic and antidepressant effects (see File et al. 2000). Therefore, consideration of these effects in relation to the reinforcing and aversive effects may contribute to a better understanding of smoking behavior in humans.

Aversive effects of nicotine: implications in understanding tobacco use in humans

It has been argued that while nicotine has reinforcing effects, it differs from other drugs of abuse because its reinforcing strength (i.e., likelihood that nicotine will function as a reinforcer under varying experimental conditions) is relatively weak (Griffiths et al. 1979; Dougherty et al. 1981; Henningfield and Goldberg 1983b; Collins 1990). In response to these observations, theories of nicotine reinforcement have proposed that nicotine may reinforce tobacco use through its primary reinforcing effects (albeit weak relative to other drugs of abuse), its secondary reinforcing effects in which neutral stimuli become conditioned reinforcers through Pavlovian conditioning (Rose and Levin 1991; Balfour 2004) and/or through its *reinforcement enhancer* effects (i.e., stimulus

effects that increase the incentive value of accompanying stimuli that are either conditioned or unconditioned reinforcers) (Chaudhri et al. 2006). While these theories may explain how nicotine, a relatively weak reinforcer, can maintain robust self-administration and tobacco-taking behaviors, a limitation of these theories is that they do not take into account that nicotine can also produce aversive effects.

Accordingly it has been proposed by some and us that self-administration of nicotine may be a function of the interaction between the reinforcing and aversive effects of the drug (Stolerman and D'Mello 1981; Katz 1989; Lynch and Carroll 2001; Riley 2011). It is well known that IV nicotine can produce negative and/or positive subjective effects concurrently in both smokers and non-smokers (see Kalman 2002; Kalman and Smith 2005), and it is believed that people's sensitivity to each of these effects is a predictor of whether people who experiment with nicotine through tobacco become regular smokers (see Pomerleau et al. 1993). Many people experience noxious effects such as nausea and dizziness on their initial experience with tobacco (Kozlowski and Harford 1976; Pomerleau 1995), and tolerance to these negative subjective effects with repeated exposure to nicotine has been proposed as mechanism in which people become smokers (see Pomerleau et al. 1993). For instance, it has been shown that nicotine can produce greater positive subjective effects and less negative effects in smokers than in non-smokers, such that these smokers were more likely to "use [nicotine] again" (Soria et al. 1996). In cigarette smoking cocaine users, it has been reported that IV cocaine produced greater positive subjective effects than IV nicotine, whereas nicotine produced greater negative subjective effects (Jones et al. 1999). Additionally, it was found that the cigarette smoking cocaine users were willing to forgo twice as much money for an injection of IV cocaine compared to nicotine in a money versus drug choice situation. This demonstrates that nicotine and cocaine can be

differentiated by their subjective and reinforcing effect. Therefore, while the reinforcing effects of nicotine contributes to an understanding of nicotine reinforcement, the degree to which nicotine is reinforcing may also reflect an interaction with the aversive effects that function to limit self-administration of nicotine.

Alternatively, it has also been proposed that unconditioned direct suppressant effects which include but are not limited to non-specific disruptions in behavior that decrease responding because animal are unable to respond may also influence rates of drug self-administration (Katz 1989; Carroll and Bickel 1998; Skjoldager et al 1991). This may be relevant to nicotine since it has been noted that doses of nicotine that are self-administered (and typically fall on the descending limb of the inverted U-shaped dose response curve) can also produce noxious effects such as emesis in squirrel monkeys (Goldberg et al. 1983) and seizures in rats (Corrigall, unpublished observations). In addition, it is proposed that rates of self-administration may be influenced by satiation of drug effects, in which the animal responds according to an optimal drug level or drug effect (Yokel 1987; Katz 1989). However, the overall shape of the dose-response curve for IV nicotine self-administration in animals tends to differ from other drugs of abuse such as cocaine and opiates such that rates at which self-administration of nicotine appears to not change as the dose increases, making the dose-response curve more flat (Dia et al 1989; Corrigall and Coen 1991). This pattern of self-administration of behavior in which responding seems to be insensitive to dose occurs across a variety of animal species, and schedules of reinforcement such as fixed ratio, fixed interval, or progressive ratio (Goldberg et al. 1981; Risner and Goldberg 1983; Corrigall and Coen 1989). Therefore, while changes in responding are seemingly related to dose, it may be that other effects are contributing to rates of self-administration. Nonetheless, consideration of the possible direct effects and/or satiation may be useful in explaining

individual differences in the rates at which cigarettes are smoked among smokers.

Tobacco Addiction is an ongoing problem

In January of 2014, the Surgeon General released a new report on the health consequences of smoking. The release of the 2014 report marks the 50th anniversary of the 1964 report, which is arguably one of the most influential publications responsible for bringing public awareness to the health consequences of tobacco smoking. Since the release of the 1964 report, the prevalence of cigarette smoking among adults in the United States has declined from 42% in 1965 to 18% in 2012 (US Department of Health and Human Services 2014). However, despite the "50 years of Progress" in tobacco-related research and policy, over 40 million Americans continue to smoke, and it is estimated that over 400,000 will die every year from smoking-related disease. The 2014 report also suggests that cigarettes today are more harmful than they were fifty years ago. Today's smokers have a higher risk of developing lung cancer than smokers in 1964, despite smoking fewer cigarettes (due in part to the changes in the composition and design of cigarettes, such as the use of ventilated filters that can lead to more inhalation of lethal materials). Therefore, while progress has been made, tobacco addiction is still a serious problem, and the use of tobacco remains a leading cause of preventable disease and premature death in the United States and other countries.

Future studies

Of the people that try cigarette smoking, it is estimated that only 20 -25% of them become smokers (Johnston et al. 2007). This suggests that while the reinforcing effects of nicotine may contribute to development and maintenance of cigarette

smoking in 75-80 % of the people who try cigarette smoking, there may be individual differences, which may predict whether a person is more vulnerable to smoking addiction than others. Since it has been proposed herein that the aversive effects of nicotine may prevent or limit self-administration of nicotine, future studies examining the effects of nicotine in humans should include an examination of the aversive effects of nicotine and determinants that contribute to punishment.

It is has been proposed that certain people become smokers depending on their sensitivity to the aversive effects of nicotine, such that people who are more sensitive are less likely to become regular smokers (see Pomerleau et al. 1993). This suggests that people with less sensitivity to the aversive effects of nicotine may be more vulnerable to developing tobacco addiction. In keeping with this, smokers with polymorphisms in the α5 nAChR subunit gene that results in a decrease function of the subunit (Bierut et al. 2008; Kuryatov et al. 2011) have been shown to smoke more cigarettes than smokers without the polymorphism (Macqueen et al. 2014). Since it has been proposed that the α5 nAChR subunit may be involved in mediating the aversive effects of nicotine (Fowler et al. 2011), it may be useful to examine whether people with polymorphisms in the α5 nAChR subunit gene are less sensitive to the aversive effects of nicotine. Additionally, development of antagonists that are selective at the α5 subunit may be useful in determining the precise role of nAChRs containing the α5 subunit in tobacco smoking, which may have implications on how to prevent and/or treat tobacco addiction. In theory, it is possible that a pharmacotherapy that selectively increases the aversive effects of nicotine while decreasing the reinforcing effects may be useful in the cessation of smoking.

Lastly, tobacco use typically begins in childhood or adolescence, such that it has been reported that 80% of smokers begin smoking by 18 years of age, and that

there is an increased risk of developing tobacco addiction in people who experiment at an earlier age (Lynch and Bonnie 1994). Exposure to nicotine in adolescence may have differential effects in the development and maintenance of nicotine self-administration compared to exposure in adulthood, because adolescence represents a unique period in which there may be an increased vulnerability to the reinforcing effects of nicotine (see Slotkin 2001; Barron et al. 2005). Therefore, future studies using a model of adolescent nicotine punishment in rats to examine whether or how the aversive effects of nicotine may affect nicotine-self administration may provide information on how tobacco addiction may differentially develop in adolescents compared to adults. These studies could contribute to an understanding why young people who try cigarettes are more likely to develop an addiction.

Conclusions

Examining the behavioral effects of nicotine has been crucial in understanding tobacco addiction. However, the effects of nicotine that contribute to tobacco-addiction are complex and remain poorly understood. The findings reported herein and in other studies show that nicotine is a complex pharmacological compound that can produce divergent effects that may be differentiated pharmacologically. Therefore, while it is recognized that the reinforcing effects of nicotine contributes to the development and maintenance of the abuse of nicotine-containing tobacco products (Young and Herling 1986; Ator and Griffths 2003), these effects alone may not determine whether nicotine is self-administered, or the rates in which nicotine is self-administered. Examining the aversive effects of nicotine may contribute to a better understanding of how nicotine can control behavior. Consideration of both the

reinforcing and aversive effects of nicotine may have practical implications in the treatment and prevention of tobacco addiction.

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Appendix

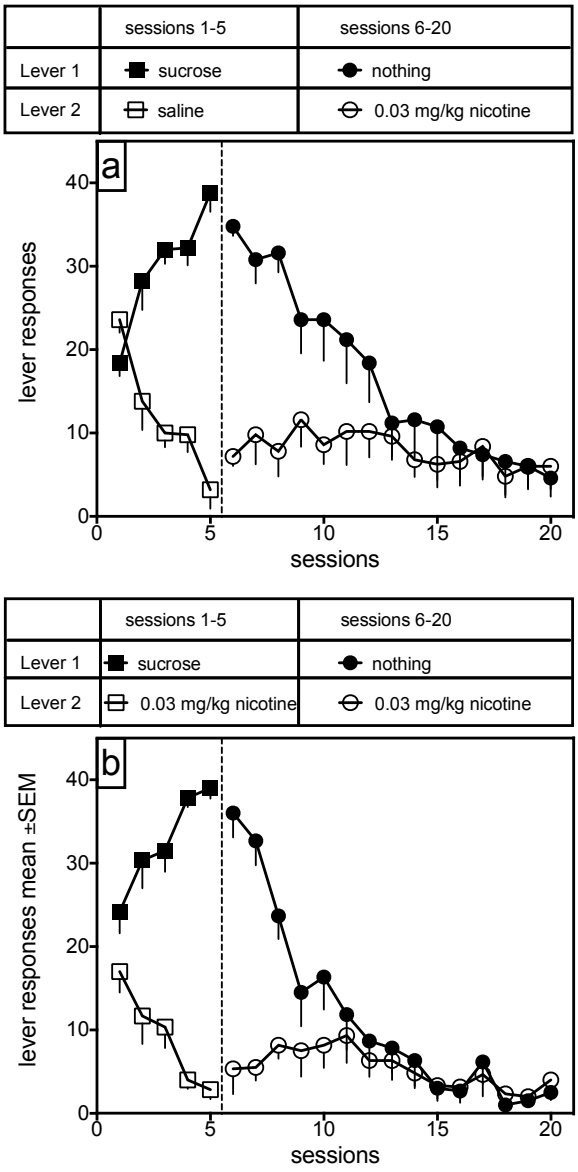

Figure A1.1 Responding on levers that delivered either sucrose, IV nicotine, IV saline or nothing. Each graph displays data from individual groups that differ by the consequence of responding on lever 2 (a) IV saline injection (b) IV 0.03 mg/kg nicotine injection across sessions 1-5 (*n*=5-6 per group). Closed and open symbols represent the mean (±SEM) responses made on lever 1 and lever 2, respectively. Legends next to the symbols listed in the table indicate the consequences of responding on lever 1 and lever 2 across sessions.

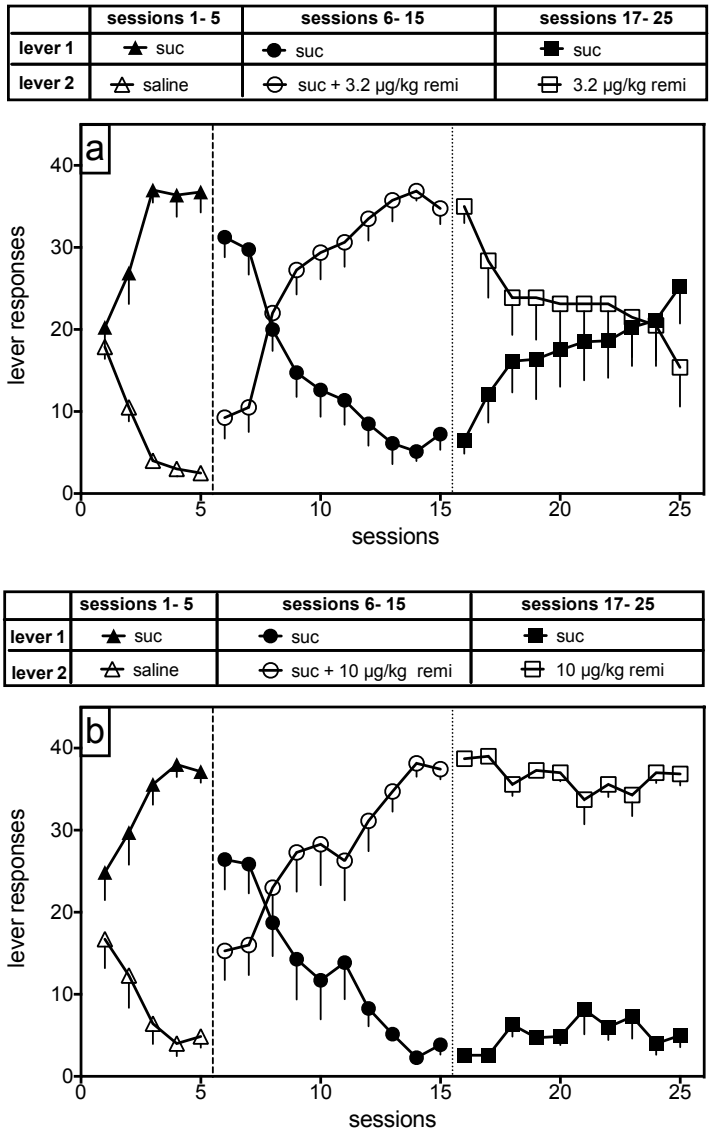

Figure A1.2 Effects of pairing remifentanil injection with sucrose delivery on lever responding. Each graph displays data from individual groups that differ by the dose of IV remifentanil (remi) injection paired with sucrose (suc) delivery across sessions 6-15 (a) 3.2 μg/kg (b) 10.0 μg/kg (*n*=8 per group). Closed and open symbols represent the mean (±SEM) responses made on lever 1 and lever 2, respectively. Legends next to the symbols listed in the table indicate the consequences of responding on lever 1 and lever 2 across sessions.