

**INDIVIDUAL DIFFERENCES IN DELAY DISCOUNTING AND NICOTINE
SELF-ADMINISTRATION IN RATS**

by

Maggie M. Sweitzer

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This thesis was presented

by

Maggie M. Sweitzer

It was defended on

June 23, 2008

and approved by

Anthony R. Caggiula, Ph.D., Professor

Stephen B. Manuck, Ph.D., University Professor

Thesis Advisor: Eric C. Donny, Ph.D., Assistant Professor

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Delay discounting—a behavioral measure of impulsivity defined as a tendency to prefer a small, immediate reward over a larger reward delayed in time—has been extensively linked with tobacco smoking. However, the causal direction of this relationship remains unclear. One possibility is that delay discounting may be a marker for an underlying vulnerability to nicotine reinforcement—a possibility which can be isolated using an animal model. In the current study, we investigated whether indifference points derived using an adjustable delay procedure of delay discounting predicted several indices of nicotine reinforcement in rats, including rate of acquisition of nicotine self-administration, break point reached on a progressive ratio schedule of reinforcement, or a shift in the dose-response curve. Stable indifference points were assessed for 63 male Sprague-Dawley rats, and extreme groups of highly impulsive (HI; n=15) and low impulsive (LI; n=11) rats were selected to self-administer nicotine. Rats responded by nose poking for infusions of 0.03 mg/kg nicotine during 1 hour daily sessions. After a 20 session acquisition period, rats completed 3 4-hour progressive ratio sessions, during which the response requirement was increased after each infusion earned. This was followed by 3 1-hour fixed ratio sessions at each of 3 nicotine doses, presented in ascending order (0.015, 0.03, and 0.09 mg/kg). All but one rat (HI group) acquired stable nicotine self-administration; however, no group differences in rate of acquisition were observed. HI and LI rats did not differ in their responses on a progressive ratio schedule or infusions earned at any dose of nicotine, although a significant

dose-response effect was observed overall. Indifference points reassessed after self-administration were highly correlated with original indifference points, and mean indifference points for each group at the second assessment did not differ significantly from baseline assessment. These results suggest that delay discounting is a highly reliable measure, but may not be a predictive marker for increased vulnerability to nicotine self-administration in rats.

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1.0 INTRODUCTION

Tobacco smoking is a behavioral risk factor for a multitude of diseases, including cardiovascular disease, cancer, and chronic obstructive pulmonary disease. Although public health efforts have resulted in a modest decline in smoking rates in recent years, an alarming number of young people continue to experiment with smoking, and many go on to become regular smokers. The pharmacological effects of nicotine are thought to underlie the process by which people become regular smokers; however, there are marked individual differences in vulnerability to smoking (Stanton et al., 2004; Chassin et al., 2000). Understanding the traits that make some individuals prone to becoming regular smokers can help to illuminate pathways of vulnerability and facilitate efforts for prevention.

Delay discounting—the tendency to choose a smaller immediate reward over a larger reward delayed in time—is one factor which may predispose some individuals to smoking. Delay discounting has been extensively linked with tobacco smoking, but the causal direction and underlying mechanisms for this relationship remain unclear. Animal models provide a valuable framework for further exploration of the relationship between delay discounting and nicotine-taking behavior, allowing extraneous variables to be carefully controlled. In this study, we investigated whether individual differences in delay discounting in rats predicted differences in nicotine self-administration. We hypothesized that rats with steeper discounting rates (i.e. a

greater preference for a smaller, immediate reward) would self-administer more nicotine, and acquire this behavior more rapidly, than rats with lower discounting rates.

1.1 DELAY DISCOUNTING AND SMOKING

Delay discounting refers to the tendency for individuals to discount the subjective value of a reward as it is delayed in time. Most people will reliably choose a large reward over a small reward when both are available immediately. However, as the larger reward is delayed in time, its subjective value decreases, so that at some point the larger magnitude is offset by the decrease in value due to delay, and the preference shifts toward the immediate small reward. Individuals discount the value of delayed rewards at different rates, and a steeper discounting curve is thought to reflect impulsive choice (Bickel & Marsch, 2001).

A large literature exists linking discounting of delayed rewards with abuse of drugs, including heroin (Madden et al., 1997; Kirby, Petry, & Bickel, 1999), cocaine (Coffey et al., 2003), alcohol (Vuchinich & Simpson, 1998; Petry, 2001), and tobacco (Mitchell, 1999; Reynolds et al., 2004). Cigarette smokers discount delayed rewards more rapidly than non-smokers, and the number of cigarettes smoked and total daily nicotine intake are both correlated with discounting rate (Ohmura, Takahashi, & Kitamura, 2005). Interestingly, although current smokers consistently make more impulsive choices on delay discounting tasks than non-smokers, ex-smokers and non-smokers don't differ (Bickel, Odum, & Madden, 1999; Skinner, Aubin, & Berlin, 2004). This could be the result of a reversible drug-induced increase in impulsivity, or a selection bias in which individuals with steeper discounting rates have greater difficulty quitting smoking.

Although the relationship between smoking and delay discounting has been clearly established, the evidence is largely correlational, and exactly how these factors are related is unclear. Three possibilities exist. First, delay discounting may be causally related to smoking. For example, highly impulsive adolescents may be more likely to try smoking with their friends despite parental warnings, and impulsive current smokers may fail to quit because immediate relief of withdrawal is valued above long term health outcomes. In this case, initiation and maintenance of smoking could be viewed as a manifestation of the same trait impulsivity which was previously manifested in other contexts in the individual's life. Although this pathway seems intuitive, evidence is indirect at best, and temporal precedence (i.e., impulsivity predicting subsequent smoking) has not been clearly established. A second explanation is that smoking may actually increase delay discounting through its pharmacological actions. Evidence suggests that drugs of abuse may increase impulsivity through their induction of neuroplastic changes in the brain (Jentsch & Taylor, 1999). Indeed, Dallery and Locey (2005) found that both acute and chronic experimenter-administered nicotine increased delay discounting in rats—an effect which endured after 30 days of abstinence. The third possible explanation is that a third variable could be contributing to both steeper delay discounting and smoking. For example, it is possible that delay discounting represents a phenotypic marker for a neurobiological vulnerability toward drug reinforcement. In this case, individual variation in the common neurocircuitry could lead to both preference for immediate reward and increased susceptibility to the reinforcing effects of nicotine. If such common neural circuitry is contributing to the relationship between delay discounting and smoking, then this relationship might be observed even in contexts in which there is not a long term trade-off for engaging in the behavior.

It is important to note that the three possible explanations for the relationship between delay discounting and smoking described above are not mutually exclusive. Indeed, individual susceptibility to nicotine reinforcement and drug-induced changes in relevant neurocircuitry may operate in conjunction to influence smoking trajectories. However, decomposing the relative contributions of each factor is important for understanding vulnerability. Animal models are particularly well-suited for this purpose, because the third variable hypothesis can be isolated from the two direct causal pathways. In animal self-administration models there are no delayed consequences to drug taking, so that failure to heed parental warnings or a disregard for future health consequences cannot be the mechanism of causality. Likewise, pharmacologically induced changes are eliminated through experimental design; delay discounting is assessed prior to drug exposure. Any relationship that remains in the animal model is therefore likely due to common neurobiological substrates influencing both processes. Assessing whether individual differences in delay discounting represent a phenotypic marker for vulnerability to self-administration was the primary objective of this study.

Preliminary support for the hypothesis of a common neural substrate comes from a recent study, in which delay discounting was found to predict faster acquisition of cocaine self-administration in rats (Perry et al., 2005). An earlier study also found a similar effect with alcohol, in which choice for the small immediate reward predicted higher levels of alcohol consumption in rats (Poulos, Le & Parker , 1995). Research on behavioral responses to alcohol administration has found results along similar lines. Outbred mice with steeper discounting rates exhibited greater locomotor activity after repeated alcohol administration, an effect thought to represent sensitization of the midbrain dopaminergic pathway (explained below) (Mitchell et al.,

2006). However, no studies have examined the relationship between delay discounting and nicotine reinforcement in an animal model.

1.2 EVIDENCE FOR A COMMON NEURAL SUBSTRATE

Research into the neural basis of delay discounting and drug reinforcement suggests that common pathways may be involved. Although the exact mechanisms vary, both natural and drug rewards increase extracellular dopamine in the region of the nucleus accumbens (Adinoff, 2004). This activation of the mesolimbic dopaminergic system is thought to be responsible for increasing goal-directed approach behavior, by attaching incentive salience to environmental cues associated with the obtained reward (Schultz, 2002; Robinson & Berridge, 1993). Sensitization of the nucleus accumbens dopaminergic response to psychomotor stimulants has been associated with increased self-administration in animals (Vezina, 2004), and individual variability in susceptibility to sensitization is thought to be a central predisposing factor in the development of addiction (Robinson & Berridge, 1993).

Recent evidence suggests that activation of the mesolimbic dopamine system may be responsible for assigning additional incentive value to rewards available immediately rather than after a delay. McClure and colleagues (2004) found increased activation of the ventral striatum when participants weighed choices that included an immediate option, relative to choices that had no immediate option. In another study, Hariri and colleagues (2006) found that individuals with steeper discounting rates exhibited greater BOLD activation in the ventral striatum in response to reward than individuals who discounted delayed rewards less steeply.

A substantial literature has also implicated serotonin (5-HT) as a critical neural substrate of impulsivity (Manuck et al., 2003). Specifically, serotonergic activity appears to be negatively associated with impulsivity (Cardinal et al., 2004), and manipulations which increase serotonin also decrease delay discounting (Bizot et al, 1999; Wolff & Leander, 2002). Although much less well studied, indirect evidence also suggests that serotonin may play a moderating role in nicotine reinforcement (Olausson, Engel, & Soderpalm, 2002). Interactions of 5-HT with the midbrain dopamine system may provide an explanation for serotonin's influence on reinforcement. For example, administration of 5-HT agonists has been consistently shown to attenuate cocaine induced increases in midbrain extracellular dopamine (Czoty, Ginsberg, & Howell, 2002), an effect which has also been found with nicotine (Pierucci, Di Matteo, & Esposito, 2004; Grottick, Corrigall, & Higgins, 2001). Together this evidence suggests that serotonergic and dopaminergic systems may be a common source of variation in both delay discounting and behavioral sensitization to drugs of abuse.

1.3 MEASURING DELAY DISCOUNTING IN RATS

Adjustable delay procedures have been widely used to assess discounting of delayed rewards in animal models (Mazur, 1987). Such procedures require animals to choose between a small reward available sooner (SS) or a large reward available later (LL). The length of the delay to the LL reward is adjusted up or down, depending on the animal's choices, until an "indifference point" is reached, indicating that the subjective value of the LL reward is equal to that of the SS reward. This approach for assessing delay discounting in animals is consistent with procedures used in humans, in which amount and delay for the LL reward are both varied across many

choices, and multiple indifference points can be derived and fitted to a hyperbolic curve (Green & Myerson, 2004). Varying both delay and amount with animals reveals that their discounting rates are also best described by hyperbolic functions, suggesting that an equivalent construct is being measured (Richards et al., 1997; Mazur, 1987).

Although hyperbolic discounting functions can be calculated for animals by assessing multiple indifference points, many studies rely on single indifference points derived from adjustable delay procedures (Perry et al., 2005; Wogar, Bradshaw, & Szabadi, 1993, Dallery & Locey, 2005; Wolff & Leander, 2002). This method does not provide the same extent of information as deriving an entire discounting curve, but it has the advantage of being much more efficient while retaining the construct validity of more comprehensive approaches. Therefore, a single indifference point derived from an adjustable delay procedure was used in this study.

1.4 CHARACTERIZING INDIVIDUAL DIFFERENCES IN NICOTINE SELF-ADMINISTRATION

Several well-established procedures have been used to assess individual differences in nicotine reinforcement. For example, acquisition of self-administration tested at low doses has been used to assess sensitivity to the reinforcing effects of a drug (e.g. Piazza et al., 1989), since only some animals will acquire at low doses. An alternative approach is to examine the rate of acquisition in terms of changes in infusions earned over time—a measure which has been shown to be related to individual differences in other outcome variables (e.g. nicotinic receptor binding; Donny et al., 2004). Stable responding on a fixed ratio (FR) schedule allows for examination of levels of nicotine intake, as well as the regulation of intake at different doses. Indeed, dose

appears to be an important moderator of individual differences in self-administration and its relationship to other factors. For example, in one study rats exhibiting high and low locomotor responses to novelty were found to differ in rate of acquisition of cocaine self-administration only at the lowest dose tested, while differences in stable FR responding were more pronounced at the highest dose tested (Mantsch, Schlussman, & Kreek, 2001). Accordingly, we assessed self-administration on a FR schedule at multiple doses. Furthermore, we utilized a relatively low dose during acquisition in order to maximize individual differences, while still supporting self-administration (Donny et al, 2004).

A progressive ratio (PR) schedule of reinforcement, in which the response requirement to obtain an infusion is increased after each infusion earned, is thought to provide an indication of motivation or incentive salience of a drug (Donny et al., 1999; Richardson & Roberts, 1996). Importantly, although infusions earned on FR and PR schedules have been shown to be related (Piazza et al., 2000), substantial unexplained variance between them suggests that they may reflect relatively distinct aspects of reinforcement (Donny et al., 1999). Therefore, both types of reinforcement schedules were included in this study.

1.5 SPECIFIC AIMS

This study tested the hypothesis that impulsive choice predicts individual differences in nicotine reinforcement. To address this question, we assessed the degree to which natural variation in delay discounting rates among outbred rats predicted multiple self-administration parameters designed to assess individual differences in nicotine reinforcement. Specifically, we evaluated group differences in the rate of acquisition of nicotine-taking behavior, break point on a PR

schedule of reinforcement, and infusions earned on an FR schedule at varying doses of nicotine. This approach has the advantage of assessing naturally occurring variation in discounting rates prior to any drug exposure, and establishing a behavioral framework for further exploration of specific neurobiological pathways.

2.0 METHOD

2.1 SUBJECTS

Subjects were 63 male Sprague-Dawley rats (Harlan farms), aged approximately 3 months and weighing 250 to 300 grams at the start of the experiment. Rats were housed individually in a temperature controlled room under a 12 hour reversed light/dark cycle (lights off at 7 a.m.). Animals were fed once per day in the home cage following experimental sessions. The amount of food was adjusted each day for each rat to account for any pellets earned during the sessions, so that food intake totaled 20 grams per day. This feeding schedule was designed to keep rats at approximately 85% of their free-feeding weight, in order to both control for differences in weight gain that might emerge based on choices in the delay discounting procedure, and to minimize the degree to which unlimited or pre-session feeding might compete with nicotine as a primary reinforcer (Donny et al, 1998). Rats were given unlimited access to water in the home cage. All sessions were conducted between the hours of 8 a.m. and 6 p.m.

2.2 DESIGN

Rats were first screened using a delay discounting procedure (explained below) to determine baseline levels of impulsive choice. Among rats reaching stable indifference points (n=50), 16

rats were classified as high impulsive (HI), and 16 rats were classified as low impulsive (LI), according to the procedure described below. This sample size is similar to those used in other investigations of individual differences (Piazza et al, 1989; Poulos, Le & Parker, 1995; Perry et al, 2005; Diergaarde et al, 2008), and provides adequate power to detect a medium effect size. Following group assignment, rats were anesthetized and jugular catheters were surgically implanted to allow for intravenous administration of nicotine. After a brief recovery period rats were then allowed to nose poke for infusions of 0.03 mg/kg of nicotine (dose reported as free base) during daily 1 hour self-administration sessions. Self-administration continued for approximately 50 sessions, and included an initial acquisition period, a progressive ratio test to assess break point, and a within-subjects manipulation of dose to determine dose-response curves. Following the self-administration phase, rats were reassessed on the delay discounting procedure to determine the stability of indifference points. Details of each phase of the study are described below.

2.3 DELAY DISCOUNTING

2.3.1 Apparatus

Behavioral testing was carried out in eighteen 25x31x28 operant test chambers (MED Associates, Inc., St. Albans, Vermont, USA), each enclosed in a sound-attenuated cubicle. During the delay discounting procedure, chambers were fitted with two retractable response levers located on the side panel, 15 cm apart and 7 cm above the floor. A nose poke was centered directly between the two levers. A 45 mg food pellet dispenser delivered pellets into a

food tray located directly across from the nose poke on the center of the opposite wall. No cue lights were present above the levers, and no house light was illuminated during the procedure.

2.3.2 Pretraining

Prior to beginning the delay discounting procedure, rats underwent several sessions designed to train the rats to perform the chained nose poke and lever press response, to train rats to discriminate the start of a new trial, and to assess any preliminary side bias toward either lever. Animals were first auto-shaped to press the levers for the delivery of food pellets; autoshaping was considered successful when rats had pressed each lever a minimum of 25 times in a single session. Rats were then trained to nose poke in order to extend the levers (which served to center them between the two levers at the start of each trial). During the nose poke training, onset of each new trial was signaled by the illumination of the nose poke light. A nose poke within the 10 second time limit resulted in extension of the levers, and a subsequent lever press was followed by retraction of the levers and delivery of a single 45 mg food pellet. Failure to respond within 10 seconds on either operant resulted in termination of the trial (extinction of the light or retraction of levers) and no food pellet delivery. Shaping of the chained nose poke and lever press response continued until rats completed 50 successful trials within a session. After successful shaping, a preference test was conducted to determine any initial bias toward one lever or the other for each rat. Side bias was assessed by calculating the percentage of responses on each lever when both were presented simultaneously and responses were not reinforced. Side bias was then considered when assigning the smaller sooner (SS) and larger later (LL) levers, so that, as a group, the degree of initial preference for the SS lever averaged 50%.

2.3.3 Behavioral procedure

The delay discounting procedure was based upon Mazur's (1987) adjustable delay paradigm described above. Rats completed one-hour sessions twice per day, five days per week. Each session consisted of a series of choice trials in which a response on one lever was followed by immediate delivery of one pellet, and response on the other lever was followed by three pellets delivered after a variable delay (2 to 74 seconds). Lever assignment was counter-balanced between rats, but remained constant for each rat for the duration of the study.

A schematic diagram of the sequence of events for each trial is shown in Figure 1 below. The start of each new trial was signaled by the illumination of the nose poke light, consistent with the cue conditions present during pre-training. In addition, the same 10 second time limit on responses was imposed. The absence of a response within this time limit was recorded as an omission. Once a lever was pressed, both levers were retracted and the 60 second inter-trial interval began. In order to avoid introducing a confounding factor that may bias selections, and to preserve novel cues for the self-administration procedure, no other cues signaled the response selection or the delivery of either the immediate or delayed reward.

Sessions consisted of 15 blocks of 4 trials each. The first two trials of each block were forced-choice trials (each lever presented alone once), and the final two trials were free-choice trials (both levers presented). The forced-choice trials were designed to insure that rats sampled each option before making a selection. Forced-choice lever presentation occurred in pseudorandom order. At the start of the first session, all delays were set at 10 seconds. At the end of each block of trials, the delay was adjusted up or down depending upon the free choices. Two LL choices resulted in a 10% increase in the delay, two SS choices resulted in a 10% decrease during the next block of trials, and one choice on each lever resulted in no change.

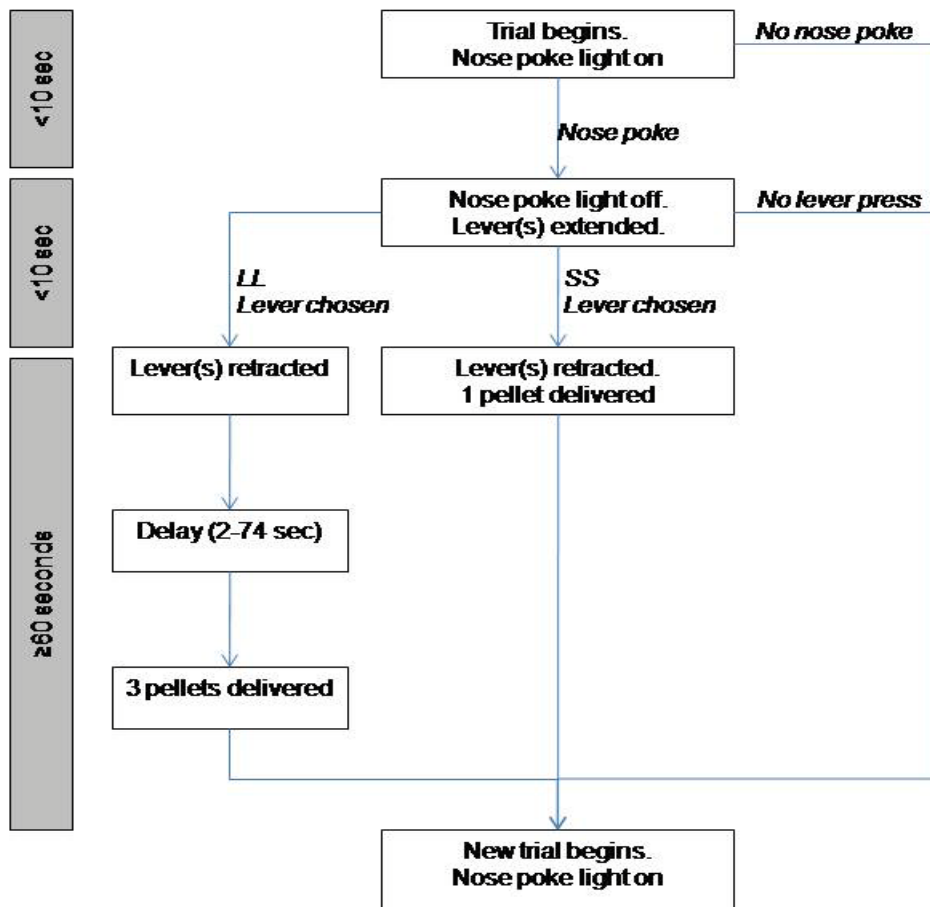


Figure 1. Procedure for each trial during delay discounting.

Each new session began with the ending delay from the previous session. Indifference points were calculated based on the average adjusting delay for each session according to the criteria described below. Testing continued for 53 sessions, until stable indifference points were reached for 50 animals. Animals reaching stability sooner completed the remaining sessions, in order to insure that all animals had equivalent experience within the operant chambers prior to self-administration.

2.3.4 Calculating indifference points

The average adjusting delay experienced by the rat was calculated for each session, and the pattern across sessions was evaluated to determine a stable indifference point. After completing a minimum of 30 sessions, stability was assessed according to the following criteria: 1) a coefficient of variation of less than 20% for the average adjusting delay across 5 days, 2) less than 20% or 5 seconds change in the average adjusting delay across the next ten days, and 3) absence of a visual linear trend over the total 15 day period. The latter criterion was imposed to allow for detection of subtle shifts over time that may not be evident when averages over multiple days were being considered. Once the stability criteria were met, the average adjusting delay across the entire 15 day period was calculated as the indifference point.

2.4 GROUP ASSIGNMENT

At the conclusion of the delay discounting assessment, animals were assigned to high and low impulsivity groups according to the following procedure. First, rats completing delay discounting training and meeting stability criteria were rank ordered by indifference points. Those with indifference points in the upper third were considered for assignment to the low impulsive (LI) group, and those with indifference points in the bottom third were considered for assignment to the high impulsivity (HI) group. To ensure that the final selected groups accurately reflected “high” and “low” impulsivity, the average adjusting delays across all completed sessions beginning with session 26 (the earliest session that could be included in the indifference point assessment) and continuing through session 53 were also considered. The

percentage of these final 28 sessions for which the average adjusting delay was higher or lower than the average indifference point for the population was calculated for each rat. Rats with more than 40% of their average adjusting delays falling below the population average were excluded from the low impulsivity group, and rats with more than 40% of their average adjusting delays falling above the population average were excluded from the high impulsivity group. This resulted in the exclusion of three rats who would have otherwise been assigned to the low impulsivity group. After these exclusions were made, the 16 rats with the lowest indifference points were assigned to the HI group, and the 16 rats with the highest indifference points were assigned to the LI group.

2.5 SURGERY

Rats assigned to the LI and HI groups (n=32) were then implanted with right jugular catheters under halothane anesthesia, and were allowed a minimum of seven days healing time before beginning self-administration. Cannulae were flushed daily with 0.1 ml of sterile saline containing heparin (30 U/ml) and Timentin (66.67 mg/ml) to maintain catheter patency and prevent infection. In addition, rats received streptokinase (8333 U/ml) for the first four days post-surgery. Catheter patency was tested twice during self-administration by observing loss of the righting reflex following an infusion of 200 mg/kg chloral hydrate.

2.6 NICOTINE SELF-ADMINISTRATION

2.6.1 Apparatus

Sessions took place in the same operant chambers used for delay discounting. However, the internal construction of the chambers was changed. The two retractable response levers and the centering nose poke were removed from the chambers, so that the side panel previously used for responding was empty of all operants, with only a red house light located at the top. On the opposite wall, the pellet trough was removed, and two nose pokes were positioned 15 cm apart and 3 cm above the floor. Nose pokes were used as the operant instead of levers to maximize the procedural differences between the delay discounting and self-administration sessions. A white stimulus light was located 5 cm above each nose poke. A 1 cm diameter hole in the top of the chamber gave access to a drug-delivery swivel system, which connected to the implanted catheters and allowed nearly unlimited movement throughout the chamber.

2.6.2 Timeline

Self-administration took place in the following three stages: acquisition, PR sessions, and FR dose-response manipulation. The timeline for these procedures is listed in Table 1 below.

2.6.3 Acquisition

Sessions were held 1 hour per day, 5 days per week, for approximately 20 sessions. Responses on the active nose poke were reinforced with an infusion of 0.03 mg/kg nicotine bitartrate (dose

Table 1. Timeline for nicotine self-administration procedures.

| Stage of Self-administration | Procedure | Nicotine Dose | Number of Sessions |
|------------------------------|-----------|---------------|--------------------|
| Acquisition | FR 2 | (0.03) | 10 |
| | FR 5 | (0.03) | 10-13 |
| PR Sessions | PR Test | (0.03) | 3 |
| | FR 5 | (0.03) | 3 |
| Dose-response Assessment | FR 5 | (0.015) | 3 |
| | FR 5 | (0.03) | 3 |
| | FR 5 | (0.09) | 3 |
| Total | | | 35-38 |

reported as free-base), paired with a 15-second illumination of the white cue light located above the active nose poke. The dose of 0.03 mg/kg was used because it has been shown to support self-administration, while maximizing individual differences in the rate of acquisition (Donny et al., 2004). The cue light paired with infusion delivery was selected because it is consistent with cue conditions used in other studies examining individual differences in nicotine self-administration (Suto, Austin, & Vezina, 2001; Le et al., 2006), and this stimulus has been shown in our laboratory to have minimal primary reinforcing value in the absence of nicotine. A 60 second unsignaled time-out period followed infusion delivery, during which responses were recorded but not reinforced. Responses on the inactive nose poke were recorded but had no consequence. Prior studies have demonstrated that rats acquire nicotine self-administration on a

FR1 schedule, and that stable and robust responding is maintained at a FR5 schedule (Donny et al., 1998; 2000). Because of the shift to a more natural operant with which the rats had prior history, we began with a FR2 for the first 10 days of acquisition. Beginning on day 11 and continuing throughout the remainder of the acquisition period, the response requirement was increased to a FR5. Typical nicotine self-administration procedures involve stepwise increases in FR requirement over multiple sessions (e.g. Donny et al., 1998; 1999), and these small increases in the FR tend to exaggerate individual differences in nicotine self-administration (Lanza et al., 2004). Acquisition of self-administration was judged to be complete when responding favored the active nose poke by a ratio of 2:1, and a minimum of 5 infusions were earned per session for the majority of the last five sessions on a FR5. Animals not acquiring self-administration ($n=1$) were excluded from further analyses.

2.6.4 Progressive ratio test

Immediately following the 20 day acquisition period, rats were switched to a PR schedule. The PR test consisted of 3 consecutive 4-hour sessions. During these sessions, cue conditions and dose of nicotine were identical to the acquisition conditions, but the response requirement was increased with each successive infusion earned according to the following schedule: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 179, 219, 268, 328, 402, 492. “Break point” was defined as the number of infusions earned prior to an hour elapsing with no infusions, or as the total number of infusions earned during the session. After 3 PR sessions, rats were returned to baseline for 3 sessions of self-administering 0.03 mg/kg nicotine on a FR5 schedule of reinforcement. Catheter patency was tested at the conclusion of the progressive ratio sessions; animals failing to demonstrate patency ($n=7$) were excluded from analyses.

2.6.5 Dose effects

Following PR testing, within-subjects dose manipulations were conducted to determine dose-dependent effects. During this phase of self-administration, sessions were conducted 7 days per week, in order to avoid a period of abstinence prior to testing. Rats spent 3 days self-administering each of 3 doses, presented in ascending order, on a FR5 reinforcement schedule. Doses were 0.015 (minimum dose that supports self-administration, which may reveal differences in sensitivity to the reinforcing effects of nicotine), 0.03 (standard self-administration dose), and 0.09 (maximizing primary reinforcing effects, Chaudhri et al., 2006). At the conclusion of the dose manipulation, rats were tested again for catheter patency. No additional rats failed patency at this time.

2.7 REASSESSMENT OF INDIFFERENCE POINTS

Following the conclusion of self-administration, rats were then reassessed on the delay discounting procedure to determine the stability of indifference points over time. Operant chambers were returned to their original configurations. Delay discounting sessions identical to those conducted during the initial assessment period resumed twice per day, five days per week, until new indifference points were determined. During the first session, the starting delay interval was reset to 10 seconds for all rats, in order to avoid biasing new assessments based on past performance. Rats were required to complete a minimum of 10 sessions, after which stability was determined according to criterion 1 described above (coefficient of variation of less than 20% for the average adjusting delay across five days). Additional criteria were not used

during reassessment since comparisons with prior indifference points were considered to be a better indication of stability. The average adjusting delay over the 5 days during which the coefficient of variation first dropped below 20% was used as the new indifference point.

2.8 STATISTICAL ANALYSES

A three-way ANOVA (Group X Day X FR) was used to assess change over time in number of infusions earned per session during acquisition. Group differences in slopes across days were compared using polynomial contrasts. In addition, planned comparisons tested for group differences on the final day of each FR (days 10 and 20), as well as the change score following an increase in FR (day 10 – day 11). A two-way ANOVA (Group X Session) with polynomial contrasts was conducted to assess group differences in break point across sessions. In addition, break points reached on the last two sessions of the PR schedule were averaged, and an independent samples t-test was used to determine group differences. Dose effects were evaluated by calculating the average number of infusions earned during the final two sessions for each dose. A two-way (Group X Dose) ANOVA was used to evaluate group differences in average number of infusions earned at each dose. In the case of a significant overall effect, independent samples t-tests were used to compare group differences at each individual dose, while paired samples t-tests were used to differences between doses. Finally, the correlation between initial indifference points and those determined during reassessment was calculated as an index of reliability. In addition, t-tests were used to determine whether any change in indifference points occurred following self-administration among high or low impulsive rats.

3.0 RESULTS

3.1 DELAY DISCOUNTING

Of the 63 rats who participated in the delay discounting procedure, 4 were removed from the study due to equipment failure which interfered with the delivery of the large reward. A fifth animal was removed from the study due to an excessive number of omitted trials (>11 per session), which resulted in early termination of more than 20% of his sessions. By comparison, the remainder of the animals failed to respond before the time limit an average of only 2.6 trials per session, and they successfully completed 99.3% of their sessions.

The remaining 58 rats completed between 49 and 53 sessions ($M=52.7$). Although all rats participated in 53 sessions, the number of successfully completed sessions was slightly lower for some animals, since a high number of omitted trials resulted in early termination of the session. Of the 58 rats completing the procedure, 52 rats met criteria 1 and 2 for establishing a stable indifference point. Two of these were excluded because they violated criterion 3, demonstrating a decreasing visual trend. The remaining 50 rats were then assigned to groups according to the procedure described above. Indifference points and standard deviations for the 50 rats meeting stability criteria are shown in Figure 2.

The mean indifference point for all 50 rats meeting stability criteria was 27.73 ($SD=10.13$). The indifference points for rats assigned to the HI group ranged from 11.10 to

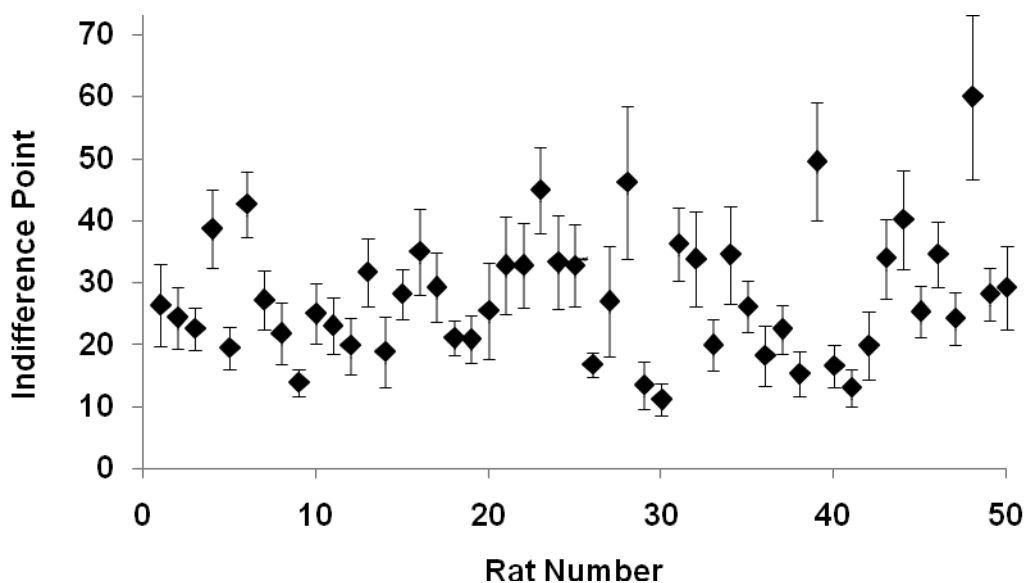


Figure 2. Indifference points (with standard deviations) for all rats meeting stability criteria during delay discounting.

21.77 seconds, and indifference points for rats assigned to the LI group ranged from 29.26 to 59.94 seconds. Data for each of these groups and the overall sample are presented in Table 2. Independent samples t-tests were used to assess differences between HI and LI rats for each measure assessed during the delay discounting procedure. Indifference points for HI rats were significantly lower than indifference points for LI rats ($t=-9.971$, $p<0.001$), verifying the validity of the group assignment procedure. LI rats took an average of 3 sessions longer than HI rats to meet criteria for establishing indifference points, a difference which was statistically significant ($t=-2.275$, $p<0.05$). Although a trend toward fewer omitted trials among LI rats was observed, this difference did not reach significance ($t=-1.723$, $p>0.05$). There was no weight difference between LI and HI rats during the time period in which stable indifference points were being reached.

Table 2. Mean (and SD) values on measures assessed during delay discounting for high impulsivity rats, low impulsivity rats, and the total sample.

| Variable | High Impulsivity (<i>n</i> =16) | Low Impulsivity (<i>n</i> =16) | Total Sample (<i>n</i> =50) |
|--------------------------------------|-------------------------------------|------------------------------------|---------------------------------|
| Indifference Point** | 17.49 (3.29) | 38.96 (7.96) | 27.73 (10.13) |
| Omissions | 2.18 (1.43) | 3.18 (1.82) | 2.50 (1.58) |
| #Sessions to Criteria* | 41.13 (2.03) | 44.13 (4.87) | 43.54 (4.34) |
| Average Weight for Sessions 40 to 48 | 305.73 (15.08) | 306.16 (18.50) | 309.68 (17.38) |

*Indicates a significant difference at the $p < 0.05$ level.

**Indicates a significant difference at the $p < 0.001$ level.

3.2 NICOTINE SELF-ADMINISTRATION

3.2.1 Acquisition

Seven of the 32 rats (2 HI; 5 LI) failed to demonstrate patent catheters at the first patency test. Their data was subsequently dropped from analysis. An additional rat (HI) failed to acquire self-administration, and his data were also dropped. This resulted in 13 rats remaining in the HI group, and 11 rats remaining in the LI group. Mean daily infusions earned by each group during acquisition are presented in Figure 3; responses on the active and inactive nose pokes are presented in Figure 4. The main effect of day on number of infusions earned was significant, $F(9,198)=35.146$, $p < .001$, as were both linear and quadratic contrasts for day (p 's $< .001$). The main effect of FR was also significant, $F(1,22)=6.857$, $p < .05$, with rats earning significantly

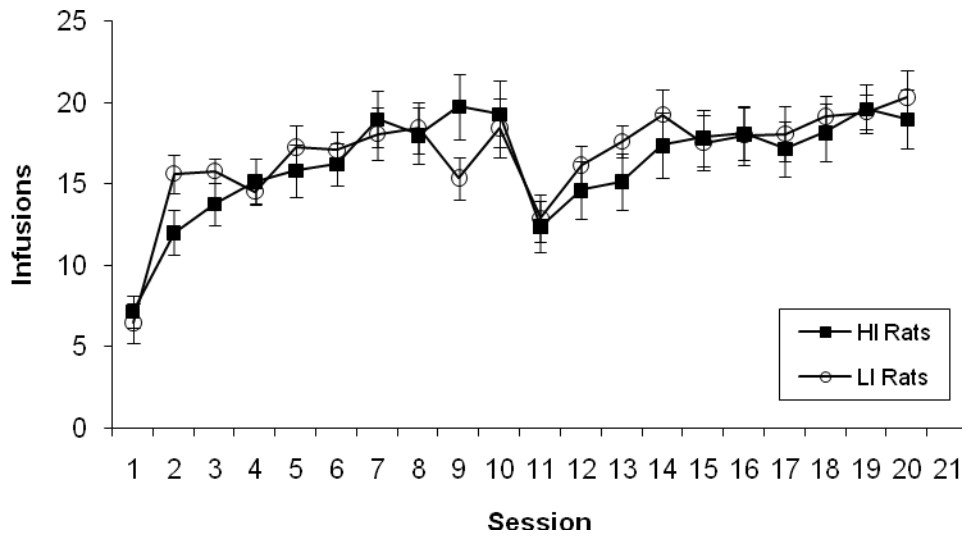


Figure 3. Mean infusions (with standard errors) for each day during acquisition for rats high and low in impulsivity.

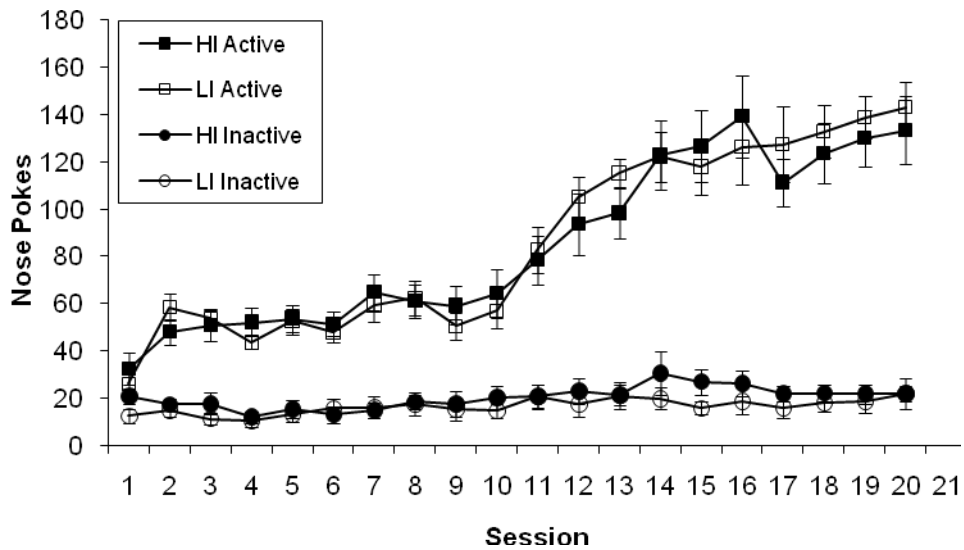


Figure 4. Mean active and inactive nose pokes (with standard errors) for each day during acquisition for rats high and low in impulsivity.

more infusions during the FR5 condition than during the FR2 condition, $t(23)=2.610$, $p<.05$. There was no main effect of impulsivity group, $F(1,22)=.091$, ns , nor was there a significant group by FR interaction, $F(1,22)=.394$, ns . There was a significant group by day interaction, $F(9,198)=2.020$, $p<.05$. However, neither linear nor quadratic contrasts were significant (both $p's>.10$). Planned comparisons revealed that there were no significant differences between high and low impulsivity groups for number of infusions earned on day 10, $t(22)=.301$, ns , or 20, $t(22)=.236$, ns , nor was there any significant group difference in the change in infusions earned between days 10 and 11, when the increase to the FR5 schedule was initiated, $t(22)=.612$, ns . Likewise, there were no significant differences between groups for number of active nose poke responses, either as a main effect or an interaction.

3.2.2 Progressive ratio test

Several rats failed to reach a break point on the progressive ratio test within the allotted time (Table 3). However, there were no significant group differences for the number of rats reaching a break point for any session ($\chi^2 = 2.970$, .839, and .509 for sessions 1, 2, and 3 respectively; all ns). Mean infusions earned prior to reaching a break point for each group are presented in Figure 5. For those rats not reaching a break point, total infusions earned during the session were used instead. There was no main effect of session number, $F(2,44)=1.685$, ns , no main effect of group, $F(1,22)=.059$, ns , and no group by session interaction, $F(2,44)=2.396$, $p>.10$. When considering just the mean of the last two sessions, the HI group earned an average of 10.85 ($SD=3.70$) infusions prior to reaching break point, while rats in the LI group earned an average of 9.86 ($SD=4.06$) infusions. This difference was not significant, $t(22)=.620$, ns .

Table 3. Number of rats (and %) from each group and the total sample failing to reach break point during each progressive ratio session.

| Progressive Ratio Session | High Impulsivity (n=13) | Low Impulsivity (n=11) | Total Sample (n=24) |
|---------------------------|-------------------------|------------------------|---------------------|
| Session 1 | 1 (7.7%) | 4 (36.4%) | 5 (20.8%) |
| Session 2 | 3 (23.1%) | 1 (9.1%) | 4 (16.7%) |
| Session 3 | 3 (23.1%) | 4 (36.4%) | 7 (29.2%) |

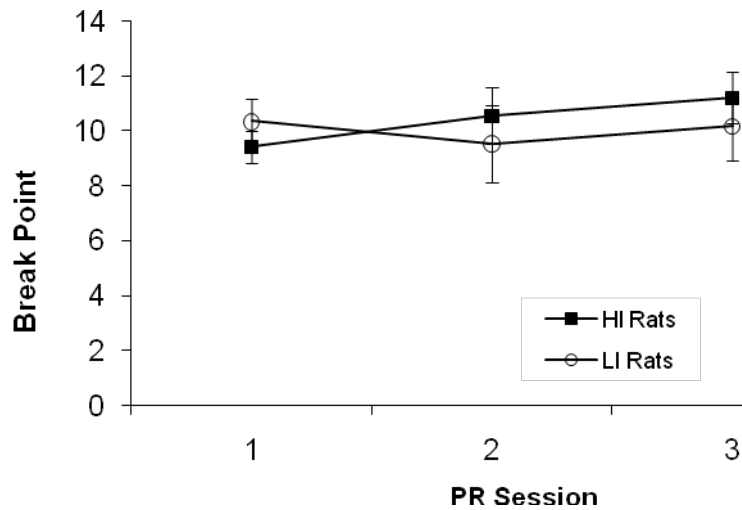


Figure 5. Mean break point (with standard errors) during each progressive ratio session for rats high and low in impulsivity.

3.2.3 Dose effects

Mean infusions earned during the last two sessions at each dose are presented in Figure 6. Repeated-measures ANOVA revealed a main effect of dose, $F(2,44)=86.675$, $p<.001$, with significant linear and quadratic contrasts (both p 's $<.001$). Post-hoc comparisons indicated that rats earned significantly more infusions at the 0.015 dose than at the 0.03 or 0.09 doses, $t(23)=3.936$ and 10.403 , respectively, both p 's $\leq.001$. In addition, rats earned more infusions at the 0.03 dose than at the 0.09 dose, $t(23)=9.808$, $p<.001$. However, there were no significant group differences, either as a main effect, $F(1,22)=.012$, ns , or an interaction, $F(2,44)=.059$, ns .

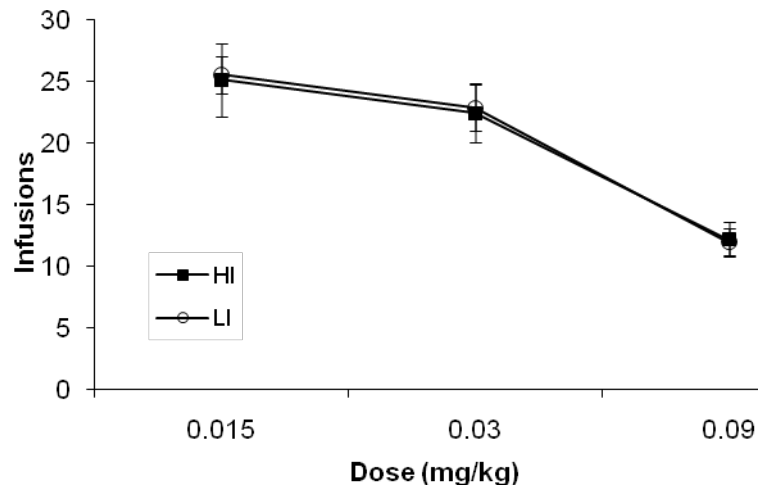


Figure 6. Mean infusions earned (with standard errors) during the last two sessions at each dose for rats high and low in impulsivity.

3.3 REASSESSMENT OF INDIFFERENCE POINTS

All but one of the 25 rats met the reassessment stability criteria described above. Rats reached stability in an average of 12.13 sessions (*S.D.*=2.71). New indifference points ranged from 12.40 to 45.36 seconds. For the HI group, indifference points ranged from 12.40 to 38.04 seconds, and for the LI group, indifference points ranged from 27.89 to 45.36 seconds. As expected, new indifference points were significantly higher in the LI group compared with the HI group, $t(22)=4.559$, $p<.001$. Comparisons between indifference point assessments at Time 1 and Time 2 are presented in Table 4. Although there was a trend toward higher indifference points at Time 2 among the HI rats, this comparison failed to reach significance. Likewise, there was no change between Time 1 and Time 2 in indifference points among rats in the LI group, or in the sample as a whole.

Table 4. Mean (and SD) indifference points assessed during delay discounting at Time 1 and Time 2, and *t* statistics for paired samples comparisons among high impulsivity rats, low impulsivity rats, and the total sample.

| Group | Indifference Point Time 1 | Indifference Point Time 2 | <i>t</i> statistic | <i>p</i> value |
|-------------------------------------|------------------------------|------------------------------|--------------------|----------------|
| High Impulsivity (<i>n</i> =14) | 17.64 (3.24) | 21.98 (8.61) | 2.079 | .058 |
| Low Impulsivity (<i>n</i> =10) | 37.30 (8.83) | 37.51 (7.65) | .053 | .959 |
| Total Sample (<i>n</i> =24) | 25.83 (11.60) | 28.45 (11.22) | 1.257 | .221 |

Individual indifference points at Time 1 were highly related to indifference points at Time 2 ($r=.599, p<.01$). However, analysis of influence statistics indicated that one rat was a statistical outlier ($Cook's D_i=2.9$). When this rat was dropped from the analysis, the correlation between Time 1 and Time 2 measures improved ($r=.771, p<.001$; Figure 7).

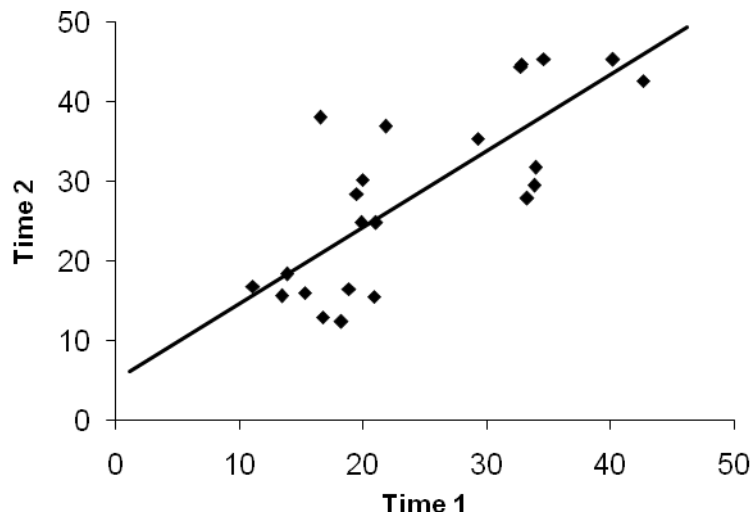


Figure 7. Indifference points reassessed at Time 2 plotted against indifference points assessed at Time 1 for rats meeting stability criteria at both time points. (One statistical outlier has been removed).

4.0 DISCUSSION

This study utilized a rat model of nicotine self-administration to evaluate whether pre-existing individual differences in delay discounting predicted several different aspects of nicotine taking behavior. Specifically, we hypothesized that steeper discounting of delayed rewards would contribute to increased infusions earned during acquisition of nicotine self-administration, higher break point on a progressive ratio schedule, and an upward shift in the dose-response curve. Contrary to our expectations, we found no differences between the high and low impulsivity groups on any measure of self-administration. This is surprising, given the literature supporting this prediction (Perry et al, 2005; Poulos, Le, & Parker, 1995; Mitchell et al., 2006).

Given the lack of association between impulsivity level and any measure of nicotine self-administration, one potential explanation is that there was insufficient variability present within the single outbred strain of rats to permit an association to be detected. However, this is unlikely given that a substantial amount of variability was observed in the measure of indifference points and in the propensity to self-administer nicotine. For example, among rats maintaining patency throughout the study, average infusions earned during week 4 of the acquisition period ranged from 11.0 to 31.4, and average break points during sessions 2 and 3 on a progressive ratio schedule ranged from 3.5 to 16.5, demonstrating variability comparable to that observed in other studies (Donny et al, 1999), with a sufficient range for examining individual differences. Furthermore, several other studies have found significant relationships between baseline

characteristics and drug self-administration outcomes using a single outbred strain of rats (Perry et al, 2005; Poulos, Parker, & Le, 1998; Diergaarde et al, 2008; Suto, Austin, & Vezina, 2001; Piazza et al, 1989).

The lack of significant findings in the present study also raises concern about the reliability and validity of the measures used. However, several points address these concerns. First, the delay discounting procedure used was quite similar to procedures used in other studies, in which associations with relevant neurocircuitry have been demonstrated (Bizot et al, 1999; Mobini et al, 2000; Wolff & Leander, 2002). The indifference points in the current study were somewhat larger than those found in other studies (e.g. Dallery & Locey, 2005; Perry et al, 2005), although strain differences and slight procedural differences are likely to have played a role. For example, the delay discounting procedure used in the current study did not employ any visual or auditory cues when a response selection was made or the reward was delivered, but rats still readily learned the procedure, and it is unlikely that these differences would detract from the validity of the measure. In addition, the observed indifference points were well within the range constrained by the task, suggesting that floor and ceiling effects did not compromise the validity of the measure.

The current study evaluated the test-retest reliability of the delay discounting procedure, and the strength of this reliability is particularly notable. Nearly nine weeks elapsed between the completion of the first delay discounting assessment and the start of the second assessment—an interval of time more than twice as long as that used in other studies to determine test-retest reliability (Diergaarde et al, 2008). This strongly supports the use of the indifference point measure obtained in the current study as a stable, trait-like measure of delay discounting. In addition, the strong association between the two assessments is unlikely to have been biased by

the testing procedure itself, since delay intervals were reset to 10 seconds at the start of the second assessment, and then were allowed to vary freely based on the rats' behavior. Because of this strategy, rats were required to shift the delays back to their preferred indifference point through choices favoring one lever, and then hold their indifference points constant through a selection pattern approximating 50% responses on each lever. Furthermore, it is notable that the strong association between indifference points obtained at Time 1 and Time 2 was not disrupted by the intervening performance of another operant procedure (self-administration), further attesting to the stability of the measure. The fact that indifference points did not shift as a consequence of the pharmacological effects of nicotine exposure is surprising, given that previous studies have observed nicotine-induced increases in delay discounting (Dallery & Locey, 2005). However, the nicotine in this study was self-administered, exposure was brief (generally 1 hour per day), and amount of exposure was not systematically controlled in any way. Given that self-administered nicotine has been shown to produce physiological effects quite different from those produced by experimenter-administered nicotine (Donny et al, 2000), further study should examine whether nicotine self-administered under carefully controlled conditions induces impulsive choice similar to that induced by experimenter-administered nicotine (Dallery & Locey, 2005).

The nicotine self-administration paradigm used in the present study has been extensively characterized by previous work, demonstrating that nicotine acts as a primary reinforcer (Corrigall & Coen, 1989; Donny et al, 1998; Donny et al, 1999). Accordingly, rats in the present study readily acquired self-administration as defined by earning a minimum of 5 infusions during the majority of sessions on a FR5, and as early as the first session rats favored the active nose poke over the inactive nose poke by a ratio of 2 to 1. In addition, rats earned infusions at a rate

similar to other studies using a comparable cue and nicotine dose (Donny et al, 1999). Individual differences in self-administration were also quite stable. For example, the average number of infusions earned per session during week 3 of acquisition was highly correlated with average infusions earned per session during week 4 ($r=.833$, $p<.001$). This suggests that individual differences in infusions earned reflect stable trait-like variability in the propensity to self-administer nicotine, rather than random error.

The progressive ratio test was designed to assess motivation to obtain nicotine or incentive salience of the drug (Donny et al., 1999; Richardson & Roberts, 1996). In the present study rats were responsive to the change in response contingency as expected, dramatically increasing their active nose poke responses relative to FR responding, while reducing the number of infusions earned. Although dose was not manipulated on the PR schedule in the present study, the replication of previous work using the 0.03 dose (e.g. Donny et al, 1999) suggests that the current PR manipulation was effective at tapping into the motivation to obtain nicotine. It is then most surprising that no group differences emerged during this phase of self-administration, as the incentive motivational properties of the drug are thought to be mediated by circuitry known to be relevant for discounting of delayed rewards (Hariri et al, 2006; McClure et al, 2004; Robinson & Berridge, 1993). Several rats failed to reach a break point during the 4 hour PR test, effectively creating a ceiling for those animals. It is possible that extending the length of the test would have revealed additional variance that may have been related to baseline impulsivity. However, given that on average over 70% of the animals did reach a break point, it is unlikely that this would have affected the results.

Rats were also sensitive to changes in nicotine dose when returned to an FR schedule, significantly increasing the number of infusions earned at the 0.015 dose and decreasing the

number of infusions earned at the 0.09 dose, effectively titrating intake in a manner similar to that observed in other studies (Donny et al, 1999). This finding is important, given that the dose-response assessment was conducted within-subjects. The similarity between the pattern of infusions earned in the present study and previous work conducted between-subjects supports the validity of a short-term within-subjects dose-response assessment.

It is important to note that the selected doses did not yield a complete dose-response curve, since the number of infusions earned was at a peak at the lowest dose. The absence of a sufficiently low dose to detect a decrease in responding prohibits us from evaluating whether or not impulsivity might be related to differences in sensitivity to nicotine. Thus, it is possible that administration of a lower dose (e.g. 0.005) might have revealed group differences in responding that were not apparent at higher doses. For example, while all rats would be expected to exhibit a decrease in infusions earned relative to the 0.015 dose, it is possible that high impulsivity could take more infusions than low impulsivity rats, demonstrating a greater sensitivity to nicotine which enables the low dose to retain some reinforcing properties. However, a true difference in sensitivity refers to a shift to the left of the dose-response curve (Piazza et al, 2000), such that group differences should have also been observed at the 0.09 dose if sensitivity to nicotine differed between groups. In any case, the hypothesized vertical shift in the dose-response curve was effectively ruled out with the doses tested in the present study, indicating that there were no group differences in overall responsiveness to the reinforcing properties of nicotine.

Finally, the lack of a significant effect was not due to inadequate power. For example, impulsivity group explained approximately 2.2% of the variance in average break point during

sessions 2 and 3 on a progressive ratio schedule. In order to achieve 80% power to detect an effect of this size, high and low impulsivity groups would have required approximately 190 rats each.

It is surprising, given adequate power and valid and reliable measures, that no relationship was observed between level of impulsivity and any measure of nicotine self-administration. This suggests that delay discounting is not related to a susceptibility to the reinforcing properties of nicotine. However, this does not necessarily preclude the possibility that smoking and delay discounting may be related to each other through a common neural substrate. Instead, it is possible it is not nicotine reinforcement, but rather some other process relevant to smoking or nicotine-taking behavior, which shares a common neural substrate with delay discounting. In order to further explore this possibility, it is important to consider how the current findings can be reconciled with investigations of other drugs of abuse and with the human smoking literature.

The demonstration of a prospective relationship between delay discounting and subsequent self-administration of cocaine and alcohol (Perry et al, 2005; Poulos, Le, & Parker, 1995) supports the hypothesis that, at least for some substances, drug self-administration and delay discounting share a common neural substrate. If this hypothesis is correct, then the results of the present study indicate that either this can only be detected under certain self-administration conditions, or it is only true for some substances. Thus, methodological differences in the measures used to assess self-administration or inherent differences between nicotine and cocaine and alcohol could both be factors contributing to the discrepancy in findings between research with other drugs of abuse and the present study. Concerning methodological differences, Perry and colleagues (2005) primarily investigated the percentage of high and low impulsivity rats

which acquired cocaine self-administration using an auto-priming procedure. In the present study, the simple nose poke operant and moderate nicotine dose used during acquisition were designed to maximize the number of rats which acquired self-administration and could undergo further testing. Thus, it is possible that individual differences related to delay discounting could have been observed under conditions designed to exaggerate the variability in the number of rats acquiring self-administration, such as by using a lower dose of nicotine or more rapidly increasing the FR schedule. However, both Perry et al (2005) and Poulos et al (1995) found significant differences in amount of cocaine and alcohol intake, respectively, between high and low impulsivity rats under stable self-administration conditions. This suggests that even when stable responding on a FR schedule is being assessed, differences between drugs may yield quite different results. For example, one potentially important difference between cocaine and nicotine is the demonstration that cocaine more readily produces dependence, as evidenced by compulsive, escalating use (Deroche, Le Moal, & Piazza, 1999).

This raises the possibility that delay discounting may be more closely related to nicotine dependence than to nicotine use per se. Indeed, this perspective is consistent with findings in the human literature. Although some studies have found an association between delay discounting and quantity of use (Ohmura, Takahashi, & Kitamura, 2005; Heyman & Gibb, 2006), other studies have failed to replicate this effect (Johnson, Bickel, & Baker, 2007; Sweitzer et al, 2008). By contrast, nicotine dependence has been shown to be relatively dissociable from measures of use (Donny & Dierker, 2007), and a recent study demonstrated that delay discounting was related to dependence as measured by the FTND but not to cigarettes smoked per day (Sweitzer et al, 2008). Although this finding awaits replication with other measures of nicotine dependence, the results are consistent with the current study. Similarly, another recent human

study found that steeper discounting predicted smoking during an abstinence reinforcement paradigm (Dallery & Raiff, 2006). This suggests that delay discounting may be an important marker for propensity to relapse—a construct central to dependence. Although the responding on a PR schedule is thought to tap motivational processes likely to be relevant for dependence, it is possible that other measures or procedures designed to assess the construct of dependence might have revealed an association with delay discounting. For example, previous studies have assessed behaviors such as persistence in responding for a drug during a signaled “no drug” period, continued self-administration despite aversive stimuli, and reinstatement of drug-seeking behavior following extinction (Deroche-Gamonet, Belin, & Piazza, 2004; Vanderschuren & Everitt, 2004). Indeed, such behaviors tend to cluster together, even when no differences in reinforcement are detected during stable limited access self-administration (Deroche-Gamonet, Belin, & Piazza, 2004). This suggests that these procedures are tapping into a common dependence construct that may not be apparent on a simple FR schedule.

Although no studies had previously evaluated the prospective relationship between impulsivity and nicotine self-administration in rats, Diergaarde and colleagues (2008) recently published a study addressing this question. They found that delay discounting failed to predict responding during self-administration on a FR schedule, but rats with steeper discounting extinguished their responses more slowly and made more active responses during reinstatement than less impulsive rats. Delay discounting also predicted increased responding during a PR test, but this effect did not emerge until the 7th session. These findings are partially consistent with the results of the current study. Our results replicated the lack of an effect of delay discounting during FR self-administration. However, contrary to the findings of Diergaarde et al, we did not find a relationship between delay discounting and infusions earned on a PR

schedule. This could be due to methodological differences, as Diergaarde et al increased the response ratio over repeated sessions, rather than after each infusion within a single session. Indeed, the authors argue that the emergence of an effect of delay discounting during later PR sessions could be less of a reflection of motivation for the drug, and may instead be more closely related to the failure to inhibit responding during extinction. The strongest effect of delay discounting observed in their study was during extinction and reinstatement, a test designed to assess persistence of drug-seeking and relapse following exposure to the drug or drug-related stimuli (Shaham et al, 2003). These findings support the interpretation that delay discounting may be related to nicotine dependence rather than nicotine reinforcement.

Collectively, this literature supports the hypothesis that smoking and delay discounting may be related to each other through a common neural substrate, and that delay discounting may be a marker for vulnerability to nicotine dependence. This is particularly important given that traditional theories of dependence have relied on the explanation that drug exposure leads to changes in the brain which contribute to the development of dependence. For example, Goldstein & Volkow (2002) posit that drug-induced deficits in prefrontal function lead to an impaired ability to inhibit drug seeking behavior—an important hallmark of addiction. By contrast, the literature reviewed above suggests that delay discounting may be a marker for a pre-existing susceptibility to dependence, which is likely linked to cortico-striatal-limbic circuitry. For example, individual variation in prefrontal inhibitory control pathways may predispose some individuals to develop dependence if they are exposed to a drug. Understanding how variation in these neural pathways may confer risk for dependence is an important area for further exploration. Phenotypic markers, like delay discounting, which may be associated with risk for dependence can provide a framework for both animal and human models to identify the

behavioral mechanisms and underlying neurocircuitry that contribute to vulnerability. Ultimately such efforts could be useful for increasing our understanding of the mechanisms underlying the development of nicotine dependence, and for facilitating the treatment and prevention of nicotine dependence for those most at risk.

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