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Differential-reinforcement-of-low-rate-schedule performance and nicotine administration: a systematic investigation of dose, dose-regimen, and schedule requirement

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Abstract

Differential-reinforcement-of-low-rate (DRL) schedules have been used to evaluate the effects of a wide variety of drugs, including amphetamines, cannabinoids, and antidepressant medication. To earn a reinforcer, organisms operating under a DRL schedule are required to withhold a response for a predetermined amount of time before responding, and therefore this schedule maintains a low rate of responding and can be viewed as a response-inhibition task. In experiment 1, three different DRL schedules (4.5, 9.5, and 29.5 s) were used to evaluate systematically a range of nicotine doses (0.0, 0.1, 0.3, and 0.5 mg/kg). The dose–response effect of nicotine then was compared with the effects of increased reinforcer magnitude on responding. Both the administration of nicotine and increased reinforcer magnitude engendered less accurate DRL-schedule performance compared with baseline conditions, and the dose and magnitude-dependent shifts were most evident on the DRL 29.5-s schedule. Experiment 2 compared the differences between acute and chronic dosing regimens (0.3 mg/kg nicotine) on DRL 29.5-s schedule responding. After 20 consecutive sessions of nicotine dosing, accuracy deteriorated significantly, demonstrating that chronic nicotine dosing leads to a behavioral sensitization apparent on the DRL 29.5-s schedule. The results from both experiments suggest that responding on the DRL 29.5-s schedule is sensitive to both dose–response and regimen-dependent effects of nicotine.

Keywords

chronic administration; differential-reinforcement-of-low-rate schedule; interresponse time greater than X schedule; nicotine; rat; reinforcer magnitude; sensitization

Introduction

Substance dependence is characterized by the search for immediate gratification and a heightened sensitivity to reward (Bickel and Marsch, 2001; Bechara and Damasio, 2002), and both of these features of drug dependence may be attributable to changes in the neurobiological systems involved in incentive sensitization (Koob, 1997; Robinson and Berridge, 2001; DiChiara, 2002). The lack of impulse control exhibited in substance dependence perpetuates use and represents a tremendous obstacle to treatment success and drug-induced impulsivity (e.g. Olmstead, 2006) is the focus of the current investigation. Evenden (1999) has noted that

impulsivity may be a product of a variety of independent and interconnected neurobiological phenomena that involve time perception, discounting of delayed rewards and response inhibition (Jentsch and Taylor, 1999). Recent evidence in rats has demonstrated that impulsive choice on an adjusting-delay procedure is the direct pharmacological result of chronically administered nicotine (Dallery and Locey, 2005), suggesting that the sensitizing effect of nicotine extends well beyond its ability to escalate global locomotor activity (Shim *et al.*, 2001; Schoffeleer *et al.*, 2002).

Differential-reinforcement-of-low-rate (DRL) schedules (e.g. McClure and McMillan, 1997) have been used to evaluate the effects of psychomotor stimulants on schedule-maintained response inhibition by reinforcing events. Behavioral inhibition represents a core feature of impulsive behavior (Evenden, 1999) and the effect of repeated and consecutive administrations of nicotine on the DRL-schedule performance has not previously been reported. However, before an analysis of the sensitization effects of chronically administered nicotine can be conducted, a systematic examination of the acute effects of nicotine on DRL-schedule performance is warranted.

The DRL schedule (also called an inter-response time greater than X schedule) requires the subject to withhold a response for at least a specific period of time (X) and then respond to obtain a reinforcer. Responses emitted before the criterion do not produce reinforcement and they reset the trial; therefore, the subject must learn to withhold responses for a certain amount of time before a response is reinforced. DRL schedules have been called models of timing behavior (Schuster and Zimmerman, 1961; Evenden, 1999), response inhibition (Popke *et al.*, 2000), and impulsive behavior (Van Den Broek *et al.*, 1987). The distribution of accumulated interresponse times (IRTs) from a session serves as the primary dependent measure obtained from DRL schedules, and the model IRT is representative of how accurately the subject can withhold the second response (McClure and McMillan, 1997). The median IRT value typically approximates the length of the DRL schedule, but longer DRL schedules tend to produce less accurate performance (Staddon, 1965; Doughty and Richards, 2002), meaning that under longer DRL-schedule values, the median IRT value occurs before the DRL criterion and the vast majority of responses are not reinforced.

Exposure to psychomotor stimulants interferes with the ability of the subject to procure reinforcement on a DRL schedule (e.g. McClure and McMillan, 1997). Some investigators have reported dose–response effects of acutely administered nicotine (Morrison, 1968; Pradhan and Dutta, 1970; Popke *et al.*, 2000), but conclusions drawn from these previous reports are limited because of the manner in which the data were presented. In some cases, the IRT distribution is not provided (Morrison, 1968) and none of the aforementioned studies evaluated a range of nicotine doses across a range of schedule requirements. McClure and McMillan (1997) used a range of DRL-schedule requirements to evaluate a variety of drugs, but nicotine was not included in their analysis.

Popke *et al.* (2000) compared the effects of nicotine across a wide variety of operant tasks including the DRL 10-s schedule and the temporal-response distribution test (TRD, also 10-s). Nicotine administrations varying from 0.30 to 0.56 mg/kg dose-dependently produced decrements in DRL-schedule accuracy and the median IRT value decreased (toward shorter IRTs) with increased doses of nicotine. The same range of nicotine dose did not significantly alter timing behavior as assessed by the TRD, and the researchers concluded that nicotine disrupts response-withholding without altering mechanisms of timing. Both TRD and DRL tasks revealed that larger doses of nicotine were associated with greater frequency of ‘bursting’ responses, which are responses that occur within the first 0.5 s of the DRL schedule. The greater frequency of bursting responses at larger doses of nicotine may be evidence of an increase in global locomotor activity rather than an increase in response inhibition, *per se*. To differentiate

the effects of psychomotor stimulants on response inhibition (and/or timing) from the escalation of global locomotor activity, other researchers (e.g. Schuster and Zimmerman, 1961) have used a 'relative' IRT frequency distribution and bursting responses then can be evaluated in proportion to total responding. Doughty and Richards (2002) discovered that larger reinforcer magnitudes (both water and food) engender poor performance on the DRL schedule, and the researchers attributed this finding to an enhanced motivational arousal produced by the interaction of a large reinforcer magnitude and the passage of time (Killeen, 1985). Nicotine may act to enhance motivational arousal produced by nondrug reinforcers, and this notion might explain why some researchers have found that nicotine administration disrupts DRL-schedule performance (Morrison, 1968; Pradhan and Dutta, 1970; Popke *et al.*, 2000). Indeed, a growing body of evidence supports the notion that nicotine enhances the reinforcing efficacy of consequential events (Caggiula *et al.*, 2002; Donny *et al.*, 2003; Olausson *et al.*, 2004a, b; Chaudri *et al.*, 2006). Furthermore, Pothuizen *et al.* (2005) discovered that lesions in the dorsolateral subdivision of the nucleus accumbens, called the core, lead to detriments in DRL-schedule maintained behavior. The dopaminergic neurons in this region have been implicated in the incentive-motivational processes involved in both reinforcement and impulsive behavior (Di Chiara and Bassareo, 2007). Therefore, nicotine may disrupt DRL-schedule performance by modifying motivational arousal created by nondrug incentives.

This investigation replicated previous research by examining the effects of nicotine on DRL-schedule maintained behavior (Morrison, 1968; Pradhan and Dutta, 1970; Popke *et al.*, 2000) and extended those findings by evaluating a range of doses (0.1, 0.3, and 0.5 mg/kg) across a range of schedule requirements (DRL 4.5-s, 9.5-s, and 29.5-s schedules). Experiment 1 was also designed to compare directly the disruptive effects of increased reinforcer magnitude (0.06 vs. 0.10 ml of sucrose solution) and nicotine administration on DRL-schedule performance and a relative analysis of bursting responses was included. The results from the first experiment demonstrate that DRL-schedule performance decrements, because of nicotine-dose and reinforcer-magnitude manipulations, were most evident on the longest of the three schedules (29.5-s).

To evaluate the consequences of chronic nicotine administration on response inhibition, a consecutive-dosing regimen was compared with an acute-dosing regimen of 0.3 mg/kg nicotine in experiment 2 using the DRL 29.5-s schedule. It was hypothesized that if nicotine acts in a manner similar to DL-amphetamine (Schuster and Zimmerman, 1961), then repeated once daily injections of nicotine should lead to behavioral tolerance and produce DRL-schedule performance similar to saline conditions. The results of experiment 2, however, show that chronically administered nicotine produced lesser response-inhibition (evidenced by fewer earned reinforcers and a leftward shift in the IRT distribution), and this result substantiated further previous evidence that repeated nicotine administration creates behavioral sensitization of impulsive-like responding (Dallery and Locey, 2005).

Methods

Experiment 1

Subjects—The subjects were nine, experimentally naive, male Sprague-Dawley rats (*Rattus norvegicus*, obtained from Charles River Labs, Inc., Montreal, Canada). They were 90 days old at the start of the experiment, with a mean body weight of 514.30 g. Housed in groups of three in a 20 × 30-in plastic home cage, they experienced a 12-h light/dark schedule, and all experimentation occurred during the light portion of the cycle. The rats had continuous access to water in their home cages and their food was restricted to 20 g of rat chow per rat per day, available 1 h after experimental testing occurred. A 6 × 12-in PVC tube and a Nylabone (Durable Chew, Neptune, New Jersey, USA) chew also were placed in their home cages. All

care and experimental procedures were approved by the Saint Michael's College IACUC before the initiation of the study.

Apparatus—Behavior was assessed using three identical operant test chambers (MED Associates, Inc., Saint Albans, Vermont, USA, model number ENV-007, 1991). Each operant chamber was housed in a PVC sound-attenuated cubicle with ventilating fan, and a houselight was located on the center of the back wall of the chamber, and two tricolor nose-poke operants on either side of the reinforcer-delivery well. To earn a mixture of sucrose and water (30 ml granulated sugar per 240ml water), subjects were required to operate the active (right side) tricolor cue light nose poke with infrared photobeam interrupts [green, red, and yellow light-emitting diodes (LEDs) situated in an equilateral triangle pattern within the nose poke; MED Associates, model number ENV-114M]. The LEDs, hereafter, are referred to as LEDg, LEDy, and LEDr (for green, yellow, and red, respectively). Responses on the left nose poke were recorded but did not result in reinforcer delivery. A liquid-reinforcement dipper (model number ENV-202M) was located in the center of the front wall, equidistant from the two nose-poke operanda. The liquid dipper contained a feeder cup that could be modified to administer either 0.06 or 0.10 ml of liquid per reinforcing event, and head-entry responses into the receptacle were monitored using an infrared photobeam. All response and reinforcement hardware were interfaced with a PC with MED-PC IV software (MED Associates, SOF-735, 1991), and all three operant chambers were operated simultaneously during the sessions.

Fixed-ratio schedule training—Each rat experienced one training session per day. During the training sessions, the houselight was illuminated as was the right nose-poke device (LEDg was illuminated). Consumption responses, or head entries into the dipper receptacle, were shaped using a variable time 30-s schedule (sugar water was delivered response independently on the average of once every 30-s). The dipper containing 0.06 ml of liquid was raised to make the reinforcer available, and rats were allowed 5 s to consume the liquid; once a head entry was detected in the dipper well by a photobeam interruption, the dipper remained in the available position for 5-s. The photobeam interrupt in the dipper well was used to ensure that the rats were making a consumption response before the dipper cup was made unavailable. The variable time 30-s schedule remained in effect until each rat made one response on the active operant nose-poke device (right side), then the rats were shifted automatically to a continuous-reinforcement schedule (fixed-ratio 1, FR1). During the FR1 schedule, LEDg terminated once a response was detected, and LEDr illuminated synchronously with the lifting of the liquid dipper to signal reinforcer availability. LEDr terminated after 5 s of liquid consumption. The next trial began with the illumination of LEDg. Sessions terminated after 50 min or once 50 reinforcing events occurred, and FR1-schedule training lasted for 10 consecutive days until all rats earned 50 reinforcers per session.

Differential-reinforcement-of-low-rate-schedule routine and training—The DRL schedule used was a discrete trial FR2 schedule with an imposed delay (Schuster and Zimmerman, 1961; Morrison, 1968; Pradhan and Dutta, 1970); as previously stated, DRL schedules commonly are referred to as 'IRT greater than X' schedules. This discrete trials procedure differs from some earlier research in which the IRT counter was initiated after the delivery of food (e.g. Doughty and Richards, 2002). Furthermore, the DRL schedules used also differed from others in which an upper-IRT limit was imposed (McClure and McMillan, 1997); no upper limit was used to restrict IRTs in this design. Rats were required to separate two responses by 'X' number of seconds to earn a reinforcer. After the format of the schedule used in FR schedule response training, LEDg and the houselight were illuminated to begin a trial. Once a response was detected, LEDg was terminated and LEDy was illuminated to signal the start of the IRT period. If the rat waited at least the appropriate predetermined amount of time (X) before making a second response, LEDy terminated and LEDr illuminated

synchronously with reinforcer delivery. Thus, the stimulus conditions during the IRT interval did not change (LEDy to LEDr) until the subject made a second response and a reinforcing event occurred. Again, a photobeam interrupt in the dipper well initiated the consumption period, and rats had 5 s to consume the 0.06 ml sucrose-water reinforcer. After 5 s elapsed, LEDr and the houselight were terminated, and the rats experienced a 5-s intertrial interval. Therefore, if a head entry in the dipper well was made immediately following reinforcer delivery, a total of 10 s would elapse before the beginning of the next trial. For nonreinforced trials, if the rat failed to wait X s before emitting a second response, then LEDy terminated synchronously with the houselight and the rat experienced a 10-s timeout before LEDg and the houselight again were illuminated, and a new trial began. Operant responses in the nose poke during the timeout period or consumption period had no effect on reinforcer availability but were recorded.

On day 11 of FR-schedule training, all rats were assigned randomly to one of three groups and responding was shaped under their appropriate DRL schedule (4.5, 9.5, or 29.5-s DRL schedules). These particular DRL schedules were chosen because they are approximately exponential derivatives of one another ($X^{1.5}$). The DRL-schedule-shaping session began with an IRT of 0.5 s, and once five reinforcers occurred, the DRL schedule was escalated by 0.5 s until five more reinforcers occurred, and so forth until each rat arrived at the appropriate schedule requirement for that group (either 4.5, 9.5-s or 29.5-s). Each session lasted 50 min, and each successive session began with the previous session's IRT. The 4.5-s DRL group reached their respective IRT within five consecutive sessions, the 9.5-s DRL group within 14 consecutive sessions, and the 29.5 group within 18 consecutive sessions; therefore, the groups differed with respect to the number of DRL-schedule training sessions. All rats continued to respond under their respective DRL schedules until stability in performance was achieved. Stability was defined as no monotonic increases or decreases in accuracy (number of reinforcers) over five consecutive sessions/day, as assessed by visual inspection and FR. Given that DRL-schedule training began on the same day for all subjects, the rats from the 4.5-s group reached stability before the other two groups, and were not exposed to experimental procedures for 7 days whereas the other two groups were brought up to the point of stability. Once stability was achieved, all rats were delivered 0.5 ml/kg of saline solution before each of 10 consecutive sessions; however, each rat experienced a 10-min blackout period before the DRL schedule was initiated.

Nicotine-administration sessions—Rats were administered 0.5 ml/kg physiological saline (0.9% solution) through subcutaneous injection for 10 sessions (as mentioned above) before all nicotine-administration sessions. Nicotine was prepared as a base to be administered in doses of 0.1, 0.3, and 0.5 mg/kg. Saline was used as the dilution for the nicotine ditartrate dihydrate (Sigma Chemical Co., St Louis, Missouri, USA), and the concentrations of nicotine varied per dose, such that the volumes of nicotine administration did not vary across doses and injections were standardized to 0.5 ml/kg.

Each rat was administered a dose of nicotine and then placed immediately into the operant chamber for a 10-min blackout, as previously experienced during saline-administration conditions in the DRL-schedule training phase. All rats were exposed to each dose of nicotine (0.1, 0.3, and 0.5 mg/kg) on at least two different occasions during experiment 1. Doses were counterbalanced across and within rats, so that on any given nicotine-dosing session/day, each rat in each experimental group received a different dose. Therefore, each rat in each group received a different order of nicotine doses. The nicotine-dosing, DRL-schedule sessions were separated by two sessions/days of saline DRL-schedule testing to allow for nicotine elimination, and this experimental phase (nicotine-dosing followed by 2 days of saline-dosing) lasted 18 sessions for all groups.

Reinforcer-manipulation sessions—Experiment 1 was designed to evaluate the effects of nicotine administration across a range of DRL schedules, and to compare the performance-interfering effects of nicotine administration to the performance-interfering effects created by large reinforcers. Therefore, once the effects of each nicotine dose were evaluated, rats experienced four DRL-schedule sessions during which the amount of liquid received per reinforcing event was increased from 0.06 to 0.10 ml. To follow the protocol established during the nicotine-administration sessions, rats in each group experienced a large-reinforcer (0.10 ml) session followed by two small-reinforcer sessions (0.06 ml). This protocol was repeated until each rat experienced four large-reinforcer sessions. All but two large-reinforcer sessions were preceded by 0.5 ml/kg saline injection; on two occasions, rats experienced a 0.3-mg/kg nicotine injection to evaluate the interactive effects of nicotine on large-reinforcer, DRL-schedule performance. During all large and small-reinforcer DRL-schedule sessions, the amount of time the rat was given to consume the reinforcer remained constant (5 s after the detection of a head entry; this 5 s consumption period was established at the outset of the experiment to allow for the consumption of the 0.10 ml reward).

Experiment 2

Subjects—The nine rats from experiment 1 were reassigned into one of three new groups, all trained and maintained under a DRL 29.5-s schedule. The saline group received only saline for the remainder of the experiment. The acute-dosing group received five nicotine-dosing sessions (0.3 mg/kg), each separated by three saline sessions, and all of these sessions were distributed over the course of 20 consecutive days. The chronic-dosing group was administered 0.3 mg/kg nicotine daily for 20 consecutive days.

One rat from each of the three aforementioned groups from experiment 1 was assigned to each group for experiment 2, such that newly established groups (saline, acute, and chronic) contained one rat from each of the 4.5-s, 9.5-s, and 29.5-s schedule/groups. Furthermore, each home cage contained one animal from each of the three new experimental groups, and each operant chamber operated with one rat from each of the three newly established groups; these controls were established to prevent housing, time-of-day, and experimental-chamber confounds. All other housing and experimental protocols were similar to experiment 1.

DRL 29.5-s schedule training—The training protocol resembled that of experiment 1 in that those rats previously maintained under 4.5-s and 9.5-s schedules were shaped to respond under a DRL 29.5-s schedule (see day 11 of behavioral training in experiment 1 for greater detail). Training continued until there were no monotonically increasing or decreasing trends in the number of reinforcers delivered per session or response rate (based on FR) for all three groups operating under the 29.5-s schedule.

Testing—Testing commenced after 17 consecutive days of 29.5-s DRL-schedule training. On day 1 of testing, the acute and chronic groups received 0.3 mg/kg subcutaneously delivered nicotine before experimental sessions, again dissolved as a base solution in accordance with the procedures established in experiment 1. The saline group received 0.5 ml/kg of saline before all testing sessions, so that all rats in all groups received a same volume of solution before each session. On day 2, only the chronic group received the nicotine injection. The acute group received nicotine on testing days 1, 5, 9, 13, and 17; thus, each nicotine-delivery session was separated by 3 days/session of saline-administration testing to allow for nicotine clearance. The chronic group received 20 consecutive doses of 0.3 mg/kg nicotine, each dose delivered before experimental testing on 20 consecutive days. The reinforcer for all sessions was 0.06 ml of sucrose solution.

Data analysis

The interresponse time cumulative frequency distribution analysis—The degree to which nicotine altered the distribution of IRTs was the primary dependent variable in this investigation. Increases in the dose (milligrams/kilogram), duration of nicotine-dose regimen, and reinforcer magnitude were expected to correspond to increases in the frequencies of short IRTs; therefore, the distribution of IRTs was expected to shift leftward as a result of each experimental manipulation. A three-step process was used to evaluate changes in the IRT distribution in response to each manipulation.

First, relative-frequency histograms (Schuster and Zimmerman, 1961; Richards *et al.*, 1993; Doughty and Richards, 2002) were constructed using 2 day/session means for each subject in each experimental condition. The relative-frequency histograms included the proportion of total IRTs per time bin (Richards *et al.*, 1993 for a more thorough description of relative-frequency histograms).

The second step in the IRT-distribution analysis involved the transformation of the relative frequency histograms into curvilinear, cumulative IRT frequency distributions (McClure and McMillan, 1997). To analyze differences across experimental manipulations, a nonlinear regression analysis was then performed on each subject's cumulative IRT frequency distribution for each condition (e.g. saline, 0.1 mg/kg) and compared with a cumulative Gaussian distribution for proportions, illustrated by:

$$\Phi(x) = \Phi\left(\frac{x - \mu}{\sigma}\right), \quad 0 < x < 1$$

μ and σ (the mean and standard deviation) are for the cumulative distribution function $\Phi(x)$ of the Gaussian equation. This equation was used because the cumulative IRT frequency distribution followed the sigmoid shape of cumulative normal curve and includes an asymptote at 1.0. The μ in the equation (0.5 on the y-axis) represented the median IRT time bin between 0.0 s and the asymptote of the cumulative curve; changes in μ from baseline/saline conditions indicated the degree of the leftward shift in the cumulative IRT distribution in response to the independent variable manipulations. The standard deviation (σ) denoted the slope of the curve and smaller numbers yielded steeper slopes.

The nonlinear regression analysis was performed on each subject's cumulative IRT frequency distribution per experimental condition using the 'cumulative Gaussian function for fractions' analysis in Prism 5 [Graphpad Software 5 for Windows (2007), San Diego, California, USA]. The results of the nonlinear regression yielded values of both free parameters (μ and σ) and a goodness-of-fit value (R^2). The values did not violate the homogeneity of variance assumption of parametric statistics (based on Mauchly's test of sphericity) and three separate analyses of variance (ANOVAs) were performed to determine whether the μ , σ , and R^2 values differed across experimental conditions. For experiment 1, a 3 (group) $\times$ 4 (saline, 0.1, 0.3, 0.5 mg/kg) ANOVA was performed to evaluate changes in the cumulative IRT frequency distribution due to nicotine dose. Furthermore, a 3 (group) $\times$ 3 (saline 0.06ml, saline 0.10 ml, and 0.3mg/kg nicotine+0.10 ml sucrose solution) ANOVA was conducted to evaluate changes across reinforcer-manipulation sessions. For experiment 2, a 3 (group: saline, acute, and chronic) $\times$ 2 (dose: predose vs. endpoint dose) mixed-design ANOVA was performed.

General performance measures—In addition to the IRT cumulative frequency distribution, performance on the DRL schedules was evaluated by several dependent measures: percent-correct trials, reinforcing events earned per 50-min session, number of time outs (trials in which no reinforcers were delivered), bursting responses relative to total responding, number

of head entries into the dipper well, and responses per minute on both the active hole poke and the inactive hole poke (adjunctive response) operants. A factorial ANOVA was performed to evaluate the effects of schedule length, nicotine dose, reinforcer magnitude, and nicotine-dose regimen on the aforementioned dependent variables. Before drug dosing in experiment 1, a repeated measures ANOVA was used to determine that stability in reinforcement rate and response rate was achieved across 5 consecutive days in the baseline phase before proceeding to the nicotine-administration phase.

Results

Experiment 1

Baseline stability—Baseline stability was defined as no monotonically increasing or decreasing trends in reinforcers earned per 50-min session across 5 consecutive days, and a repeated-measures ANOVA was performed to verify that each group had met the stability criteria ($F=1.0$, $P>0.1$). Stability was achieved by the 28th sessions of DRL-schedule responding for all three groups.

The interresponse time cumulative frequency distribution analysis, nicotine-administration sessions—The mean IRT cumulative frequency distributions for each group are displayed in Fig. 1. As each nicotine-dosing session/day was separated by two saline sessions/day, the cumulative distribution for the control for each group was created using data from 14 sessions of saline dosing (two sessions before nicotine dosing, two sessions after the last nicotine-dosing session, and 10 intradosing saline sessions). The data from 14 saline sessions were collapsed because nicotine seems to have little effect on the cumulative IRT distribution 24-h after administration (Fig. 2b).

The cumulative IRT frequency distributions for each nicotine dose represent the average of two nicotine-administration sessions per rat per group. Nonlinear-regression analysis was performed on each subject's cumulative IRT frequency distribution for each condition. A significant main effect of dose was discovered for the parameter values of μ [$F(3,18)=3.94$, $P<0.05$, $\eta^2=0.40$]. No significant main effects were discovered for the σ parameter or goodness-of-fit (R^2) values [$F(3,18)=0.25$ and 1.07 , respectively, NS]. Therefore, the cumulative Gaussian equation was a useful and reliable quantitative tool for distinguishing leftward shifts (indicated by changes in μ) of IRT distributions. See Table 1 for a list of mean parameter and R^2 values as well as the results from the least-significant difference (LSD) post-hoc multiple comparison across experimental conditions within each group.

Significant between-group main effects were discovered for both free parameters, μ and σ [$F(2, 6)=8.33$ and 42.46 , respectively, $P<0.01$, $\eta^2>0.73$] but not R^2 values [$F(2,6)=2.64$, $P>0.1$]. Given that the median IRT values associated with each group were dependent upon the DRL criteria for each group, this effect was not surprising. Tukey's HSD post-hoc multiple comparison revealed that the 29.5-s group differed with respect to μ values compared with the 4.5-s and 9.5-s groups, but no significant differences were discovered among σ values across groups. Significant dose $\times$ group interactions were not discovered either for parameter values or goodness-of-fit value [$F(6,18)<1.91$, NS]. The results of both between and within-group comparisons suggest that the goodness-of-fit of the nonlinear regression model does not significantly vary across experimental conditions.

Visual inspection of the 4.5-s group's cumulative IRT distribution fails to provide evidence that nicotine influenced DRL-schedule performance, and the nonlinear analysis substantiates the failure to reject the null hypothesis. The cumulative IRT distributions for the 9.5-s (Fig. 1c) and 29.5-s (Fig. 1e) schedule groups both reveal differences between the doses of nicotine and the control/saline condition. For the 9.5-s schedule group, significant differences were

apparent between the 0.3 and 0.5 mg/kg nicotine doses and saline (Tables 1 and 2). Nicotine doses of 0.1 mg/kg and above also significantly altered the distribution of responses for the 29.5-s schedule group.

The question remains whether the shifts in the IRT cumulative frequency distributions for the 9.5 and 29.5-s schedule groups were predictable in a dose-dependent manner. For the 29.5-s schedule group, a multiple pairwise comparison (LSD) of the μ values associated with each dose-revealed differences between the 0.1mg/kg dose and both the 0.3 and 0.5 mg/kg doses, but no significant difference between 0.3 and 0.5 mg/kg was discovered, nor were there any significant differences across the range of nicotine doses for the 9.5-s schedule group. In response to nicotine administration, both the 9.5-s and 29.5-s schedule groups showed changes in DRL-schedule performance; however, dose-dependent effects were evident only under the 29.5-s schedule.

The interresponse time cumulative frequency distribution analysis, reinforcer-manipulation sessions—For each group, cumulative IRT frequency distributions were created from four sessions in which the magnitude of the sucrose reinforcer was increased from 0.06 to 0.10 ml per reinforcing event (Fig. 1b, d, and f). Two of these four sessions were preceded by 0.3 mg/kg nicotine administration, and the other two were preceded by saline. The ANOVA revealed no significant main effect of reinforcer manipulation for either parameter value or R^2 value [$F(2,12) < 1.67$, NS], but there was a significant between-groups main effect for μ and σ values [$F(2,6) = 27.79$ and 127.83 , respectively, $P < 0.01$, $\eta^2 > 0.90$]. No between-group difference was discovered for goodness-of-fit [$F(2,6) = 2.54$, NS]. A significant group $\times$ reinforcer magnitude interaction was discovered for μ values [$F(4,12) = 31.72$, $P < 0.05$, $\eta^2 = 0.43$] suggesting that the effectiveness of the reinforcer-magnitude manipulation on DRL performance was dependent upon the schedule requirement.

Values for each parameter are listed in Table 1, as well as the results from a LSD post-hoc multiple comparison. These results suggest, under saline conditions, increased reinforcer magnitude shifted significantly the cumulative frequency IRT distribution leftward for 29.5-s group, but not for the 4.5-s or 9.5-s groups. The combination of nicotine injection and increased reinforcer magnitude failed to shift significantly the distribution further leftward for the 29.5 group.

General performance measures—Table 2 displays the means for each group and each dosing condition for a wide variety of secondary dependent variables. These secondary measures can be divided between accuracy measures (e.g. the number of reinforcers earned) and response rate measures (e.g. total operant responses). The number of reinforcers earned per session and the number of time-out events per session were used to obtain ‘percent-correct trials’ to parallel the performance measure of accuracy in McClure and McMillan (1997). ‘Percent bursting’ refers to the percent of IRTs allocated to the first time bin relative to the total number of IRTs. Absolute values for bursting responses could not be used because the total number of responses increased under nicotine conditions.

Using the means obtained for each rat (two sessions per condition), a 3 (schedule requirement) $\times$ 6 (manipulation: saline/control, 0.1, 0.3, 0.5 mg/kg, large reward, large-reward/0.3 mg/kg interaction) mixed-design ANOVA was performed including percent-correct trials, percent bursting, operant responses, adjunctive responses, and head-entry responses. A significant main effect of schedule requirement was revealed [$F(2,6) = 96.29$, $P < 0.05$, $\eta^2 = 0.97$], suggesting that the DRL-schedule requirement affected some of these secondary measures. Tukey’s HSD post-hoc multiple comparison showed that the groups differed from one another with respect to percent-correct trials and response rate ($P < 0.05$), but not with respect to the number of head entries, adjunctive responses, nor to percent bursting. The comparison of response-rates across

groups demonstrates that the schedules were indeed controlling the rate of operant responding. Figure 2 illustrates the effect of dose on operant response rate for each schedule requirement. A dose–response effect is evident at the 4.5-s DRL requirement but not the other two DRL schedules.

Significant main effects of dose were evident for each of the aforementioned dependent variables except bursting and adjunctive responding [$F(5,30) > 2.99$, $P < 0.05$, $\eta^2 > 0.33$]. Pairwise multiple comparison (LSD) was used to compare the effects of each dose to the saline condition, and a general trend was discovered showing a decrease in percent-correct trials ($P < 0.05$) with increases in nicotine dose across groups (Table 2). A significant group $\times$ dose interaction was apparent for response rate [$F(10, 30) = 4.77$, $P < 0.05$, $\eta^2 = 0.61$] confirming the results depicted in Fig. 2a that dose–response effects of nicotine on response rate were only evident on the 4.5-s schedule. Group $\times$ dose interactions were not significantly different for head entries, adjunctive responses or percent bursting (all $P > 0.05$).

The results from the ANOVA demonstrate that several dependent measures can be used to describe the effects of nicotine on DRL-schedule performance. The present statistical analyses revealed that (i) accuracy, as measured by percent-correct trials per session, was sensitive to changes in dose and reinforcer magnitude; (ii) bursting responses, relative to total responses, were not significantly altered as a result of nicotine dosing; (iii) head-entry responses were not constrained by schedule requirement; and (iv) a dose effect of nicotine on response rate was only evident at the 4.5-s schedule. The shifts in IRT distributions under the DRL 9.5-s and 29.5-s schedules were created by a true leftward shift in the IRT distribution, and bursting responses and response rate measures were not altered systematically by either nicotine-dose or reward-magnitude manipulations.

Experiment 2

Baseline stability—For all three groups, baseline stability (in terms of reinforcement and response rate) under saline-dosing control conditions was achieved after 17 sessions of DRL 29.5-s schedule experience ($F = 1.0$, $P > 0.1$). Figure 3a illustrates the mean cumulative IRT frequency distributions for all three groups on day 17 of DRL 29.5-s schedule training, and these distributions are similar.

The interresponse time cumulative frequency distribution analysis—The mean IRT cumulative frequency distributions for each group are displayed in Figs. 3 and 4. Once again, the cumulative Gaussian equation for nonlinear analysis (Graphpad Prism 5, 2007) was used to evaluate the leftward shifts in the cumulative IRT frequency distributions for each group and condition. The control for each group was that group's average IRT distribution for the 5 days before the commencement of testing, labeled 'predose' in Fig. 3a. Visual inspection of the data from the saline group (Fig. 3b) shows no change in the cumulative IRT frequency distribution over the course of 20 testing days. The 'predose' IRT distributions were obtained from an average of the five saline-administration (baseline) sessions before the first dose of nicotine for any group. The 'endpoint-dose' IRT distributions were obtained from all five acute-dose sessions for the acute group, and the final five saline-dose and nicotine-dose sessions for the saline and chronic groups, respectively.

A significant main effect of dose was discovered for μ [$F(1,6) = 5.11$, $P < 0.05$, $\eta^2 = 0.46$], suggesting that 0.3 mg/kg nicotine significantly shifted the median of the IRT distributions to the left from predose/baseline conditions. No significant main effect of dose was revealed for σ [$F(1,6) = 0.91$, NS] and R^2 approached significance [$F(1,6) = 3.70$, $P = 0.07$]. A significant between-groups main effect was significant in terms of σ [$F(2,6) = 6.71$, $P < 0.05$, $\eta^2 = 0.46$] but not for μ nor R^2 [$F(2,6) = 0.90$ and 0.06 , respectively, NS]. No dose $\times$ group interactions were significant, although μ approached significance [$F(2,6) = 4.48$, $P = 0.06$]. Collapsing across

groups, pairwise multiple comparisons (LSD) revealed that values for μ and R^2 differed ($P < 0.05$) between endpoint and predose conditions. This result suggests that the steep slope associated with chronic nicotine dosing may have led to a significantly poorer goodness-of-fit value, but this value was still quite high (0.85) (Table 3). Tukey's HSD revealed significant differences across σ values between the chronic and saline groups ($P < 0.05$), but all other between-group comparisons were not significant.

Figure 3 depicts the average of all five nicotine-dosing sessions for the acute group, compared with the average of five predosing sessions for that group, and this result replicated the effect of acute dosing on DRL 29.5-s schedule performance in experiment 1. Figures 3 and 4 illustrate the leftward shifts in the cumulative IRT distribution resulting from chronic nicotine dosing. The average of the first 5 chronic-injection days resembles the performance from the acute group, but the data from the final five sessions (testing days 16 through 20) exemplify poorer DRL-schedule accuracy for the chronic group. Thus, repeated, consecutive days of nicotine dosing led to less accurate responding under the DRL 29.5-s schedule. Figure 4 illustrates the time-course of the sensitization effect, and the greatest leftward change in the cumulative IRT distribution seems to have occurred between days 6 and 8 of consecutive daily dosing.

General performance measures—ANOVA was performed on percent-correct trials, percent bursting, response rate on both the active and inactive operants (adjunctive responses), and head-entry responses. The 'predose' averages were obtained from the five saline-administration (baseline) sessions before the first dose of nicotine for the acute and chronic groups. The 'endpoint dose' averages were obtained from all five acute-dose sessions for the acute group, and the final five saline-dose and nicotine-dose sessions for the saline and chronic groups, respectively. No significant main effects of group for any general-performance measure [all $F(2,6) < 1.20$, NS] were observed; see Table 3 for means and standard errors for each group for each general-performance measure. Significant main effects of dose regimen were apparent for the number of head entries and response rate per minute [$F(1,6) = 11.13$ and 85.56 , $P < 0.05$, $\eta^2 = 0.65$ and 0.93 , respectively], but not for percent-bursting or adjunctive responses [$F(1,6) > 0.07$, NS]. The main effect for percent-correct trials approached significance [$F(1,6) = 5.07$, $P = 0.06$, $\eta^2 = 0.46$]. Significant group $\times$ dose interactions were revealed for percent-correct trials [$F(2,6) > 5.24$, $P < 0.05$, $\eta^2 = 0.64$], operant response rate per minute [$F(2,6) = 12.76$, $P < 0.05$, $\eta^2 = 0.81$], and head entries [$F(2,6) = 6.54$, $P < 0.05$, $\eta^2 = 0.69$], but not adjunctive responses nor for the percent-bursting responses ($P > 0.50$).

Percent-correct trials, reported in Table 3, echo the graphic results depicted in Fig. 4 in that chronic administrations of nicotine led to greater impairments in DRL-schedule responding than did acute administrations. Head entries and responses per minute escalated under nicotine-administration conditions, but paired t -tests within each group across predosing and endpoint-dosing conditions failed to verify that response rate differed significantly. Percent bursting and adjunctive responding were unaltered as a result of nicotine delivery. Furthermore, the leftward shift of the cumulative IRT frequency distribution in response to acutely delivered 0.3 mg/kg nicotine seems to be a robust and reliable phenomenon. Poor DRL-schedule performance accuracy in the chronic group is evidenced by an extreme distortion of the IRT frequency curve relative to predosing and acute-dosing sessions. Furthermore, the difference in the cumulative IRT distribution between acute and chronic groups cannot be attributable to a sensitization of global locomotor behavior (Shim *et al.*, 2001) because response rate does not differ between groups, although some differences were apparent within each group compared with baseline/saline conditions (Table 3).

Discussion

Experiment 1

The results demonstrate that the disruptive influence of nicotine on DRL-schedule performance is more evident at longer schedules and emphasize the importance of examining a range of doses across a range of schedule requirements. A dose–response relationship on the cumulative IRT frequency distribution was evident among those rats maintained at a DRL 29.5-s schedule, but not the 9.5-s nor the 4.5-s schedules, and these results further substantiate the finding that perturbations in DRL-schedule responding are more evident at longer schedule requirements (Doughty and Richards, 2002). Although the DRL 9.5-s schedule was sensitive to the administration of nicotine, differences among doses were not apparent using the cumulative IRT frequency distribution analysis. The possibility exists that doses above 0.5 mg/kg would provoke significantly greater perturbations on DRL 9.5-s responding, but Popke *et al.* (2000) found that doses of 0.75 mg/kg produced DRL 10-s performance equal to the effects of 0.42 mg/kg nicotine.

The cumulative IRT frequency distribution for DRL 4.5-s schedule was insensitive to both nicotine and reinforcer-magnitude manipulations. As shorter DRL schedules seem to be less vulnerable to increases in reinforcer magnitude (Doughty and Richards, 2002), the failure to disrupt responding under the DRL 4.5-s schedule with nicotine was not surprising. Dose-dependent or magnitude-dependent differences, however, could potentially be discovered under the DRL 4.5-s schedule with greater experimental resolution achieved by increasing the subject population or the number of IRT bins. McClure and McMillan (1997) found that behavior maintained under a DRL 4-s schedule was responsive to doses of methamphetamine, and their analysis included four rats and 0.4-s IRT bins. This study used three rats and 0.5-s IRT bins; therefore, the results from the two studies indeed may be comparable. Morrison (1968) stated that nicotine and low doses of amphetamine have similar effects on DRL 20-s schedule performance, so one might conclude that the effects of amphetamine and nicotine are distinguishable on short (e.g. 4 or 4.5 s) DRL-schedule requirements.

Response rate differed significantly with increasing doses of nicotine for the DRL 4.5-s schedule, but not the longer 9.5-s or 29.5-s schedules. This result is perplexing given that the cumulative IRT frequency distributions for the 4.5-s schedule were not altered with increases in doses, but the distributions for the 9.5-s and 29.5-s schedules shifted leftward as a result of dose. A plausible explanation for this finding is that as responses emitted during the consumption, blackout, or intertrial period were included in the response-rate measure, response rate was only partially controlled by reinforcement on the DRL schedule. Given that subjects operating the DRL 4.5-s schedule earned more reinforcers, they also had a greater opportunity to respond during consumption and intertrial intervals.

Bursting responses, as measured relative to the total amount of operant responding, were not dose-dependently altered as a result of nicotine administration. These results suggest that increases in the absolute frequency of bursting responses may be evidence of an increase in global locomotor behavior in response to nicotine. When bursting is evaluated relative to total operant responses (as in this report), there seems to be little difference in the degree of bursting across schedule requirements and there is no discernable pattern of increases or decreases in percent bursting because of different doses of nicotine.

The purpose of the reinforcer-magnitude manipulation was not to test whether nicotine enhances the motivation- arousal engendered by food reinforcement, but rather to compare modestly the disruptive effects of two manipulations (nicotine dose and reinforcer magnitude) at various schedule requirements. At a very basic level, the comparison demonstrates that a variety of experimental manipulations can create perturbations in DRL-schedule performance,

and conclusions regarding underlying motivational mechanisms cannot be asserted without more substantial evidence. In this experiment, three doses of nicotine and only two levels of reinforcer magnitude were used. Perhaps a greater diversity of reinforcer magnitudes and doses would help to elucidate functional differences of these two manipulations on the DRL-schedule performance and/or lend some support to an enhanced motivational arousal hypothesis regarding the behavioral effects of nicotine.

Experiment 2

Experiment 2 was performed to determine how repeated and consecutive administrations of nicotine would alter DRL-schedule performance. The results demonstrate the development of a behavioral sensitization effect in response to once-daily administrations of 0.3 mg/kg nicotine rather than the emergence of tolerance; the chronic group's IRT distribution shifted away from reinforced IRTs with successive administrations of the drug. This result stands in stark contrast to those results reported by Schuster and Zimmerman (1961) with DL-amphetamine, showing that DRL 17.5-s schedule accuracy improved with consecutive once daily dosing. Balcells-Olivero *et al.* (1997), however, used a DRL 72-s schedule and found that twice weekly dosing with amphetamine leads to sensitization. The inconsistencies in the literature on DRL-maintained behavior in response to repeated administration of amphetamine may be because of either the different lengths of DRL schedules used or the different intervals separating drug administrations (Balcells-Olivero *et al.*, 1997).

In experiment 2, rats in the acute group received several nicotine doses over 20 consecutive days, but each dose was followed by three sessions of saline administration. As the acute group did not demonstrate the deteriorating performance exhibited by the chronic group, after each acute injection with 3 days of saline dosing must have allowed for drug elimination sufficiently; therefore, the evidence would suggest that spaced nicotine dosing prevents the development of sensitization. As experiment 2, however, did not compare directly the effects of spaced dosing versus the overall frequency of dosing, it is not possible to say conclusively that spaced dosing prevents the development of sensitization. Furthermore, the rats in the chronic group had experience with intermittent dosing in experiment 1. Therefore, the sensitizing effect of nicotine on the DRL 29.5-s schedule evident in experiment 2 may not be due entirely to consecutive daily dosing intervals. Future experimentation should include a comparison of intermittent versus consecutive dosing conditions between groups and control for the overall frequency of dosing to resolve these questions.

General discussion

These data replicate results reported elsewhere that acutely delivered injections of nicotine alter DRL-schedule-maintained behavior (Morrison, 1968; Pradhan and Dutta, 1970; Popke *et al.*, 2000) and extend those findings with a systematic evaluation of the effects of nicotine at a range of doses and schedule requirements. Compared with saline conditions, the administration of nicotine produced less accurate DRL-schedule performance under both the 9.5-s and 29.5-s schedule requirements, but failed to alter behavior significantly under the 4.5-s schedule. Furthermore, only the DRL 29.5-s schedule was sensitive to differences among doses, and the cumulative IRT frequency distribution was an informative index of DRL-schedule performance.

Experiment 1 was designed not only to assess the disruptive effects of a range of nicotine doses, but also to compare the effects of nicotine with the disruptive effects of increased reinforcer magnitude. Consonant with previous findings (Doughty and Richards, 2002) increases in reinforcer-magnitude-produced DRL-schedule performance decrements in a manner that was not dissimilar to the effects of acutely delivered nicotine. Doughty and Richards (2002) demonstrated a schedule-dependent effect of reinforcer magnitude on DRL-schedule accuracy.

These researchers suggest that arousal induced by the larger reinforcer magnitude interferes with accurate DRL-schedule performance, and that DRL-schedule maintained behavior at longer schedules is particularly sensitive to manipulations of reinforcer magnitude. The results reported here further substantiate those findings because the reinforcer-magnitude manipulation was particularly successful in disrupting responding under the longest of the three schedules used.

The most robust, and perhaps the most surprising, discovery in this investigation is that chronic nicotine exposure engendered a greater leftward shift in the cumulative IRT distribution than did acute administration. Furthermore, after 20 consecutive sessions of 0.3 mg/kg nicotine administration, rats in the chronic group exhibited poorer accuracy than those rats in the acute group. The deteriorating performance in the chronically administered group appears to be evidence of a sensitization effect resulting from repeated nicotine delivery. Shim *et al.* (2001) demonstrated that chronic nicotine exposure creates a sensitizing effect on general locomotor activity, so the fact that response rate failed to differ between the chronic and acute groups in this investigation is noteworthy. The leftward shift in the cumulative IRT distribution in this study is evidence of a sensitization effect of nicotine involving a variable other than global response rate.

Chronic administration, defined here as once daily dosing of 0.3 mg/kg nicotine, led to a lower reinforcement rate as DRL-schedule accuracy declined. The diminished density of reinforcement experienced by the chronic group may have contributed to the deterioration of DRL-schedule accuracy, possibly due to frustration effects (Amsel and Surridge, 1964) induced by diminished reinforcer value. In experiment 1, however, decreased density of reinforcement produced by smaller reinforcer magnitudes engendered more accurate DRL performance. Therefore, it is unlikely that the chronic group's deteriorating performance is because of a diminished reinforcement density alone. Experiment 2 did not equate reinforcement rate across the saline, acute, and chronic groups, and to avoid this potential limitation in interpretation, future investigations of drug effects on DRL-schedule maintained behavior should include methods to do so.

Nicotine administration disrupted DRL-schedule-maintained behavior in each of the two aforementioned experiments, and these data may represent a form of drug-induced impulsivity (cf. Olmstead, 2006). DRL schedules also have been described as evaluative of timing mechanisms (Schuster and Zimmerman, 1961), and an examination of the literature on the effects of reinforcer magnitude and nicotine on temporal behavior is necessary. As measured by assays other than the DRL schedule, there are mixed and limited results regarding reinforcer-magnitude effects on timing (MacEwen and Killeen, 1991; Grace and Nevin, 2000; Ludvig *et al.*, 2007). With peak-interval procedures, the most reliable finding is that larger reinforcer magnitudes engender shorter wait times (Ludvig *et al.*, 2007), and this result may be congruent to the effects of larger reinforcer magnitudes on DRL-schedule performance (cf. Doughty and Richards, 2002; Ludvig *et al.*, 2007). Overall, however, there are few reliable effects of reinforcer magnitude across studies in measures (e.g. peak times and stop times) that might characterize timing (e.g. Gallistel *et al.*, 2004). The effects of nicotine on timing (Bizot, 1997; Popke *et al.*, 2000) mirror the timing results just discussed in that there are limited results and minimal evidence for robust effects.

Given that chronically administered nicotine produces greater delay discounting (Dallery and Locey, 2005) and poorer DRL-schedule performance (this study), these two different behavioral assays may be united by a common underlying process. Adjusting-delay procedures (Dallery and Locey, 2005) and DRL schedules (Van Den Broek *et al.*, 1987) have been described and used as tests of impulsive behavior, and although other factors may play a role in each of these tasks, they both seem to be sensitive to dose-regimen-dependent effects of

nicotine. Dallery and Locey (2005) found that 65 consecutive days of dosing (0.3 mg/kg) produces behavioral sensitization on an adjusting-delay procedure, whereas in the current investigation, the sensitization effect occurred within 20 days on the DRL 29.5-s schedule.

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Fig. 1.

Results from experiment 1 are depicted as cumulative interresponse time greater than (IRT) frequency distributions with time bins on the horizontal axis and percent cumulative frequency on the vertical axis. (a, c, and e) (on the left) Saline, 0.1, 0.3, and 0.5 mg/kg nicotine doses for each of the 4.5-s, 9.5-s and 29.5-s differential-reinforcement-of-low-rate (DRL)-schedule requirements, and subjects were responding for 0.06 ml of sugar water solution. Each line represents the results from the nonlinear regression model and the data points represent an average from three animals per condition, and two administrations per dose. Open boxes for all figures illustrate the same data for animals responding under saline conditions to obtain 0.06 ml of sugar water solution. Dose-dependent alterations in IRT patterning are evident for

subjects operating the 9.5-s and 29.5-s DRL schedule. (b, d, and f) Averages obtained from the reward-manipulation sessions during which subjects experienced four sessions of increased magnitudes of reward per reinforcing event (0.10 ml rather than 0.06 ml of sugar water); half of these sessions were preceded by saline and the other two sessions were preceded by 0.3 mg/kg nicotine injection.

Fig. 2.

Mean rate of responses per minute, per group for experiment 1 is depicted in (a). A dose-effect relationship on response rate was evident in the differential-reinforcement-of-low-rate (DRL) 4.5-s group only; *Significant within-group differences due to dose. (b) The cumulative interresponse time greater than (IRT) distributions, per schedule requirement, for the two saline-administration sessions/day that separated each nicotine-dosing day in experiment 1. The figure illustrates the similarity between the cumulative IRT frequency distributions across all saline conditions. 'Baseline' performance illustrates the IRT distribution for the five saline administrations before the first dose of nicotine. '24-h after' represents the average data obtained from the first saline-administration session/day after a nicotine administration day,

and the next successive day of saline administration is labeled '48-h after'. The results depicted in (b) demonstrate that nicotine failed to alter the IRT distributions 24-h after administration.

Fig. 3.

Data from experiment 2 are represented as cumulative interresponse time greater than (IRT) frequency distributions (a–d) and the line plots are the results from the nonlinear regression analysis. The ‘predose’ comparison (a) is the average of the final 5 days of differential-reinforcement-of-low-rate (DRL) 29.5-s training for all three groups. The saline group’s performance was unaltered by further exposure to the DRL schedule (b). (d) The dramatic shift in the chronic group’s performance with repeated administrations of nicotine; after consecutive nicotine-dosing sessions, the vast majority of IRTs are not reinforced. (c) The reliability of nicotine-dependent leftward shifts in the IRT distribution for the acute group, and this figure contains an average line plot from all five nicotine-dosing sessions for this group. Nicotine-dosing sessions for the acute group were separated by 5 days/session of saline dosing to allow for drug elimination.

Fig. 4.

Time-course analysis of the cumulative IRT frequency distribution for the chronic group. The leftward shift occurs between days 6 and 8 of consecutive daily 0.3 mg/kg nicotine dosing; little difference is apparent between days 10 and 20 of consecutive daily dosing.

Table 1

Nonlinear-regression parameter and goodness-of-fit values for each group and condition

Condition and group	μ	σ	R^2
DRL 4.5-s schedule			
Saline	2.01 ± 0.66	2.50 ± 0.33	0.97 ± 0.02
0.1 mg/kg	2.30 ± 1.42	2.49 ± 0.95	0.96 ± 0.03
0.3 mg/kg	2.56 ± 1.06	2.42 ± 1.15	0.96 ± 0.02
0.5 mg/kg	2.02 ± 0.66	2.50 ± 0.33	0.97 ± 0.02
Large reward	3.23 ± 0.72	2.55 ± 1.07	0.96 ± 0.02
Large reward + 0.3 mg/kg	2.71 ± 0.94	1.88 ± 0.37	0.98 ± 0.01
DRL 9.5-s schedule			
Saline	3.67 ± 2.97	6.28 ± 1.23	0.98 ± 0.01
0.1 mg/kg	1.86 ± 1.59	5.89 ± 2.39	0.98 ± 0.02
0.3 mg/kg	0.49 ± 1.11 *	6.61 ± 1.75	0.94 ± 0.05
0.5 mg/kg	0.79 ± 2.59 *	4.77 ± 2.86	0.97 ± 0.02
Large reward	3.13 ± 1.25	5.12 ± 1.96	0.99 ± 0.01
Large reward + 0.3 mg/kg	2.95 ± 2.34	4.27 ± 1.13	0.99 ± 0.00
DRL 29.5-s schedule			
Saline	12.53 ± 2.67	14.41 ± 3.57	0.97 ± 0.00
0.1 mg/kg	8.23 ± 1.64 *	13.31 ± 4.19	0.98 ± 0.01
0.3 mg/kg	5.09 ± 1.91 *	15.91 ± 6.79	0.97 ± 0.02
0.5 mg/kg	3.26 ± 2.06 *	18.71 ± 9.25	0.98 ± 0.01
Large reward	5.09 ± 3.59 *	12.11 ± 5.51	0.98 ± 0.01
Large reward + 0.3 mg/kg	6.11 ± 3.87 *	14.50 ± 3.49	0.98 ± 0.01

A least-significant difference multiple comparison (LSD) revealed significant within-group differences due to nicotine and reinforcer-magnitude manipulations for the μ parameter only.

DRL, differential-reinforcement-of-low-rate; LSD, least-significant difference.

* $P < 0.05$ compared with saline/baseline conditions.

Table 2
A list of general performance measures per experimental group per condition, n = 3 per group/schedule requirement

Manipulation	Accuracy			Response rate		
	Percent correct trials	Number of reinforcing events	Number of time outs	Percent bursting	Number of adjunctive responses	Number of head entries
DRL 4.5-s schedule						
Saline	28.63 ± 6.36	37.17 ± 7.63	92.67 ± 14.26	26 ± 2.9	1.25 ± 0.56	239.67 ± 10.38
0.1 mg/kg	21.86 ± 5.77*	30.83 ± 6.06	110.2 ± 13.91	18 ± 3.6	1.75 ± 1.06	287.83 ± 36.75*
0.3 mg/kg	19.03 ± 7.13*	31.5 ± 1.81	134 ± 10.25	23 ± 4.6	0.5 ± 0.45	372.17 ± 40.88*
0.5 mg/kg	15.12 ± 3.10*	26.83 ± 4.15	150.67 ± 3.96	19 ± 1.9	1.5 ± 0.58	369.33 ± 37.9*
Large reward	31.98 ± 8.33	38.17 ± 4.61	81.17 ± 9.93	20 ± 4.0	0.5 ± 0.26	263.33 ± 21.47*
Large reward + 0.3 mg/kg	21.21 ± 8.29*	35.67 ± 5.58	132.5 ± 23.11*	17 ± 2.0	0.75 ± 0.67	402.67 ± 49.12*
DRL 9.5-s schedule						
Saline	13.05 ± 4.30	16.33 ± 1.74	108.83 ± 8.37	29 ± 5.3	1.75 ± 0.22	254 ± 10.54
0.1 mg/kg	10.11 ± 3.22	13.83 ± 2.42	123 ± 19.8	28 ± 1.7	1.5 ± 0.77	263.67 ± 44.52
0.3 mg/kg	8.82 ± 2.93*	11.83 ± 2.2	122.33 ± 17.48	37 ± 4.6	2.75 ± 1.23	279.33 ± 42.66
0.5 mg/kg	8.23 ± 3.68*	12.17 ± 2.49	135.67 ± 9.79	39 ± 5.6	2.75 ± 1.26	289.83 ± 58.12*
Large reward	13.58 ± 5.11	18.17 ± 1.68	115.67 ± 6.69	30 ± 3.0	1.25 ± 0.85	274.67 ± 35.83
Large reward + 0.3 mg/kg	6.09 ± 3.13*	8.67 ± 0.37	133.67 ± 10.95	30 ± 5.0	1.75 ± 0.76	403.83 ± 77.19*
DRL 29.5-s schedule						
Saline	10.22 ± 4.58	7.17 ± 1.04	63 ± 4.77	22 ± 2.6	1.5 ± 0.45	163.52 ± 18.66
0.1 mg/kg	6.18 ± 1.10*	5.5 ± 0.55	83.5 ± 3.61	20 ± 4.6	1.25 ± 0.43	209.48 ± 11.71*
0.3 mg/kg	7.86 ± 1.47*	6.17 ± 1.21	72.33 ± 5.83	25 ± 5.6	1.75 ± 1.28	194.54 ± 37.86
0.5 mg/kg	7.04 ± 1.01*	6 ± 1.1	79.17 ± 5.69	28 ± 5.8	1.25 ± 0.56	214.57 ± 28.68*
Large reward	7.33 ± 1.00*	6.5 ± 0.88	82.17 ± 7.17	26 ± 5.0	1.75 ± 0.99	184.17 ± 16.96*
Large reward + 0.3 mg/kg	3.95 ± 2.36*	3.67 ± 0.92	89.33 ± 8.05	25 ± 6.0	3.5 ± 1.39	260.17 ± 31.3*

Group means and standard errors are presented for each variable, and these means depict performance across two sessions per condition. 'Percent-correct trials' represents the total average number of reinforcing events per session divided by the total number of trials. 'Percent bursting' refers to the percentage of interresponse times within the first time bin (0–0.5 s). 'Number of adjunctive responses' represents the number of nose-poke responses into the left, inactive nose-hole operant. 'Head entries' refers to the total number of consumption responses made into the dipper well. 'Responses per minute' refers to

operant responses emitted on the active, right nose-hole poke. Post-hoc pairwise comparison (LSD) revealed significant within-group differences due to nicotine and reinforcer-magnitude manipulations in percent-correct trials, head entries, and responses per minute. Number of reinforcing events and number of time outs were not included in the analysis of variance because both variables were included in the percent-correct trials variable.

DRL, differential-reinforcement-of-low-rate; LSD, least-significant difference.

* $P < 0.05$ compared with saline/baseline conditions.

Table 3

General performance measures and parameter values from experiment 2

	Saline			Acute			Chronic		
	Predose	Endpoint	Change (%)	Predose	Endpoint	Change (%)	Predose	Endpoint	Change (%)
μ	9.63 ± 1.20	9.16 ± 3.88	- 4.88	10.88 ± 1.47	5.30 ± 6.67*	- 51.29	12.33 ± 2.56	2.37 ± 4.24*	- 80.78
σ	14.56 ± 5.27	12.68 ± 6.80	- 12.90	11.40 ± 3.97	9.31 ± 3.61	- 18.33	11.21 ± 2.70	7.08 ± 1.08	- 36.85
R^2	0.91 ± 0.06	0.89 ± 0.29	- 2.20	0.94 ± 0.17	0.93 ± 0.30	- 1.06	0.97 ± 0.03	0.85 ± 0.08*	- 12.37
Percent-correct trials	9.36 ± 2.60	11.99 ± 3.05	+ 28.10	9.43 ± 1.07	5.08 ± 1.01*	- 46.13	10.94 ± 2.55	3.92 ± 1.62*	- 64.17
Head entries	149.04 ± 27.00	147.01 ± 19.70	- 1.34	140.85 ± 27.10	283.00 ± 58.0*	+ 100.92	129.30 ± 25.00	161.22 ± 42.00	+ 24.81
Responses/min	2.80 ± 0.50	3.10 ± 0.51	+ 10.71	2.67 ± 0.60	4.21 ± 1.00	+ 49.81	3.30 ± 0.31	4.10 ± 0.30	+ 24.24
Adjunctive responses	1.30 ± 2.10	1.50 ± 1.80	+ 15.38	1.62 ± 0.90	1.80 ± 2.10	+ 11.11	2.02 ± 1.00	2.00 ± 0.80	0.00
Percent-bursting responses	23.00 ± 13.00	24.05 ± 10.10	+ 4.35	26.67 ± 3.50	28.11 ± 9.00	+ 4.99	18.43 ± 9.10	14.04 ± 8.10	- 22.22

'Predose' values are group means and standard errors obtained from the five consecutive sessions before nicotine dosing. 'Endpoint' values represent the averages obtained from all five nicotine-dosing sessions for the acute group, the final five consecutive nicotine-dosing sessions for the chronic group, and the final five consecutive saline-dosing sessions for the saline group. Paired-samples *t*-tests were used to compare predose and endpoint values within each group.

* Significant differences ($P < 0.05$).