COMPLEX INTERACTIONS BETWEEN NICOTINE AND NONPHARMACOLOGICAL STIMULI REVEAL A NOVEL ROLE FOR NICOTINE IN REINFORCEMENT

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Although considerable progress has been made we do not yet fully understand the behavioral and neurobiological bases of nicotine reinforcement, and without this knowledge treatment strategies aimed at reducing smoking remain deficient. This dissertation provides an original perspective on nicotine reinforcement, which arises from substantial evidence of complex interactions between nicotine and nonpharmacological stimuli. The present experiments tested the hypothesis that nicotine reinforcement derives from at least two sources: 1) the primary reinforcing properties of nicotine, an action that requires response-dependent drug administration, and 2) the more prominent ability of nicotine to enhance behavior maintained by salient non-nicotine stimuli, an action that does not require a contingent relationship between drug administration and reinforced operant responding. Although novel for nicotine, this hypothesis has origins in an extensive literature on the reinforcing properties of psychostimulant drugs. Empirical support for the application of this hypothesis to nicotine reinforcement will be presented. By investigating the interaction between nicotine and nonpharmacological stimuli within the context of drug self-administration in rats, the present research has generated new insights into the paradox of how nicotine, an apparently weak primary reinforcer, can sustain the robust behavior observed in self-administration and in smoking. Hypotheses generated by these data provide important direction for future investigations into the neurobiology of nicotine reinforcement.

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ORGANIZATION OF DISSERTATION

Chapters in this dissertation have either been published in peer-reviewed journals, or are currently being prepared for publication. Experiments in Chapter 2 were conducted by N. Chaudhri, and the manuscript was co-authored by N. Chaudhri and EC. Donny.

Chapters 1 and 5

Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Liu X, Sved AF. Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement (submitted as an invited review paper to *Psychopharmacology*)

Chapter 2

Donny EC, Chaudhri, N, Caggiula AR, Evans-martin FF, Booth S, Gharib MA, Clements LA, Sved AF (2003) Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology,* 169: 68-76

Chapter 3

Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Booth S, Gharib MA, Craven LA, Sved AF. Self-administered and noncontingent nicotine enhance reinforced operant responding in rats: the impact of dose and reinforcement-schedule (submitted to *Psychopharmacology*)

Chapter 4

Chaudhri N, Caggiula AR, Donny EC, Booth S, Gharib MA, Craven LA, Sved AF. Primary reinforcement and the reinforcement-enhancing actions of nicotine can be dissociated by manipulating the strength of a response-contingent conditioned stimulus (submitted to *Psychopharmacology*)

SALUTATIONS

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1. BACKGROUND AND INTRODUCTION

The primary purpose of this chapter is to acquaint the reader with a broad literature on a behavioral animal model of nicotine reinforcement, the nicotine self-administration paradigm. A historical perspective is maintained in this section to reveal how the development of this model has engendered a more comprehensive understanding of the reinforcing actions of nicotine. This summary also provides a foundation for the experiments that comprise this dissertation.

1.1. GENERAL INTRODUCTION

The drug self-administration paradigm, in which animals perform an operant response such as nose poke or lever press to obtain intravenous drug infusions, was developed as a model to study the behavioral and neurobiological underpinnings of drug reinforcement (Schuster and Thompson 1969; Caggiula et al. 2001; Di Chiara 2002; Schindler et al. 2002; See et al. 2003). Central to this paradigm is the premise that behavior leading to drug delivery is reinforced by the direct, pharmacological actions of that drug within the central nervous system. This principle is analogous to theories of drug use and dependence in humans: in both cases repeated drug-taking behavior is strengthened by the ability of the drug to serve as a primary, positive reinforcer (Wise 1987; Meisch and Lemaire 1993; Di Chiara 1999; Glautier 2004).

Since its introduction (Weeks and Collins 1976; Weeks and Collins 1978), the drug self-administration paradigm has evolved to accommodate the complex factors that describe drug dependence in humans. For example, the observation that environmental stimuli frequently associated with drugs can induce craving and relapse after prolonged abstinence in humans (Childress et al. 1999; Conklin and Tiffany 2002) has prompted the inclusion of nonpharmacological stimuli in the drug self-administration paradigm, to test their ability to induce reinstatement of drug-seeking in animals (Shaham et al. 2003; See 2002). A more recent example is the recognition that protracted drug use in humans produces hallmark behavioral changes (e.g., continued use in the face of negative outcomes, persistent drug-seeking during periods of drug absence, and increased motivation to obtain drug), which has triggered methodological refinements in the animal model to assess the emergence of similar outcomes in rats (Deroche-Gamonet et al. 2004; Vanderschuren and Everitt 2004).

While research on drug addiction in humans has influenced the design and parameters of the self-administration model in animals, the converse is also valuable. Investigations of the behavioral and neurobiological substrates of drug reinforcement in animals are instrumental in shaping theories about drug dependence. This dissertation will review the utility of the drug self-administration paradigm in extending our understanding of nicotine reinforcement. Recent data demonstrating an interaction between nicotine and nonpharmacological stimuli will be presented to illustrate the novel hypothesis that nicotine reinforcement derives from at least two sources: 1) the primary reinforcing properties of nicotine, an action that requires response-dependent drug administration, and 2) the more prominent ability of nicotine to enhance operant responding for reinforcing non-nicotine stimuli, an action that does not require a contingent relationship between drug administration and reinforced operant behavior (see Table 1; Donny et al. 2003).

The implications of this hypothesis for smoking will be discussed, and parallel theories on the reinforcing properties of other stimulant drugs will be described to demonstrate that although new for nicotine, the notion that drug reinforcement stems from multiple behavioral effects is well established in the psychostimulant literature.

Table 1: Behavioral effects of the primary reinforcing and reinforcement-enhancing properties of nicotine

Primary Reinforcer	**Reinforcement-Enhancer**
Hypothesis: Maintains operant behavior in the absence of a contingent non-nicotine stimulus	*Hypothesis:* Increases operant behavior maintained by reinforcing non-nicotine stimuli.
Evidence: Caggiula et al. 2002b; Donny et al. 2003; Chaudhri et al. 2005b	*Evidence:* Donny et al. 2003; Chaudhri et al. in press; Palmatier et al. 2005
Hypothesis: Establishes non-nicotine stimuli as conditioned reinforcers.	*Hypothesis:* Enhances approach evoked by appetitive conditional stimuli and responding for conditioned reinforcers.
Evidence: Cohen et al. 2005; Unpublished Data	*Evidence:* Olausson et al. 2004a, 2004b
	Hypothesis: Prevents or interferes with the extinction of conditioned approach and conditioned reinforcement.
	Evidence: No studies reported

1.2. ADVANCEMENTS IN THE NICOTINE SELF-ADMINISTRATION MODEL

1.2.1. Nicotine self-administration is obtained under finite experimental conditions

Nicotine is the major psychoactive constituent of tobacco that produces widespread tobacco dependence and persistent smoking (USDHHS 1988). However, initial attempts to establish voluntary, intravenous nicotine self-administration in animals produced equivocal results. While some experiments achieved reliable nicotine self-administration, others suggested that nicotine was unable to reinforce behavior leading to its delivery (Deneau and Inoki 1967; Clark 1969; Dougherty et al. 1981).

Early published reports of successful nicotine self-administration employed schedule-induction as a necessary addition to standard self-administration protocols, because intermittent food delivery at regular fixed intervals facilitated lever pressing for intravenous nicotine infusions (Lang et al. 1977; Latiff et al. 1980; Smith and Lang 1980; Singer et al. 1982; Slifer and Balster 1985). Fundamental to the validity of schedule-induced self-administration procedures was the hypothesis that the dose of self-administered drug would not initially function as a positive reinforcer, and would therefore be unable to independently support operant responding (Slifer 1983). Indeed, rats maintained on free-feeding conditions did not acquire nicotine self-administration, while rats maintained at a reduced body weight by restricted food access only gradually acquired low rates of nicotine self-administration in the absence of concurrent schedule-induction (Lang et al. 1977). The fragility of nicotine self-administration in these pioneering experiments was underscored by observations that there were no clear dose effects of nicotine on responding (Slifer 1983); overall levels of within-session nicotine intake

were low (Cox et al. 1984); and changing urinary pH (which affected rates of nicotine excretion) disrupted levels of behavior (Latiff et al. 1980). Further evidence that nicotine was a weak primary reinforcer came from comparisons between nicotine and cocaine self-administration which demonstrated that unlike cocaine, nicotine was not readily self-administered and did not produce rates of responding that were as reliable or robust as those achieved for cocaine (Pickens and Thompson 1968; Ator and Griffiths 1983; Risner and Goldberg 1983; Collins 1990).

Current protocols for nicotine self-administration have adopted techniques that facilitate consistently high levels of operant responding for nicotine. They include, but are not limited to (a) testing self-administration using fixed ratio reinforcement schedules with limited daily access to nicotine (but see Valentine et al. 1997, and Brower et al. 2002 for nicotine self-administration using extended access schedules) (b) increasing the response requirements necessary to produce nicotine infusions (c) delivering nicotine in combination with a nonpharmacological stimulus (d) decreasing the infusion duration to mimic the nicotine 'bolus' that is delivered rapidly, via smoking (Balfour et al. 2000) and (e) training rats to lever press for food delivery prior to self-administration sessions (Corrigall and Coen 1989; Shaham et al. 1997; Tessari et al. 1995; Donny et al. 1995). Additional parametric considerations such as maintaining rats on a restricted diet and testing them during the dark phase of their light/dark cycle, which engender more robust nicotine self-administration, have also been incorporated into contemporary self-administration procedures (Donny et al. 1998).

The use of stringent test conditions such as these has led to the demonstration of reliable nicotine self-administration in a number of species including rats (Corrigall and Coen 1989; Donny et al. 1995; Shoaib et al. 1997; Valentine et al. 1997), mice (Picciotto et al. 1998; Rasmussen and Swedberg 1998; Stolerman et al. 1999), dogs (Henningfield and Goldberg

1983b; Risner and Goldberg 1983), monkeys (Spealman et al. 1981; Ator and Griffiths 1983; Slifer and Balster 1985) and humans (Henningfield et al. 1983; Rose and Corrigall 1997). Nevertheless, the hypothesis that nicotine is a relatively weak primary reinforcer is grounded in the observation that high and stable rates of nicotine self-administration in animals are achieved only within a relatively restricted range of experimental parameters (Henningfield and Goldberg 1983b). These observations along with recent data (Donny et al. 2003; Chaudhri et al. 2005b) highlight an important question regarding the role of nicotine in reinforced behavior: *how can nicotine, a drug with apparently weak primary reinforcing properties, support the establishment of smoking, one of the most addictive behaviors worldwide?*

In answer to this question a sizeable literature suggests that smoking is maintained by both the primary reinforcing properties of nicotine and its related ability to establish associated environmental stimuli as conditioned stimuli, which can subsequently induce craving and provoke drug-seeking (Rose and Levin 1991; Perkins 1999; Caggiula et al. 2001; Cohen et al. 2005). Research by our laboratory concurs fully with this hypothesis, but extends it to include a distinct behavioral property of nicotine - its capacity to enhance responding for an already reinforcing non-nicotine stimulus (Donny et al. 2003; Chaudhri et al. in press). Empirical support for these actions of nicotine will be presented in subsequent chapters to illustrate the following important points. First, primary reinforcement from nicotine can sustain self-administration in rats, and is a probable contributor to smoking in humans. However, these effects are relatively weak when compared to the ability of nicotine to interact with nonpharmacological stimuli and augment the magnitude of reinforcement conferred to behavior that results in stimulus delivery. Second, the specific interactions between nicotine and nonpharmacological stimuli are largely determined by the intrinsic reinforcing value of those stimuli.

1.2.2. Nonpharmacological stimuli contribute significantly to nicotine reinforcement

Goldberg and colleagues (Goldberg et al. 1981) were first to investigate the impact of nonpharmacological stimuli in nicotine reinforcement. They tested the hypothesis that self-administration might be more effectively maintained if operant behavior produced not just nicotine, but also an environmental stimulus associated with nicotine. Using a second-order reinforcement schedule where animals responded on a fixed ratio schedule for a light that was subsequently paired with nicotine on a fixed interval schedule, they were able to demonstrate high overall rates of operant responding. More importantly, removing the stimulus caused a substantial decrease in responding despite the continued availability of nicotine, providing preliminary evidence that nonpharmacological stimuli incorporated into models of nicotine reinforcement have a notable impact on behavior.

Discrete, drug-paired stimuli are now well integrated into current models of nicotine self-administration, and converging evidence from several laboratories suggests that they contribute considerably to various stages of nicotine reinforcement. For example, nicotine self-administration is greatly facilitated when drug delivery is combined with a nonpharmacological stimulus compared to responding for either nicotine alone (Caggiula et al. 2002a; Caggiula et al. 2002b; Chaudhri et al. 2005b), or the stimulus alone (Cohen et al. 2005; Caggiula et al. 2002a; Chaudhri et al. 2005b). A comparison of the dose-response relationships for nicotine self-administration when drug delivery is either associated with, or delivered in the absence of a discrete non-drug stimulus reveals two significant effects. First, overall responding for nicotine is markedly reduced when infusions are not combined with a nonpharmacological stimulus. Second, the peak of the dose-response function in this condition is shifted to the right (0.06

mg/kg/inf; free base) relative to behavior reinforced by nicotine paired with a non-drug stimulus, where responding peaks at 0.02-0.03 mg/kg/inf (Corrigall and Coen 1989; Donny et al. 2000; Donny et al. 2003; Chaudhri et al. 2005b). These data suggest that nicotine self-administration is less reliable in the absence of concurrent non-drug stimuli, and support the prior hypothesis that the primary reinforcing effects of nicotine alone are relatively weak.

The efficacy of nonpharmacological stimuli in promoting nicotine self-administration depends on their contingent relationship to lever pressing and/or nicotine infusions; noncontingent stimulus presentations to rats that respond only for nicotine do not enhance self-administration (Caggiula et al. 2002a). Similarly, others have demonstrated that non-drug stimuli delivered noncontingently fail to potentiate cocaine and heroin seeking on a second-order reinforcement schedule (Di Ciano and Everitt 2003). Response-dependent presentations of nicotine-paired stimuli during saline substitution sustain reduced but stable lever pressing (Caggiula et al. 2001), in some cases at higher levels than behavior reinforced by the same stimulus that was never previously associated with nicotine (Cohen et al. 2005). The latter finding suggests that repeated response-dependent pairings between nicotine and a non-drug stimulus can enhance the reinforcing efficacy of the stimulus via Pavlovian conditioning, and subsequently increase its control over operant behavior. Removing the stimulus after animals have maintained responding for it during saline substitution causes a further decrease in lever pressing, providing additional support for this hypothesis (Cohen et al. 2005; Caggiula et al. 2001; Donny et al. 2000). Finally, following extinction of responding induced by removing nicotine and concurrent non-drug stimuli, reinstatement of lever pressing can be stimulated by either priming infusions of nicotine (Chiamulera et al. 1996; Shaham et al. 1997; Andreoli et al. 2003), or by presentations of a non-drug stimulus that was previously combined with nicotine

(Caggiula et al. 2001; Lesage et al. 2004; Paterson et al. 2005). These data are consistent with a large body of clinical evidence that environmental stimuli associated with nicotine intake via smoking not only trigger craving and induce relapse in abstinent smokers (Caggiula et al. 2001; Brody et al. 2002; Heishman et al. 2004; Rose and Levin 1991), but also greatly influence reinforcement derived from smoking (Perkins et al. 2001). They provide parallel support within the context of an animal model, for the hypothesis that non-drug factors associated with nicotine become an important part of the stimulus complex that sustains nicotine reinforcement.

1.2.3. Nicotine interacts synergistically with nonpharmacological stimuli

While these experiments imply an interaction between nicotine and non-drug stimuli, they do not explain the nature of this effect, and do not demonstrate how a weak primary reinforcer, nicotine, can support high levels of behavior in the presence of response-contingent stimulus delivery. To address these issues Caggiula and colleagues tested the independent and combined effects of nicotine and nonpharmacological stimuli on operant responding. Separate groups of rats were allowed to acquire lever pressing for either nicotine paired with a compound visual stimulus (VS: onset of white cue light for 1 second, followed by offset of white house light for 1 minute. House light offset signaled a time-out period when responding was recorded but not reinforced), saline paired with the VS, nicotine in the absence of the VS (nicotine alone), and saline alone (Donny et al. 2003). The principal outcome from this study was that associating nicotine with the VS produced a synergistic, not just additive enhancement of self-administration; i.e., response rates generated by the combination of VS and nicotine were more than twice the sum of response rates

produced by either the VS alone, or nicotine alone (see Figure 1). A second, equally important finding was that although nicotine alone did not establish self-administration at the dose tested, the VS in the absence of nicotine supported moderate but stable behavior. In a subsequent experiment the ability of the VS to impact lever pressing when presented in combination with nicotine after rats had acquired self-administration of nicotine alone was tested (Chaudhri et al. 2005b). This study replicated the observation that rats only acquired nicotine self-administration in the absence of a concurrent non-drug stimulus when larger doses of nicotine were available. More importantly, combining the VS with nicotine in rats with a prolonged history of responding for nicotine alone produced an immediate, sizeable increase in lever pressing, and this effect was prominent at low doses (0.03 and 0.06 mg/kg/inf) but did not occur at the highest dose tested (0.15 mg/kg/inf).

This research using a standard model of self-administration provides important insight into why nicotine reinforcement is so much more pronounced in the presence of concurrent non-drug stimuli: it is not the drug alone, but the synergistic interaction between nicotine and nonpharmacological stimuli that produces a significant increase in positive reinforcement. A potential mechanism for this effect can be conceptualized based on the following observations. First, nicotine elevated responding for a non-drug stimulus that functioned as an unconditioned reinforcer without any prior association with nicotine (Donny et al. 2003; Chaudhri et al. 2005b). Second, the synergistic interaction required stimulus delivery to be contingent upon behavior; it did not occur when rats responded for nicotine and received noncontingent VS presentations (Caggiula et al. 2002a). These data suggest that the synergy between nicotine and the VS that resulted in elevated lever pressing was a consequence of nicotine enhancing the reinforcing properties of, and therefore behavior maintained by an already reinforcing nonpharmacological

stimulus. Alternatively, or perhaps in conjunction, the interaction could have resulted from nicotine establishing the concurrent VS as a conditioned reinforcer via Pavlovian conditioning. These hypotheses can be differentiated experimentally by dissociating nicotine delivery from operant responding maintained by the VS. If Pavlovian conditioning is the central mechanism underlying the interaction between nicotine and non-drug factors, then responding for the VS should not be elevated when nicotine delivery is independent of the animal's behavior, and therefore unrelated to the VS. However, if nicotine enhances reinforcement through non-associative mechanisms, then an increase in responding for the VS should be preserved under this condition. The latter hypothesis also predicts that nicotine should elevate responding for reinforcing nonpharmacological stimuli other than the VS (e.g., conditioned reinforcers, discriminative stimuli, intrinsically reinforcing stimuli), and that this effect should also be independent of a temporal relationship between nicotine delivery, and both presentations of the stimulus and the behavior that it controls.

While these predictions apply to stimuli that reinforce behavior in the absence of drug, a separate set of predictions can be generated for the interaction between nicotine and relatively neutral stimuli. Nicotine, functioning non-associatively as a reinforcement-enhancer, should have less impact on responding for non-drug stimuli that are neutral or only very weakly reinforcing. However, as a primary reinforcer, nicotine can establish associated neutral stimuli as conditioned reinforcers (Rose and Levin 1991). Therefore, nicotine should not increase responding for a neutral stimulus if drug delivery is unrelated to either stimulus presentations or operant behavior; in contrast, when nicotine and the stimulus are combined and delivery is contingent upon lever pressing, then the repeated association should confer some reinforcement to the stimulus via

Pavlovian conditioning. Subsequently, responding for the stimulus, now potentially a conditioned reinforcer should be further enhanced by nicotine (see Table 2).

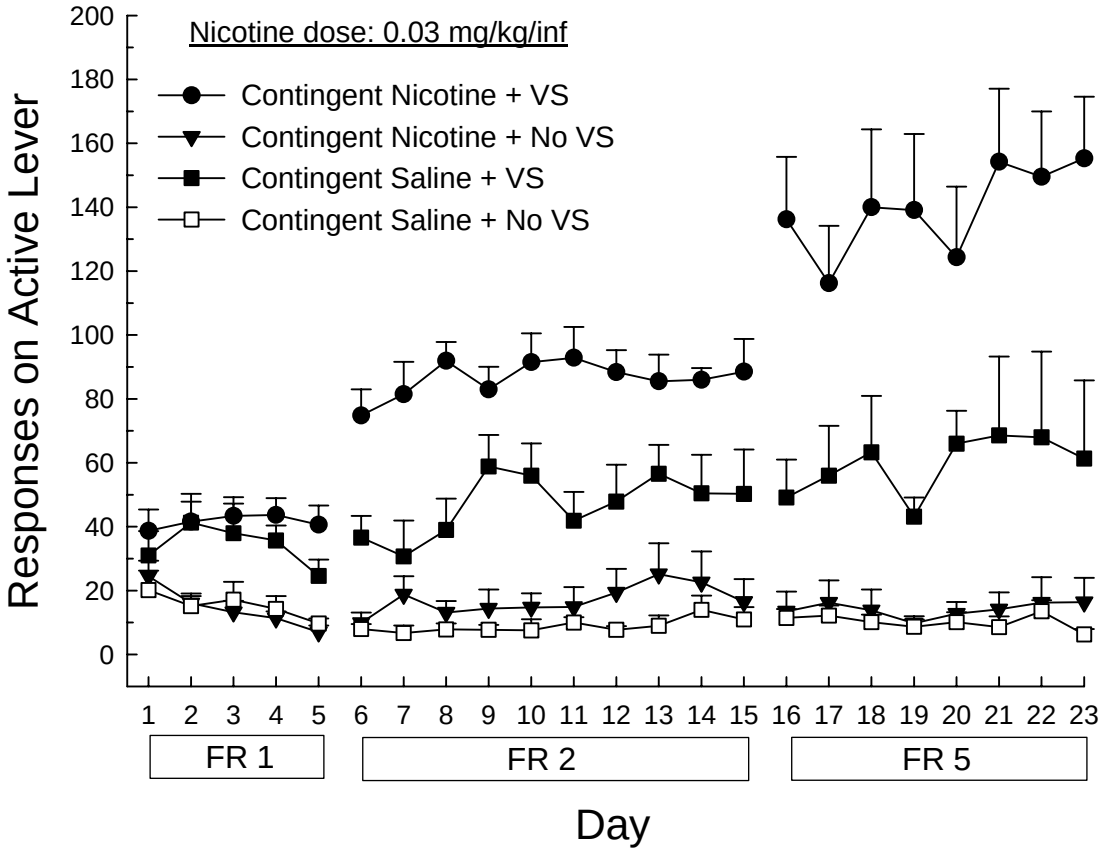


Figure 1: Nicotine self-administration in the presence and absence of a visual stimulus (VS). Active lever responding (including responding during the time-out period) is depicted. Results are means (± SEM) for data obtained from 7-10 animals per group. Schedule of reinforcement is indicated below the abscissa (modified from Donny et al. 2003)

Table 2: The dual-reinforcing actions of nicotine. Manipulating drug contingency can differentiate the behavioral effects resulting from the primary reinforcing and reinforcement-enhancing properties of nicotine

	Response-dependent nicotine (Contingent)	**Response-independent nicotine (Noncontingent)**
Primary Reinforcement	i) *Can* maintain operant behavior in the absence of non-nicotine stimuli	i) *Is unable to* maintain operant behavior in the absence of contingent, reinforcing non-nicotine stimuli
	ii) *Can* establish concurrent non-nicotine stimuli as conditioned reinforcers	ii) *Is unable to* establish concurrent non-nicotine stimuli as conditioned reinforcers
Reinforcement-Enhancer	iii) *Can* elevate behavior maintained by reinforcing non-nicotine stimuli	iii) *Can* elevate behavior maintained by reinforcing non-nicotine stimuli

2. OPERANT RESPONDING FOR A VISUAL REINFORCER IN RATS IS ENHANCED BY NONCONTINGENT NICOTINE: IMPLICATIONS FOR NICOTINE SELF-ADMINISTRATION AND REINFORCEMENT

2.1. ABSTRACT

Current conceptualizations of drug reinforcement assume that drug-taking behavior is a consequence of the contingent, temporal relationship between the behavior and drug reward. However, stimulant drugs also potentiate the rewarding effects of other reinforcers when administered noncontingently. Here we determined whether noncontingent nicotine enhances the reinforcing properties of a nonpharmacological reinforcer, and whether this direct effect facilitates operant behavior within the context of a nicotine self-administration procedure. Rats self-administered nicotine or food, or received noncontingent nicotine, saline, or food either with or without a response-contingent, unconditioned reinforcing visual stimulus (VS). Noncontingent nicotine, whether delivered as discrete injections based on a pattern of self-administered nicotine or as a continuous infusion, increased response rates maintained by the VS. There were no significant differences in responding by animals that received contingent compared to noncontingent nicotine when a VS was available. This increase was not observed in the absence of the VS or as a consequence of noncontingent food delivery. Operant behavior was equally attenuated and reinstated by the removal and subsequent replacement of contingent and noncontingent nicotine. Nicotine supported self-administration in the absence of response-

contingent, nicotine-paired stimuli; however, response rates were drastically reduced compared to nicotine self-administration with the VS. These results suggest that nicotine influences operant behavior in two ways: by acting as a primary reinforcer when it is contingent upon behavior, and by directly potentiating the reinforcing properties of other stimuli through a non-associative mechanism. Nicotine self-administration and smoking may be largely dependent on this later action.

2.2. INTRODUCTION

A basic tenet of behavioral research on addiction is that drug-taking behavior of both humans and animals is the result of a predictable temporal relationship between the behavior and drug reward. This dictum has been widely employed as the principle explanation for why people smoke tobacco – smoking results in the rapid delivery of nicotine to the brain, and the consequent neuropharmacological effects of nicotine reinforce continued smoking behavior (USDHHS 1988).

The hallmark test for drug reinforcement in laboratory animals is self-administration. The principal of contingency is a critical component of this test; response-contingent presentation of the drug should engender more robust operant behavior than response-independent drug delivery (Meisch et al. 1993). The demonstration that drug-delivery must be contingent on the animal's behavior in order to support self-administration illustrates a central feature of instrumental behavior (Balleine and Dickinson 1998) and helps to eliminate alternative, non-associative, explanations of drug-seeking behavior (e.g., non-specific locomotor activation; Meisch and

Lemaire 1993). Nicotine, like other drugs of abuse, is self-administered by a variety of animal species (Corrigall and Coen 1989; Goldberg et al. 1981; Henningfield and Goldberg 1983a; Rose and Corrigall 1997). Nicotine self-administration is dose- and schedule- dependent (Corrigall and Coen 1989; Donny et al. 2000; Shoaib et al. 1997), extinguishes when nicotine is replaced with saline (Corrigall and Coen 1989; Shoaib et al. 1997), and, in the absence of other reinforcing stimuli, is dependent on nicotine being response-contingent (Donny et al. 1998). Models of nicotine self-administration are well established and have been used to investigate the behavioral, environmental and neurophysiological underpinnings of nicotine reinforcement (e.g., Caggiula et al. 2001; Corrigall et al. 1992; Picciotto et al. 1998).

However, other research suggests that some drugs can enhance responding for reinforcing stimuli by a mechanism that does not depend on a contingent relationship with either the stimuli or the behavior. For example, it is well established that nicotine and other stimulants can directly increase low rates of schedule-controlled behavior (Byrd 1979; Dews 1958; Hendry and Rosecrans 1982). Furthermore, noncontingent administration of psychostimulants such as amphetamine, cocaine and pipradrol can enhance responding for stimuli that have previously been associated with primary rewards (Beninger et al. 1981; Hill 1970; Robbins et al. 1983; Robbins 1978; 1977; 1976). Phillips and Fibiger (1990) reviewed the literature on the reward-enhancing properties of cocaine and concluded that these effects may provide an additional mechanism driving cocaine abuse that is distinct from the primary reinforcing effects of the drug. With some notable exceptions (Whitelaw et al. 1996), relatively little attention has been paid to the possibility that the reward-enhancing effects of stimulants may contribute to stimulant self-administration.

The aim of the present studies was to determine if nicotine produced reward-enhancing effects like those observed for cocaine and amphetamine, and to begin to evaluate whether such effects might contribute to operant responding within the context of an animal model of self-administration. We examined the rate of responding maintained by either self-administered (contingent) or response-independent (noncontingent) nicotine under conditions in which an unconditioned reinforcing visual stimulus (Caggiula et al. 2002a) was either present or absent. The results demonstrated that noncontingent administration of nicotine greatly enhanced responding for reinforcing stimuli. These findings have important implications for both understanding the factors controlling nicotine-seeking behavior and interpreting standard laboratory evaluations of drug reinforcement.

2.3. MATERIALS AND METHODS

2.3.1. Subjects

Male, Sprague-Dawley rats (Harlan Farms), 41-44 days old and weighing between 200 and 225 g upon arrival were individually housed in a temperature-controlled environment on a 12-hr reversed light/dark cycle. Upon arrival, all animals were placed on an unrestricted diet during one week of habituation to the laboratory. After training, (described below) and for the remainder of the study, all animals received 20 g of food per day. Unlimited access to water was available throughout all experiments. Animals were 60+ days old at the start of the experiments. Separate cohorts of animals were used for each of the experiments described below.

2.3.2. Apparatus

Lever training and all subsequent experimental sessions took place in a 25x31x28 cm^3 operant conditioning chamber (BRS/LVE Model # RTC-020) with identical inactive and active levers, a white cue light located 5 cm above the active lever, an overhead house light, and a pellet trough. For experimental sessions, all animals were connected to a drug-delivery swivel system that allowed nearly unrestricted movement in the chamber. An interfaced computer software package (Med Associates, MED-PC IV) was used to record active lever responses, inactive lever responses, and reinforcements. A constant background noise of approximately 75 db that was produced by exhaust fans located within each sound-attenuating chamber masked the auditory cues associated with food/drug delivery and ambient noise.

2.3.3. Food training

Following habituation to the colony room, rats were food deprived for 24 hrs and then trained to lever press on the right (active) lever for 45 mg food pellets. Training consisted of a single 20 minute habituation session in the experimental chamber, a 25 minute magazine training session, and a session that began with hand shaping, during which animals received approximately 20 pellets as a consequence of responding on the active lever, and ended with a programmed fixed ratio (FR) 1 with a maximum of 75 food reinforcements. Responding on the left (inactive) lever had no scheduled consequence. In all of the experiments described here, rats were trained in the absence of any scheduled changes in stimulus conditions (i.e., the cue light remained off and a dim red house light was illuminated to allow monitoring by the experimenters).

2.3.4. Surgery

After training all animals were anesthetized with halothane and implanted with jugular catheters. Rats were allowed at least 7 days to recover from surgery prior to the start of the experimental sessions. For the first 2 weeks after surgery, rats were treated with both heparin and streptokinase in order to help maintain catheter patency, and the antibiotic ticarcillan plus clavulanate to reduce post-surgical infections (see Donny et al. 1999 for details). Thereafter, catheters were flushed once daily with 0.1 ml sterile heparinized saline (30 U/ml) on non-testing days (weekends), and both prior to (10 U/ml) and following (30 U/ml) each session on testing days.

2.3.5. Experimental design

Experiment 1: The effects of contingent and noncontingent (i.e., yoked) nicotine on operant responding in the presence or absence of a behaviorally contingent visual stimulus.

The purpose of Experiment 1 was to: 1) determine whether noncontingent nicotine administration potentiates responding maintained by an unconditioned, reinforcing, visual stimulus, and 2) begin to evaluate the potential influence of this effect on nicotine self-administration. Animals in Experiment 1 were divided into six groups following food training. In one group (Contingent NIC + VS), fulfillment of the schedule requirement resulted in the delivery of an intravenous infusion of 0.03 mg/kg nicotine bitartate (all infusions were delivered in a volume of 0.1 ml/kg over approximately 1 sec; dose reported as free base weight) and a visual stimulus (VS) paired with each infusion. A schematic of the relationship between operant behavior, infusions, and changes in the VS is shown in Figure 2.

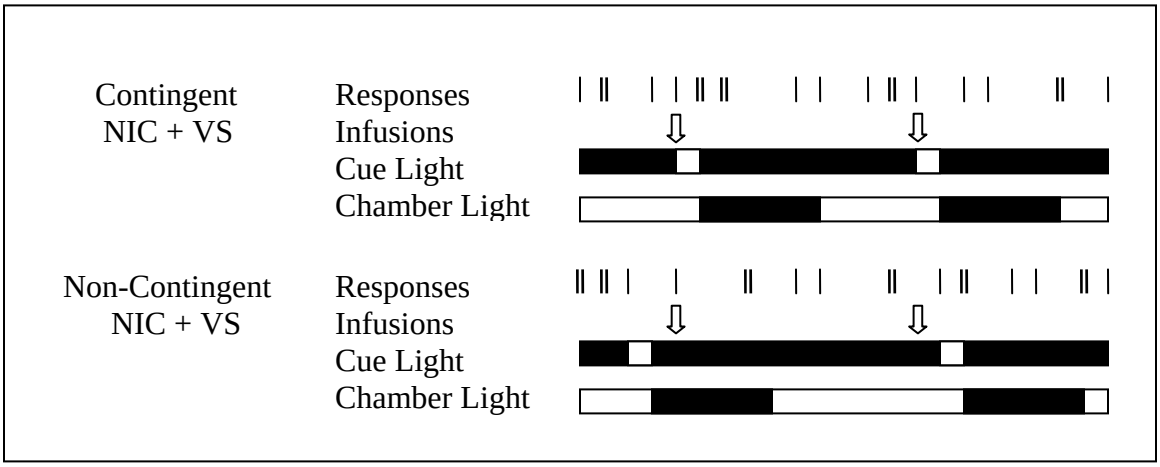


Figure 2: Schematic illustration of the relationship between operant responding on the active lever, infusions, and stimulus changes in the Contingent NIC + VS and Noncontingent NIC + VS conditions, on an FR 5 reinforcement schedule

In the Contingent NIC + VS condition, nicotine and the VS were always presented together and dependent upon the animal's behavior (see Figure 2). The VS, used previously in studies of nicotine self-administration (Donny et al. 2000; Donny et al. 1998), consisted of the onset of a white cue light for 1 second and the concurrent offset of a white house light for 1 minute. A second group (Contingent NIC + No VS) also self-administered 0.03 mg/kg nicotine, but without any scheduled changes in stimulus conditions. The third and fourth groups were allowed to self-administer saline either paired with the VS (Contingent SAL + VS) or without the VS (Contingent SAL + No VS). Animals in the fifth group (Noncontingent NIC + VS) were paired with individuals in the Contingent NIC + VS group and received the same number of nicotine infusions at identical times during each session (i.e. yoked). Figure 2 illustrates the differences

between this condition and the Contingent NIC + VS group. Nicotine infusions in this group were contingent upon the partner's responding and not upon their own lever pressing. However, lever pressing by animals in this group resulted in response-contingent presentation of the VS. A sixth group also received yoked nicotine infusions (matched to the same individual animals in the Contingent NIC + VS condition), but lever pressing had no scheduled consequence (Noncontingent NIC + No VS). The schedule of reinforcement for nicotine/saline and VS presentations was increased sequentially from a FR 1 (days 1-5), through a FR 2 (days 6-13) to a FR 5 (days 14-23). All response-contingent reinforcements (i.e., contingent nicotine, saline and/or VS) were followed by a 1 minute time out period during which responding had no consequence. Noncontingent presentations of nicotine or saline were not followed by a time out period. All experimental sessions lasted one hour.

Experiment 2: Replication of the effects of noncontingent (i.e., yoked) nicotine and comparison to noncontingent saline and food

The purpose of Experiment 2 was to: 1) further evaluate the tendency for noncontingent nicotine to elevate responding for the VS by comparing these effects with noncontingent saline control conditions, 2) re-examine the similarities and/or differences between contingent and noncontingent nicotine in the presence of the VS, and 3) determine whether noncontingent food would produce a similar elevation in response rates. Animals in Experiment 2 were divided into seven groups following food training. Two of these groups were identical to those described in Experiment 1 (Contingent NIC + VS and Noncontingent NIC + VS). Two additional groups received noncontingent infusions of saline that were yoked to the Contingent NIC + VS group while their responding resulted in either the presentation of the VS (Noncontingent SAL + VS)

or no consequence (Noncontingent SAL + No VS). A fifth group of animals lever pressed for 45-mg food pellets paired with the VS (Contingent Food + VS). Individuals in the sixth group (Noncontingent Food + VS) received yoked food pellets (i.e., controlled by animals in the Contingent Food + VS condition) while responding for contingent presentations of the VS. The seventh group (Noncontingent Food + No VS) also received yoked food pellets, but lever pressing in this group had no consequence. The schedule of reinforcement for contingent nicotine, food and VS presentations was an FR 1 for days 1-5, an FR 2 for days 6-13, and an FR 5 for days 14-20. A 1 minute time out period followed all response-contingent reinforcers. All experimental sessions lasted one hour.

Experiment 3: Changes in responding after the removal and replacement of self-administered versus yoked nicotine.

The purpose of Experiment 3 was to: 1) further evaluate the direct effects of noncontingent nicotine on VS-maintained responding by substituting saline for nicotine in a within-subjects design, and 2) compare these effects to extinction and reacquisition in animals self-administering nicotine. Animals were divided into two groups described in Experiment 1 (Contingent NIC + VS and Noncontingent NIC + VS). Following a 20 day acquisition period identical to Experiment 1, saline was substituted for nicotine for three days, and then nicotine was reinstated from days 24-28 in both groups. The response-contingent presentation of the VS remained available throughout the experiment. The schedule of reinforcement for nicotine/saline and for the VS was an FR 5 throughout the maintenance, extinction, and reacquisition phases. A 1 minute time out period followed all response-contingent reinforcers. All experimental sessions lasted one hour.

Experiment 4: The effects of continuously infused nicotine on stimulus-maintained responding.

The purpose of Experiment 4 was to: 1) determine whether a continuous intravenous infusion of nicotine would potentiate responding maintained by the VS in a manner similar to pulsed infusions of nicotine, and 2) evaluate changes in VS-maintained responding during saline substitution. Animals were divided into four groups. Three of the groups were identical to groups reported in Experiment 1 and 3, including a Contingent NIC + VS group, a Noncontingent NIC + VS group, and a Noncontingent SAL + VS group. The fourth group received a noncontingent, continuous infusion of nicotine while responding was reinforced by the VS (Continuous NIC + VS). Due to experimenter error, the cumulative dose of continuous nicotine administered each session was approximately one-third the dose self-administered by animals given access to 0.03 mg/kg/inf. Therefore, direct comparisons of either the Contingent NIC + VS or Noncontingent NIC + VS and the Continuous NIC + VS conditions should be made with caution since the conditions differ across multiple parameters (e.g., methods of nicotine delivery, dose). The total nicotine delivery per 1-hr session was 0.039 mg/kg for days 1-2, 0.19 mg/kg for days 3-5, and 0.23 mg/kg for days 6-29. The concentration of nicotine base dissolved in physiological saline was 0.03, 0.1, and 0.1 mg/ml and the flow rate was 1.3, 1.9, and 2.3 ml/hr for days 1-2, 3-5, and 6-29, respectively. Following a 20 day acquisition period, saline was substituted for nicotine for six days, and then nicotine was replaced from days 27-29 in all three nicotine groups. The Contingent SAL + VS group was not run after day 26. The response-contingent presentation of the VS remained available throughout the experiment. The schedule of reinforcement for contingent nicotine/saline and/or VS presentations was increased sequentially from a FR 1 (days 1-5), through a FR 2 (days 6-13) to a FR 5 (days 14-29). A 1 minute time out period followed all response-contingent reinforcers. All experimental sessions lasted one hour.

Experiment 5: A dose-effect analysis of nicotine self-administration without cues.

The purpose of Experiment 5 was to determine whether response-contingent nicotine maintained operant behavior in the absence of other stimuli. Four groups of animals acquired nicotine self-administration without any programmed visual or auditory stimuli under the following schedule of reinforcement: FR 1 (days 1-5), FR 2 (days 6-8), FR 5 (days 9-20). A 1 minute time out period followed all nicotine injections. Each group was assigned a different dose of nicotine (0.015, 0.03, 0.06 or 0.09 mg/kg/infusion) that remained constant throughout the study. Otherwise all conditions were identical to those described for Contingent NIC + No VS above. All experimental sessions lasted one hour.

2.3.6. Statistical Analyses

Statistical analyses were conducted using the mean of the last 2-3 days of each schedule of reinforcement (FR 1, FR 2, FR 5) or test phase (maintenance, extinction or re-acquisition). This approach allowed for comparison across conditions with an unequal number of sessions and focused on stable behavior. It is important to point out that this approach does not capture the dynamic changes in behavior that occur over time within each schedule of reinforcement or test phase (e.g., the rate of extinction). The 3 day mean was used in all experiments except Experiments 3 and 5; in these cases, a two-day mean was used to avoid using the first day of a 3 day extinction period (Experiment 3) or the first day of 3 days on an FR 2 (Experiment 5).

Data from all experiments were analysed using ANOVA with either Schedule of Reinforcement (Experiments 1, 2, 4 and 5) or Extinction Phase (Experiment 3 and 4) as the within-subjects factor and Group (Experiments 1-4) or Dose (Experiment 5) as the between-

subjects factor. Analysis of extinction data from Experiment 4 did not include data from Contingent Sal + VS. Pre-planned comparisons between groups utilized targeted two-factor ANOVAs (Schedule/Phase and Group/Dose) with the between-subject factors confined to the two conditions of interest, followed by paired and independent sample t-tests. The α level was set to 0.05.

2.4. RESULTS

Experiment 1

Lever pressing by both the Contingent NIC + VS and the Noncontingent NIC + VS groups rose rapidly across the 20 day acquisition period, reaching robust, stable rates that were greater than those maintained by nicotine or the VS alone (Figure 3). ANOVA indicated that active response rates significantly differed by Group [$F(5,43)=23.54$, $p<0.001$], Schedule [$F(2,86)=28.09$, $p<0.001$] and the Group by Schedule interaction [$F(10,86)=8.88$, $p<0.001$]. Response-contingent presentation of the VS increased active response rates compared to the no VS conditions both in the presence of contingent saline ($p<0.01$) and noncontingent nicotine ($p<0.001$), indicating that the VS was functioning as a reinforcer. Noncontingent nicotine tended to facilitate responding for the VS compared to Contingent SAL + VS, although this difference only reached statistical significance on an FR 2 ($p<0.05$). The facilitating effects of noncontingent nicotine on response rates for the VS were significantly different from those of contingent nicotine on an FR 1 ($p<0.05$); however the groups converged by the end of FR 2 and did not significantly differ throughout the remainder of the study.

Active response rates in the Noncontingent NIC + No VS were low throughout the experiment and did not differ from either the Contingent SAL+ No VS or the Noncontingent SAL + No VS from Experiment 2 (see below). Similarly, at this dose (see Experiment 5 for full dose-response analyses), contingent nicotine in the absence of the VS failed to significantly increase active lever response rates over Contingent SAL + No VS. The mean (± SEM) number of nicotine infusions in the Contingent NIC + VS (and the yoked, noncontingent nicotine groups) was 23.0 (± 1.68), 27.4 (± 1.25), and 24.3 (± 1.81) for the last 3 days of FR 1, FR 2 and FR 5, respectively. In contrast, the mean (± SEM) number of infusions by Contingent NIC + No VS was 6.2 (± 0.85), 6.5 (± 1.38), and 2.4 (± 0.63) and similar to the rate of infusions maintained in the Contingent SAL + No VS condition. Mean (± SEM) response rates on the inactive lever during FR 5 were 33.8 (± 3.6), 16.7 (± 2.3), 7.3 (± 1.3), 9.7 (± 1.8), 1.5 (± 0.4), and 5.0 (± 0.6) for the Contingent NIC + VS, Noncontingent NIC + VS, Noncontingent NIC + No VS, Contingent NIC + No VS, Contingent SAL + VS, and Contingent SAL + No VS, respectively.

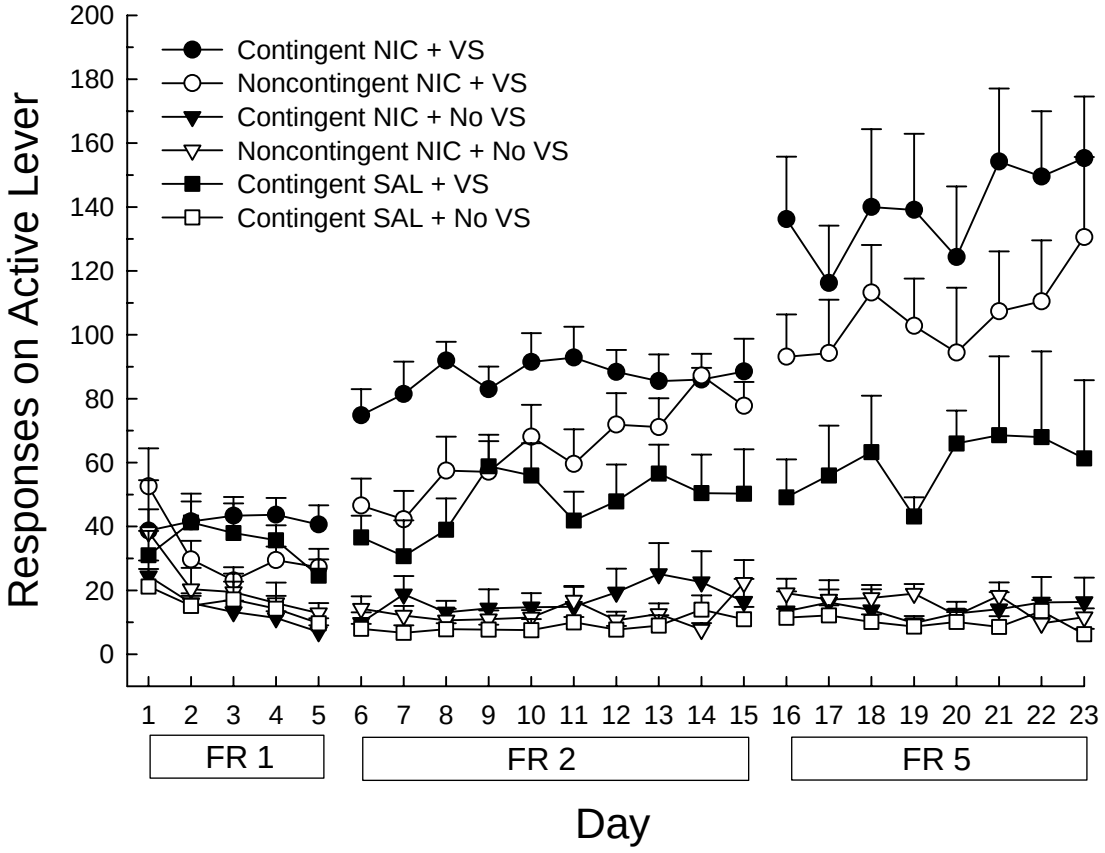


Figure 3: Effects of nicotine (NIC), saline (SAL) and the visual stimulus (VS) on active lever responding. Responses during the time out period are included. Results are mean (± SEM) of data from 7-10 animals per group. Schedule of reinforcement is indicated below the abscissa

Experiment 2

Experiment 2 replicated the finding that responding maintained by contingent and noncontingent nicotine was similar in the presence of the VS. This study also clearly demonstrated that responding for the VS was higher when accompanied by noncontingent nicotine compared to noncontingent saline; in contrast, noncontingent food produced rates of behavior that were similar to noncontingent saline. The nicotine and food data are presented in separate panels (Figure 4A and 4B) to accommodate the large differences in the maximal rates of behavior. ANOVA revealed that active response rates significantly differed by Group [$F(6,55)=73.72$, $p<0.001$], Schedule [$F(2,110)=61.49$, $p<0.001$] and the Group by Schedule interaction [$F(12,110)=20.53$, $p<0.001$]. As in Experiment 1, the VS functioned as a reinforcer, potentiating responding in the presence of both noncontingent saline ($p<0.05$) and noncontingent food ($p<0.01$). Active response rates in the presence of the VS were not statistically different in the contingent or noncontingent nicotine conditions and were significantly potentiated compared to Noncontingent SAL + VS ($p<0.005$ for overall group effect, FR 2, and FR 5; Figure 4A).

Rats receiving response-contingent food pellets paired with the VS responded at rates (Figure 4B) that were substantially higher than Noncontingent Food + VS ($p<0.001$ for overall group effect, group by schedule interaction, and all FR schedules). The delivery of noncontingent food pellets in the presence of the response-contingent VS yielded response rates that were not significantly different from the rate of responding in the Noncontingent SAL + VS condition. Mean (± SEM) response rates on the inactive lever during FR 5 were 20.9 (± 3.5), 14.1 (± 2.1), 7.4 (± 1.9), 5.2 (± 1.5), 5.7 (± 1.5), 4.2 (± 0.8), and 4.7 (± 0.5) for Contingent NIC + VS, Noncontingent NIC + VS, Noncontingent SAL + VS, Noncontingent SAL + No VS, Contingent Food + VS, Noncontingent Food + VS, and Noncontingent Food + No VS, respectively.

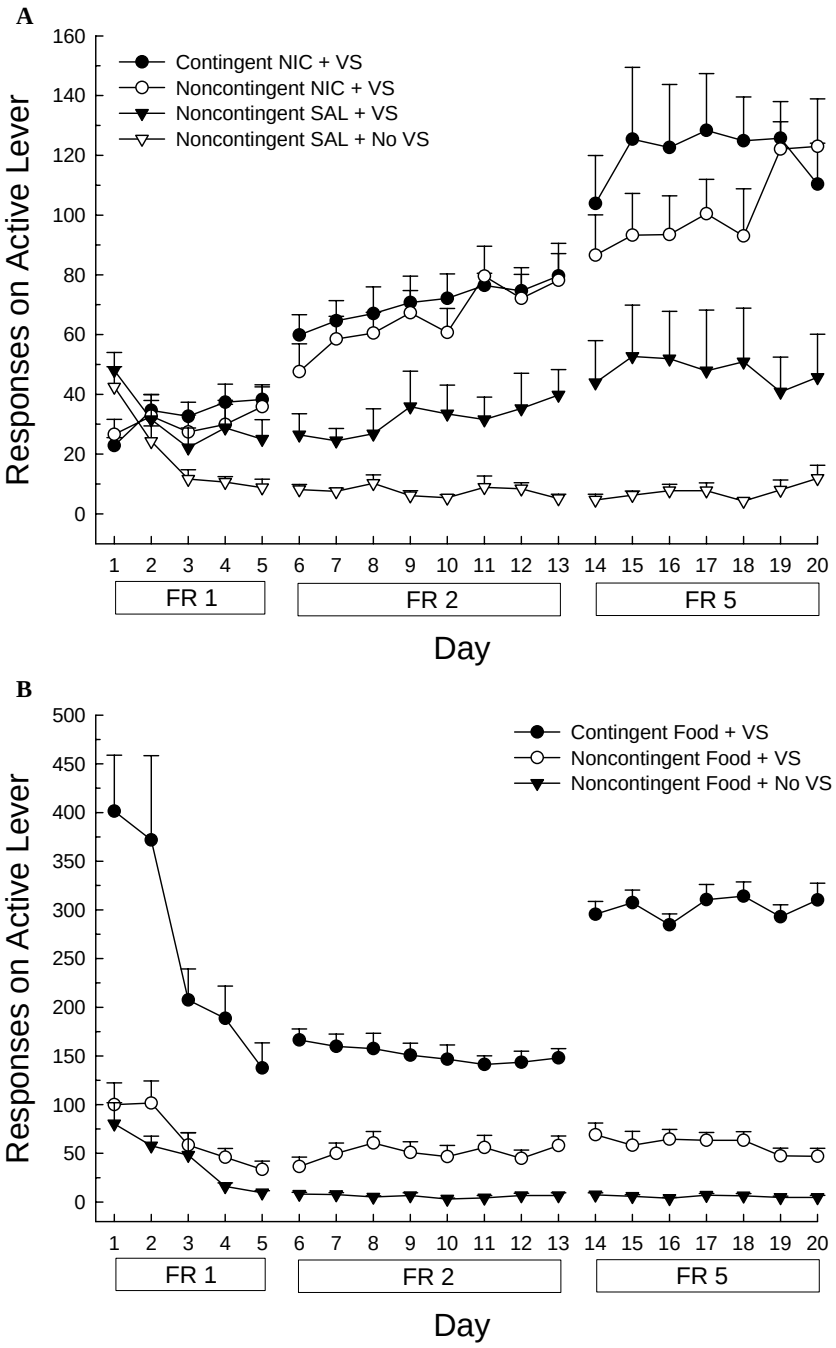


Figure 4: Effects of nicotine (NIC), saline (SAL), food and the visual stimulus (VS) on active lever responding. Results are mean (± SEM) of data from 8-10 animals per group. Upper panel (A) illustrates the effects of nicotine and or saline. Lower panel (B) illustrates the effects of food. Schedule of reinforcement is indicated below the abscissa

Experiment 3

Responding for the VS was similarly attenuated in the Contingent NIC + VS and Noncontingent NIC + VS conditions when saline was substituted for nicotine and reinstated when nicotine was reintroduced (Figure 5). ANOVA on active lever responding revealed a significant effect of Phase [$F(1,15)=78.15$, $p<0.001$], but no effect of Group or the Group by Phase interaction ($p<0.05$). In both conditions, the rates of responding on the reinforced lever decreased significantly when nicotine was substituted with saline ($p<0.001$) and increased after nicotine was reintroduced ($p<0.001$); there were no significant differences between the groups. Responding on the inactive lever was similar and significantly lower than active responding for both groups ($p<0.001$).

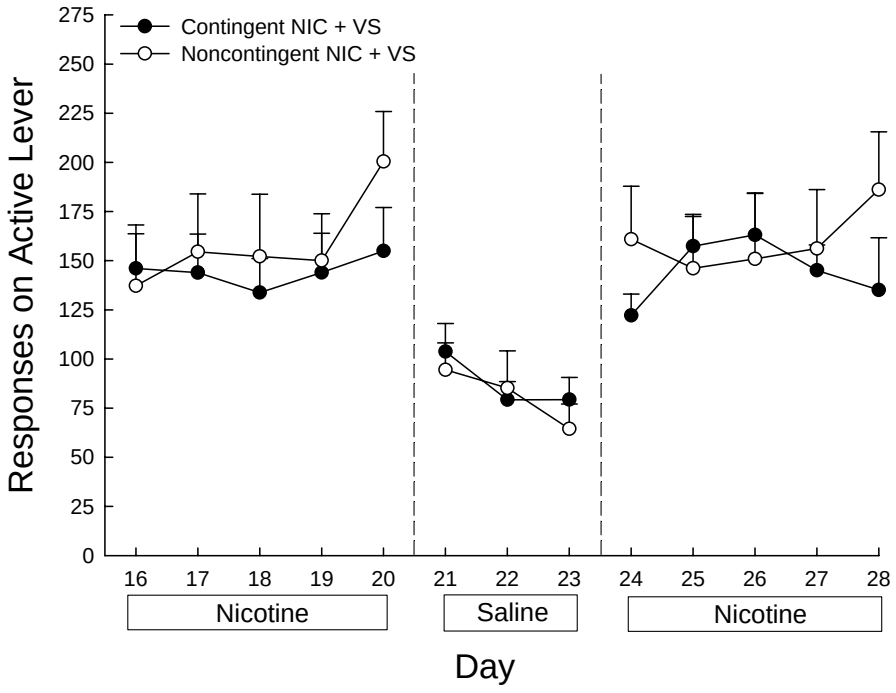


Figure 5: Effects of removing and subsequently replacing nicotine on active lever responding. Results are mean (± SEM) of data obtained from 8-9 animals per group

Experiment 4

The continuous infusion of a relatively low dose of nicotine resulted in a potentiation of responding maintained by the VS that was similar to the effect of larger doses of both response-contingent and yoked nicotine (Figure 6). ANOVA on active responses during acquisition revealed a significant effect of Group [$F(3,27)=11.72$, $p<0.001$], Schedule [$F(2,54)=189.21$, $p<0.001$], and the Group by Schedule interaction [$F(6,54)=11.58$, $p<0.001$]. Pairwise comparisons of acquisition data revealed that Continuous NIC + VS increased response rates compared to Contingent SAL + VS ($p<0.001$ overall and at each FR schedule). As observed in the previous experiments the Contingent NIC + VS and Noncontingent NIC + VS did not significantly differ from each other. Furthermore, both groups potentiated responding during individual schedules of reinforcement (FR 1 – Contingent NIC + VS only: $p<0.05$; FR 2: $p<0.05$; FR 5: $p<0.001$) compared to Contingent SAL + VS. The similarities between Contingent NIC + VS and Noncontingent NIC + VS remained when time-in and time-out active responses were considered separately. The percentage of active responses that occurred during the time out period decreases over the acquisition period in all three nicotine conditions (contingent, noncontingent and continuous nicotine), representing approximately 19-26% of the total number of responses made during the last three days on a FR 5. Inactive responding in the Contingent NIC + VS and Contingent SAL + VS was low (7.6 ± 1.0 and 3.9 ± 0.1 on an FR 5, respectively). Noncontingent NIC + VS and Continuous NIC + VS displayed elevated rates of inactive responding (37.2 ± 12.6 and 48.5 ± 12.4 on an FR 5, respectively) as a result of two animals in each condition responding at unusually high rates (<100 responses). The remaining animals displayed relatively low rates of inactive responses.

Analysis of extinction confirmed that it was the continuous infusion of nicotine that increased response rates in animals responding for the VS (Figure 6). ANOVA of maintenance, extinction, and reacquisition revealed a significant effect of Phase [$F(2,38)=97.25$, $p<0.001$], but no effect of Group [$F(2,19)=0.064$, n.s.] or the Group by Phase interaction [$F(4,38)=0.722$, n.s.]. Substituting saline for nicotine decreased the rate of responding in the Contingent NIC + VS, Noncontingent NIC + VS, and Continuous NIC + VS groups ($p<0.001$ compared to maintenance) and replacing nicotine increased the rate of operant responding in all three nicotine groups ($p<0.005$ compared to extinction). Inspection of data from the Contingent SAL + VS condition revealed continued stable rates of active lever responding from day 14 through day 26.

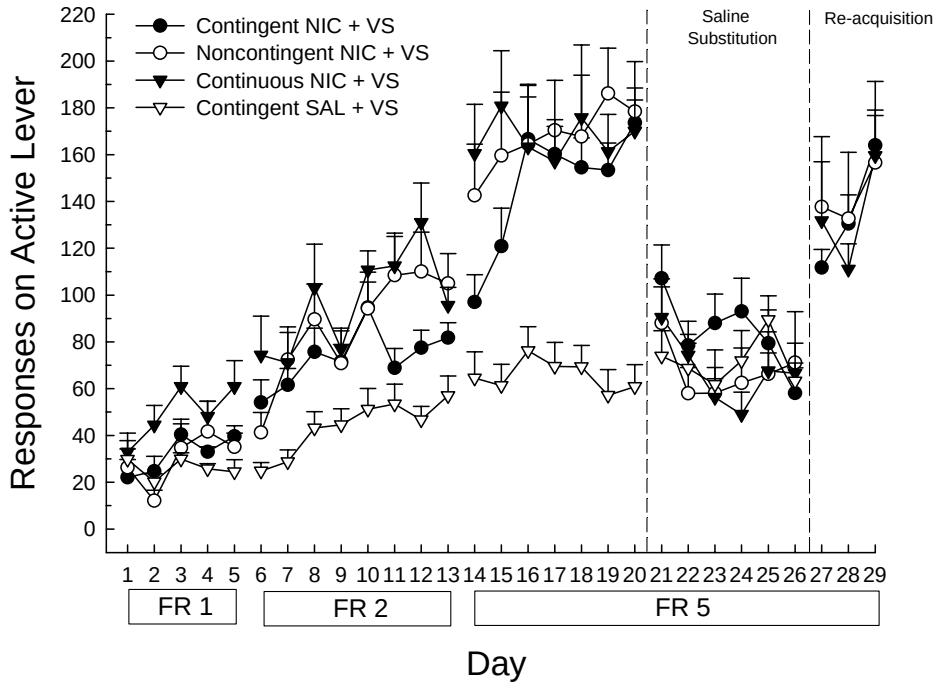


Figure 6: Effects of response-contingent, yoked, or continuous nicotine (NIC) or contingent saline (SAL) on active lever responding for VS. Saline substitution was conducted from days 21-26. Results are mean (± SEM) of data obtained from 7-9 rats per group

Experiment 5

Nicotine self-administration in the absence of cues was dose-dependent, producing an "inverted U" shaped curve that peaked at 0.06 mg/kg/infusion (Figure 7). ANOVA of active responses revealed the following main effects and interactions: Dose [$F(3,26)=4.37$, $p<0.05$], Schedule [$F(2,52)=19.54$, $p<0.001$], Dose by Schedule [$F(6,52)=2.64$, $p<0.05$]. The lowest dose, 0.015 mg/kg/infusion, maintained relatively low rates of active responding that were not significantly different from inactive lever response rates. The two middle doses (0.03 and 0.06 mg/kg/infusion) supported more active than inactive lever responding on all schedules of reinforcement ($p<0.05$). The largest dose, 0.09 mg/kg/infusion, resulted in active response rates that were only significantly greater than inactive responses on an FR 5 ($p<0.01$). Pairwise comparisons between doses revealed no differences between 0.015 and 0.03 mg/kg/infusion. There were significantly more active responses in the 0.06 mg/kg/infusion group compared to 0.015 mg/kg/infusion across schedules of reinforcement ($p<0.001$) as well as for FR 2 and FR 5 when analyzed separately ($p<0.05$). Comparison of 0.09 mg/kg/infusion to 0.015 mg/kg/infusion indicated significant group differences that varied across schedule of reinforcement (i.e., group by schedule interaction; $p<0.05$), reaching significance on a FR 2 ($p<0.05$).

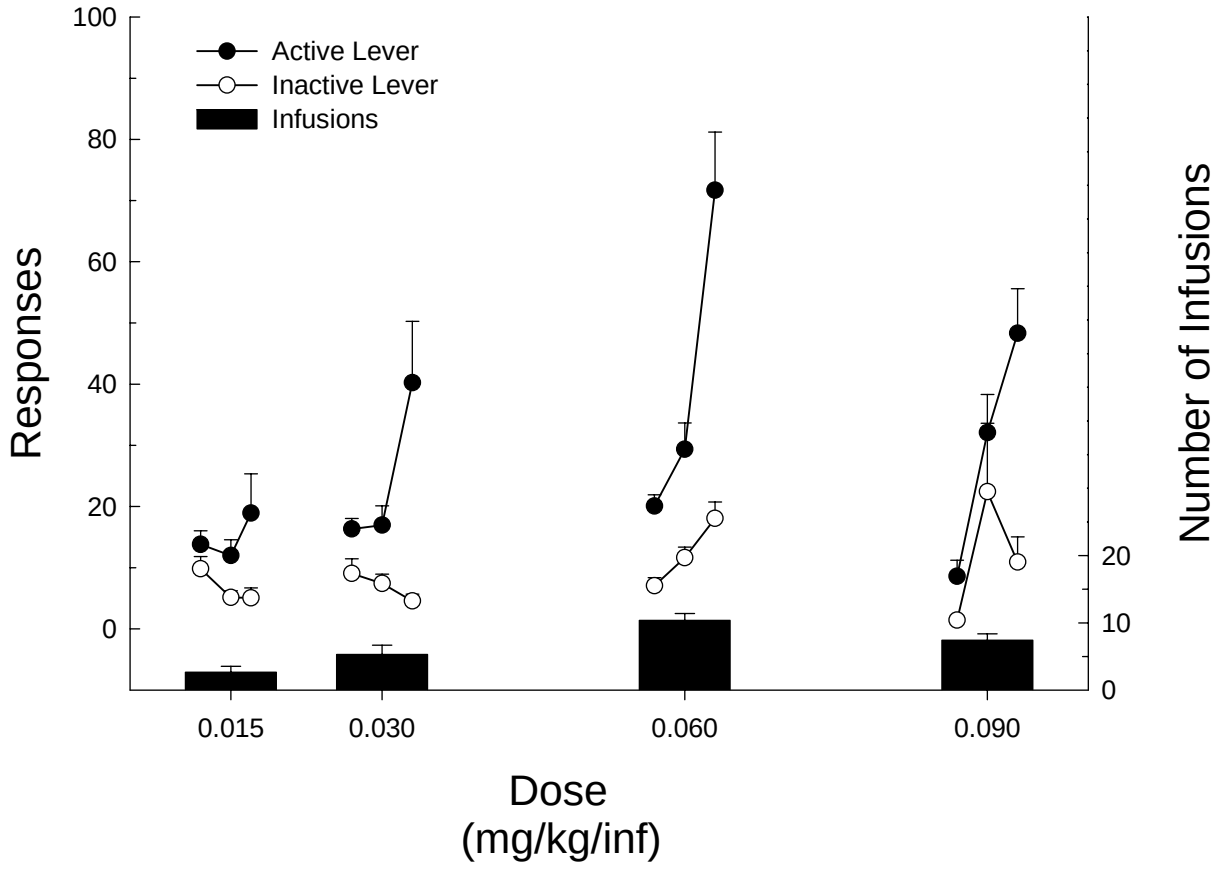


Figure 7: Self-administration of different doses of nicotine without additional programmed stimuli. Results are means (± SEM) of data obtained from 6-8 rats per group. Adjacent symbols represent the mean active lever response rates (including responding during the time out period) on the last two days on an FR 1, FR 2 and FR 5 schedule of reinforcement. Bars represent infusions obtained during the last two days on and FR 5 schedule

2.5. DISCUSSION

These findings support the hypothesis that nicotine enhances the reinforcing properties of other stimuli. This action of nicotine was demonstrated by the nicotine-induced increase in responding for a concurrently available, reinforcing, VS. The increase in responding was dependent on the availability of the VS, did not occur with noncontingent food delivery, and was under the control of nicotine delivery as demonstrated by saline substitution. The critical observation made here was that operant responding was maintained at high levels by nicotine that was neither temporally nor causally associated with behavior, indicating that this effect is distinct from the actions of nicotine as a primary reinforcer (Phillips and Fibiger 1990). This finding does not contradict the hypothesis that nicotine is the "primary psychoactive ingredient driving smoking," (USDHHS 1988) but rather suggests that nicotine may support behavior in two ways: by acting as a primary reinforcer and by directly potentiating the reinforcing effects of other stimuli.

Previous research has shown that psychostimulants such as amphetamine, cocaine, and pipradrol (Hill 1970; Phillips and Fibiger 1990; Robbins and Koob 1978; Stein 1964) can enhance the reinforcing effects of other stimuli through non-associative mechanisms. The present results suggest that nicotine may also have reinforcement-enhancing effects that may contribute to its control over behavior. This effect was observed both when nicotine administration was yoked to mimic the dose and pattern of self-administered nicotine, and when a relatively low dose of nicotine (e.g., approximately one-third the cumulative self-administered dose at 0.03 mg/kg/infusion) was slowly infused throughout the experimental session. The effectiveness of both yoked pulsed and continuous infusions of nicotine strongly supports the notion that the reinforcement enhancing effects of nicotine were non-associative in nature and not the result of

intermittent, chance associations between nicotine delivery and either operant behavior or presentation of the VS. Furthermore, although the comparison to food reinforcement is limited by the fact that food requires consumatory behavior, the observation that noncontingent food delivery failed to alter responding suggests that the increase in response rates was a direct, pharmacological action of nicotine and not a property of all reinforcers.

Although the present study examined an unconditioned reinforcing light stimulus (see Caggiula et al. 2002a for detailed discussion of this stimulus condition), the reinforcement-enhancing effects of nicotine likely extend to conditioned reinforcers. Indeed, most evidence that psychomotor stimulants enhance the effectiveness of other reinforcers has focused on conditioned reinforcement (e.g., Robbins 1978; 1976; Taylor and Horger 1999). A similar effect of nicotine would be important since nicotine-related stimuli are hypothesized to play a critical role in both nicotine self-administration in animals and smoking in humans (Caggiula et al. 2001; Rose and Levin 1991). Nicotine self-administration in animals and smoking behavior in humans is assumed to result from the combined primary reinforcing effects of nicotine and the conditioned reinforcing effects of nicotine-related stimuli. However, the present data suggest a critical interaction. The strength of the sensory stimuli as reinforcers may be greatly potentiated by nicotine, not only because nicotine has been repeatedly paired with these stimuli, but because nicotine acts directly to potentiate the reward value of those stimuli. Phillips and Fibiger (1990) recognized a similar effect in their review of the reward-enhancing properties of cocaine. After noting that the "conditioned stimuli" used in these studies often exhibit little or no reinforcing effects when presented alone, they pointed out that "the effects of conditioning are quite evident under the influence of the drug." They proposed that "under certain conditions there are latent conditioned rewarding effects that are only revealed after administration of a psychomotor

stimulant" (Phillips and Fibiger 1990, p.275). Likewise, the influence of nicotine-related stimuli may be greatest in the presence of the reinforcement-enhancing effects of nicotine.

Whether nicotine produces lasting changes in the reinforcing effects of other stimuli is unclear. Research has demonstrated that smoking cues continue to elicit positive subjective effects and reduce craving and withdrawal in the absence of nicotine (e.g., Pickworth et al. 1999; Rose et al. 2000). The generally accepted explanation for these effects is that smoking stimuli have become conditioned reinforcers as a consequence of repeated pairings with nicotine. However, these stimuli have always been experienced in the presence of nicotine, making it impossible to disentangle the simple conditioned reinforcing effects from a history of experiencing those effects in the presence of nicotine. Indirect evidence presented here suggests that some carry-over effects of nicotine may be present. A history of responding for the VS in the presence of nicotine (i.e., Noncontingent NIC + VS), yielded response rates without nicotine (i.e., first day of "Extinction") that were more than 40% greater than those maintained by Noncontingent SAL + VS (i.e., when animals had never experienced the VS in conjunction with nicotine). Although additional studies are required to rule out alternative explanations (e.g., behavioral momentum), these data suggest that reinforcing stimuli that are consistently experienced in the presence of nicotine may develop a greater reinforcing value than would be reached without this history.

These findings have important implications for models of drug self-administration. Most models of drug self-administration employ drug-paired, visual and/or auditory stimuli that would be expected to either have primary reinforcing effects (Stewart and Hurwitz 1958; Stewart 1960) or acquire conditioned reinforcing properties (e.g., Everitt et al. 2001). The reinforcement-enhancing effects of a drug may facilitate operant behavior within the context of the self-

administration model in a manner that is not dependent on the primary reinforcing properties of the drug per se (Phillips and Fibiger 1990). If this is true, changes in nicotine and other drug self-administration that occur as a consequence of neurophysiological, pharmacological, and behavioral manipulations may be attributable to changes in the reinforcement-enhancing effects of the drug, and not necessarily to its primary reinforcing effects.

Data presented here, as well as previous research (Caggiula et al. 2002a), demonstrate that nicotine supports self-administration in the absence of other reinforcers; however, there are large differences in self-administration behavior with and without response-contingent stimuli (Caggiula et al. 2001). A moderate dose of 0.03 mg/kg/infusion nicotine functions effectively as a robust reinforcer in the presence of the VS (e.g., Donny et al. 2000; Donny et al. 1998), but is only marginally reinforcing in its absence (Caggiula et al. 2002a; data presented here). In Experiment 5, a full analysis of nicotine self-administration without a contingent stimulus revealed response rates that peaked at a dose (0.06 mg/kg/infusion) that was three times larger than the peak dose in the presence of the VS (Donny et al. 2000). Furthermore, the maximal rate of nicotine self-administration is 2-3 times greater with the VS (Donny et al. 2000) than without it (Experiment 5). Additional research from our laboratory has confirmed that the dose-response function for nicotine self-administration without cues is shifted sharply downward and to the right compared to when nicotine is paired with the VS (unpublished observations). These observations are consistent with reports in humans that puff-sized doses of intravenous nicotine (i.e., without smoking cues) produce only small increases in satisfaction and liking (Rose et al. 2000; Westman et al. 1996), but that larger doses produce moderate increases in positive subjective effects (Garrett and Griffiths 2001) and self-administration (Henningfield and Goldberg 1983b).

The degree to which nicotine-paired stimuli potentiate nicotine self-administration is likely related to the unconditioned reinforcing value of those stimuli and the ability of nicotine to potentiate their effects. A recent study by Caggiula and colleagues (Caggiula et al. 2002a) examined the acquisition of nicotine self-administration behavior under a variety of cue conditions. Stimuli that were relatively neutral (i.e., did not support operant behavior when tested alone) produced a small increase in self-administration, presumably because of their repeated association with nicotine. In contrast, unconditioned reinforcing stimuli produced a large increase in responding. These findings support the notion that, under certain stimulus conditions, a substantial portion of self-administration may be determined by a direct effect of nicotine on behavior reinforced by other stimuli.

Other evidence supports the notion that nicotine potentiates the reinforcing properties of other reinforcers. In animals, nicotine increases motivation to obtain food (Popke et al. 2000), potentiates alcohol and cocaine self-administration (Clark et al. 2001; Potthoff et al. 1983), and lowers the threshold for brain reward stimulation (Bauco and Wise 1994). Likewise, clinical studies have found that smoking often occurs in conjunction with other reinforced behavior (e.g., drinking alcohol; Bien and Burge 1990). Although these effects are often interpreted as being pharmacologically specific (e.g., nicotine-alcohol interactions), an alternative interpretation is that nicotine acts more broadly, potentiating the rewarding effects of reinforcing stimuli. Supporting this hypothesis, recent neurophysiological evidence is also consistent with a more general effect of nicotine; the net GABAergic and glutamatergic influence on brain dopamine systems that modulate the reinforcement may shift towards a more excitable state following nicotine exposure (Mansvelder et al. 2002).

In conclusion, these studies support the proposal of an alternate action of nicotine that may operate in conjunction with its primary reinforcing effects to drive smoking behavior. Our data indicate that nicotine enhances the reinforcing value of other, nonpharmacological stimuli in a manner that is not dependent on a close temporal association between nicotine and either the stimuli, or the behavior controlling their delivery. The demonstration that nicotine produces both primary reinforcing effects and potent enhancement of the reinforcing effects of other stimuli suggests that basic research and treatment strategies predicated on nicotine acting principally as a primary reinforcer may be deficient.

3. SELF-ADMINISTERED AND NONCONTINGENT NICOTINE ENHANCE OPERANT RESPONDING FOR A VISUAL STIMULUS IN RATS: IMPACT OF DOSE AND REINFORCEMENT SCHEDULE

3.1. ABSTRACT

Nicotine reinforcement has recently been attributed to both the primary reinforcing effects of nicotine, and its ability to enhance reinforcement from non-nicotine stimuli. In support of the latter, nicotine infusions that are either self-administered (contingent) or response-independent (noncontingent) increase lever pressing for reinforcing nonpharmacological stimuli in rats. The present experiment examined the impact of contingent and noncontingent nicotine on responding for a moderately reinforcing visual stimulus in rats, across a range of nicotine doses on both fixed ratio and progressive ratio reinforcement schedules. Rats lever pressed for a visual stimulus with contingent nicotine, noncontingent nicotine or contingent saline. Separate groups responded for saline or nicotine without the visual stimulus. Three doses of nicotine (0.01, 0.03 and 0.09 mg/kg/inf, freebase) were tested in a between-groups design. After responding on an escalating fixed ratio reinforcement schedule rats were tested on a progressive ratio schedule. Compared to responding for the visual stimulus with saline, both contingent and noncontingent nicotine equally elevated lever pressing for the stimulus at each dose on fixed and progressive ratio schedules. In the absence of the stimulus, only the highest nicotine dose sustained self-administration. The ability of noncontingent nicotine to elevate responding for a reinforcing

visual stimulus occurs across a range of doses. Furthermore, self-administered and noncontingent nicotine equally increase motivation to obtain a reinforcing nonpharmacological stimulus, as reflected by performance on a progressive ratio schedule. Finally, in the absence of a contingent stimulus primary reinforcement from nicotine only weakly supports self-administration in rats.

3.2. INTRODUCTION

Theories of tobacco dependence and smoking have advanced considerably from the initial hypothesis that smoking is sustained largely by the direct primary reinforcing effects of nicotine, the key psychoactive ingredient in tobacco (USDHHS 1988). Current research has expanded with the awareness that smoking is a complex behavior, influenced by a combination of nicotine and non-nicotine factors. One line of investigation that has gained increasing attention focuses on the role of environmental stimuli in nicotine reinforcement. There is mounting support for the hypothesis that nonpharmacological stimuli interact with nicotine to enhance the reinforcing effects of smoking in humans (Rose and Levin 1991; Perkins et al. 1994), and nicotine self-administration in animals (Caggiula et al. 2001; Caggiula et al. 2002b; Donny et al. 2003; Chaudhri et al. in press; Chaudhri et al. 2005b).

Intravenous nicotine infusions reinforce operant responding (e.g., nose poke or lever press) in a variety of animal species (Ator and Griffiths 1983; Corrigall and Coen 1989; Tessari et al. 1995; Donny et al. 1995; Rose and Corrigall 1997; Picciotto et al. 1998). However, behavior is considerably elevated, particularly at low nicotine doses, when drug delivery is combined with a discrete nonpharmacological stimulus (Goldberg et al. 1981; Donny et al. 1999;

Caggiula et al. 2002a; Donny et al. 2003; Chaudhri et al. 2005b). In the absence of a contingent nonpharmacological stimulus only moderate levels of responding are achieved for nicotine, and larger nicotine doses are required (Donny et al. 2003; Chaudhri et al. 2005b). The importance of contingent stimuli in nicotine self-administration is further highlighted by evidence that removing the stimulus markedly reduces lever pressing for nicotine (Caggiula et al. 2001), and contingent presentations of the stimulus after nicotine is replaced with saline continue to support reduced but stable responding (Caggiula et al. 2001; Cohen et al. 2005). Finally, while removing both nicotine and the stimulus causes behavior to decrease to negligible levels, reintroducing the stimulus alone elicits rapid reinstatement of operant responding (Caggiula et al. 2001; Lesage et al. 2004; Paterson et al. 2005; Cohen et al. 2005). This body of research supports two important hypotheses. First, in the absence of a contingent nonpharmacological stimulus the primary reinforcing properties of nicotine only weakly support operant behavior. Second, nicotine and nonpharmacological stimuli interact to generate a substantial increase in positive reinforcement (Donny et al. 2003).

We have recently proposed that this interaction can be attributed to both the primary reinforcing effects of contingent nicotine which can establish concurrent environmental stimuli as conditioned reinforcers (Rose and Levin 1991; Cohen et al, 2005), and the potentially more salient capacity of nicotine to enhance the reinforcing properties of contingent non- nicotine stimuli (Donny et al. 2003; Chaudhri et al. in press). In support of the later effect, response-independent (noncontingent) subcutaneous (Olausson et al. 2004a; and unpublished data) and intravenous nicotine infusions (Donny et al. 2003) increase lever pressing for moderately reinforcing nonpharmacological stimuli in rats. In a study conducted by Caggiula and colleagues, rats that self-administered (contingent) nicotine combined with an unconditioned compound

visual stimulus (VS) also controlled the delivery of noncontingent nicotine or saline to separate rats that lever pressed only for the VS. Compared to responding maintained by the VS with saline, both contingent and noncontingent nicotine equally and robustly elevated lever pressing for the VS (Donny et al. 2003). This outcome strongly suggests that nicotine can enhance reinforcement derived from non- nicotine stimuli, through a mechanism that does not require a temporal relationship between nicotine delivery, stimulus presentations, or the behavior controlled by the stimulus.

This result was obtained using a dose of nicotine (0.03 mg/kg/in; freebase) that maintains peak levels of self-administration on an escalating fixed ratio (FR) reinforcement schedule when drug delivery is combined with a nonpharmacological stimulus (Corrigall and Coen 1989; Donny et al. 1998). Therefore, the first aim of the present experiment was to compare dose-response functions for the impact of self-administered and noncontingent nicotine on responding maintained by the VS on an FR schedule. If the ability of nicotine to enhance the reinforcing properties of non- nicotine stimuli is an essential component of nicotine reinforcement then contingent and noncontingent nicotine should have equal effects on responding for the VS across a range of doses. Alternatively, differences between the dose-response functions would suggest that the contributions of primary reinforcement (tested with contingent nicotine) and the reinforcement-enhancing effects of nicotine (tested with noncontingent nicotine) are potentially dissociable.

The second part of this study addressed the hypothesis that the behavioral effects of noncontingent nicotine (described above) reflect an increase in the reinforcing properties of the stimulus. Support for this hypothesis comes from the observation that noncontingent nicotine has little impact on responding for a minimally reinforcing tone-light stimulus (unpublished data).

However, noncontingent nicotine could also increase reinforced behavior by enhancing sensory components of the stimulus (Terry et al. 2002), facilitating the learning and memory of behavior driven by the stimulus (Levin 2002; Addy et al. 2003), or increasing attention towards the stimulus (Grilly et al. 2000; Stolerman et al. 2000; Rezvani et al. 2002). To determine if the behavioral effects of noncontingent nicotine on responding for the VS reflect an increase in the reinforcing value of the stimulus we examined the effects of contingent and noncontingent nicotine on responding for the VS on a progressive ratio (PR) reinforcement schedule, across a range of doses. PR schedules, in which the response requirements for the delivery of each consecutive reinforcement is increased during the session, provide a valuable index of how hard animals will work to obtain a reinforcer (Markou et al. 1993). Consequently, they have been widely used to demonstrate the motivational impact of reinforcers on behavior (Depoortere et al. 1993; Donny et al. 1999; Risner and Goldberg 1983; Stafford et al. 1998; Barr and Phillips 1999; Nicola and Deadwyler 2000). If nicotine increases the reinforcing strength of non- nicotine stimuli then it should also enhance motivation to obtain them. Therefore, both contingent and noncontingent nicotine should elevate responding for the unconditioned VS on a PR schedule.

3.3. MATERIALS AND METHODS

3.3.1. Subjects

This experiment used two cohorts of male, Sprague-Dawley rats (60 rats per cohort: Harlan Farms: 175-200 g on arrival) that were individually housed in a temperature-controlled environment (21°C) on a 12 hour reversed light/ dark cycle (lights off at 0700 hr). For 7 days prior to testing rats were habituated to the colony room, where they had unrestricted access to food and were weighed and handled daily. Following habituation, rats received 20 g rat chow per day for the remainder of the study. During self-administration testing food was provided in their home cages after each daily session. Rats had unlimited access to water at all times.

3.3.2. Apparatus

Training and experimental sessions occurred in 25x31x28 cm^3 operant conditioning chambers, which were outfitted with identical retractable levers, a food pellet trough directly in-between the levers, a white cue light above each lever, and an overhead house light located directly above the pellet trough near the roof of the chamber. During self-administration sessions rats were connected to a drug-delivery swivel system that allowed nearly unrestricted movement in the chamber. Responses on the active lever, inactive lever, and reinforcements (VS presentations and/or infusions) earned were recorded using an interfaced computer software package (Med Associates, MED-PC IV). Exhaust fans within each sound-attenuating chamber produced a

constant background noise (~ 75 db), which masked ambient noise as well as auditory cues associated with food and drug delivery.

3.3.3. Food training

Prior to testing, animals were habituated to the operant chambers in a single 20 min session. After overnight food deprivation they were allowed to consume 75 food pellets (45 mg) in a single magazine training session during which both levers were retracted. Next, they were food deprived overnight and hand-shaped to press the right (active) lever for 75 food pellets on a continuous reinforcement (FR 1) schedule in a single session. Rats that did not attain the criterion of responding for and consuming 50 pellets were re-shaped the following day. Responses on the left (inactive) lever had no scheduled consequences. During magazine and shaping sessions a dim red house light, to which Sprague-Dawley rats are relatively insensitive, remained illuminated. No scheduled changes occurred in either visual or auditory stimuli.

3.3.4. Surgery

Following food training rats were anesthetized with halothane, implanted with jugular catheters (Donny et al. 1999), and allowed at least 7 days to recover prior to the start of self-administration sessions. For two weeks after surgery rats were treated with heparin and streptokinase to help maintain catheter patency, and the antibiotic ticarcillan plus clavulanate (Timetin) to reduce post-surgical infections. Thereafter, catheters were flushed once daily with 0.1 ml sterile heparinized saline containing Timetin (30 U/ml) on weekends, and both prior to (10 U/ml) and following (30

U/ml) each session on testing days. Catheter patency was determined on days 15 and 27 by infusing a small volume of chloral hydrate through the catheter to induce a temporary loss of muscle tone.

3.3.5. Self-administration sessions

Prior to self-administration rats were randomly divided into 11 groups. Animals in these groups lever pressed for a compound visual stimulus (VS) with contingent or noncontingent nicotine, or lever pressed for nicotine infusions in the absence of the contingent VS. The delivery of noncontingent nicotine infusions was controlled by the self-administering rats, in a yoked design (Donny et al. 2003). Control rats responded for the VS combined with saline or saline infusions in the absence of the VS. Three nicotine doses (0.01, 0.03 and 0.09 mg/kg/inf; freebase) were tested in a between-groups design. This range of nicotine doses supports robust self-administration when nicotine delivery is combined with a nonpharmacological stimulus (Corrigall and Coen 1989; Shoaib et al. 1997; Donny et al. 2000). All infusions were delivered in a volume of 0.1 ml/kg over ~ 1 sec. The VS consisted of the onset of a white cue light for 1 sec, followed by the offset of a white house light for 1 min. The house light offset signaled a time-out period during which responding on the active lever was recorded but not reinforced (Donny et al. 2003). Rats that lever pressed for nicotine or saline without the VS received an unsignalled 1 min time-out following each infusion.

Self-administration sessions began on a Monday and were conducted on weekdays during the dark phase of the light/dark cycle. FR sessions lasted for 60 min each day according to an escalating reinforcement schedule (FR 1, days 1-5; FR 2, days 6-8; FR 5, days 9-17). The same

rats were then tested on a PR reinforcement schedule for 12 days. PR sessions lasted for 220 min and used the formula 5xEXP(0.2xinfusion number) – 5 (Depoortere et al. 1993; Donny et al. 1999), which results in the following sequence of required responses per reinforcement earned: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 179, 219, 268, 328.

3.3.6. Statistical analyses

Prior to statistical analyses data were removed from 19 (out of 120) rats that failed one or both chloral hydrate tests for catheter patency, and 6 additional rats that were identified as statistical outliers using SPSS (v 11). The outlier analysis constructed separate box plots for each group to determine the median and interquartile range of active lever responses on each day of self-administration. Rats were identified as outliers if they exhibited extreme values (>3 box plot lengths from the upper or lower edge of the interquartile range) on 30 % of self-administration test days (Lehman 1995).

Separate analyses were conducted on responses (active and inactive) and number of reinforcements (VS presentations or infusions) earned. Because each group was represented approximately equally in both cohorts of the study, data were first analyzed for a main effect of Cohort. There was no main effect of Cohort for FR data. Therefore, subsequent analyses were conducted on data collapsed across both replications. The main effect of Cohort for PR data was followed up for each group by an analysis of variance (ANOVA) with Cohort as the between- and Day as the within- subjects factor. Significant comparisons are reported in the text. However, in the absence of a Cohort by Day interaction, PR data were collapsed across cohorts for subsequent analyses.

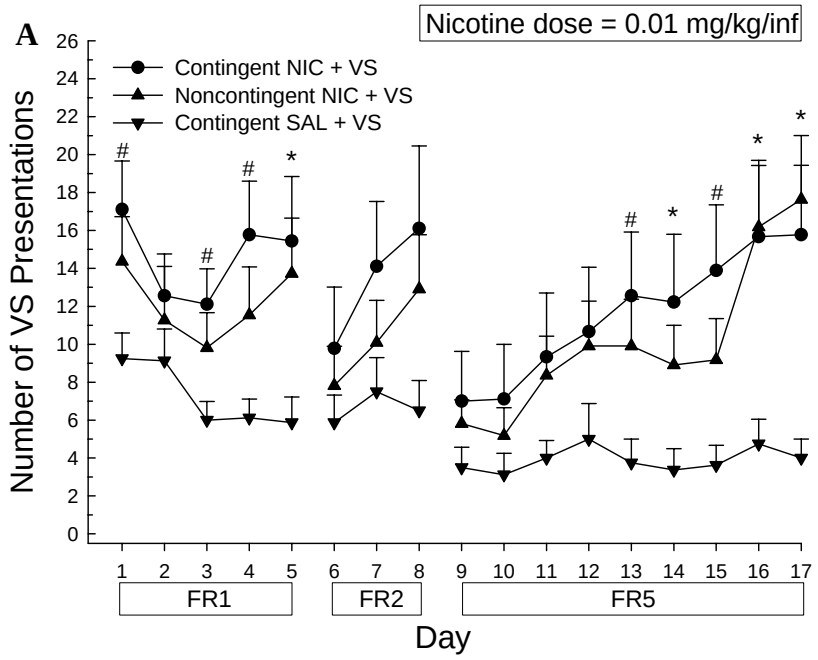
FR and PR data were analyzed separately, using ANOVA with Group as the between- and Lever and Day as the within- subjects factors for responses, and Group as the between- and Day as the within- subjects factors for reinforcements earned. With the exception of three groups (Contingent SAL + no VS; Contingent NIC + no VS, 0.01 mg/kg/inf; and Contingent NIC + no VS, 0.03 mg/kg/inf) responding on the active lever was significantly higher across Day compared to the inactive lever. Therefore, only data on reinforcements earned are presented. Pre-planned comparisons between groups utilized targeted three- and two- factor ANOVAs followed by t-tests for paired and independent samples. The α level was set to 0.05.

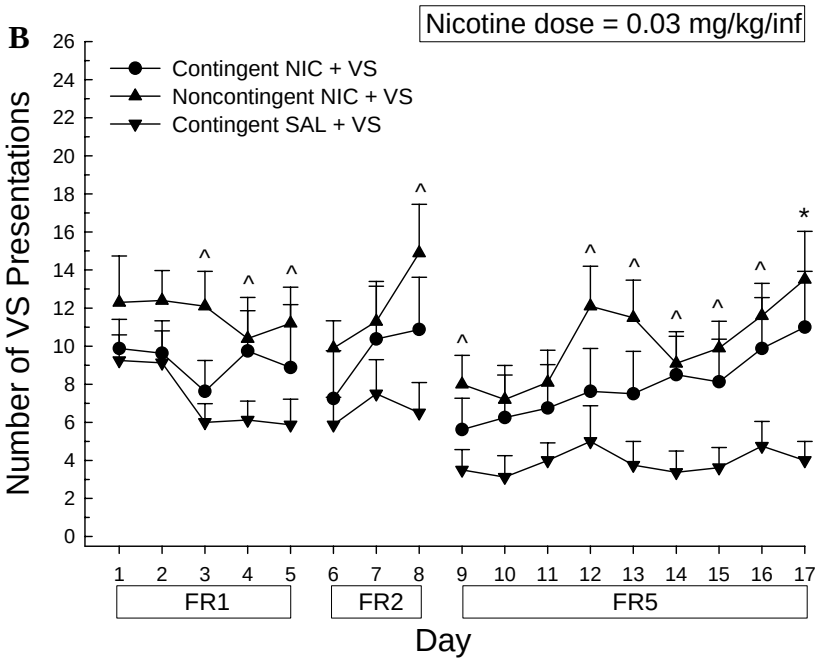
3.4. RESULTS

The number of VS presentations earned on an FR schedule was significantly elevated by both contingent nicotine [Group $F(1,15)=6.60$, $p<0.05$] and noncontingent nicotine [$F(1,17)=6.48$, $p<0.05$] at 0.01 mg/kg/inf, compared to SAL + VS (Figure 8A). This effect of nicotine was equivalent for both groups [$F(1,18)=0.47$, n.s.]. Similarly, at 0.03 mg/kg/inf, noncontingent nicotine enhanced the number of VS presentations earned compared to Contingent SAL + VS [$F(1,16)=13.33$, $p<0.01$; Figure 8B]. The comparison between Contingent NIC + VS and SAL + VS was not significant at this dose [$F(1,14)=2.42$, n.s.], although there was also no difference between the two nicotine conditions [$F(1,16)=1.26$, n.s.]. Further analysis revealed that 2 rats in the Contingent NIC + VS (0.03 mg/kg/inf) group earned < 2 VS presentations during each session on an FR 5 schedule, whereas the average for the remaining 6 rats was 11.53 (SEM $\pm$ 0.89). A subsequent comparison between this group and Contingent SAL + VS after excluding

the 2 low responders resulted in a significant main effect of Group [$F(1,12)=6.26$, $p<0.05$]. At the highest dose (0.09 mg/kg/inf) contingent nicotine [$F(1,16)=5.64$, $p<0.05$] and noncontingent nicotine [Day by Group interaction, $F(16,272)=4.82$, $p<0.001$] increased the number of VS presentations earned compared to Contingent SAL + VS (Figure 8C), and the impact of nicotine under both conditions was equivalent [$F(1,19)=0.05$, n.s.]. Finally, response contingent presentations of the VS with SAL supported higher levels of behavior compared to Contingent SAL + no VS [$F(1,15)=18.35$, $p<0.001$]. There was a main effect of Day ($p<0.05$) for each of the above comparisons, indicating that the number of VS presentations earned increased across the escalating FR reinforcement schedule. However, there was no main effect of Dose in comparisons between contingent and noncontingent nicotine groups.

In the absence of a contingent VS there was no difference in the number of infusions earned when responding was reinforced by saline or the two lowest doses of nicotine (0.01 and 0.03 mg/kg/inf; Figure 9). However, at 0.09 mg/kg/inf rats self-administered significantly more nicotine infusions compared to either saline [$F(1,13)=15.19$, $p<0.01$], or the two lowest doses of nicotine [0.01 mg/kg/inf; [$F(1,10)=8.84$, $p<0.05$: 0.03 mg/kg/inf; $F(1,10)=5.55$, $p<0.05$].


A
Nicotine dose = 0.01 mg/kg/inf
Contingent NIC + VS
Noncontingent NIC + VS
Contingent SAL + VS
Number of VS Presentations
FR1
FR2
FR5
Day


B
Nicotine dose = 0.03 mg/kg/inf
Contingent NIC + VS
Noncontingent NIC + VS
Contingent SAL + VS
Number of VS Presentations
FR1
FR2
FR5
Day

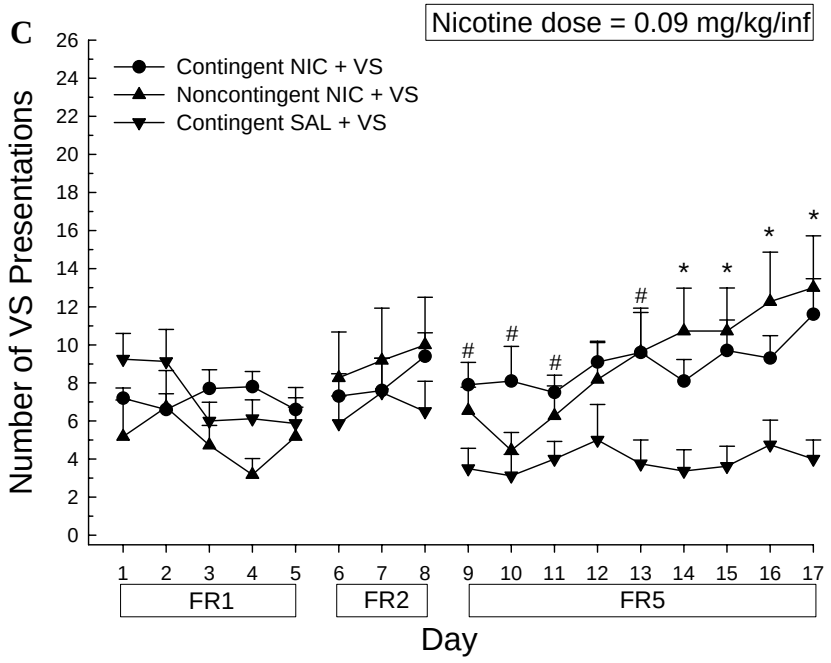


Figure 8: Effects of nicotine (NIC) or saline (SAL) on responding for the VS on an escalating fixed ratio reinforcement schedule. Data are mean (± SEM) VS presentations earned. (A) 0.01 mg/kg/inf (B) 0.03 mg/kg/inf (C) 0.09 mg/kg/inf. Schedule of reinforcement is indicated below the abscissa. * contingent and noncontingent NIC greater than SAL ($p<0.05$). # contingent NIC greater than SAL ($p<0.05$). ^ noncontingent NIC greater than SAL ($p<0.05$)

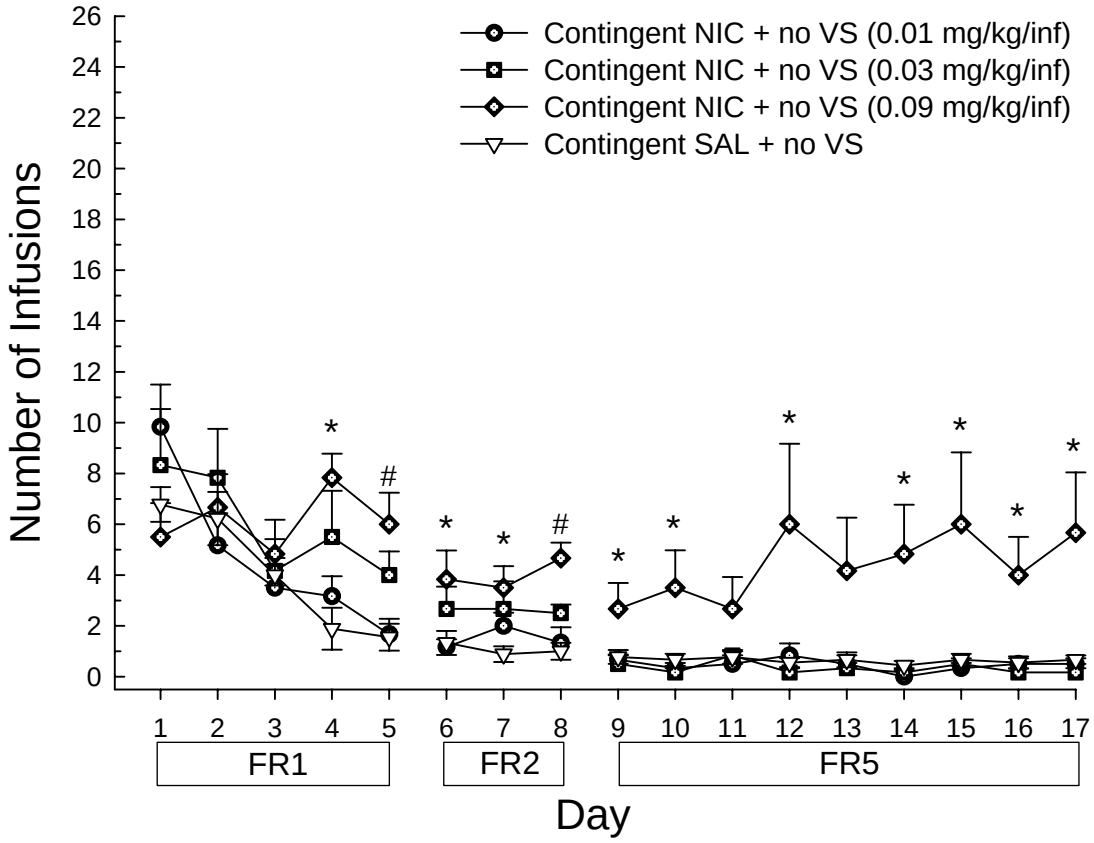


Figure 9: Dose effects of NIC and SAL on lever pressing in the absence of the contingent VS on an escalating fixed ratio reinforcement schedule. Data are mean (± SEM) number of infusions earned. Schedule of reinforcement is indicated below the abscissa. * 0.09 mg/kg/inf greater than SAL ($p<0.05$). # 0.09 and 0.03 mg/kg/inf greater than SAL ($p<0.05$)

The analysis of reinforcements (VS presentations or infusions) earned on a PR schedule resulted in a significant main effect of Cohort [$F(1,72)=5.38$, $p<0.05$], but no interaction between Cohort and Day [$F(11,110)=1.52$, n.s.]. Subsequent analyses within each group revealed that the main effect of Cohort was driven by higher response rates on a PR schedule, obtained for the second cohort of this study for the following groups: Contingent NIC + VS (0.03 mg/kg/inf); Noncontingent NIC + VS (0.03 mg/kg/inf); Contingent SAL + VS; Contingent NIC + no VS (0.01 mg/kg/inf). The direction of these effects did not impact significant outcomes in key between-group comparisons. Therefore, PR data were collapsed across cohort for all analyses.

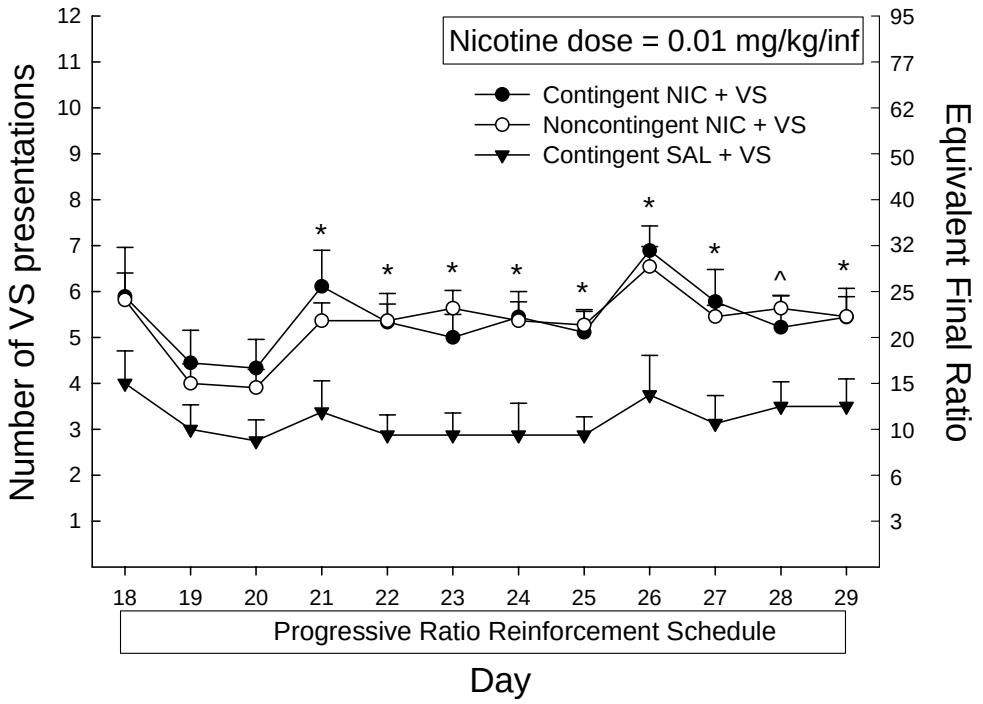
Compared to Contingent SAL + VS, both contingent and noncontingent nicotine equally elevated the number of VS presentations earned at 0.01 mg/kg/inf [Figure 10A; contingent, $F(1,15)=9.46$, $p<0.05$; noncontingent, $(F1,17)=15.27$, $p<0.001$), 0.03 mg/kg/inf [Figure 10B; contingent, $F(1,14)=6.79$, $p<0.05$; noncontingent, $(F1,16)=6.48$, $p<0.05$] and 0.09 mg/kg/inf [Figure 10C; contingent, $F(1,16)=12.14$, $p<0.05$; noncontingent, $(F1,17)=17.17$, $p<0.001$). The analysis of Dose for Contingent NIC + VS across day on a PR schedule indicated a significant Day by Dose interaction for the comparison between 0.03 and 0.09 mg/kg/inf [$F(11,176)=3.27$, $p<0.05$]; the number of reinforcements earned by rats self-administering 0.09 mg/kg/inf was significantly greater compared to 0.03 mg/kg/inf on days 21-24. No other dose comparisons were significant. In the absence of the contingent VS the number of nicotine infusions earned at the two lowest nicotine doses was no different from saline controls on a PR schedule (Figure 10 D). However, rats self-administering 0.09 mg/kg/inf earned more nicotine infusions compared to Contingent SAL + no VS [$F(1,13)=11.33$, $p<0.01$], as well as 0.01 mg/kg/inf [$F(1,10)=8.17$, $p<0.05$] and 0.03 mg/kg/inf [$F(1,10)=8.13$, $p<0.05$]. There was no dose-dependent difference between the two lowest doses.

Compared to Contingent NIC + no VS, combining nicotine delivery with the VS significantly increased self-administration at 0.01 mg/kg/inf [FR 5, $F(1,13)=11.41$, $p<0.01$; PR, ($F(1,13)=57.93$, $p<0.001$] and 0.03 mg/kg/inf [FR 5, $F(1,12)=7.96$, $p<0.05$; PR, $F(1,12)=59.25$, $p<0.001$]. This effect was also significant for 0.09 mg/kg/inf on an FR 5 schedule [$F(1,14)=6.84$, $p<0.05$], but not on a PR schedule. Finally, the number of reinforcements earned by SAL + VS rats on a PR schedule was significantly greater compared to rats that responded for SAL + no VS [$F(1,15)=17.03$, $p<0.001$].

There was a dose-dependent difference in the total amount of nicotine (mg/kg) that rats self-administered in combination with the VS on both FR 5 and PR schedules (Figure 11A). Although the two lowest doses did not differ on an FR 5 schedule, rats responding for 0.03 mg/kg/inf nicotine took more nicotine compared to 0.01 mg/kg/inf on a PR schedule [$F(1,15)=51.10$, $p<0.001$]. Furthermore, a significant Dose by Day interaction [$F(11,187)=2.19$, $p<0.05$] suggests that the amount of nicotine self-administered at 0.03 mg/kg/inf increased across PR sessions. Finally, on both reinforcement schedules, nicotine intake was significantly higher at 0.09 mg/kg/inf, compared to 0.01 mg/kg/inf (FR 5, $F(1,17)=35.16$, $p<0.001$; PR, $F(1,17)=68.80$, $p<0.001$] and 0.03 mg/kg/inf [FR 5, $F(1,16)=18.42$, $p<0.001$; PR, $F(1,16)=38.63$, $p<0.001$].

In the absence of the VS, nicotine intake was equivalent at the two lowest nicotine doses on an FR 5 schedule (Figure 11B). However, on a PR schedule rats self-administering 0.03 mg/kg/inf achieved marginally higher nicotine intake values compared to 0.01mg/kg/inf [$F(1,10)=5.01$, $p<0.05$]. Finally, nicotine intake was higher at 0.09 mg/kg/inf, compared to both 0.01 [FR 5, $F(1,10)=6.00$, $p<0.05$; PR, $F(1,10)=10.97$, $p<0.01$] and 0.03 mg/kg/inf [FR 5, $F(1,10)=5.84$, $p<0.05$; PR, $F(1,10)=10.26$, $p<0.01$].

A


Nicotine dose = 0.01 mg/kg/inf
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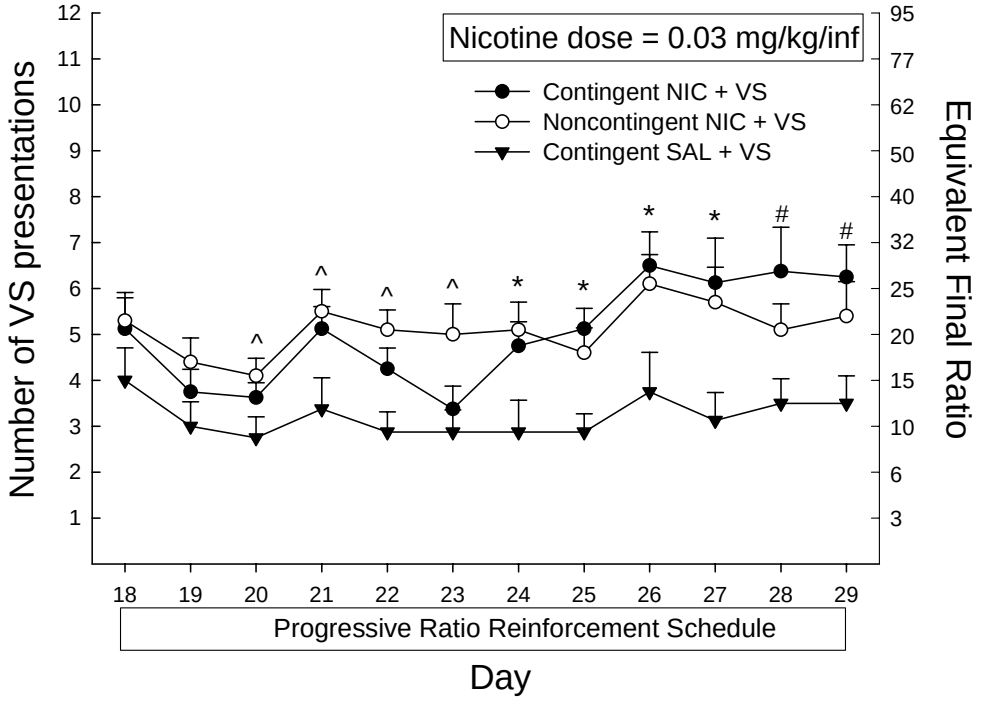
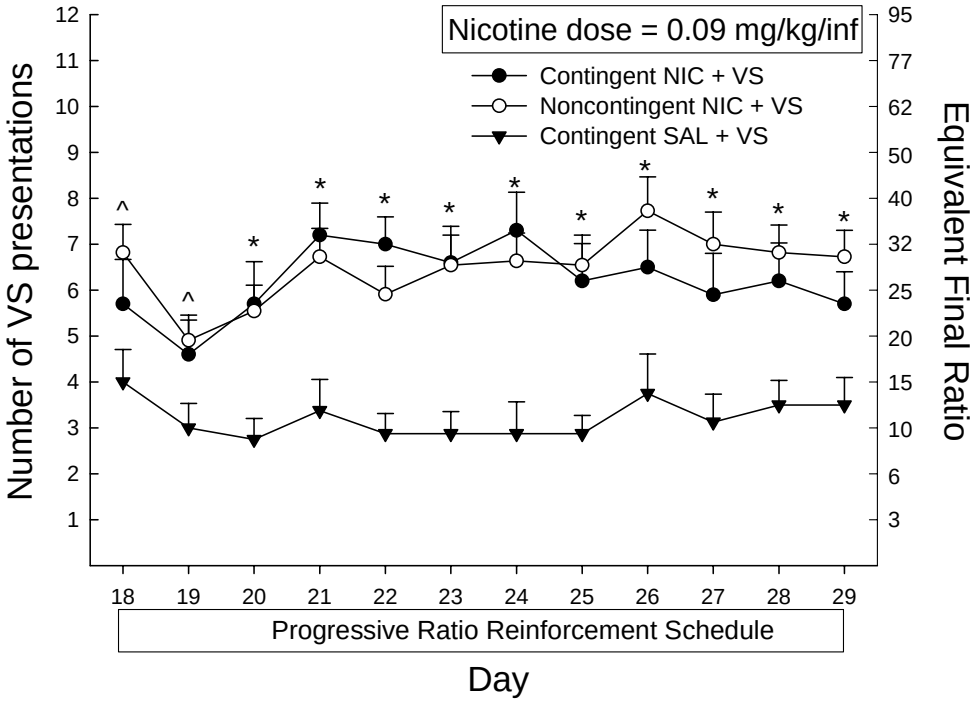
Noncontingent NIC + VS
Contingent SAL + VS
Number of VS presentations
Equivalent Final Ratio
Progressive Ratio Reinforcement Schedule
Day

B


Nicotine dose = 0.03 mg/kg/inf
Contingent NIC + VS
Noncontingent NIC + VS
Contingent SAL + VS
Number of VS presentations
Equivalent Final Ratio
Progressive Ratio Reinforcement Schedule
Day

C


Nicotine dose = 0.09 mg/kg/inf
Contingent NIC + VS
Noncontingent NIC + VS
Contingent SAL + VS
Number of VS presentations
Equivalent Final Ratio
Progressive Ratio Reinforcement Schedule
Day

D

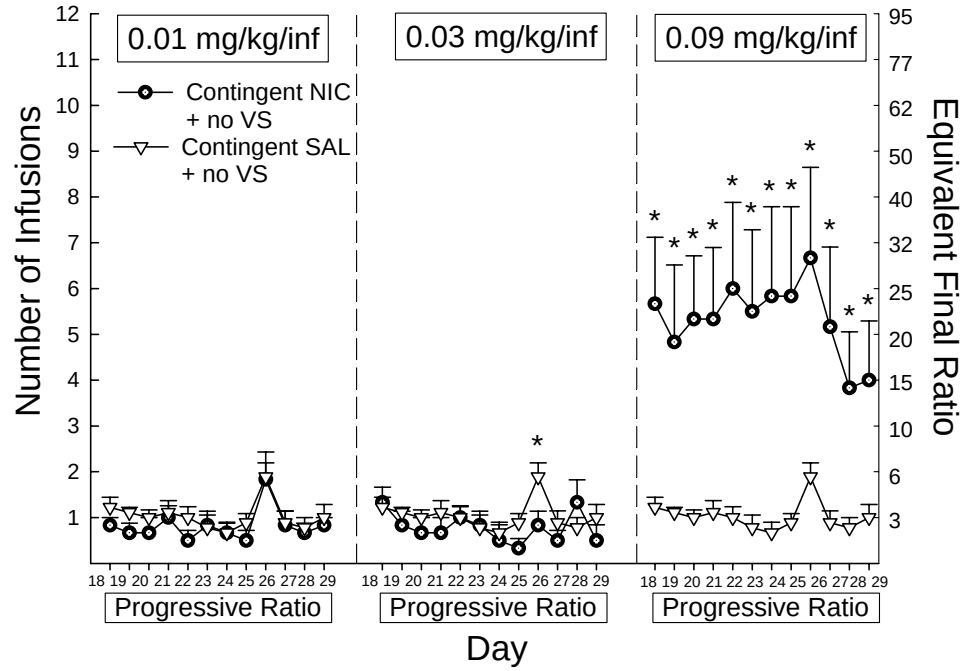

0.01 mg/kg/inf
0.03 mg/kg/inf
0.09 mg/kg/inf
Contingent NIC + no VS
Contingent SAL + no VS
Number of Infusions
Equivalent Final Ratio
Progressive Ratio
Progressive Ratio
Progressive Ratio
Day

Figure 10: Effects of nicotine (NIC) and saline (SAL) on responding for the VS on a progressive ratio reinforcement schedule. Right margin indicates highest ratio of responding achieved. (A) 0.01 mg/kg/inf (B) 0.03 mg/kg/inf (C) 0.09 mg/kg/inf. Data are mean (± SEM) number of VS presentations earned. * contingent and noncontingent NIC greater than SAL ($p<0.05$). # contingent NIC greater than SAL ($p<0.05$). ^ noncontingent NIC greater than SAL ($p<0.05$). (D) Dose effects of NIC or SAL (replicated in each panel) on lever pressing in the absence of the VS on a progressive ratio reinforcement schedule. Data are mean (± SEM) number of infusions earned. * 0.09 mg/kg/inf greater than SAL ($p<0.05$)

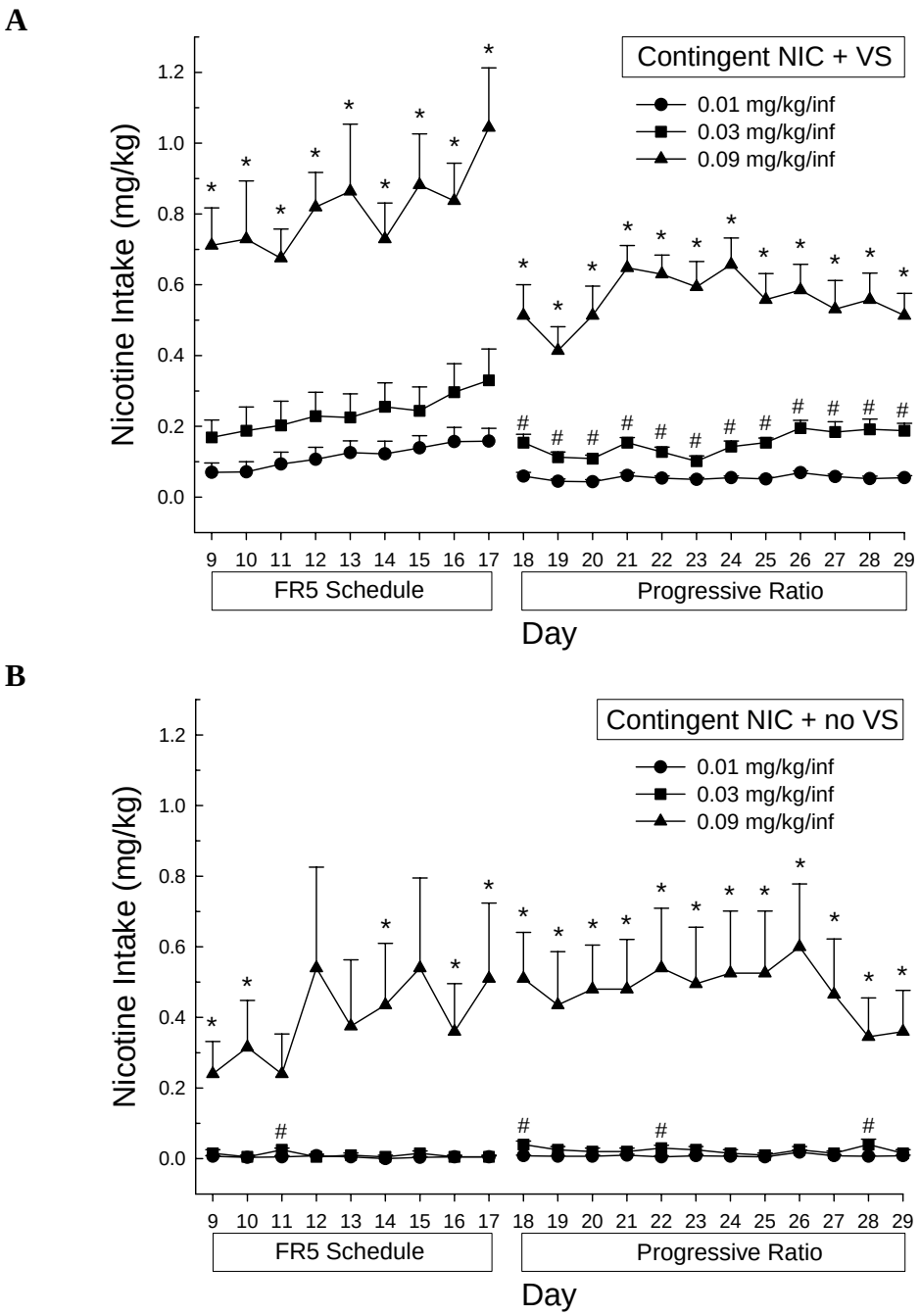


Figure 11: Total NIC intake (mean ± SEM; mg/kg) for rats that lever pressed for (A) contingent NIC + VS or (B) Contingent NIC + no VS on FR 5 and progressive ratio reinforcement schedules. * 0.09 greater than 0.01 and 0.03 mg/kg/inf ($p<0.05$). # 0.03 greater than 0.01 mg/kg/inf ($p<0.05$)

3.5. DISCUSSION

In the present experiment both self-administered (contingent) and response-independent (noncontingent) nicotine infusions elevated lever pressing for a moderately reinforcing, unconditioned VS. This effect was observed across a range of nicotine doses on both FR and PR reinforcement schedules. In the absence of the contingent VS only the highest nicotine doses (0.09 mg/kg/inf) sustained infusions rates above saline.

We have previously determined that a low dose of nicotine administered continuously throughout a 60 min FR session also enhances responding for the VS to a similar extent as pulsed infusions of contingent or noncontingent nicotine (Donny et al. 2003). Furthermore, lever pressing in the absence of a contingent VS with noncontingent nicotine is no different from the low levels of responding obtained with saline alone (Donny et al. 2003). These converging results demonstrate that nicotine can increase behavior maintained by moderately reinforcing non-drug stimuli, and strongly suggest that this action occurs through a non-associative mechanism.

The interpretation that an increase in operant responding for a contingent stimulus reflects an increase in the positive reinforcing value of that stimulus is prevalent in behavioral theories of reinforcement (Stewart 1960; Wise 1987). The present data demonstrate the ability of noncontingent nicotine to enhance responding for the VS using two distinct reinforcement schedules (FR and PR), which have been postulated to provide unique but complementary information on the processes that govern reinforcement. While FR schedules are thought to measure the hedonic impact of a drug, PR schedules have been used to index the motivational strength of pharmacological, natural and nonpharmacological reinforcers (Markou et al. 1993; Depoortere et al. 1993; Donny et al. 1999; Risner and Goldberg 1983; Stafford et al. 1998;

McGregor and Roberts 1995; Barr and Phillips 1999; Donny et al. 1999; Nicola and Deadwyler 2000). The present data suggest that regardless of contingency, nicotine can increase motivation to obtain the VS, which may reflect a corresponding increase in the incentive salience of the stimulus induced by nicotine.

Nicotine increased responding for the VS on a PR schedule, even though overall nicotine intake was markedly reduced on a PR schedule compared to the preceding FR 5 schedule. This result suggests that the ability of nicotine to enhance reinforcement can be elicited by relatively small quantities of nicotine. It is notable that even the smallest dose of nicotine administered, 0.01 mg/kg/inf, was sufficient to increase VS reinforced responding on both FR and PR schedules. Similarly, a small amount of subcutaneous nicotine delivered prior to testing can facilitate responding for the VS (unpublished data), and for a nonpharmacological conditioned stimulus (Olausson et al. 2004a). Further support for the hypothesis that only small amounts of nicotine are required to increase responding for reinforcing stimuli comes from a study in which rats responded on one lever for nicotine (0.06 mg/kg/inf), and a second lever for the VS (Palmatier et al. 2005). The number of responses on the nicotine lever was no different from that of control rats that only had access to contingent nicotine and responded at a fairly low level. However, responding on the VS lever was significantly higher than the behavior of control rats that only had access to the VS, and more closely approximated the behavior of rats that had access to an inactive lever and a lever that controlled the delivery of nicotine combined with the VS (i.e., a standard self-administration condition). These data demonstrate that the robust ability of nicotine to enhance responding for the VS can be achieved with small amounts of self-administered nicotine.

The present study provides additional confirmation of previous findings that nicotine and nonpharmacological stimuli interact to generate a substantial increase in self-administration, compared to nicotine administered without a contingent stimulus (Caggiula et al. 2002a; Donny et al. 2003; Chaudhri et al. in press; Chaudhri et al. 2005b). They also illustrate a recurrent observation in our research, that the interaction between nicotine and the VS appears to be stronger at lower, compared to higher nicotine doses (Chaudhri et al. 2005b; and present data). In the present study, the two low doses of contingent nicotine did not support self-administration without the VS, whereas 0.09 mg/kg/inf sustained moderate, albeit relatively variable infusion rates. However, combining contingent nicotine with the VS profoundly increased self-administration at the two lowest doses on both reinforcement schedules, and was significant (although less dramatic) only on an FR schedule for 0.09 mg/kg/inf. These outcomes indicate that the primary reinforcing effects of contingent nicotine can sustain moderate levels of nicotine reinforcement at high, but not low nicotine doses. They also suggest that the interaction between nicotine and a contingent non-drug stimulus is greater at nicotine doses that demonstrate only weak primary reinforcing effects, whereas the additional contribution of nonpharmacological stimuli is less apparent at nicotine doses that sustain moderate self-administration on their own.

The dose response function for contingent nicotine in combination with a nonpharmacological stimulus is typically a shallow, inverted 'U' shape, where responding peaks at low doses (0.01 – 0.03 mg/kg/inf) and then declines with increasing dose (Corrigall and Coen 1989; Shoaib et al. 1997; Donny et al. 1998; Rasmussen and Swedberg 1998; Chaudhri et al. 2005b). The lack of a dose effect in the impact of contingent and noncontingent nicotine on responding for the VS in the present experiment can be accounted for in at least two ways. First, in contrast to our previous research in which acquisition on an FR schedule is typically tested

over 20-25 days (Donny et al. 1999; Donny et al. 2003), rats in the present study had only 17 days of FR responding prior to the PR schedule. It is conceivable that dose effects would have emerged had responding been given longer to stabilize on an FR 5 schedule, which may consequently have translated into dose-dependent behavior on a PR schedule. Second, overall levels of behavior obtained in the present experiment were somewhat lower than previous observations, as is evident in responding maintained by the VS with saline. For example, in an earlier study male Sprague-Dawley rats earned 7.14 (SEM ± 0.15) VS presentations on an FR 5 schedule (Chaudhri et al. 2005b), whereas in the present study this average was reduced to 3.2 (SEM ± 0.22). This difference may be attributed to the specific shipments of animals used in this study, or to external conditions (e.g., noise levels generated by ongoing construction) that were beyond the control of the experimenters. Regardless, main effects in the present experiment were similar to pilot studies (unpublished data) and previously reported data (Caggiula et al. 2002a; Donny et al. 2003; Chaudhri et al. 2005b).

A divergence in the dose-response curves for the two nicotine conditions would have implied that the primary reinforcing effects of contingent nicotine can be dissociated from the reinforcement-enhancing effects common to both contingent and noncontingent nicotine, based simply on nicotine dose. While this conclusion cannot be drawn from the present findings, we have recently demonstrated that the primary reinforcing and reinforcement-enhancing effects of nicotine *can* be dissociated by varying the reinforcing strength of the nonpharmacological stimulus (Chaudhri et al. 2005a). In that experiment a tone-light stimulus was either repeatedly paired with sucrose pellets or explicitly unpaired from sucrose delivery prior to self-administration. Subsequently, responding for the stimulus from both training conditions was tested with contingent nicotine, noncontingent nicotine or noncontingent saline. In the presence

of saline, rats responded more for the sucrose-paired stimulus compared to the sucrose-unpaired stimulus, indicating that repeated prior association with sucrose had rendered the stimulus as a conditioned reinforcer, and that the two stimulus conditions – paired and unpaired – differed markedly in reinforcing strength. Contingent and noncontingent nicotine equally increased responding for the sucrose-paired (conditioned) stimulus on FR and PR schedules, and contingent nicotine also increased responding for the less reinforcing sucrose-unpaired stimulus. However, noncontingent nicotine was less effective in enhancing behavior maintained by the weaker sucrose-unpaired stimulus, particularly on a PR schedule. This interaction in the effects of contingent and noncontingent nicotine with the less reinforcing sucrose-unpaired stimulus has two important implications. First, it suggests that the primary reinforcing actions of contingent nicotine can enhance behavior maintained by weak nonpharmacological reinforcers, an effect that could result from the ability of contingent (but not noncontingent) nicotine to establish concurrent stimuli as conditioned reinforcers. Second, it suggests that the capacity of nicotine to enhance reinforcement is less robust if the stimulus itself is not a strong positive reinforcer. This study provides initial evidence for a model in which the primary reinforcing and reinforcement-enhancing properties of nicotine can be behaviorally dissociated.

In summary, the present experiment demonstrates that the ability of nicotine to enhance reinforced behavior is not limited by dose or reinforcement schedule. These findings corroborate the hypothesis that nicotine has dual roles in reinforcement: it can function as a weak primary reinforcer when administered contingently, and can increase reinforced responding, an action that does not require response-dependent nicotine delivery (Rose and Levin 1991; Cohen et al. 2005; Donny et al. 2003; Olausson et al. 2004a; Chaudhri et al. in press). In this respect, nicotine parallels psychostimulant drugs such as cocaine and amphetamine, which have been extensively

studied and shown to exhibit analogous dual effects in reinforcement (Robbins 1976; Taylor and Robbins 1984; Robbins et al. 1989; Phillips and Fibiger 1990). The insight that nicotine can enhance reinforcement through non-associative mechanisms extends our evolving understanding of how nicotine, a relatively weak primary reinforcer, can mediate complex behaviors such as self-administration and smoking.

4. PRIMARY REINFORCEMENT AND THE REINFORCEMENT-ENHANCING ACTIONS OF NICOTINE CAN BE DISSOCIATED BY MANIPULATING THE STRENGTH OF A RESPONSE-CONTINGENT CONDITIONED STIMULUS.

4.1. ABSTRACT

Nicotine infusions that are either self-administered (contingent) or response-independent (noncontingent) increase responding for an unconditioned nonpharmacological stimulus in rats, suggesting that nicotine reinforcement is partly derived from its ability to enhance the reinforcing properties of non-nicotine stimuli. In addition, nicotine is a weak primary reinforcer, capable of supporting moderate behavior in the absence of concurrent nonpharmacological stimuli. Here we attempted to dissociate the primary reinforcing effects of nicotine from its ability to enhance reinforcement from non-drug stimuli, using the nicotine self-administration paradigm. Rats were exposed to a stimulus that was either paired with or explicitly unpaired with sucrose pellets, and the ability of the stimulus to reinforce new learning was tested. Subsequently, rats in each training condition lever pressed for the stimulus in the presence of contingent nicotine, noncontingent nicotine (0.06 mg/kg/inf, freebase) or noncontingent saline. Another group of rats with sucrose-paired training self-administered nicotine without the stimulus. After acquisition on a fixed ratio schedule, rats were tested on a progressive ratio reinforcement schedule. Prior association with sucrose made the stimulus a stronger reinforcer, compared to sucrose-unpaired training. Contingent and noncontingent nicotine elevated responding equally for the more reinforcing (sucrose-paired) stimulus; however for the less reinforcing (sucrose-unpaired) stimulus, contingent nicotine was more effective than noncontingent nicotine on both fixed and progressive ratio schedules. These data indicate that the reinforcing strength of the stimulus determines whether noncontingent nicotine can (non-

associatively) enhance its reinforcing effects, and suggest that for weakly reinforcing stimuli, associative processes derived from the primary reinforcing effects of contingent nicotine are also required.

4.2. INTRODUCTION

There is mounting support for the hypothesis that non-pharmacological stimuli play an important role in the maintenance of smoking behavior and its relapse after quitting (Rose and Levin 1991; Rose et al. 2000; Caggiula et al. 2001; Caggiula et al. 2002b). For example, exposure to visual stimuli (cues) associated with smoking significantly elevates the urge to smoke (Drobes and Tiffany 1997), and blocking visual and olfactory smoking cues decreases reinforcement derived from smoking (Perkins et al. 2001). Such smoking cues have also been shown to strongly activate brain regions correlated with reward, arousal, and motivation (Brody et al. 2002; Due et al. 2002). Analogous results are obtained with behavioral models of nicotine reinforcement in animals. Contingent nonpharmacological stimulus presentations facilitate the acquisition (Goldberg et al. 1981; Caggiula et al. 2002a; Donny et al. 2003) and retard the extinction of nicotine self-administration (Caggiula et al. 2001; Cohen et al. 2005). Additionally, environmental stimuli associated with nicotine can reinstate drug seeking behavior after extinction (Caggiula et al. 2001; Lesage et al. 2004; Paterson et al. 2005) and activate prefrontal cortex and limbic areas in rats, as determined by patterns of immediate early gene expression (Schroeder et al. 2001).

These and other findings (Rose et al. 2000; Caggiula et al. 2002a; Heishman et al. 2004) suggest that nicotine and nonpharmacological stimuli interact to generate high rates of nicotine

self-administration in animals, and smoking in humans. Research on this interaction by our laboratory has led us to propose that nicotine reinforcement has at least two components; 1) the primary reinforcing effects of nicotine which can establish originally non-reinforcing environmental stimuli as conditioned reinforcers and requires response-contingent drug administration, and 2) reinforcement-enhancing effects by which nicotine increases behavior maintained by reinforcing environmental stimuli, an action that does not require a contingent relationship between drug administration and reinforced operant responding (Donny et al. 2003; Chaudhri et al. in press).

Accordingly, the interaction between nicotine and non-drug stimuli could result from repeated associations between the stimulus and the relatively weak primary reinforcing effects of simultaneous nicotine (Donny et al. 2003), which can increase the reinforcing capacity and subsequent behavioral control of the stimulus (Rose and Levin 1991; Caggiula et al. 2001; Cohen et al. 2005). Alternatively, nicotine could facilitate behavior driven by nonpharmacological stimuli through non-associative mechanisms (Donny et al. 2003; Chaudhri et al. in press). Converging studies indicate that noncontingent nicotine can increase responding for conditioned reinforcers (Olausson et al. 2004a; Olausson et al. 2004b), as well as intrinsically reinforcing nonpharmacological stimuli in rats (Donny et al. 2003). In the latter experiment, rats lever pressed for a moderately reinforcing compound visual stimulus with contingent saline, contingent nicotine, or noncontingent nicotine (delivery of noncontingent nicotine was controlled by the self-administering rats in a yoked design). Compared to saline, lever pressing was substantially and equivalently enhanced by both self-administered (contingent) nicotine, and by infusions of response-independent (noncontingent) nicotine. This outcome strongly suggests that

nicotine can enhance the reinforcement derived from salient nonpharmacological stimuli through non-associative mechanisms.

To fully appreciate the impact of nicotine in reinforcement, we sought to determine the extent to which primary reinforcement and the reinforcement-enhancing properties of nicotine contribute to nicotine self-administration in rats. The relative involvement of these two properties can be differentiated experimentally by dissociating nicotine delivery from operant responding maintained by nonpharmacological stimuli of distinct reinforcing capacities. For example, the dual-reinforcement hypothesis described above predicts that the reinforcement-enhancing effects of nicotine will increase behavior maintained by moderately reinforcing non-nicotine stimuli, be less effective for weakly reinforcing non-nicotine stimuli, and be ineffective for neutral stimuli. Furthermore, if this property is mediated through a non-associative mechanism then both contingent and noncontingent nicotine should be equally effective. The relationship between nicotine delivery and stimulus presentations becomes more meaningful when the stimuli are neutral or only weakly reinforcing. Pavlovian conditioning principles suggest that the reinforcing value of such stimuli will be strengthened by repeated associations between the stimulus and the primary reinforcing effects of contingent nicotine (Rose and Levin 1991). Consequently, the control over behavior exerted by this stimulus, now potentially a conditioned reinforcer, should be further enhanced by nicotine.

The present experiment addresses these predictions within the context of a nicotine self-administration paradigm in rats. Specifically, we investigated the impact of contingent and noncontingent nicotine on lever pressing maintained by a compound light-tone stimulus that was rendered either strongly or weakly reinforcing in the following way. Prior to testing with nicotine, one group of rats received stimulus presentations combined with the delivery of sucrose

pellets, while a second group received presentations of the same stimulus that were explicitly unpaired from sucrose pellets. The ability of the stimulus to reinforce lever pressing was then tested to demonstrate that the sucrose-paired stimulus had acquired conditioned reinforcing properties, and was therefore a stronger reinforcer compared to the same stimulus that was repeatedly unpaired with sucrose. Subsequently, rats from both training conditions were allowed to lever press on an escalating fixed ratio reinforcement schedule for stimulus presentations with contingent nicotine, noncontingent nicotine or noncontingent saline. To examine the primary reinforcing effects of nicotine alone, an additional group of rats with sucrose-paired training acquired self-administration of nicotine in the absence of a nonpharmacological stimulus. Rats were then tested on a progressive ratio reinforcement schedule to determine if the predictions outlined above would also be substantiated with a more rigorous reinforcement schedule, that is used largely to assess the motivational control of reinforcers on behavior (Markou et al. 1993; Stafford et al. 1998).

4.3. MATERIALS AND METHODS

4.3.1. Subjects

Male, Sprague-Dawley rats (Harlan Farms: 175-200 g) were individually housed in a temperature-controlled environment (21°C) on a 12 hour reversed light/ dark cycle (lights off at 0700 hr). For 7 days prior to testing they were habituated to the colony room where they had unrestricted access to food, and were weighed and handled daily. Following habituation, rats

received 18 g rat chow per day for the remainder of the experiment. During training and self-administration phases, food was provided in their home cages after each daily testing session. Rats had unlimited access to water at all times.

4.3.2. Apparatus

Training and experimental sessions occurred in 25x31x28 cm^3 operant conditioning chambers, which were outfitted with identical retractable levers, a food pellet trough directly in-between the levers and an overhead house light located directly above the pellet trough near the roof of the chamber. During self-administration sessions rats were connected to a drug-delivery swivel system that allowed nearly unrestricted movement in the chamber. Responses on the active (reinforced) lever, inactive (non-reinforced) lever, and reinforcements earned (stimulus presentations and/or infusions) were recorded using an interfaced computer software package (Med Associates, MED-PC IV). Exhaust fans within each sound-attenuating chamber produced a constant background noise (~ 75 db), which masked ambient noise as well as auditory cues associated with food and drug delivery.

4.3.3. Pavlovian training and test for conditioned reinforcement

Initially, rats were placed in the operant chambers for a single 20 minute habituation session during which the fans were turned on and levers were retracted. After overnight food deprivation they were given a single magazine training session during which the fans were turned on; levers were retracted; a dim red house light illuminated the chamber; and 45 sucrose pellets (45-mg)

were delivered on a fixed-interval 20 second schedule. Rats that consumed fewer than 40 pellets were re-trained the following day. Pilot studies indicated that prior exposure to sucrose pellets facilitates the attainment of this criterion. Therefore, rats were given small quantities of sucrose pellets in addition to their daily food ration for 5 days prior to magazine training.

Conditioning sessions were conducted for 14 days, Monday through Friday. Animals were randomly assigned to one of two groups in which a compound light-tone stimulus was either paired with (final n=41), or explicitly unpaired with (final n=25) the delivery of sucrose pellets. The same stimulus was used in both conditions, and consisted of the onset of a tone for 5 seconds (2900 Hz; 10 dB above the background noise of ~ 75 dB produced by the chamber fans) and the concurrent offset of a white house light. Rats were placed in the operant chambers with levers retracted, and the onset of the white house light signaled the start of each session. In the paired training condition the stimulus was presented according to a random-time (RT) 40-second schedule, and 2 sucrose pellets were rapidly delivered 2.5 seconds after stimulus onset. In the unpaired condition distinct RT 40-second schedules controlled the delivery of sucrose and the stimulus. Introducing a 3 second delay after both the stimulus and two sucrose pellets (delivered in rapid succession) ensured that they were explicitly unpaired. For each group, sessions ended after rats received 30 stimulus presentations and 60 sucrose pellets.

Following training, the ability of the stimulus to support lever pressing in the absence of sucrose was tested to determine its reinforcing properties (Robbins and Koob 1978; Di Ciano and Everitt 2004). Rats were placed in the operant chambers, a white house light came on to signal the start of the session, and 3 seconds later two response levers were extended into the chambers. The first lever an animal pressed was designated the active lever and produced the training stimulus, which was shortened to 3 seconds to maximize the number of presentations earned, and

therefore total exposure to the stimulus. Responding on the remaining lever (inactive) had no consequence. Initially, active lever presses were reinforced on a continuous reinforcement schedule. After 3 stimulus presentations, active lever pressing was reinforced on a variable-ratio 1:3 schedule. Sessions terminated 30-minutes after the first lever press.

4.3.4. Surgery

Subsequently, rats were anesthetized with halothane, implanted with jugular catheters, and allowed at least 7 days to recover prior to the start of self-administration sessions (Donny et al. 1995). For two weeks after surgery rats were treated with heparin and streptokinase to help maintain catheter patency, and the antibiotic ticarcillan plus clavulanate (Timetin) to reduce post-surgical infections. Thereafter, catheters were flushed once daily with 0.1 ml sterile heparinized saline (30 U/ml) containing Timetin on weekends, and both prior to (10 U/ml) and following (30 U/ml) each session on testing days. Catheter patency was determined on day 24 of the experiment by infusing a small volume of chloral hydrate through the catheter to induce a temporary loss of muscle tone.

4.3.5. Self-administration sessions

After recovery, rats were given 3 additional days of Pavlovian training according to the procedures described above. Self-administration sessions were initiated on the fourth day. Rats in each training condition were allowed to lever press for the training stimulus (5 second tone onset and concurrent house light offset) with contingent nicotine, noncontingent nicotine, or

noncontingent saline, in a between-groups design. Within each stimulus condition, rats that responded for the stimulus combined with contingent nicotine also controlled the delivery of noncontingent nicotine and noncontingent saline to separate animals that responded only for the stimulus, in a yoked design (Donny et al. 2003). An additional set of rats with sucrose-paired training lever pressed for nicotine in the absence of contingent stimulus presentations.

Self-administration was tested on weekdays in daily 60 minute sessions. During acquisition rats responded on an escalating fixed-ratio reinforcement schedule (FR 1, days 1-5; FR 2, days 6-8; FR 5, days 9-19). Following acquisition rats were put back on an FR 1 schedule for 5 additional days (days 20-24), and then tested on a progressive ratio schedule for 3 days (days 25-27). Progressive ratio sessions lasted for 180 minutes each day, and used the formula 5xEXP(0.2xinfusion number) – 5 (Depoortere et al. 1993; Donny et al. 1999). This formula resulted in the following sequence of required responses per reinforcer earned: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 179, 219, 268, 328. Because the progressive ratio schedule was used to determine if contingent and noncontingent nicotine influenced motivation to obtain the stimulus, rats that acquired nicotine self-administration without the stimulus were not tested.

The dose of nicotine used was 0.06 mg/kg/inf (calculated as freebase). This dose was selected because it has previously been shown to reinforce nicotine self-administration in the absence of a contingent nonpharmacological stimulus (Donny et al. 2003; Chaudhri et al. 2005b), but also has robust effects when self-administered in combination with a nonpharmacological stimulus (Donny et al. 1998). All infusions were delivered intravenously in a volume of 0.1 ml/kg over approximately 1 second. Stimulus presentation and contingent nicotine infusions were followed by a 1 minute unsignalled time-out period, during which responding on the active

lever was recorded but not reinforced. The active lever during self-administration was the first lever that rats pressed during the test for conditioned reinforcement.

4.3.6. Statistical analyses

Prior to statistical analyses data were removed from 3 rats that died during catheter implant surgery, 8 rats that failed the chloral hydrate test, and 7 rats that were identified as statistical outliers. A standard outlier analysis, conducted with the statistical package SPSS (v 11), constructed separate box plots for each group to determine the median and interquartile range of active lever responses on each day of self-administration. Rats were identified as outliers if they exhibited extreme values (>3 box plot lengths from the upper or lower edge of the interquartile range) on 15% of self-administration test days (Lehman 1995).

ANOVA with Group as the between- and Lever as the within-subjects factor was used to analyze the ability of the stimulus to reinforcer lever pressing during the test for conditioned reinforcement. A one-way ANOVA was also conducted on the number of stimulus presentations earned by each group. Group differences across the acquisition of self-administration (days 1-24) were analyzed for responses on the active and inactive levers, as well reinforcements earned (stimulus presentations and/or nicotine infusions). The ANOVA of responses used Group as the between- and Lever and Day and the within-subjects factors. The ANOVA conducted on reinforcements earned used Group as the between- and Day as the within-subjects factors. Similar main effects and interactions were observed in both analyses, and responding on the active lever was significantly greater than the inactive lever in all groups except Contingent NIC + no stimulus. Therefore, only data on number of reinforcements earned are presented. Extreme

differences in variance between groups were found in the stimulus-unpaired condition, which suggested a bimodal distribution for the noncontingent nicotine group. Therefore, a chi square analysis was conducted on the proportion of rats that earned fewer than 5 presentations of the sucrose-unpaired stimulus (a criterion previously used to define significant self-administration; Donny et al. 1995) for contingent and noncontingent nicotine. Data for this analysis were average stimulus presentations earned by each rats across the last 2 days of each reinforcement schedule.

ANOVA with Group as the between- and Day (25-27) as the within-subjects factors was conducted on the number of stimulus presentations earned (break points) during the progressive ratio schedule. Additionally, the interaction between drug-contingency (contingent vs. noncontingent) and stimulus-pairing (sucrose-paired vs. sucrose-unpaired) was examined for the nicotine groups using ANOVA with Contingency and Pairing as the between- and Day (26-27) as the within-subjects factors. Only data from the last two days were used in this analysis, to exclude potentially unstable behavior resulting from the switch to a progressive ratio schedule. All main effects and interactions were further examined with targeted three- and two-factor ANOVAs, followed by t-test for paired and independent samples. The α level was set to 0.05.

4.4. RESULTS

The conditioned reinforcing properties of the compound light-tone stimulus that was repeatedly paired with sucrose are illustrated by the finding that in the test for conditioned reinforcement, rats with sucrose-paired training responded more on the stimulus reinforced lever, compared to

the inactive lever and to rats with unpaired training [Figure 12; Lever by Group, $F(1,64)=9.04$, $p<0.01$]. Consequently, rats in the sucrose-paired group earned more stimulus presentations than rats with unpaired training [Figure 12; $F(1,64)=9.99$, $p<0.01$].

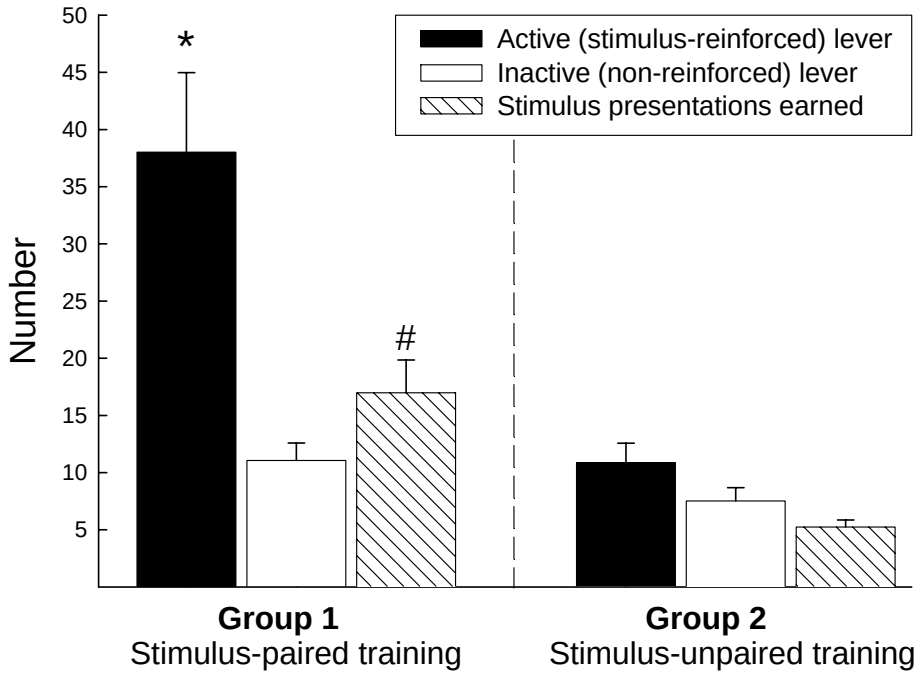


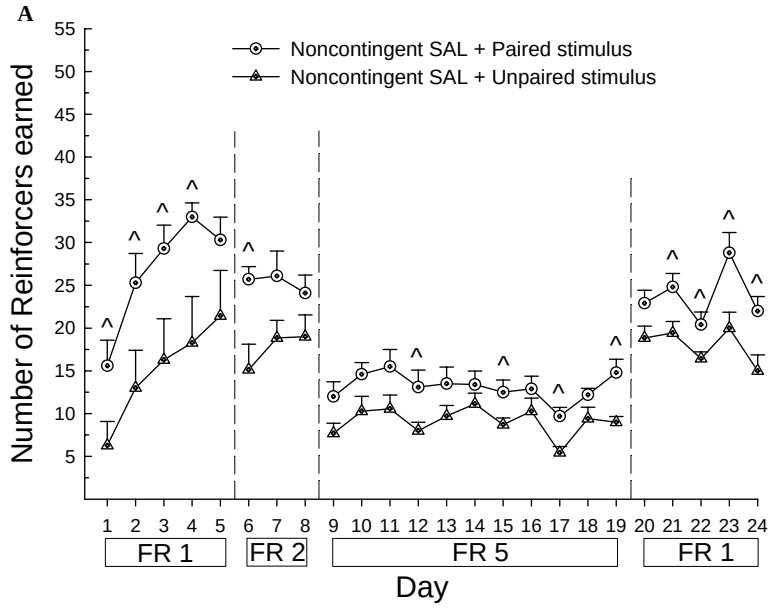
Figure 12: Conditioned reinforcing properties of the light-tone stimulus. Mean (± SEM) behavioral outcomes during a 30 minute test session by rats trained with a stimulus that was paired with sucrose (n=41), or the same stimulus that was explicitly unpaired with sucrose (n=25). * significant difference compared to inactive lever for rats with stimulus-paired training ($p<0.0001$). # significant difference in number of stimulus presentations earned ($p<0.01$)

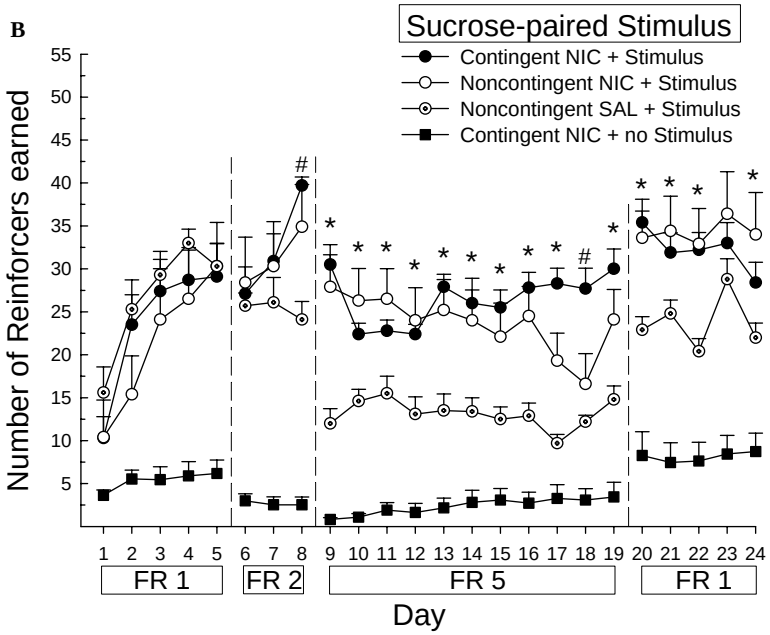
Regardless of prior training, rats in both groups continued to respond at moderate levels for the stimulus with noncontingent saline throughout the self-administration phase on an FR reinforcement schedule (Figure 13A). However, rats with sucrose-paired training earned consistently more stimulus presentations compared to rats in the unpaired group [$F(1,15)=11.92$, $p<0.01$], and t-tests for independent samples at each day indicate that this effect was most pronounced during the initial and final FR 1 reinforcement schedules.

Responding for the sucrose-paired stimulus during acquisition was significantly increased by both contingent nicotine [$F(1,18)=19.97$, $p<0.001$] and noncontingent nicotine [Day by Group, $F(23,414)=4.63$, $p<0.001$], compared to noncontingent saline (Figure 13B). Similarly, responding for the sucrose-unpaired stimulus was significantly elevated by both contingent nicotine [$F(1,14)=25.39$, $p<0.001$] and noncontingent nicotine [Day by Group, $F(23,322)=3.68$, $p<0.01$], compared to saline (Figure 13C). Individual comparisons between nicotine and saline groups on each day indicate that noncontingent nicotine significantly increased the number of sucrose-paired stimulus presentations earned on the first day of an FR 5 schedule, and on 14 of the remaining 16 FR sessions (Figure 13B). The impact of noncontingent nicotine on presentations of the weaker sucrose-unpaired stimulus was only significant on 3 of the 11 days on an FR 5 schedule, and 4 of the 5 days on the second FR 1 schedule (Figure 13C). In contrast, the effect of contingent nicotine on the sucrose-unpaired stimulus was evident much sooner than that of noncontingent nicotine, at the start of the FR 2 schedule, and continued until the final FR session (Figure 13C).

Combining contingent nicotine with the stimulus resulted in a significant increase in the number of reinforcers earned for both the sucrose-paired [$F(1,19)=176.35$, $p<0.001$] and sucrose-unpaired stimulus [$F(1,18)=113.71$, $p<0.001$], compared to contingent nicotine alone (Figures

13B and 13C). Furthermore, this effect was synergistic and not simply additive: for both stimulus-pairing conditions, the number of reinforcers earned when nicotine and the stimulus were combined was more than the sum of infusions earned by rats that responded for nicotine alone, plus stimulus presentations earned by rats that responded for the stimulus alone.





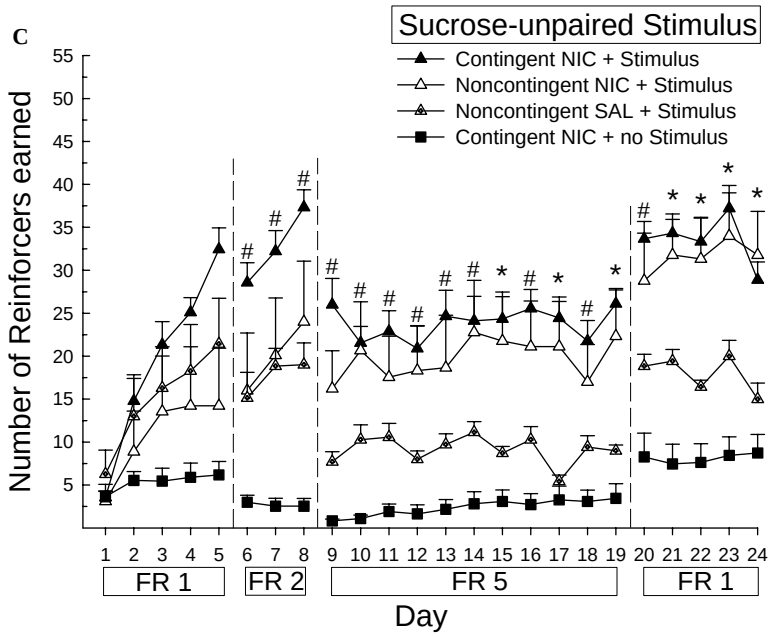


Figure 13: Effects of nicotine (NIC) or saline (SAL) on fixed ratio responding for a light-tone stimulus that was either previously paired with, or explicitly unpaired with sucrose pellets. Data are mean (±SEM) stimulus presentations or NIC infusions earned. (A) sucrose-paired or sucrose-unpaired stimulus (B) sucrose-paired stimulus; rats responded for the stimulus with contingent or noncontingent NIC, or for NIC without the stimulus (n=11) (C) sucrose-unpaired stimulus (NIC infusions for rats with sucrose-paired training that responded for NIC without the stimulus are duplicated). Schedule of reinforcement is indicated below the abscissa. ^ significant difference between noncontingent SAL + stimulus groups ($p<0.05$). # contingent NIC + stimulus greater than noncontingent SAL + stimulus ($p<0.05$). * both contingent and noncontingent NIC + stimulus greater than noncontingent SAL + stimulus ($p<0.05$)

Levene's test for equal variances between groups indicated that for the sucrose-unpaired stimulus condition, rats receiving noncontingent nicotine exhibited more variable behavior than both the contingent nicotine and noncontingent saline groups that received the unpaired stimulus. To examine this effect further, mean stimulus presentations earned by individual rats during the last two days of each schedule were analyzed. Figure 14 illustrates that rats receiving noncontingent nicotine in the sucrose-unpaired condition (n=9) can be separated into two distinct sub-populations based on the number of stimulus presentations earned. Across acquisition, the number of rats with fewer than 5 stimulus presentations is 6 (FR 1), 4 (FR 2), 3 (FR 5), and 1 (FR 1). A chi-square analysis conducted on the proportion of rats with fewer than 5 presentations of the sucrose-unpaired stimulus in the noncontingent nicotine and contingent nicotine groups revealed that the two groups were significantly different on the first FR 1 schedule [X^2 (1)=9, $p<0.01$] and on the FR 2 schedule [X^2 (1)=5.14, $p<0.05$]. This outcome suggests that noncontingent nicotine was initially less effective than contingent nicotine at elevating behavior driven by the sucrose-unpaired stimulus.

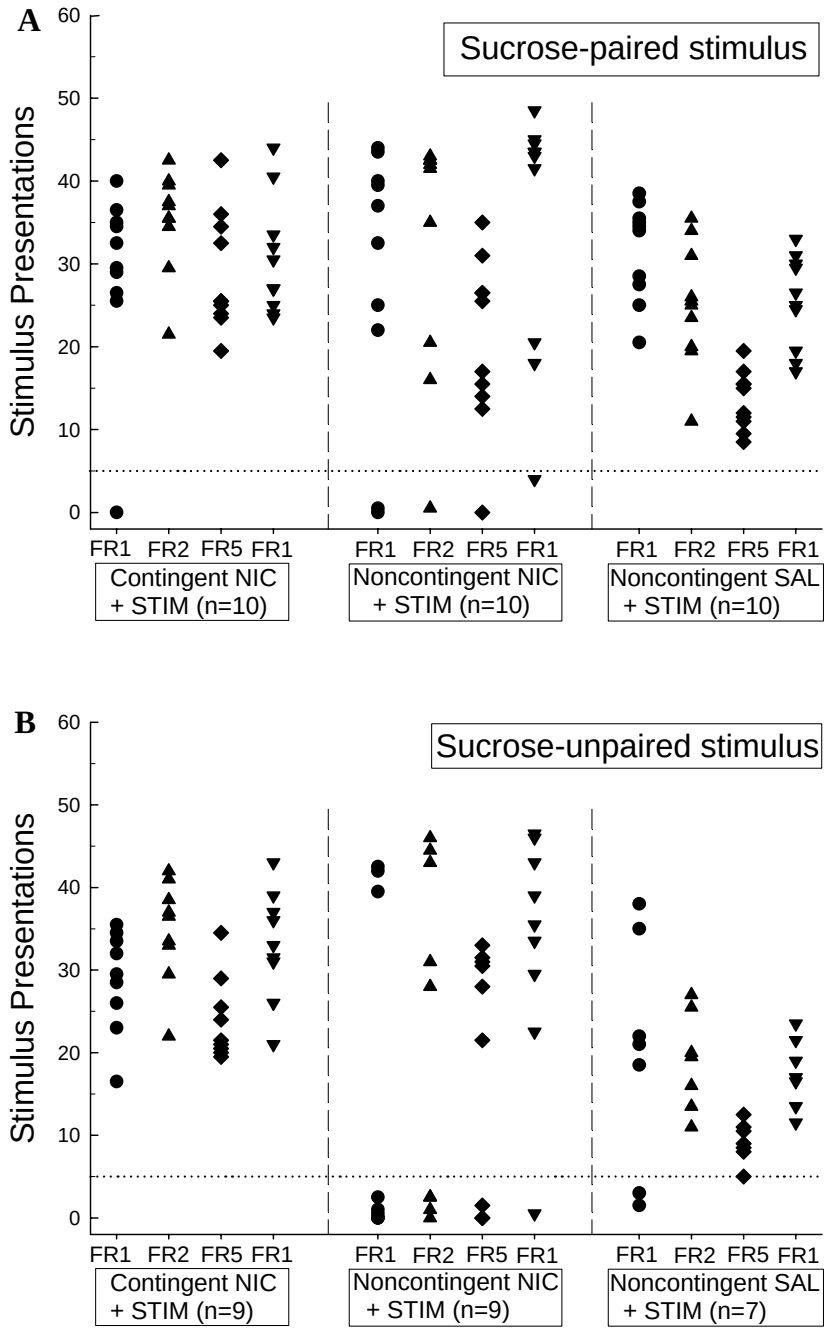
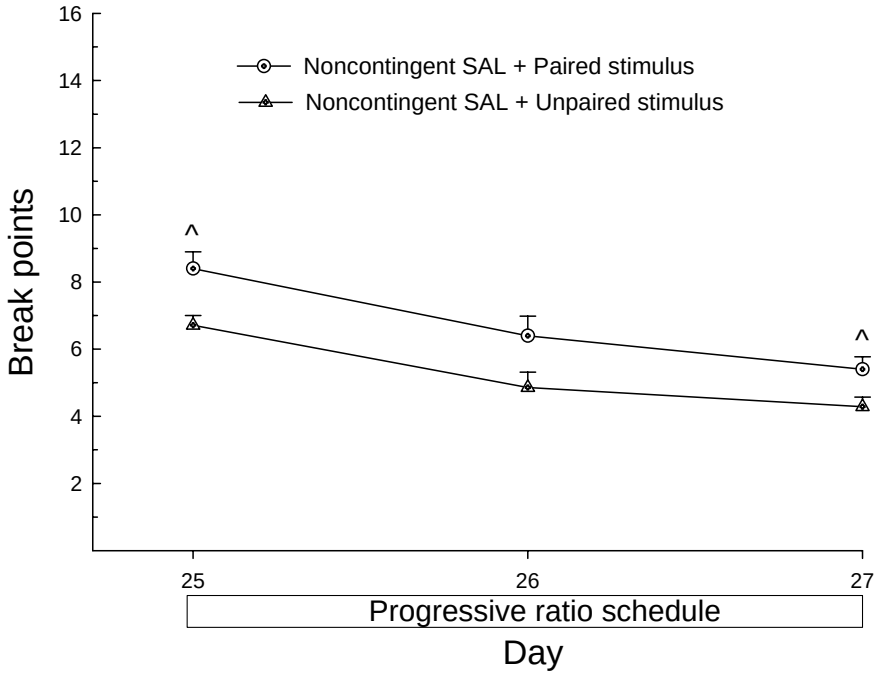


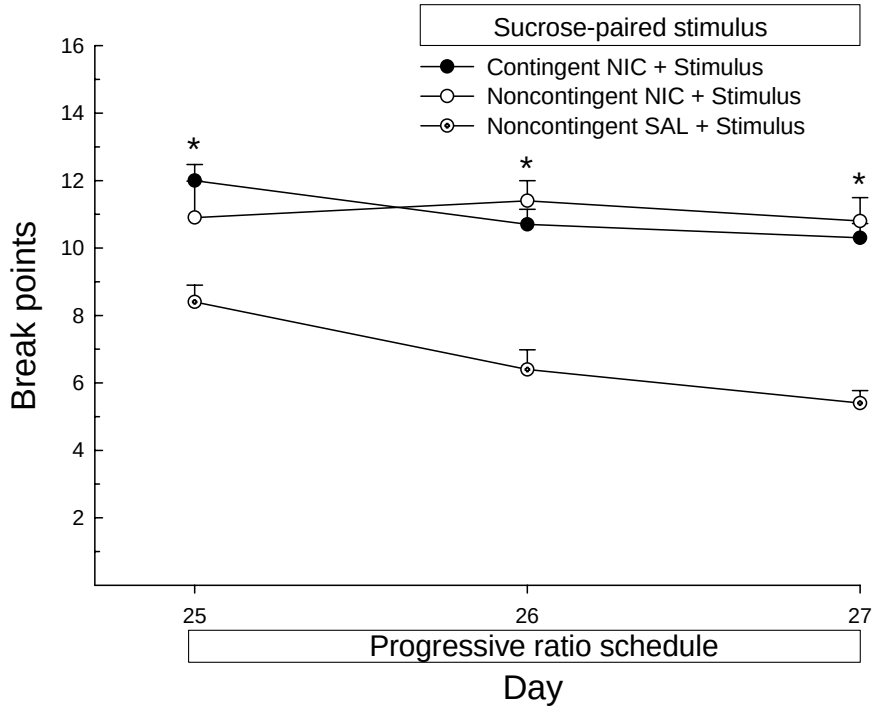
Figure 14: Number of stimulus presentations (mean ± SEM) earned across the last two days of each reinforcement schedule for individual rats in each group. (A) sucrose-paired stimulus (B) sucrose-unpaired stimulus. Schedule of reinforcement is indicated below the abscissa

On a progressive ratio schedule, rats with sucrose-paired training continued to earn more stimulus presentations, and therefore attained higher break points compared to rats with sucrose-unpaired training in the presence of noncontingent saline [Figure 15A; $F(1,15)=8.41$, $p<0.01$]. Both contingent nicotine [$F(1,18)=64.79$, $p<0.001$] and noncontingent nicotine [$F(1,18)=31.04$, $p<0.001$] increased break points equally for the sucrose-paired stimulus, compared to noncontingent saline (Figure 15B). Similarly, both contingent nicotine [$F(1,14)=42.38$, $p<0.001$] and noncontingent nicotine [$F(1,14)=11.996$, $p<0.01$] elevated break points for the sucrose-unpaired stimulus, compared to noncontingent saline (Figure 15C). However, a significant interaction between drug-contingency and stimulus-pairing [Contingency by Pairing, $F(1,34)=4.84$, $p<0.05$] indicated that although contingent nicotine increased break points equivalently for both the sucrose-paired and unpaired stimuli, noncontingent nicotine was less able to enhance break points for the weaker sucrose-unpaired stimulus (Figure 16).

A


Noncontingent SAL + Paired stimulus
Noncontingent SAL + Unpaired stimulus
Break points
16
14
12
10
8
6
4
2
^
^
25
26
27
Progressive ratio schedule
Day

B


Sucrose-paired stimulus
Contingent NIC + Stimulus
Noncontingent NIC + Stimulus
Noncontingent SAL + Stimulus
Break points
16
14
12
10
8
6
4
2
*
*
*
25
26
27
Progressive ratio schedule
Day

C

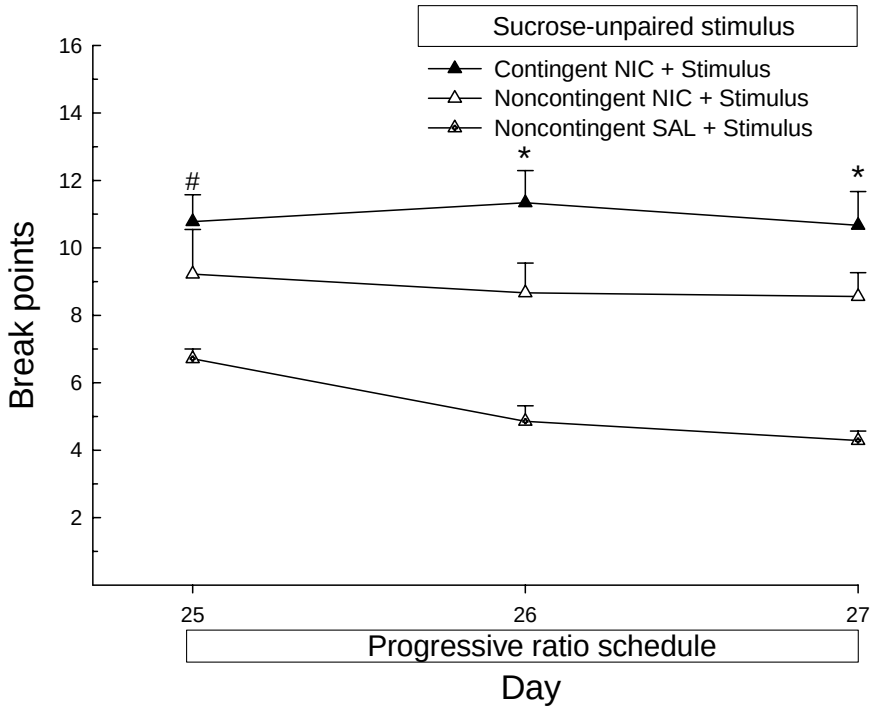


Figure 15: Effects of NIC or SAL on progressive ratio responding for a light-tone stimulus that was either previously paired with or explicitly unpaired with sucrose-pellets. Data are mean (± SEM) break points (stimulus presentation earned). (A) sucrose-paired or sucrose-unpaired stimulus (B) sucrose-paired stimulus, (C) sucrose-unpaired stimulus. ^ significant difference between noncontingent SAL + stimulus groups ($p<0.05$). # contingent NIC + stimulus greater than noncontingent SAL + stimulus ($p<0.05$). * both contingent and noncontingent NIC + stimulus greater than noncontingent SAL + stimulus ($p<0.05$)

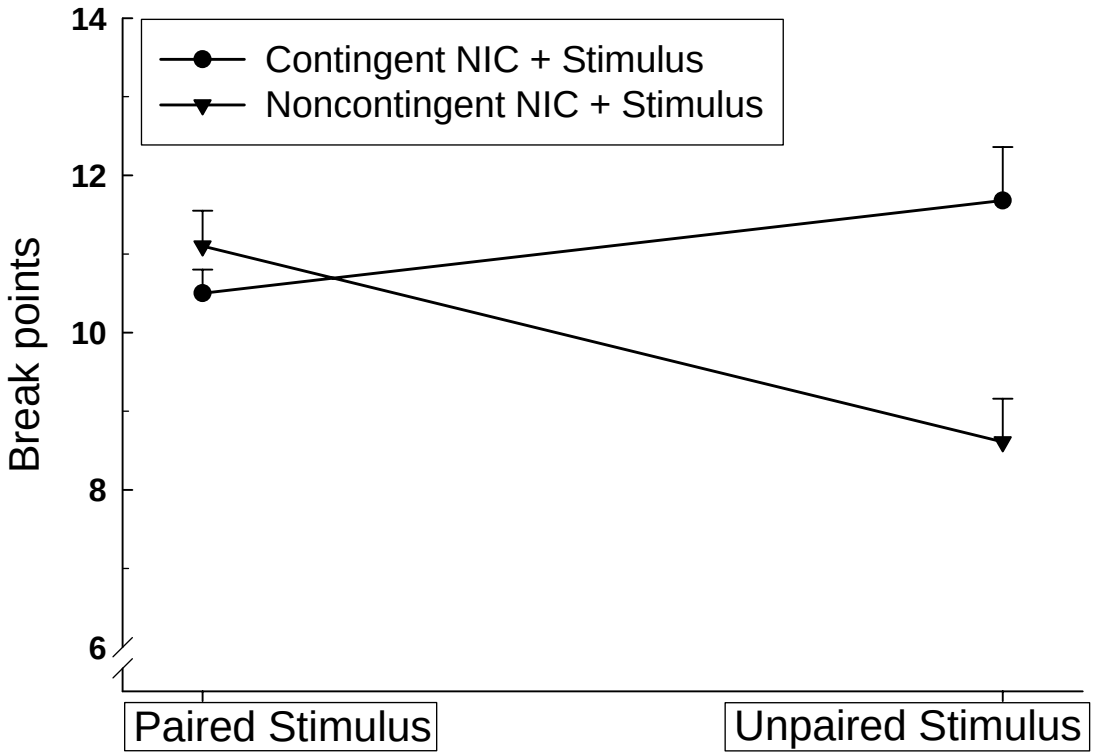


Figure 16: Interaction between stimulus-training condition (sucrose-paired vs. sucrose-unpaired) and drug contingency (contingent NIC vs. noncontingent NIC). Data are mean (± SEM) VS presentations earned on the last 2 days of the progressive ratio schedule

4.5. DISCUSSION

Repeated prior association with sucrose pellets made the compound light-tone stimulus a robust conditioned reinforcer compared to the same stimulus that was explicitly unpaired with sucrose. Contingent nicotine delivered in combination with the stimulus equally increased the number of stimulus presentations earned regardless of prior training. By contrast, noncontingent nicotine more effectively synergized with and increased responding for the conditioned (sucrose-paired) stimulus, when compared with the less reinforcing sucrose-unpaired stimulus. Finally, rats that self-administered nicotine without a nonpharmacological stimulus earned fewer infusions compared to rats that self-administered contingent nicotine combined with a stimulus. The interpretations and theoretical implications of these results are discussed below.

Conditioning sessions conducted prior to self-administration succeeded in manipulating the reinforcing strength of the tone-light stimulus. Initially, more lever pressing was maintained by the sucrose-paired stimulus compared to the unpaired stimulus, indicating that repeated association with sucrose established the stimulus as a conditioned reinforcer. This ability of conditioned stimuli to support the acquisition of a new response is widely-accepted as a stringent test for conditioned reinforcement (Robbins and Koob 1978; Taylor and Robbins 1984; Fletcher et al. 1999; Olausson et al. 2004a; b).

Regardless of training history the ability of the stimulus (with noncontingent saline) to reinforcer lever pressing increased rapidly during the first 5 days of self-administration, suggesting that either the stimulus had some unconditioned reinforcing properties or the unpaired condition of training nevertheless imparted some conditioning, and that the reinforcing capacity of the stimulus developed as rats learned the contingent relationship between lever pressing and

stimulus presentations (Stewart 1960). Nevertheless, the lasting impact of conditioning was evident throughout self-administration; in the presence of noncontingent saline, rats with sucrose-paired training earned significantly more stimulus presentations than rats with unpaired training. This effect was particularly robust during the initial and final FR 1 phases, suggesting that behavioral control exerted by conditioned stimuli may be better observed on a continuous reinforcement schedule. Similar results illustrating the long lasting impact of conditioned reinforcers have been obtained in other laboratories (Di Ciano and Everitt 2004; Ciccocioppo et al. 2004; Cohen et al. 2005). Finally, the relative strength of the sucrose-paired stimulus was also demonstrated on a progressive ratio schedule, where rats with sucrose-paired training achieved significantly higher breakpoints compared to rats in the unpaired condition.

Both contingent and noncontingent nicotine equally elevated behavior maintained by the sucrose-paired stimulus, on both fixed ratio and progressive ratio reinforcement schedules. This finding extends previous observations that noncontingent nicotine delivered intravenously (Donny et al. 2003) or subcutaneously (unpublished data) increases operant responding for an unconditioned reinforcing visual stimulus, and acute and chronic subcutaneous nicotine elevates responding for a conditioned visual stimulus (Olausson et al. 2004a; Olausson et al. 2004b). These converging outcomes suggest that nicotine can facilitate behavior maintained by distinct categories of reinforcing non-nicotine stimuli. Additionally, they suggest that the reinforcement-enhancing effect of nicotine is largely independent of the relationship between nicotine delivery and operant responding, and support the hypothesis that this effect is non-associative in nature (Donny et al. 2003; Chaudhri et al. in press; Chaudhri et al. 2005b).

Conversely, the impact of contingent and noncontingent nicotine on responding for the weaker sucrose-unpaired stimulus was less uniform; contingent nicotine more effectively

increased lever pressing for the sucrose-unpaired stimulus compared to noncontingent nicotine. On a fixed ratio schedule, while contingent nicotine first increased the number of stimulus presentations earned on an FR 2 schedule and had a consistently robust impact throughout acquisition, the reinforcement-enhancing effects of noncontingent nicotine with the sucrose-unpaired stimulus were only apparent towards the end of acquisition. This observation was supported by a nonparametric comparison of individual data from rats at each schedule. The analysis revealed that the proportion of rats with fewer than 5 presentations of the sucrose-unpaired stimulus was significantly higher with noncontingent nicotine compared to contingent nicotine on an FR 1 and FR 2 schedule; this difference decreased as the animals progressed to an FR 5 and the latter FR 1 schedule. The effectiveness of noncontingent nicotine at increasing behavior maintained by the sucrose-unpaired stimulus over time, which is suggested by this analysis, could result from an interaction between the reinforcement-enhancing effects of nicotine and an increase in the reinforcing strength of the stimulus as more rats learned the contingent relationship between lever pressing and stimulus delivery. Finally, when the schedule requirement for stimulus presentations was dramatically increased on a progressive ratio schedule noncontingent nicotine less effectively enhanced break points for the unpaired stimulus compared to contingent nicotine. This effect has two important implications. First, it suggests that repeated associations between contingent nicotine and the concurrent sucrose-unpaired stimulus increased the conditioned reinforcing properties of, and therefore behavior maintained by the stimulus. Second, it demonstrates that the reinforcement-enhancing property of nicotine, assessed with noncontingent drug administration, functions in parallel with the reinforcing strength of the contingent non-nicotine stimulus.

These interpretations are corroborated by a separate study in which operant responding for a distinct minimally reinforcing nonpharmacological stimulus (concurrent 5 second tone and cue light onset) was dramatically increased by contingent nicotine, whereas noncontingent nicotine had no impact on stimulus-reinforced behavior (unpublished data). This outcome is consistent with the hypothesis that while the reinforcement-enhancing effect of nicotine decreases as the reinforcing strength of the stimulus decreases, repeated associations between contingent nicotine and either a weakly reinforcing or neutral stimulus can establish the conditioned reinforcing properties of the stimulus. Subsequently, it is conceivable that the reinforcement-enhancing action of nicotine further elevated responding for this now conditioned stimulus. To test the latter hypothesis within the same experiment, drug contingency for both nicotine groups was reversed after responding on an FR 5 reinforcement schedule had stabilized. As predicted, noncontingent nicotine now maintained the high response levels achieved by the same rats that had previously lever pressed for contingent nicotine and the stimulus. This outcome provides compelling evidence that repeated prior associations with the primary reinforcing effects of contingent nicotine established the conditioned reinforcing properties of the stimulus, which were subsequently enhanced by noncontingent nicotine. Combining contingent nicotine with the stimulus in rats that had previously responded for the stimulus with noncontingent nicotine produced a steady increase in lever pressing, such that there was eventually no difference in responding between the two nicotine groups (unpublished data). This result suggests the gradual development of conditioning caused by the concurrent delivery of contingent nicotine and the stimulus.

The present experiment was designed to dissociate the actions of primary reinforcement and the reinforcement-enhancing properties of nicotine within the context of the self-

administration paradigm in rats. The procedures used to make this assessment were based on specific predictions regarding the interactions between contingent and noncontingent nicotine and a nonpharmacological stimulus that either demonstrated conditioned reinforcement, or was less able to reinforce operant responding. Specifically, we predicted that while both contingent and noncontingent nicotine would elevate responding for the reinforcing conditioned stimulus, only contingent nicotine would enhance lever pressing for the stimulus when it was a comparatively weaker positive reinforcer. The present results support these predictions and suggest three important conclusions. First, repeated association between the primary reinforcing effects of nicotine and a non-drug stimulus impacts behavior, and this effect is more pronounced when the stimuli themselves are not potent reinforcers. Second, the reinforcement-enhancing effects of nicotine assessed with noncontingent nicotine, are dependent on the reinforcing strength of the nonpharmacological stimulus. Third, both contingent and noncontingent nicotine increased responding for a conditioned reinforcer.

This latter effect has significant implications for smoking, as the sensorimotor components of smoking likely acquire conditioned reinforcing properties as a function of their association with nicotine (Rose and Levin 1991). Additionally, it has direct relevance for conditioned stimuli associated with other abused substances such as alcohol and cocaine. If the reinforcement-enhancing effect of nicotine impacts a range of salient nonpharmacological stimuli, then it could also enhance the ability of environmental stimuli conditioned to other drugs to elicit craving and drug seeking. In support of this hypothesis, nicotine has been shown to augment cocaine craving produced by cocaine-related cues (Reid et al. 1999), and this effect is reduced by the nicotinic receptor antagonist, mecamylamine (Reid et al. 1999). Smoking cues can also stimulate craving for alcohol (Palfai et al. 2000). In animal models of reinforcement,

pre-exposure to nicotine can facilitate the acquisition of cocaine self-administration (Horger et al. 1992), and increase ethanol intake (Potthoff et al. 1983; Clark et al. 2001) in rats. The potential for nicotine delivered via smoking to increase the incentive-motivational properties of alcohol- or cocaine-conditioned cues has direct bearing on treatment strategies, which often focus on the target substance but do not include a concurrent plan for smoking cessation.

The hypothesis that nicotine has dual effects in reinforcement has origins in a similar explanation for the behavioral effects of psychostimulants in reinforcement. Cocaine and amphetamine, in addition to being primary reinforcers that can establish salient environmental stimuli as conditioned stimuli, also enhance behavior driven by conditioned reinforcers (Stein and Ray 1960; Hill 1970; Beninger et al. 1980; Robbins et al. 1989; Phillips and Fibiger 1990). The neurobiology of these distinct actions has been well elucidated for cocaine and amphetamine (Taylor and Robbins 1984; Taylor and Robbins 1986; Taylor and Horger 1999; Fletcher and Korth 1999; Fletcher et al. 1999; See et al. 2001). While basic circuits underlying nicotine reinforcement have been extensively investigated (Corrigall et al. 2000; Corrigall et al. 1994; Corrigall et al. 1992; Corrigall and Coen 1991; 1989; Jose Lanca et al. 2000; Lanca et al. 2000), only limited research has been conducted on the neural underpinnings for the interactions between nicotine and nonpharmacological stimuli (Liu et al. 2004; Cohen et al. 2005; Paterson et al. 2005). The present paradigm has potential utility in such investigations, because it provides a means to dissociate the primary reinforcing and reinforcement-enhancing effects of nicotine. Future experiments that target the neural mechanisms for these distinct behavioral actions of nicotine will undoubtedly advance our appreciation of nicotine reinforcement, and bring us closer to developing successful treatments for smoking cessation.

5. DISCUSSION

The following sections summarize main findings from the preceding chapters, and provide a broader theoretical context within which to interpret the results. Parallels between the behavioral actions of nicotine and other psychostimulant drugs are presented, and the implications of the present data for the neurobiology of nicotine reinforcement, and for smoking are discussed.

5.1. SUMMARY OF RESULTS

The overarching hypothesis investigated in this dissertation was that nicotine reinforcement arises from at least two sources; 1) primary reinforcement, an action that requires contingent nicotine administration, and 2) the capacity of nicotine to enhance reinforcement from non-nicotine stimuli, and action that occurs through non-associative mechanisms and therefore does not require response-dependent nicotine administration.

These data demonstrate that while nicotine in the absence of a contingent non-drug stimulus can reinforce moderate lever pressing at high doses, responding is substantially increased when nicotine delivery is combined with a discrete nonpharmacological stimulus. This interaction between nicotine and nonpharmacological stimuli is synergistic in nature, and can account for a considerable amount of the behavior obtained in nicotine self-administration.

Lever pressing for an unconditioned, reinforcing visual stimulus (VS) is equally enhanced by infusions of self-administered (contingent) or response-independent (noncontingent) nicotine. Noncontingent nicotine delivered in the absence of contingent VS presentations does not facilitate lever pressing. Furthermore, a low dose of nicotine delivered as a continuous infusion throughout the 1 hour test session produces a similar enhancement of behavior; like animals that received noncontingent pulsed nicotine, these rats also maintain elevated lever pressing for the VS, thereby negating the possibility that partial reinforcement from chance pairings between behavior and noncontingent pulsed nicotine delivery enhanced VS responding. The ability of nicotine to increase lever pressing for reinforcing non-drug stimuli is a specific property of stimulant drugs (e.g., nicotine, cocaine and amphetamine), because noncontingent delivery of food pellets does not impact responding for the VS, compared to lever pressing for the VS alone. Replacing either noncontingent or self-administered nicotine with saline produces an equal drop in lever pressing, and reinstating nicotine under both conditions results in an equivalent return to pre-extinction levels of responding. Moreover, the enhancement of responding for the VS by noncontingent nicotine is observed across a range of doses, and on both fixed ratio and progressive ratio reinforcement schedules. The latter condition, where the number of responses required for each reinforcer increases within each session, is an acknowledged test of the motivational impact of reinforcers on behavior. Collectively this evidence substantiates the hypothesis that one mechanism by which nicotine impacts reinforcement is by increasing the incentive value of reinforcing non-nicotine stimuli.

Finally, the primary reinforcing and reinforcement-enhancing actions of nicotine can be dissociated using nonpharmacological stimuli that have differing strengths as reinforcers. Contingent nicotine interacts with and elevates responding for conditioned stimuli and stimuli

that are weaker reinforcers. However, the reinforcement-enhancing ability of nicotine (assessed with noncontingent nicotine) elevates responding for stimuli that are reinforcing, but is less effective with weakly reinforcing or neutral stimuli.

5.2. PSYCHOSTIMULANTS INCREASE CONDITIONED REINFORCEMENT

The research described above strongly supports the hypothesis that nicotine has dual roles in reinforcement; in addition to its primary reinforcing effects these data suggest that nicotine can increase behavior maintained by reinforcing non-nicotine stimuli. While novel for nicotine, a similar hypothesis has been proposed to explain the impact of psychostimulant drugs (e.g., cocaine, amphetamine, pipradrol) on reward. Phillips and Fibiger (Phillips and Fibiger 1990) advocated that the behavioral effects of cocaine are controlled by two factors: its correlated ability to act as a strong primary reinforcer and establish environmental stimuli that are associated with cocaine delivery as conditioned reinforcers, and its capacity to enhance behavior controlled by cocaine-conditioned stimuli.

The ability of psychostimulants to establish nonpharmacological stimuli as conditioned reinforcers has been systematically investigated (Kruzich et al. 2001; Di Ciano and Everitt 2004; Ciccocioppo et al. 2004). Similarly, the facilitation of reward by psychostimulants has been extensively demonstrated with a number of behavioral models. Initial evidence came from studies of brain stimulation reward, where both amphetamine (Stein and Ray 1960; Stein 1961) and cocaine (Crow 1970) lowered thresholds for brain self-stimulation reward in rats. A comparable impact of nicotine on brain stimulation reward has also been demonstrated (Ivanova

and Greenshaw 1997; Bozarth et al. 1998a; Bozarth et al. 1998b; but see also Clarke and Kumar 1983). In particular, nicotine was shown to induce parallel leftward shifts in rate-frequency functions for both lateral hypothalamic and midline mesencephalic brain stimulation, which could reflect a synergism between nicotine and the reinforcing properties of brain stimulation (Bauco and Wise 1994). Apart from informing theories on common neurobiological substrates that mediate reinforcement from both abused drugs and intracranial self-stimulation, these data underscore the analogous behavioral effects of a relatively weak primary reinforcer, nicotine, and more salient primary reinforcers such as cocaine and amphetamine.

The observation that psychostimulants increase the reinforcing impact of sub-threshold frequencies of brain stimulation reward provides one source of evidence for the hypothesis that these drugs facilitate reward. A second line of support comes from an extensive literature demonstrating that like nicotine, psychostimulants also increase operant responding for conditioned reinforcers (Hill 1970; Bugelski 1938; Beninger et al. 1980). One of the most widely utilized behavioral models to study the reinforcement enhancing properties of stimulants is the acquisition of a new response procedure (described above). Using this paradigm, Robbins (Robbins 1976) demonstrated a potent, specific facilitation of responding on a lever that produced a conditioned reinforcer in rats injected with the psychostimulant pipradrol prior to testing. This effect was dose-dependent, and was not observed when the same stimulus was either never previously paired with water (the primary reward), or was only randomly correlated with water and therefore did not support conditioned reinforcement. Repeated treatment with pipradrol across six successive test sessions progressively enhanced responding for conditioned reinforcement, suggesting that the impact of psychostimulants on conditioned reinforcement is sensitive to neuroadaptations resulting from repeated drug exposure (Robbins 1978). In a

separate experiment pipradrol dose-dependently increased responding for a stimulus that was predictive of brain stimulation reward, and not for a distinct stimulus correlated with the unavailability of brain stimulation reward (Robbins and Koob, 1978).

To investigate the reinforcement-enhancing properties of cocaine within the context of self-administration, we conducted an experiment to determine if noncontingent cocaine delivery produced a similar enhancement in lever pressing for a nonpharmacological visual stimulus that has been observed previously with noncontingent nicotine (Chaudhri et al. 2003). Rats that self-administered cocaine infusions in combination with a reinforcing compound visual stimulus (VS; described above) also controlled the delivery of noncontingent cocaine to separate animals that lever pressed only for the VS. Like nicotine, both self-administered and noncontingent cocaine elevated responding for the VS, compared to rats that lever pressed for the VS alone. These data contribute to a growing literature on the ability of stimulants such as amphetamine, nicotine and cocaine to enhance the reinforcing properties of, and therefore behavior maintained by reinforcing nonpharmacological stimuli.

5.3. THE NEUROBIOLOGY OF NICOTINE REINFORCEMENT

The present research suggests that nicotine reinforcement derives from the primary reinforcing properties of nicotine, which include its ability to establish conditioned reinforcers, and the capacity of nicotine to enhance the reinforcing properties of salient non-nicotine stimuli. Before attempting to understand the neural circuitry underlying these specific behavioral effects, it is necessary to identify basic pathways that have been broadly implicated in nicotine

reinforcement. Extensive research suggests that the mesolimbic dopamine system and its afferent inputs from the pedunculopontine tegmentum (PPTg) are a candidate circuit. Systemically administered dopamine antagonists (Corrigall and Coen 1991), lesions of midbrain dopamine neurons (Corrigall et al. 1992), and microinfusions of either GABA receptor agonists (Corrigall et al. 2000) or the nicotinic receptor antagonist dihydro-beta-erythroidine (DHßE; Corrigall et al. 1994) into the ventral tegmental area (VTA) attenuate nicotine self-administration in rats. Lesions of cholinergic PPTg neurons that project to the VTA, and infusions of DHßE into an intact PPTg also reduce nicotine self-administration (Lanca et al. 2000), suggesting that cholinergic modulation of the mesolimbic system resulting from actions of nicotine at the PPTg impacts nicotine reinforcement. This hypothesis is strengthened by evidence that acute nicotine activates immediate early gene expression in the PPTg, although this effect occurs primarily in noncholinergic (possibly glutamatergic or gabaergic) projection- and inter-neurons (Jose Lanca et al. 2000).

Although this circuit is a proposed target for the actions of nicotine, it is not known if the same pathways mediate the interactions between nicotine and nonpharmacological stimuli. Compared to other abused drugs, the neurobiology underlying stimulus-drug interactions has not been well elucidated for nicotine. Within the context of self-administration a broad spectrum of receptor antagonists block cue-induced nicotine seeking in rats (Liu et al. 2004; Paterson et al. 2005; Cohen et al. 2005). However, little attention has been paid to identifying distinct neural structures that mediate either the establishment of conditioned reinforcers by nicotine, or the recently identified reinforcement-enhancing properties of nicotine. Therefore, the remainder of this section will focus on research that addresses similar questions for other psychostimulants, in an effort to provide testable hypotheses for future explorations on the neurobiology of nicotine

reinforcement. This strategy has considerable merit given the plentiful evidence that cocaine and nicotine produce many comparable neurobiological effects. For example, both drugs elicit immediate early gene expression in overlapping brain regions (Pich et al. 1997). Like cocaine, nicotine increases VTA dopamine neuron firing (Imperato et al. 1986; Peoples and Cavanaugh 2003) and elicits dopamine release in the nucleus accumbens (Di Chiara 2000; Balfour 2002); activation of this system has been widely implicated in the reinforcing properties of abused drugs (Wise and Bozarth 1987; Robinson and Berridge 1993) including nicotine (Balfour et al. 2000; Di Chiara 2000). Finally, environmental stimuli previously associated with either nicotine or cocaine activate analogous brain regions in human addicts (Childress et al. 1999; Due et al. 2002), and both nicotine and natural rewards induce similar patterns of localized immediate early gene expression in laboratory animals (Schroeder et al. 2001).

A key neuronal structure implicated in the ability of psychostimulants to enhance the behavioral control of conditioned stimuli is the nucleus accumbens (NAcc). Infusions of d-amphetamine into NAcc but not the caudate putamen or thalamus dose-dependently potentiate responding for conditioned stimuli (Taylor and Robbins 1984). Dopaminergic signaling is important for this effect, as suggested by evidence that responding for conditioned reinforcers is accelerated by intra-NAcc dopamine infusions (Robbins et al. 1989), and reduced by dopamine depletion of the NAcc (Taylor and Robbins 1986; Parkinson et al. 2002). Conversely, serotonin depletion augments the potentiation of conditioned reinforcement induced by intra-NAcc amphetamine (Fletcher et al. 1999) and in separate studies prior infusion of serotonin (5-HT) or prior activation of 5-HT_{1B} receptors in the NAcc attenuated the behavioral effects of intra-NAcc amphetamine (Fletcher and Korth 1999). These data suggest a potential role for serotonin in inhibiting the enhancement of responding for conditioned stimuli by psychostimulants. Finally,

prolonged exposure to either nicotine or cocaine before training in which a stimulus is associated with a primary reinforcer not only increases responding for that conditioned stimulus in the absence of drug, but also potentiates the enhancement of this effect by intra-NAcc amphetamine (Taylor and Horger 1999; Olausson et al. 2004b). Therefore, it is plausible that neuroadaptations resulting from repeated drug exposure further impact the reinforcement-enhancing properties of psychostimulants, adding another layer of complexity to the issue of exactly where and how associations between drugs and nonpharmacological stimuli are formed.

Converging evidence suggests that the establishment of such associations is mediated by the basolateral amygdala (BLA). BLA lesions and infusions of dopamine receptor antagonists into an intact BLA prevented cue-induced relapse of cocaine seeking (Meil and See 1997; See et al. 2001) and heroin-seeking in rats (Fuchs and See 2002). Animals with BLA lesions fail to acquire basic second-order conditioning (Lindgren et al. 2003), and do not readily acquire cocaine self-administration on a second-order reinforcement schedule (Whitelaw et al. 1996). Furthermore, BLA lesions reduce the potentiation of conditioned reinforcement by intra-NAcc amphetamine (Cador et al. 1989). In addition to the BLA, the orbitofrontal cortex is also thought to influence the execution of behavior reinforced by conditioned stimuli (Pears et al. 2003).

To the extent that neurobiological explanations for the behavioral effects of psychostimulants are comparable to those of nicotine, it is conceivable that the establishment of conditioned stimuli by nicotine is also mediated by the BLA, and that nicotine-induced dopaminergic signaling in the NAcc also enhances the reinforcing properties of non-nicotine stimuli. These experiments have yet to be conducted. However, in understanding the neurobiology of nicotine reinforcement it is necessary to consider an important effect of nicotine that occurs at the nicotinic acetylcholine receptor (nAChR). A vast literature demonstrates that

after initial activation, nAChRs rapidly desensitize and the duration of this inactivation varies according to the sub-unit composition of the receptor (Ochoa et al. 1990; Rosecrans and Karan 1993; Mansvelder and McGehee 2002). In light of this effect it has remained a paradox that heavy smokers, with fairly high circulating blood-levels of nicotine (Rose et al. 1999), demonstrate persistent smoking each day. If nAChRs go through periods of desensitization and functional inactivity, then what drives these remarkably high levels of smoking?

This review suggests that nicotine functions as more than just a primary reinforcer, and that a very prominent component of nicotine reinforcement is its ability to magnify the salience of non-nicotine stimuli. Therefore, it is plausible that the high levels of behavior observed in self-administration and in smoking are maintained by the enhanced reinforcing effects of non-nicotine factors, and that only small amounts of nicotine are necessary to drive this effect. In support of this hypothesis we found that when given a choice, rats typically self-administer very small amounts of nicotine controlled by one lever, which is enough to potentiate responding on a second lever for a reinforcing visual stimulus (VS; discussed above). However, in most cases the response patterns generated on each lever throughout the test session were parallel, although of different magnitudes, suggesting that the enhancement of responding for the VS resulted from a fairly continual interaction with nicotine. Actual levels of circulating nicotine were not determined in this study, which may have provided insight into the functional state of neuronal nAChRs. In a separate, preliminary study a single subcutaneous infusion of nicotine (0.4 mg/kg, freebase) increased responding maintained by the VS compared to saline (unpublished data). Together with the observation that acute nicotine increases responding for conditioned reinforcers (Olausson et al. 2004a), these data provide further support for the hypothesis that the reinforcement-enhancing effects of nicotine can be elicited by relatively small doses of nicotine.

5.4. IMPLICATIONS FOR SMOKING

A prevalent argument in the clinical literature is that smoking is maintained by both the direct primary reinforcing properties of nicotine and the related ability of nicotine to establish paired non-nicotine stimuli as conditioned reinforcers (Rose and Levin 1991; Caggiula et al. 2001). However, this theory only *partly* explains how nicotine, a demonstrably weak primary reinforcer, can exert the robust control over behavior that is observed in self-administration and in smoking. The present research extends this hypothesis and presents substantial empirical support for a novel role for nicotine in reinforcement. We propose that in addition to its primary reinforcing effects, nicotine can also enhance the reinforcing properties of non-nicotine stimuli through non-associative mechanisms, a property which has critical implications for the role of nicotine reinforcement in smoking.

The impact of discrete nonpharmacological stimuli on nicotine reinforcement has been widely established for smoking. For example, exposure to stimuli that are associated with smoking (smoking cues) significantly enhances the urge to smoke (Perkins et al. 1994; Drobes and Tiffany 1997; Tiffany et al. 2000). Smoking denicotinized cigarettes (i.e. receiving cues alone) produces comparable levels of smoke intake, satisfaction, and reduction of craving and withdrawal as smoking nicotine-containing cigarettes (i.e. cues plus nicotine) (Gross et al. 1997; Shahan et al. 1999; Rose et al. 2000; Shahan et al. 2001; Dallery et al. 2003). Similarly, the removal of visual and olfactory cues associated with cigarettes reduce overall measures of reinforcement derived from smoking (Perkins et al. 2001).

These select examples from a vast clinical literature exemplify the powerful impact of nicotine-related stimuli on behavior and motivation. The comprehensive hypothesis for nicotine reinforcement presented in this dissertation predicts that in addition to imparting value to non-

nicotine stimuli via Pavlovian conditioning, nicotine can directly increase reinforcement derived from both nicotine-conditioned stimuli, as well as other reinforcing non-nicotine components of smoking. An important consequence of these effects is that the reinforcement-enhancing properties of nicotine could also impact the behavioral effects of environmental stimuli that become conditioned reinforcers for other co-abused drugs, such as alcohol and cocaine. In support of this theory, Reid and colleagues (Reid et al. 1998) demonstrated that cue-induced cocaine craving was augmented by nicotine, and that the nicotinic receptor antagonist mecamylamine reduced this effect (Reid et al. 1999). Along similar lines, alcohol cues induce craving for tobacco smoking in subjects who abuse both drugs (Gulliver et al. 1995; Rohsenow et al. 1997; Drobes 2002), and smoking cues also elicit craving for alcohol (Palfai et al. 2000). In animal models, pre-exposure to nicotine has been shown to facilitate acquisition of cocaine self-administration (Horger et al. 1992), and increase ethanol intake (Potthoff et al. 1983; Clark et al. 2001) in rats. The potential for nicotine delivered via smoking to increase the incentive-motivational properties of alcohol- or cocaine-conditioned cues has considerable bearing on treatment strategies, which often focus on the target substance but do not include a concurrent plan for smoking cessation.

5.5. FUTURE DIRECTIONS

The research in this dissertation provides compelling support for the hypothesis that in addition to its primary reinforcing properties, nicotine can enhance behavior maintained by reinforcing non-nicotine stimuli through non-associative mechanisms. The implications of this hypothesis

for future studies on the neurobiology of nicotine reinforcement, and its relevance to smoking have been discussed. However, there remain at least two additional issues that when addressed, will expand our theoretical framework for understanding the interactions between nicotine and nonpharmacological stimuli.

First is the question - how well does the reinforcement-enhancing action of nicotine generalize to various classes of non-nicotine stimuli? We have demonstrated this effect with an unconditioned reinforcing visual stimulus, as well as a nonpharmacological conditioned reinforcer. The pronounced enhancement in operant responding for these stimuli with both contingent and noncontingent nicotine suggests that nicotine can increase the reinforcing value of stimuli that are positive reinforcers. However, to fully understand the breadth of this effect it is necessary to test the impact of contingent and noncontingent nicotine on a wider range of reinforcers. For example, does nicotine elevate behavior maintained by pharmacological or natural rewards, and will it increase operant responding for additional categories of nonpharmacological stimuli, such as negative reinforcers or discriminative stimuli?

A second important issue that was not addressed in the present experiments is impact of sex differences in the dual reinforcing actions of nicotine. The possibility that there may be sex differences in the contribution of nicotine and non-nicotine factors to smoking is supported by clinical outcomes which suggest that smoking may be less strongly driven by the direct effects of nicotine for women than men (Perkins et al. 1999; Perkins et al. 2002). Conversely, female smokers appear to show greater sensitivity than males to nonpharmacological stimuli that are associated with cigarette use (Perkins et al. 2001). The latter finding is substantiated by studies of nicotine self-administration which demonstrate that female rats acquire nicotine self-administration more rapidly than males (Donny et al. 2000), and may also be more sensitive than

males to the interaction between nicotine and nonpharmacological stimuli (Chaudhri et al. 2005b). These data highlight the importance of considering sex differences in the impact of both nicotine and nonpharmacological factors in smoking, and prompt the hypothesis that sex differences exist in the reinforcement-enhancing actions of nicotine.

5.6. CONCLUDING COMMENTS

Traditional theories on drug reinforcement maintain that the continued occurrence of drug seeking behavior is a direct consequence of the neuropharmacological actions of that drug (primary reinforcement). The present body of research has identified an alternative interpretation that calls this fundamental tenet into question. Nicotine has been repeatedly demonstrated to exert only weak effects as a primary reinforcer, and indeed, the ability of nicotine to enhance the reinforcing actions of contingent nonpharmacological stimuli may play a more prominent role in nicotine self-administration in rats and smoking in humans. The hypothesis that nicotine has multiple behavioral actions in reinforcement should be taken into account in current investigations of tobacco addiction and smoking.

APPENDIX A

THE DRUG SELF-ADMINISTRATION PARADIGM IN RATS

APPENDIX B

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