Modeling Acquisition of Nicotine Self-administration in Rats

by

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ABSTRACT

Nicotine is thought to underlie the reinforcing and dependence-producing effects of tobacco-containing products. Nicotine supports self-administration in rodents, although measures of its reinforcing effects are often confounded by procedures that are used to facilitate acquisition, such as food restriction, prior reinforcement training, or response-contingent co-delivery of a naturally reinforcing light. This study examined whether rats acquire nicotine self-administration in the absence of these facilitators. A new mathematical modeling procedure was used to define the criterion for acquisition and to determine dose-dependent differences in rate and asymptote levels of intake. Rats were trained across 20 daily 2-h sessions occurring 6 days/week in chambers equipped with active and inactive levers. Each active lever press resulted in nicotine reinforcement (0, 0.015, 0.03, 0.06 mg/kg, IV) and retraction of both levers for a 20-s time out, whereas inactive lever presses had no consequences. Acquisition was defined by the best fit of a logistic function (i.e., S-shaped) versus a constant function (i.e., flat line) for reinforcers obtained across sessions using a corrected Akaike information criterion (AICc) as a model selection tool. The results showed an inverted-U shaped function for dose in relation to the percentage of animals that acquired nicotine self-administration, with 46% acquiring at 0.015 mg/kg, 73% at 0.03 mg/kg, and 58% at 0.06 mg/kg. All saline rats failed to acquire as expected. For rats that acquired nicotine self-administration, multiple model comparisons demonstrated that the asymptote (highest number of reinforcers/session) and half learning point (h; session during which half the

assymptote had been achieved) were justified as free parameters of the reinforcers/session function, indicating that these parameters vary with nicotine dose. Asymptote exhibited an inverted U-shaped function across doses and half learning point exhibited a negative relationship to dose (i.e., the higher the dose the fewer sessions to reach h). These findings suggest that some rats acquire nicotine self-administration without using procedures that confound measures of acquisition rate. Furthermore, the modeling approach provides a new way of defining acquisition of drug self-administration that takes advantage of using all data generated from individual subjects and is less arbitrary than some criteria that are currently used.

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Chapter 1

INTRODUCTION

Nicotine is the pharmacological agent thought to be responsible for the use of, and addiction to, tobacco-related products. Surprisingly, nicotine has relatively weak intrinsic reinforcing effects compared to other drugs of abuse despite having a high addiction liability. Some researchers maintain that it is necessary to provide response-contingent cues along with nicotine infusions to establish and maintain robust nicotine self-administration in animals (Palmatier *et al.*, 2006). During nicotine self-administration, light cues facilitate acquisition as well as progression to more demanding partial reinforcement schedules (Caggiula, Donny, White, *et al.*, 2002). Stimuli commonly used in rodent drug self-administration paradigms, such as the onset or termination of a light, possess mild primary reinforcing effects (Deroche-Gamonet, Piat, Le Moal, & Piazza, 2002; Palmatier *et al.*, 2006; Palmatier *et al.*, 2007; Raiff & Dallery, 2009). Interestingly, Palmatier *et al*. (2006) found that control of a light cue is more reinforcing in rats than nicotine itself. Furthermore, non-contingent (i.e., experimenter delivered) nicotine enhances responding for a light stimulus, demonstrating that the primary reinforcing effects of visual stimuli are enhanced while under the influence of nicotine (Chaudhri *et al.*, 2007). After a withdrawal period, nicotine-seeking is reinstated more robustly by using the cues previously paired with the nicotine delivery than by using a nicotine priming injection (LeSage, Burroughs, Dufek, Keyler, & Pentel, 2004). A light cue also facilitates acquisition of cocaine reinforcement; however, it is important to note that a visual

cue does not directly modify the primary reinforcing effects of cocaine (Deroche-Gamonet *et al.*, 2002) as it does with nicotine (Chaudhri, Caggiula, Donny, Palmatier, *et al.*, 2006). Thus, primary reinforcing effects of response-contingent light cues may hinder interpretation of nicotine reinforcement when co-presented during self-administration sessions.

Several criteria have been used to operationally define acquisition of nicotine self-administration. Some researchers use stability criteria (e.g., less than ±15% variation over 2 consecutive days) with a requirement for minimum number of reinforcers obtained (Belluzzi, Wang, & Leslie, 2005; Chaudhri *et al.*, 2005; Yoshimura *et al.*, 2007). Others determine acquisition by relative rates or ratios of active to inactive lever presses (Brower, Fu, Matta, & Sharp, 2002; Chen, Matta, & Sharp, 2007; Donny *et al.*, 2003; Harris, Pentel, & LeSage, 2009; LeSage, Keyler, Collins, & Pentel, 2003; Valentine, Hokanson, Matta, & Sharp, 1997; Wang, Chen, & Sharp, 2008), while others use statistically significant differences between groups (Chaudhri, Caggiula, Donny, Booth, *et al.*, 2006; Levin *et al.*, 2007; O'Dell *et al.*, 2007). These criteria have been useful for identifying factors that influence acquisition of nicotine self-administration, however, some drawbacks are that (1) in some cases acquisition criteria are somewhat arbitrary, (2) the rate of acquisition is based on a final endpoint rather than the entire acquisition curve, and/or (3) there is little information regarding performance of individual subjects.

Few studies have employed mathematical modeling approaches to examine acquisition of nicotine self-administration. For instance, growth curve

modeling has been used to examine individual differences in acquisition of nicotine self-administration, as well as factors that influence the trajectory and shape of acquisition curves (Donny *et al.*, 2004; Lanza, Donny, Collins, & Balster, 2004). However, food pre-training, food restriction and drug-associated light stimuli were employed which reduced natural individual variability in responding and therefore affected acquisition rates. Inferences from acquisition curves must be drawn from individual animals, because the average of those curves is often very different from the curve of the average animal (Estes, 1956).

This study examined an alternative mathematical modeling approach for defining an acquisition criterion for nicotine self-administration. Male rats were trained across 20 sessions during which they had response-contingent access to one of three doses of intravenous nicotine. We then used a curve-fitting analysis to determine whether individual reinforcement rate data met the acquisition criterion of reasonable goodness of fit to a sigmoid (logistic, S-shaped) model. Acquisition curves are typically S-shaped as there is an initial baseline before learning has occurred, followed by a change in performance that reaches an asymptote over time (Hartz, Ben-Shahar, & Tyler, 2001). Corrected Akaike Information Criterion (AICc) was used to select between two possible models of response data for each rat: a logistic function demonstrating acquisition or a constant function (i.e., a flat line) demonstrating that no acquisition occurred. For those rats that demonstrated acquisition, a multi-model selection approach was applied to determine which logistic parameters (baseline, half-life, or asymptote) varied significantly across doses of nicotine. We hypothesized that there would be

dose-dependent differences in the parameter estimates of half-life (i.e., the day at which 50% total learning occurred) and asymptote obtained from the multi-model selection. To allow for individual differences in rate of acquisition, no response-contingent light cues were presented with reinforcer delivery nor was prior operant conditioning with a natural reinforcer or food motivation (i.e., food restriction or lever baiting) used in this experiment as these manipulations likely confound measures of nicotine reinforcement *per se* (Caggiula, Donny, Chaudhri, *et al.*, 2002; Clemens, Caille, & Cador, 2010; Donny *et al.*, 1998).

Chapter 2

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 240–280 g were pair-housed pre-surgery, then single housed post-surgery in a climate-controlled colony with a 12-h reverse light/dark cycle (lights off at 7:00 AM). Rats arrived on post-natal day (PND) 38 and were acclimated to handling for a few min each for 11 consecutive days. Care of the animals was in accordance with the Guide for the Care and Use of Laboratory Animals (Clark, Gebhart, Gonder, Keeling, & Kohn, 1997) and all procedures were approved by the IACUC at Arizona State University.

Drugs

(-)Nicotine hydrogen tartrate (Sigma, St. Louis, MO) was dissolved in bacteriostatic saline, adjusted to a pH of 7.4 ± 0.1, and then filtered through a 0.2 µm filter fresh daily. Doses were delivered IV at a volume of 0.1 ml and are based on the concentration of nicotine base.

Surgery

On PND 50-52, catheters were implanted intravenously as described by Pentkowski *et al*., (2009) under isoflurane gas (2-4%) anesthesia. The catheters were tunneled subcutaneously along the neck, exited through an incision across the skull, and were secured to the top of the skull using dental acrylic and anchor screws. To maintain catheter patency, a 0.1 ml solution of bacteriostatic saline containing heparin sodium (70 USP U/ml; Baxter Healthcare Corporation, Deerfield, IL) and ticarcillin disodium (20 mg/ml; GlaxoSmithKline, Research

Triangle Park, NC) was administered daily. For the first three to five days after surgery, the solution also contained the thrombolytic agent urokinase (66.67 mg/ml; ImaRX Therapeutics, Inc., Tucson, AZ). Rats were given buprenorphine, a partial opioid agonist, immediately prior to surgery (0.05 mg/kg SC). Catheter patency was confirmed by infusing 0.3 ml methohexital sodium (16.67 mg/ml, IV; Sigma), which produces brief anesthetic effects only when administered IV.

Apparatus

Self-administration took place in operant conditioning chambers (20 × 28 × 20 cm), each contained inside a sound-attenuating chamber, and equipped with two levers mounted on the front wall (Med Associates, St. Albans, VT). The self-administration chambers also had a cue light above one lever and a house light mounted on the top center of the back wall, but these features were not used in this experiment. Infusion pumps (Razel, St. Albans, VT) were located outside the chambers and contained 30 ml syringes attached via Tygon tubing to liquid swivels (Instech, Plymouth Meeting, PA). The outlet of the swivels was fastened to the catheters via Tygon tubing that ran through a metal spring leash (Plastics One, Roanoke, VA).

Nicotine self-administration dose-response without food restriction, lever baiting, or drug-paired light cues

All rats were given *ad libitum* access to rat chow and water in the home cage for the duration of this experiment. After 5–6 days of recovery from surgery, rats were randomly divided into groups that were either trained to press a

lever reinforced by nicotine infusions (0.015, 0.03, or 0.06 mg/kg/0.1 ml, IV; n=17, 15, 15 respectively) or saline infusions (Saline group; n = 11) beginning on post natal day 58. Training occurred daily for 2 h, at approximately the same time of day for a total of 20 sessions conducted 6 days/week. During the sessions, active lever presses on a fixed ratio 1 (FR1) schedule of reinforcement resulted in both levers retracting, followed 0.5 s later by a 0.1 ml infusion of the assigned drug over the duration of 1.2 s. Upon completion of the 1.2-s infusion, the levers remained retracted for a 20–s time out. The house light was off for the entire session. Responses on the inactive lever were recorded but had no scheduled consequences. Designation of the right versus left lever as the active lever was counterbalanced.

Acquisition Models

The following logistic model was used to estimate acquisition parameters of nicotine self-administration in individual subjects using Solver in Microsoft Excel:

$$R(t) = \frac{(A - B)}{1 + \exp\left[\frac{-(t - h)}{s}\right]} + B, \quad (1)$$

where R is the number of nicotine reinforcers collected each day t, A is the asymptotic number of reinforcers after acquisition; B is the baseline number of reinforcers before acquisition; h is the half-life of the acquisition process (i.e., the day when $R(h) = (A + B) / 2$); s is the slope of the acquisition process. Figure 1 illustrates how each individual parameter contributes to the shape of the logistic function. The corrected Akaike Information Criterion (AICc) was used to

determine whether the 4 free parameters of Equation 1 were justified in describing each rat's data over the single free parameter of a constant function, $R(t) = c$ (see Appendix A for details of the AICc analysis; for further details see (Burnham & Anderson, 2002). In essence, this approach allowed us to determine whether the data were best described by an S-shaped curve that demonstrates an improvement in performance relative to an initial baseline (i.e., acquisition) versus a straight line demonstrating no systematic change from baseline. AICc provides a selection criterion based on model likelihood that corrects for overfitting, favoring goodness of fit and punishing free parameters (Burnham & Anderson, 2002).

To infer that acquisition had taken place, Equation 1 had to be selected as the best fit (i.e., dAICc = AICc (constant) – AICc (logistic) > 4) and the estimated asymptote (A) had to be greater than the estimated baseline (B) by a minimum of 10 reinforcers (i.e., $A - B > 10$). Because some animals had not yet reached asymptotic performance by the last session, estimates of A were limited to ten percent above the maximum number of reinforcers obtained for each dose group across all self-administration sessions (0.015 mg/kg = 60.5, 0.03 mg/kg = 47.3, and 0.06 mg/kg = 36.3). Without visible asymptotic performance, uncapped estimates of A were often unrealistically high. B was also limited to 10 reinforcers to eliminate the possibility of exponential curves, and s was set to a minimum of 0.1 sessions, which indicated a step-shaped learning curve. It is important to note that animals did not exceed 10 reinforcers the first day of training. As a result of this curve-fitting procedure, animals that displayed a legitimate curve for reinforcers obtained and thus learned to self-administer were

designated as part of the 'acquired' group, whereas animals that did not demonstrate adequate self-administration learning were designated as part of the 'not acquired' group.

Dose-effect Models

Nicotine dose effects were further examined in the animals that had acquired self-administration using a *multi-model comparison approach*. The function of total reinforcers obtained across self-administration sessions for the acquired group was used to determine which model best represented the data by dose group. It is important to note that the parameter values for *A*, *B*, *h* and *s* only hold relevance for the rats that acquired and have no interpretable meaning for the rats that did not acquire or that received saline, and therefore subjects in these groups were not included in the multi-model selection analysis. Model selection assessed which parameters (*A*, *B*, *h*) were justified to vary freely and which were likely to remain constant across dose groups. We compared 8 different models that allowed all combinations of mean parameter estimates to vary freely across dose groups. Specifically, the mean of none, only *A*, only *B*, only *h*, *A* and *B*, *A* and *h*, *B* and *h*, or all three parameters were allowed to vary freely across dose groups. It is important to note that parameters for individual rats were always allowed to vary freely; what distinguished one model from another were the *mean* parameter estimates that were constrained to be constant across groups. In this analysis, *B* served as our negative control parameter (i.e., we didn't expect it to vary between groups). *B* is an initial baseline before acquisition, therefore one would not expect differences between groups when there is very little responding.

This comparison makes no claims regarding *s*, and therefore its mean was allowed to vary freely across dose groups in all models. The model that postulated no change in mean parameter estimates across doses served as the "null hypothesis." Residual sum of squares (RSS) and corrected AIC (AICc) values were computed for each model (see Appendix A). The lowest AICc ($AICc_{MIN}$) indicated the most likely model, corrected for the number of free parameters. AICc scores for each model ($AICc_j$, where *j* indexes the model) were then rescaled as their difference relative to $AICc_{MIN}$ ($\Delta AICc_j = AICc_j - AICc_{MIN}$; $\Delta AICc_{MIN} = 0$). $\Delta AICc_j$ thus indicated the likelihood of each model *j*, relative to the most likely model, after correcting for free parameters; lower ΔAICc were indicative of higher likelihood. Based on standard practice, the model with fewer free parameters and a $\Delta AICc < 4$ was selected; its parameters were taken as the best estimate of the learning parameters under each dose.

Some attrition occurred due to loss of catheter patency. The final number of subjects in each dose group was $n = 11$ for saline, $n = 13$ for 0.015 mg/kg dose, $n = 11$ for 0.03 mg/kg dose, and $n = 12$ for 0.06 mg/kg dose.

Chapter 3

RESULTS

Twenty-one rats acquired self-administration out of 36 rats that had access to nicotine. The percentage of rats that acquired across dose groups exhibited an inverted U-shaped pattern with less than half acquiring at the lowest dose and the highest percentage acquiring at the intermediate dose (see Table 1). Rats that acquired nicotine self-administration had mean dAICc values of 28.07 ± 2.8 (indicative of the logistic model as the best fit) relative to the rats that did not acquire who had mean dAICc values of -2.49 ± 1.5 (indicative of the constant model for the best fit). As expected, a constant function best fit the data of all saline control rats, which was indicative of a lack of acquisition in this group. The mean number of reinforcers during the last five days of self-administration, when most rats had achieved asymptote, is shown in Figure 2. These data also exhibited an inverted U-shaped pattern. Saline controls displayed the lowest number of reinforcers obtained, with the animals that received 0.015 mg/kg nicotine obtaining the highest number of reinforcers, followed by the 0.03 mg/kg group, and then the 0.06 mg/kg group.

Data from individual rats that had acquired nicotine self-administration across the 20 training sessions was then used to conduct a model comparison. The multi-model comparison was conducted to determine potential parameter differences between dose groups. Figure 3 depicts the mean group data and the mean predictions for animals that acquired and did not acquire nicotine self-administration, respectively, as well as all saline animals. As we had expected, of

the 8 possible candidate models, the model in which *B* remains constant while *A* and *h* are able to vary freely was determined to be the best fit model for individual subjects, as well as groups, that had acquired nicotine self-administration (Table 2). This model selection justifies holding the baseline (*B*) constant. Furthermore, *A* and *h* vary freely in the selected model, indicating that there are dose-dependent differences in these two parameters. The second-best model assumed that *A, B,* and *h* varied with doses, but this model, despite having a $\Delta AICc < 4$ had more free parameters than the selected model.

The model selected produced mean parameters that allowed for more individual subject variability.

The mean parameter estimates among groups is shown in Table 3. *h* values represent the session during which 50% of asymptotic learning had been acquired and *A* represents asymptotic reinforcement rates. Both of these parameters exhibited a negative relationship with dose, with animals in the high dose group having the lowest *h* estimates and asymptotic reinforcement rates and those in the lowest dose groups having the highest *h* estimates and asymptotic reinforcement rates. Thus, the group receiving the highest dose of nicotine most readily acquired self-administration but took fewer infusions per session at asymptote, whereas the group receiving the lowest dose of nicotine acquired self-administration the most slowly but took a higher number of infusions per session at asymptote.

Chapter 4

DISCUSSION

The goal of the present study was to devise a method to determine whether or not an animal acquired nicotine self-administration when given 20 days of access without response-contingent cue lights or food motivation manipulations. Therefore, we posed the question of whether a given rat's individual data was most likely described by a flat line (i.e., constant function) or by an S-shaped, logistic function that grows over time, indicative of learning. As expected, all rats assigned to the saline group failed to acquire self-administration using this criterion. Nearly half to three-fourths of animals that were given access to a given dose of nicotine acquired self-administration based on individual data that best fit a logistic curve, whereas the others best fit a constant function that closely resembled the saline control group data (Table 1 and Figure 3). The data from the animals that maintained robust responding demonstrate acquisition of nicotine self-administration in the absence of prior training, food deprivation, lever baiting, or light conditioned cues.

To compare the patterns of nicotine self-administration across doses, we used a multi-model comparison, using ΔAICc to select the best model based on goodness of fit and punishment of free parameters. The model selection generated group parameter estimates based on the best-fitting model to the data. The baseline was the only parameter constrained in the selected model, whereas the asymptote (*A*) and half-life (*h*) of the function were justified to vary freely across groups. *B* was held constant at the overall mean of the baseline prediction

for each dose group. The need for *B* to remain constant is intuitive to an acquisition curve. In other words, one would not expect differences in response rates in the initial self-administration session because acquisition is not likely to have occurred. As sessions progress, rats obtain more reinforcers and therefore learn the response-nicotine reinforcement contingency leading to a rise in reinforcement rates. Allowing the *A* parameter to vary freely gave information regarding how much nicotine groups obtained at each dose after acquisition reached asymptote whereas the *h* parameter estimates the point in time at which animals exhibited 50% of asymptotic reinforcement rate, which may reflect how quickly learning took place. Other factors that could potentially affect *h* are the incentive motivation for nicotine in each dose group or rate of tolerance to aversive effects that may initially inhibit responding. Indeed, any of these factors may influence the rising phase of the acquisition curve.

The dose-dependent differences observed in the present study are consistent with previously published data reporting differences in responding for nicotine in the rodent self-administration paradigm (Chen *et al.*, 2007; Corrigall & Coen, 1989; Cox, Goldstein, & Nelson, 1984; Donny *et al.*, 1998; Donny *et al.*, 2000; Latiff, Smith, & Lang, 1980; Watkins, Epping-Jordan, Koob, & Markou, 1999). The observed differences in the *A* and *h* parameter values across groups was expected since previous research has shown that drug dose plays a role in acquisition and reinforcement rates in drug self-administration. For instance, dose is positively correlated with rate of acquisition of cocaine self-administration (Carroll & Lac, 1997; Gerrits & van Ree, 1995). Some studies have failed to find

dose-dependent differences in acquisition rates, likely due to the schedule of reinforcement employed. For instance, Lanza *et al*., (2004), did not find differences in the rate of acquisition or the shape of the curves between doses, whereas our study did; however we used only one schedule of reinforcement, whereas they built up to a partial reinforcement schedule. Donny *et al*. (1998) found that mean reinforcement rates during acquisition increased more quickly for animals receiving 0.03 mg/kg compared to 0.06 mg/kg of nicotine on both an FR1 and FR2, but not an FR5 schedule of reinforcement. In contrast, rats receiving 0.06 mg/kg nicotine in the present study on an FR1 schedule exhibited a lower *h* value, suggesting more rapid acquisition. Similar to Donny *et al*., (2004), we believe that parameters yielded from modeling acquisition of nicotine self-administration allow a precise depiction of how acquisition differs between individual animals, as well as information regarding differences in acquisition between dose groups.

In evaluating the utility of the present approach for defining acquisition of drug self-administration, there are some limitations that need to be considered. One limitation stems from the forced constraint of the parameter estimates for *B* and *A*. In some cases where animals had begun to show learning but had not yet reached asymptote, it was necessary to create an artificial cap for the *A* parameter in order for the fitted curve to yield realistic values. We constrained *A* to 10% above the maximum number of reinforcers obtained within a given dose group. We posit that the applied constraints to the *A* estimates were within the biologically relevant range that any given animal in a dose group would naturally

meet if allowed more time to reach asymptotic responding. The present study also made no claims or interpretations regarding our *s* parameter. The *s* parameter gives information regarding slope of the entire curve, and therefore may not yield specific information regarding the acquisition process over and above the information obtained from the *B*, *h*, and *A* values.

Interestingly, the number of animals that exhibited acquisition in our study differs from other reports of nicotine self-administration in the fact that fewer animals displayed acquisition overall. Not more than 46.2 to 72.7% of animals in each nicotine dose group acquired self-administration. This is not surprising considering only approximately one-third of individuals who initiate smoking ever become addicted (Anthony, Warner, & Kessler, 1994). In addition, we likely added more opportunity for natural variability in behavior to manifest during the acquisition process by not using prior training, food restriction, lever baiting or drug-paired light cues that promote acquisition thereby reducing variability in behavior (Caggiula, Donny, Chaudhri, *et al.*, 2002; Caggiula, Donny, White, *et al.*, 2002; Clemens *et al.*, 2010; Donny *et al.*, 1998).

Compared to most literature on nicotine self-administration the acquisition functions obtained in the present study reflect a slower acquisition process and fewer animals acquiring overall. These differences are likely due to the absence of food deprivation, operant pre-training using natural reinforcers, or light cues in our paradigm that are known to obscure natural individual differences. Food deprivation and weight restriction both facilitate self-administration of abused drugs (Lang, Latiff, McQueen, & Singer, 1977; Singer, Simpson, & Lang, 1978).

Prior training for natural rewards are generally employed to facilitate acquisition of operant responding for the drug upon subsequent training sessions (Ahmed, Walker, & Koob, 2000; Baker *et al.*, 2003; Di Ciano, Blaha, & Phillips, 2001; Meil & See, 1996; Sutton *et al.*, 2003; Weiss *et al.*, 2000) which is reflected as higher response rates on the first day of self-administration (Bongiovanni & See, 2008; Donny *et al.*, 1998) likely resulting in a more rapid acquisition process, especially when the drug is response-contingent (Donny *et al.*, 1998).

Perhaps the most important feature of our approach is that we employed lever retraction rather than a light CS+ cue to signal nicotine delivery and the time out. As mentioned previously, visual light cues are known to enhance the acquisition, maintenance, and reinstatement of nicotine-seeking as well as to promote the resistance to extinction of lever pressing (Caggiula, Donny, Chaudhri, *et al.*, 2002; Caggiula *et al.*, 2001; Caggiula, Donny, White, *et al.*, 2002; Donny *et al.*, 1999; LeSage *et al.*, 2004; Liu, Caggiula, Palmatier, Donny, & Sved, 2008; Liu *et al.*, 2006). When these stimuli are used in self-administration paradigms, high rates of nicotine reinforcement are observed across sessions (Chaudhri *et al.*, 2007); however, it is important to note that these stimuli possess greater reinforcing properties when presented alone compared to access to nicotine alone and their intrinsic reinforcing effects are enhanced while animals are under the influence of nicotine (Palmatier *et al.*, 2006; Palmatier *et al.*, 2007). In contrast, lever retraction used in the present study appears to be a neutral cue because it did not elicit responding in the absence of nicotine (i.e., failed acquisition of saline self-administration). Similarly, Sorge *et al.*, (2009)

used a motivationally neutral stimulus (i.e., white noise) as a cue to promote response contingency learning, without interfering with the primary reinforcing effects of nicotine. The animals that received the neutral white noise cue with saline infusions did not maintain robust responding; however, the animals that received a white noise cue paired with nicotine infusions demonstrated robust self-administration over and above nicotine alone, suggesting that the cue may have acquired some nicotine-conditioned reinforcing effects. We did not specifically test whether discontinuing lever retraction affected nicotine reinforcement rates once animals had acquired self-administration, and therefore, we cannot completely rule out the possibility that lever retraction acquired conditioned reinforcing effects.

Our approach maximizes individual differences using a limited access paradigm. Increased time spent in the self-administration chamber (i.e., extended access) may facilitate learning by allowing subject more opportunity to self-administer nicotine, resulting in higher rates of nicotine self-administration. This effect may be due to rapid development of nicotine dependence and subsequent motivation to obtain the drug (O'Dell *et al.*, 2007; O'Dell & Koob, 2007; Paterson & Markou, 2004). It is possible that by extending the length of the animal's self-administration session, acquisition would be facilitated, while still preserving the advantages of the current method and analysis.

The approach used in the present study makes full use of an individual subject's entire acquisition curve and offers advantages in characterizing how individual subject data changes over time without using manipulations designed

to facilitate acquisition that may ultimately confound the measure of nicotine as a primary reinforcer and/or reduce variability across subjects. This feature is important for identifying how a given variable affects acquisition, such as effects of age, sex, hormone cycle, drug pre-exposure, etc., that may not be detected by null-hypothesis testing procedures, especially when procedures that facilitate acquisition are used. Therefore, the present approach is well-suited for examining factors that may enhance or impede the nicotine self-administration acquisition process.

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APPENDIX A

CORRECTED AKAIKE INFORMATION CRITERION

The corrected Akaike Information Criterion (AICc) was computed for each individual rat and within each model, as

$$\text{AICc} = 2k + n\ln(\text{RSS}/n) + \left(\frac{2(k)(k+1)}{n-k-1}\right), \quad \text{(A1)}$$

where k equals the number of free parameters in the model, n is the number of observations and RSS is the residual sum of squares,

$$\text{RSS} = \sum_{i=1}^{n} \hat{\varepsilon}_i^2 \quad \text{(A2)}$$

Acquisition Models

The AICc obtained from a sigmoid model was subtracted from the AICc obtained from a flat line model to determine the dAICc value. The criteria for rats that acquired self-administration was determined by the dAICc value: If the difference between the asymptote minus baseline values (A-B) was greater than 10, then a dAICc value > 4 was used as a criterion for successful acquisition of nicotine self-administration.

Dose-effect Models

In the multi-model comparison, the RSS, k, and AICc values were computed for each model. The lowest AICc (AICc_{MIN}) indicated the most likely model, corrected for the number of free parameters. AICc scores for each model (AICc_j, where j indexes the model) were then rescaled as their difference relative to AICc_{MIN} ($\Delta\text{AICc}_j = \text{AICc}_j - \text{AICc}_{\text{MIN}}$; $\Delta\text{AICc}_{\text{MIN}} = 0$). ΔAICc_j thus indicated the likelihood of each model j, relative to the most likely model, after correcting for free parameters; lower ΔAICc were indicative of higher likelihood. The model with fewer free parameters with $\Delta\text{AICc} < 4$ was selected; its parameters were taken as the best estimate of the learning parameters under each dose regimen.

Table 1.
Number of animals meeting the acquisition criterion by dose group.

Group	Animals acquired / total number in group	Percent acquired
Saline	0/11	0%
0.015 mg/kg	6/13	46.15%
0.03 mg/kg	8/11	72.70%
0.06mg/kg	7/12	58.33%

Note. The number and percentage of animals that met criterion for acquisition for a given dose group are displayed above. The criterion of a Δ-AICc value ≥ 4 was used as a criterion for successful acquisition of nicotine self-administration.

Table 2.
Model Comparison

Model	RSS	k	ΔAICc
None	8122.45	79	32.36
A	7734.11	81	17.91
B	8074.72	81	36.01
h	7652.79	81	13.47
A, B	7707.85	83	22.68
A, h	7302.73	83	0.00
B, h	7622.09	83	17.98
A, B, h	7253.05	85	3.41

Note. The first column specifies which parameter estimates were assigned to vary freely within the model. The row labeled none gives statistics when all parameters were fixed across group data. RSS is the residual sum of squares over all subjects, k is the number of free parameters in the model. As each model was fitted to individual subject data from a specific dose group, a set of free parameters was estimated for each dose group. $AICc=2k+n \ln(RSS/n) + [2k(k+1)/(n-k-1)]$, where n is the total number of observations = 21 rats x 20 self-administration session totals for reinforcers obtained = 420. Data for the model with the lowest AICc are underlined, and thus is the best fitted model in the comparison.

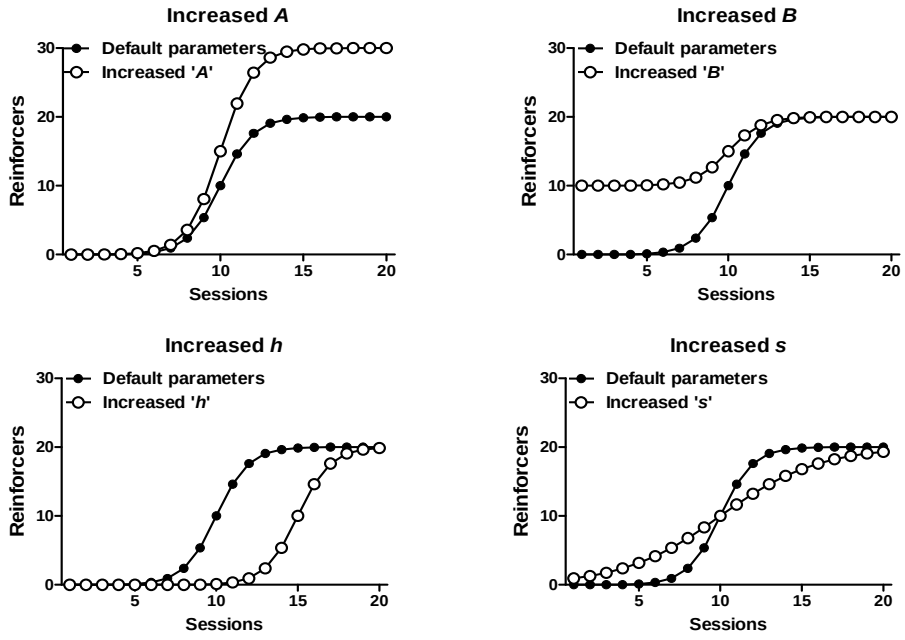
Table 3.
Parameter values from best fitting mean parameters when mean *A* and *h* varied freely across dose group (Model *A*, *h* in Table 2).

Dose group	*A* (reinforcers)	*h* (sessions)	*B* (reinforcers)
0.015 mg/kg	31.44	12.35	3.83
0.03 mg/kg	26.15	11.76	3.83
0.06 mg/kg	23.02	10.15	3.83

Note. Parameter values from the best fitting model in the 8-model comparison are displayed above. The first column specifies the dose group comprised of the animals that 'acquired' self-administration according to the curve-fitting criterion. Asymptote (*A*), Baseline (*B*), and half-life or the day at which 50% learning occurred (*h*) parameters were obtained from the model comparison. For the model that specified *B* must remain constant, but *A* and *h* could vary freely, a dose-dependent difference in *A* and *h* were observed.

Theoretical Parameter Fluctuations

in the logistic function



$$R(t) = \frac{(A-B)}{1+\exp\left[\frac{-(t-h)}{s}\right]} + B$$

Figure 1. Theoretical parameter fluctuations in the Logistic function. The example logistic function is represented using the *black lines* and the parameter values that comprise example function are set at A = 20 reinforcers (asymptote), B = 0 reinforcers (baseline), h = 10 sessions (half-life of the acquisition process) and s = 1 session (slope), where R is the number of nicotine reinforcers collected at each day t. The *white lines* represent change in the function as a result of parameter value fluctuations. The 'Increased *A*' graph depicts the function when the asymptote parameter is increased from 20 to 30 reinforcers, 'Increased *B*'

depicts a baseline parameter increase from 0 to 10 reinforcers, ‘Increased *h*’ depicts a half-life increase from 10 to 15 sessions, and the ‘Increased *s*’ depicts a slope parameter increase from 1 to 3 sessions.

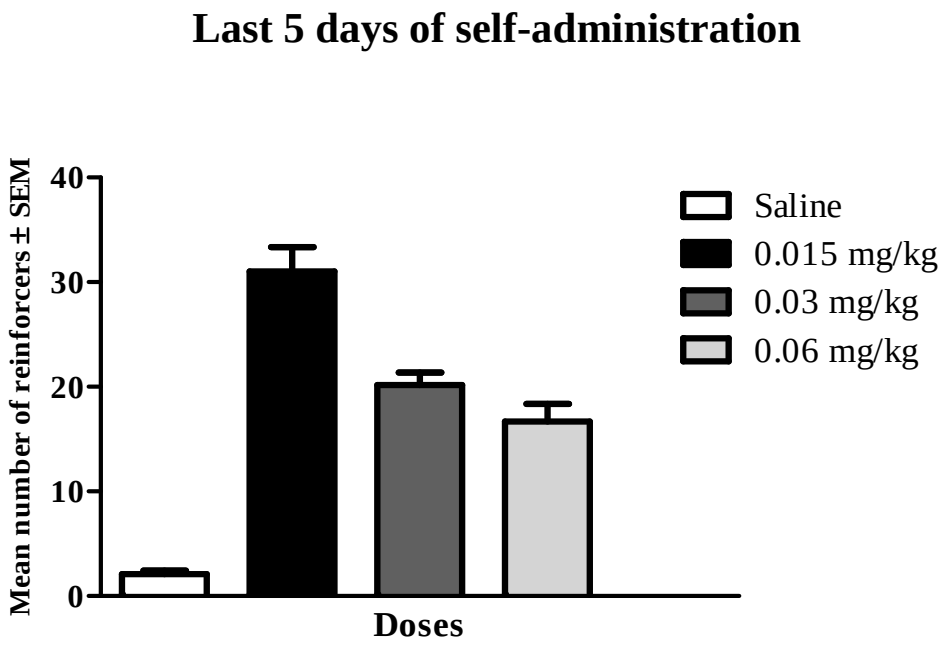


Figure 2. Mean number of reinforcers obtained (± SEM) during the last five days of self-administration for animals that acquired nicotine self-administration in addition to all saline animals tested. As expected, an inverted U-shaped dose response curve is observed.

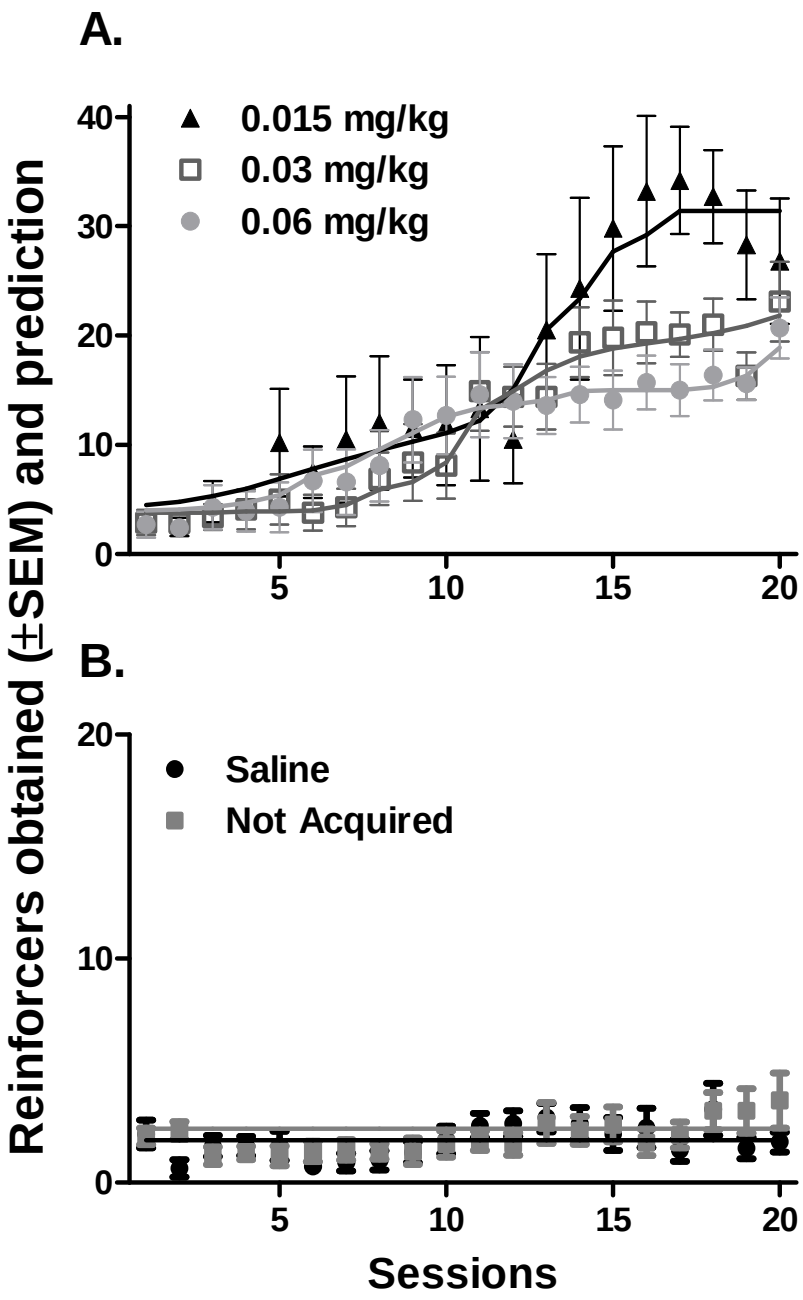


Figure 3. **A**. Mean number of reinforcers obtained (± SEM) for animals that acquired in each nicotine group across 20 days of training are represented by *points*. Animals that acquired self-administration according to our curve-fitting

procedure were divided by drug dose (i.e. 0.015, 0.03, and 0.06 mg/kg). The mean group predictions for the best logistic model holding B constant and letting h and A vary for the actual data are represented as *solid lines*. As anticipated, dose-dependent effects are visible for the number of reinforcers obtained, with the number of reinforcers taken being greater for the lowest dose followed by the medium and high doses. **B**. All saline animals failed to acquire self-administration. To illustrate that there were no differences between the saline animals and the remaining nicotine animals that failed to acquire self-administration, a separate group was labeled 'not acquired.' These nicotine animals that did not acquire self-administration mirror the saline animals for their reinforcers obtained across days of training. Mean number of reinforcers obtained (± SEM) for 'not acquired' and 'saline' groups across 20 days of training are represented by *points*. Mean prediction of the constant model for the data are represented as *solid lines*.