A HISTORY OF HIGH DOSE NICOTINE SELF-ADMINISTRATION INCREASES THE RATE OF SELF-ADMINISTRATION AT LOW NICOTINE DOSES

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Introduction: FDA-mandated product standards that drastically reduce nicotine content in cigarettes may result in decreased smoking and thus, improved health outcomes for millions of U.S. smokers. One issue is whether the rate of smoking at reduced nicotine contents would be different for current smokers from individuals who start smoking for the first time at the reduced content. *Method:* 48 rats were given the opportunity to self-administer at one of four low nicotine doses (15, 7.5, 3.75, 0.0 µg/kg/infusion) before and after self-administering a higher dose of nicotine (60 µg/kg/infusion). A second group of 57 rats acquired self-administration at the high nicotine dose before experiencing reduction. A cocktail of other cigarette constituents was included in the vehicle and remained constant across the study. *Results:* The rate of self-administration across the low doses (including vehicle) was higher following self-administration of a high dose. Rates of self-administration following reduction from the high nicotine dose were the same regardless of whether the rats originally acquired at a low dose or the high dose. The effect of self-administering a high nicotine dose was highest for a threshold dose (7.5 µg/kg/infusion). *Discussion:* The present study suggests rate of self-administering low nicotine doses may be increased by having a history of high dose self-administration. The large effect at a threshold nicotine dose may indicate a shift in the threshold for maintaining behavior as a result of experience with higher doses of nicotine. These data would support the idea that current smokers may smoke at a higher intensity following nicotine reduction than individuals who

begin smoking for the first time at the reduced rate. Furthermore, the rate of self-administration following reduction was the same regardless of whether rats acquired at the high dose of nicotine or experienced a low dose before being changed over to a high nicotine dose.

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1.0 INTRODUCTION

New policies and strategies are needed to reduce tobacco use. Cigarette smoking results in about 443,000 deaths each year, but despite the widely publicized health risks, 19.3% of Americans are still smokers (CDC, 2011). Two thirds of smokers express that they would like to quit smoking, but only 6.2% of smokers actually quit each year (CDC, 2011). The difficulty in quitting smoking is generally attributed to an addiction to nicotine (Stolerman & Jarvis, 1995).

In 2009 Congress passed the Family Smoking Prevention and Tobacco Control Act, which permits the regulation of tobacco products and their constituents, including the reduction of nicotine to any non-zero level (US Congress, 2009). Benowitz and Henningfield (1994) suggested nicotine reduction as a strategy for reducing the prevalence of smoking almost 20 years ago, but the idea has recently gained more attention with the new legislation. The outcome of a nicotine reduction policy is uncertain because some evidence suggests that smokers compensate for the decrease in nicotine level-- attempting to maintain a specific level of nicotine (Scherer, 1999). Indeed, evidence from human research and non-human self-administration paradigms shows that relatively small decreases in nicotine dose result in changes in behavior to maintain, at least partially, nicotine intake (Harris, Pentel, & LeSage, 2009; Scherer, 1999). However, there are some theories and experimental evidence to suggest that compensatory increases in behavior are likely to exist only over a range of doses and that at very low nicotine doses, smoking behavior will decrease (Benowitz et al., 2012; Benowitz et al., 2007;

DeGrandpre, Bickel, Hughes, & Higgins, 1992; Hatsukami et al., 2010; Kozlowski & Herman, 1984).

Clinical research will help identify the nicotine dose necessary to maintain dependence in current smokers. However, a history of smoking may shift the dose-response curve for the reinforcing effects of nicotine to the left (sensitization) or to the right (tolerance). In this case, a nicotine reduction policy may impact individuals who start smoking at the reduced content differently from those who were smoking prior to policy implementation. Those individuals without a history of smoking cigarettes at higher nicotine contents may have much higher or lower intensity of smoking at the reduced contents. Research regarding the impact of prior smoking experience is critical in determining how best to implement a reduction in nicotine content.

Experimental research cannot ethically investigate the rate of smoking at low nicotine contents using subjects who have not yet started smoking. Thus, a policy implementing nicotine reduction will likely rely on non-human research for information about how a history of smoking might shift the rate of smoking across a range of nicotine doses. The self-administration paradigm, in which animals engage in a response that results in delivery of drugs, is likely to be the most informative. Modeling smoking status in a rat may never be possible, but methodological preparations can incorporate many of the most important elements. Environmental stimuli can be paired with nicotine infusions in the same way that smokers experience cues paired with their smoking. Additionally, some cigarette constituents other than nicotine may promote self-administration on their own or increase the rates of self-administration when combined with nicotine (Bardo, Green, Crooks, & Dwoskin, 1999; Belluzzi, Wang, & Leslie, 2005; Clemens, Caille, Stinus, & Cador, 2009; Guillem et al., 2005; Villegier, Lotfipour,

McQuown, Belluzzi, & Leslie, 2007). A cocktail of these other constituents can be included along with nicotine infusions to better mimic the experience of a smoker. Even with these methodological considerations, the specific doses in either of the two dose-response curves will not translate between non-human animals and humans. However, the relation between the dose-response curves of the two populations may tell us about the functional effects of a history of self-administering a higher dose of nicotine.

Substantial research exists regarding the dose-response curve for acquisition of nicotine self-administration in naïve animals (Chen, Matta, & Sharp, 2007; Cox, Goldstein, & Nelson, 1984; Donny et al., 1998; Sorge & Clarke, 2009). However, studies are not typically designed to compare acquisition and maintenance dose-response curves, and comparing across studies is difficult because of methodological differences across studies including strain of rat, nicotine-paired environmental stimuli, schedule(s) of reinforcement, and access schedule. One small study provides evidence that may be useful in determining how a history of self-administration shifts the dose response curve for low-dose nicotine self-administration. Cox et al. (1984) found that following acquisition, a nicotine dose reduction produced an increase in behavior even though the dose used for reduction failed to produce acquisition in a separate group of rats. Hence, these results suggest that prior self-administration experience may reduce the threshold nicotine dose required to maintain dependence. However, research examining the effect of a history of experimenter-administered nicotine exposure on the reinforcing effectiveness of nicotine is mixed. Some studies have found that nicotine pre-exposure results in increased sensitivity to nicotine (Adriani et al., 2003; Shoaib, Stolerman, & Kumar, 1994; Tammimaki et al., 2008), while others have found the opposite (Adriani, Deroche-Gamonet, Le Moal, Laviola, & Piazza, 2006; Shoaib, Schindler, & Goldberg, 1997).

The present experiment directly examined how self-administration behavior at low doses of nicotine is changed by a history of self-administering a relatively high dose of nicotine. Rats were given the opportunity to self-administer one of four low nicotine doses (15, 7.5, 3.75, and 0 μg/kg/infusion) before and after self-administering a relatively high nicotine dose (60 μg/kg/infusion). A separate group of rats acquired self-administration at the higher nicotine dose (60 μg/kg/infusion) before reduction.

2.0 METHOD

2.1 SUBJECTS

Male Sprague-Dawley rats (Harlan-Farms, IN), weighing between 188 and 217 grams upon arrival (approximately post-natal day 49) were used as subjects (N=105). Rats were individually-housed in wire-mesh, hanging cages in a temperature-controlled room kept between 68 and 70 degrees Fahrenheit. Rats were kept on a reverse light-dark 12:12 hour schedule (lights off: 7AM). Rats received free access to water in their home cages throughout the experiment, and were given unlimited access to Purina Rat Chow for the first week after arrival. Once surgeries began, rats were restricted to 20 g/day of chow for the remainder of the experiment. In this past, this food schedule has been shown to result in weight gain of approximately 10 g/week (Donny, Caggiula, Knopf, & Brown, 1995).

2.2 APPARATUS

Thirty four commercial operant chambers (30.5cm X 24.1 cm X 21.0 cm; ENV-008CT; Med-Associates) were used in the present experiment. Each chamber was closed inside a sound-attenuating cubicle with a ventilation fan. Chambers contained two nosepoke holes, each 2.5 cm in diameter, on the right side of the chamber located 5 cm from the chamber base to the base of

the hole. A white stimulus light, 3.5 cm in diameter, was located 6.25 cm above the top of each nosepoke. Each chamber also contained a houselight on the same wall. The houselight was 2 cm in diameter, illuminated red, and located 1 cm below the ceiling of the chamber. During sessions, rats were connected to a swivel system that delivers IV infusions while allowing for unrestricted movement within the chamber.

2.3 DRUGS

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO), and a cocktail of other cigarette constituents including: acetaldehyde (Sigma, St. Louis, MO), harman (Sigma, St. Louis, MO), norharman (Sigma, St. Louis, MO), anabasine (Sigma, St. Louis, MO), anatabine (Toronto Research Chemicals, Inc), myosmine (Sigma, St. Louis, MO), cotinine (Sigma, St. Louis, MO), and nornicotine (Sigma, St. Louis, MO) were dissolved in 0.9% saline. The doses of nicotine used for self-administration were 60, 15, 7.5, and 3.75 μg/kg/infusion. At the beginning of the experiment, a dosing error was made and rats were also exposed to doses of nicotine one tenth of the intended dose for 8 sessions. The doses of nicotine were chosen to be a range that will capture the threshold for maintaining behavior after reduction. The doses of the other constituents were 16 μg/kg/infusion (acetaldehyde), 0.1 μg/kg/infusion (harman), 0.3 μg/kg/infusion (norharman), 0.9 μg/kg/infusion (anabasine and nornicotine), 0.09 μg/kg/infusion (anatabine, myosmine and cotinine) (free-base concentration) . The constituent doses were chosen based on past studies that found an effect of the constituents (Belluzzi et al., 2005; Clemens et al., 2009) or to be proportional to the amount found in cigarette smoke given 30 μg/kg/inf nicotine (harman and norharman) (Herraiz, 2004). All drug solutions were adjusted to

7.0 (±0.2) pH with dilute NaOH. All solutions, including the vehicle, were sterilized by being passed through a 0.22 μm filter. During sessions, drugs were delivered in less than 1 s at a volume of 0.1 ml/kg/infusion.

2.4 PROCEDURES

2.4.1 Surgery

During surgery, rats were anesthetized with isoflurane and implanted with jugular catheters as described previously (Donny et al., 1999). After surgery, rats recovered for a minimum of 5 days in the home cages. For the first three days after surgery, the cannulae were flushed once daily with 0.1 mL of sterile saline containing heparin (30 U/mL), timentin (66.67 mg/ml) and streptokinase (9333 U/mL) to maintain catheter patency and prevent infection. After this initial post-surgery time period, the flushing solution contained only the heparin and timentin. Only rats that passed a chloral-hydrate patency test (60 mg) following the last self-administration session are included in the data presented.

2.4.2 Habituation

Each rat was placed in their assigned operant chamber for a 20-min period during which time a red house light illuminated the chamber and the nosepokes were removed from the wall.

2.4.3 Self-Administration

Table 1 summarizes the dose order by group. The required number of pokes into the active (right) nosepoke resulted in one infusion of the assigned nicotine dose along with a cocktail of the other constituents discussed above. Each infusion resulted in the 15-s presentation of a stimulus light located above the nosepoke (cued paradigm) and a 1-min time out. Left (inactive) nosepokes had no scheduled consequences. Sessions lasted 1 hour and were conducted 7 days/week.

Table 1. Nicotine doses experienced in each phase for each group

Group	Final n	Phase1 µg/kg/infusion	Phase 2 µg/kg/infusion	Phase 3 µg/kg/infusion
Low-High-Low 15 (LHL 15)	14	15	60	15
Low-High-Low 7.5 (LHL 7.5)	10	7.5	60	7.5
Low-High-Low 3.75(LHL 3.75)	8	3.75	60	3.75
Low-High-Low 0.0 (LHL 0.0)	10	0.0	60	0.0
High-Low 15 (HL 15)	14	60	60	15
High-Low 7.5 (HL 7.5)	14	60	60	7.5
High-Low 3.75 (HL 3.75)	14	60	60	3.75
High-Low 0.0 (HL 0.0)	14	60	60	0.0

2.4.3.1 Phase 1

At the beginning of the self-administration phase, rats were randomly assigned to one of 5 acquisition doses of nicotine: 60 µg/kg/infusion (57 rats), 15 µg/kg/infusion (14 rats), 7.5 µg/kg/infusion (12 rats), 3.75 µg/kg/infusion (10 rats), or 0.0 µg/kg/infusion (vehicle; 12 rats). As noted above, due to a technical error, all rats first experienced 8 sessions of exposure to one-

tenth the assigned nicotine dose (FR 1, 1 session; FR 2, 7 sessions). Upon discovery of the technical error, all rats were returned to FR1 at their intended dose. On the first day of the experiment one nose-poke was required to receive one infusion (an FR1 schedule of reinforcement). Rats experienced 1 session on an FR1 schedule of reinforcement and 7 sessions on an FR2 schedule of reinforcement before the ratio was escalated to an FR5 for 25 sessions.

2.4.3.2 Phase 2

After 25 sessions of self-administration on an FR5 schedule of reinforcement, all rats receiving one of the four lower nicotine doses (15, 7.5, 3.75, 0.0 µg/kg/infusion), began receiving 60 µg/kg/infusion nicotine with each infusion. Rats that had already been receiving 60 µg/kg/infusion continued to receive that dose during this phase, meaning that these rats received 25 more FR5 sessions at the 60 µg/kg/infusion dose. All rats remained at this dose for 15 sessions.

2.4.3.3 Phase 3

Following Phase 2, the 57 rat exposed to the 60 µg/kg/infusion dose during the acquisition portion of the experiment (Phase 1) were assigned to one of four groups matched for number of infusions over the past three sessions. Rats were assigned to have their nicotine dose reduced to either 15 (15 rats), 7.5 (14 rats), 3.75 (14 rats), or 0.0 (vehicle; 14 rats) µg/kg/infusion. Rats exposed to one of the four lower doses in the acquisition portion of the experiment had their nicotine dose reduced to the same dose used in Phase 1. All rats remained at these doses for 16 sessions. Groups that first experienced one of the four lower nicotine doses in Phase 1 are referred to as Low-High-Low (LHL) groups along with their reduction dose (e.g

LHL7.5 for Low-High-Low group that experienced 7.5 μg/kg/infusion in Phases 1 and 3). Groups that experienced 60 μg/kg/infusion during Phase 1 are referred to as High-Low using the same shorthand.

2.5 DATA ANALYSIS

Data analyses focused on assessing 1) the number of rats meeting a criterion for self-administration in each Phase of the experiment, 2) whether earned infusions at the end of Phase 3 were different from Phase 1 for LHL groups, 3) whether the change in earned infusions were different for any nicotine dose than for vehicle, and 4) whether earned infusions in Phase 3 were different for HL and LHL groups. To assess the proportion of rats self-administering nicotine in each phase (the first objective), a criterion was set at an average of at least 25 active responses and twice as many mean active as inactive responses over the last three sessions of each Phase. All rats that failed to meet the criterion at the end of Phase 2 were excluded from this analysis from Phase 3 because they did not experience the manipulation of interest (high nicotine dose self-administration). Proportion of rats meeting the criterion in Phase 1 is reported both with and without those failing to meet the criterion in Phase 2. Rats meeting the criterion in Phase 2 are included in all analyses (regardless of whether they met or did not meet the criterion in other phases. Rats failing to meet the criterion in Phase 2 are excluded from all of the remaining analyses.

To accomplish the second objective, a 4X2 mixed ANOVA compared earned infusions in the LHL groups between doses (between-subjects factor, 4 levels) before and after Phase 2 (within-subjects factor, 2 levels). It was expected that infusions earned before and after Phase 2 may

differ for vehicle self-administration, so the third objective was to assess how any differences in self-administration for the nicotine groups compared to this difference in vehicle self-administration. Three 2X2 ANOVAs were conducted comparing each nicotine group to saline (between-subjects factor, 2 levels) before and after Phase 2 (within-subjects factor, 2 levels). A significant interaction suggests that the effect of high nicotine self-administration on responding for low nicotine doses was greater than the effect on responding for vehicle.

To determine whether the HL and LHL groups were equivalent at the end of Phase 2 (prior to reduction but after assignment of the HL groups) four independent-samples t-tests compared earned infusions over the last three sessions of Phase 2 for each dose. To determine how the dose used for acquisition and the number of sessions at the high nicotine dose affect responding following reduction, a 4X2 between-subjects ANOVAs compared the average number of earned infusions over the last three sessions of Phase 3 for each nicotine dose (4 levels) and each history type (2 levels: LHL, HL).

3.0 RESULTS

Before the dosing error was corrected (when rats were exposed to doses 10 times less than their intended acquisition dose), very few rats met the criteria for self-administration. The average earned infusions over the last three days before the dosing error was corrected (at an FR2) for the LHL15, LHL7.5, LHL3.75, LHL0.0, and HL groups were 5.19 (SE=0.74), 5.50 (SE=0.84), 5.23 (SE=0.95), 4.92 (SE=0.71), and 5.87 (SE=0.46), respectively. Once rats were switched to the correct acquisition doses, rats in the LHL15 and the HL groups readily acquired self-administration (Figure 1 shows the earned infusions over Phase 1). Rats in the LHL7.5 group began to acquire self-administration after approximately 15 sessions on an FR5 schedule. At the end of Phase 1, 79%, 58%, 30%, and 16% of rats met the criterion for self-administration in the LHL15, LHL7.5, LHL3.75, and LHL0.0 groups, respectively. Of the rats in the HL groups, 95% met the criterion for self-administration (60 μg/kg/infusion in Phase 1).

Figure 1. Earned infusions across acquisition for all groups

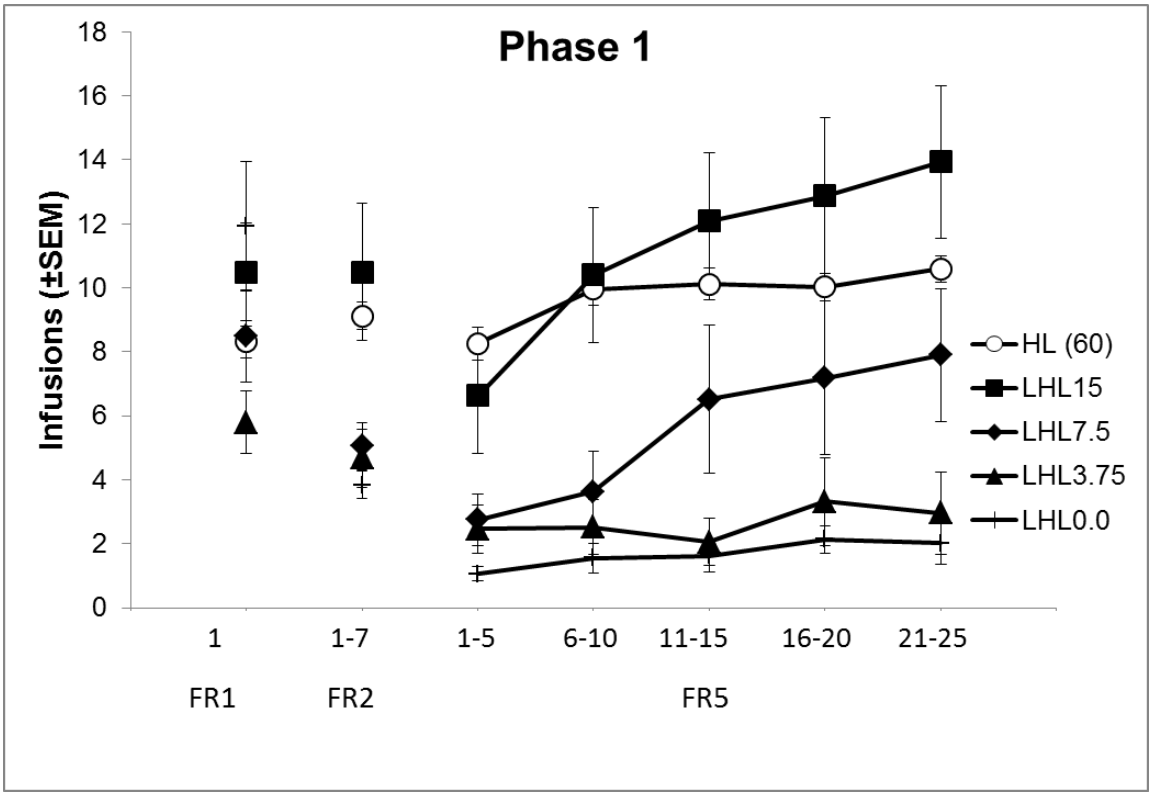

Figure 1. Data points are averages for the number of sessions indicated on the x axis. Error bars represent standard errors.

In Phase 2, 13% of rats in the LHL groups failed to meet the criterion for self-administration. The rats may not have been earning enough infusions in Phase 1 to experience the dose change in Phase 2. Of the 6 LHL rats failing to meet the criterion, 3 did not experience any infusions during Phase 2, and the other 3 experienced 25, 21, and 1 infusion over the 10 sessions. Because these rats did not experience the manipulation of interest (self-administration of a higher nicotine dose) they were excluded from the remaining analyses. Seven rats were excluded from the LHL and HL groups combined (LHL7.5, 2 rats; LHL3.75, 2 rats; LHL0.0, 2 rats; HL15, 1 rat). Following this exclusion, earned infusions of 60 μg/kg/infusion between LHL and HL groups were not significantly different at the end of Phase 2 (ps >0.339).

Figure 2 shows the average earned infusions at each of the four doses at the end of Phase 1 (LHL groups) and Phase 3 (All groups). Earned infusions at the end of Phase 2 are also shown for both LHL and HL groups. LHL rats earned significantly more infusions in Phase 3 following self-administration of 60 μg/kg/infusion nicotine than in Phase 1, $F_{1,38}=17.052$, $p<0.01$. There was also a significant main effect of dose, $F_{3,38}=10.920$, $p<0.01$ and a dose by phase interaction, $F_{3,38}=3.082$, $p<0.05$. The impact of Phase 2 experience was greater on responding for the 7.5 μg/kg/infusion than on responding for vehicle, $F_{1,18}=10.109$, $p<0.01$. Interactions were not significant at any other nicotine doses, $p>0.05$. Earned infusions in Phase 3 were not significantly different between LHL and HL groups, $F_{1,90}=0.037$, $p>0.05$.

Figure 2. Earned infusions at each nicotine dose in Phase 1 (LHL groups), Phase 2, and Phase 3 (LHL & HL groups)

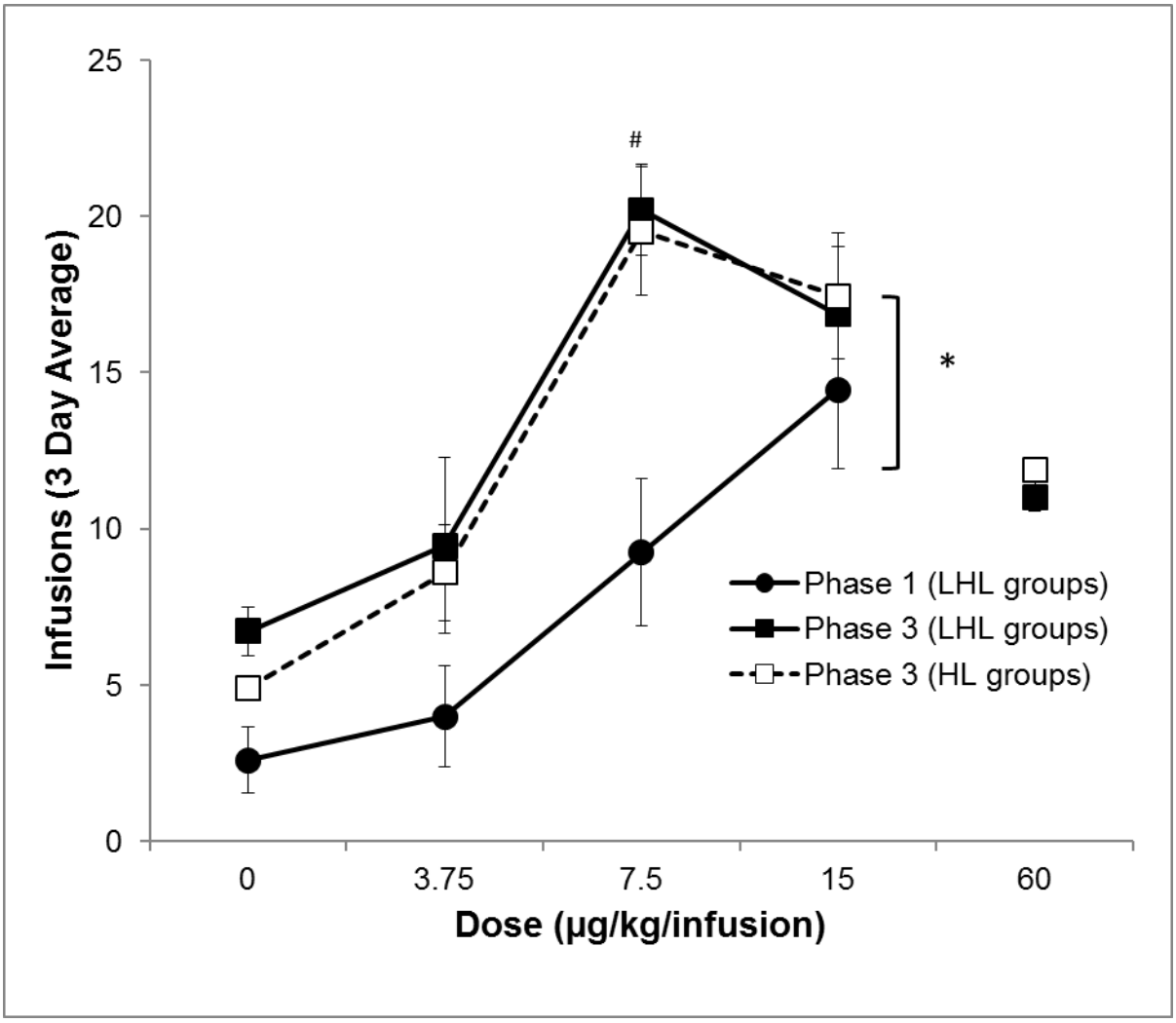

Figure 2. Earned infusions over the last three sessions of Phase 1(acquisition phase) (LHL groups, filled circles) and Phase 3 (reduction following exposure to 60 µg/kg/infusion nicotine) (LHL groups, filled squares; HL groups, open squares). Earned infusions at 60 µg/kg/infusion dose are from the last three sessions of Phase 2 for both LHL and HL groups and contain all rats for each history type. Bars represent standard errors. Significant effect of phase on LHL groups is represented by *. Significant nicotine X phase interaction is represented by #.

Figure 3 shows the average earned infusions for each of the LHL groups and individual rats at the end of each phase (average of last three sessions). The distribution at the 15 and 7.5 µg/kg/infusion doses was highly variable in both Phases 1 and 3, with some rats failing to meet

the criterion for self-administration and other rats having high rates of self-administration. The percentage of rats meeting the criterion for self-administration at the end of Phase 3 was 93%, 100%, 63%, and 80% for the LHL15, LHL7.5, LHL3.75, and LHL0.0 groups, respectively. The percentage of rats meeting the criterion for the HL groups was 100%, 100%, 79%, and 50% for the HL15, HL7.5, HL3.75, and HL0.0 groups, respectively. For comparison to Phase 1, after excluding the rats that failed to meet the criterion in Phase 2, the percentage of rats meeting the criterion in Phase 1 for the LHL15, LHL7.5, LHL3.75, and LHL0.0 groups was 79%, 70%, 38%, and 20%.There was little variability in any of the LHL groups during Phase 2 when rats were exposed to 60 μg/kg/infusion. The average earned infusions in the LHL3.75 μg/kg/infusion group during Phase 3 was increased as a result of one rat that had a very high rate of self-administration.

Figure 3. Earned infusions for individual rats in LHL groups in each phase of the experiment.

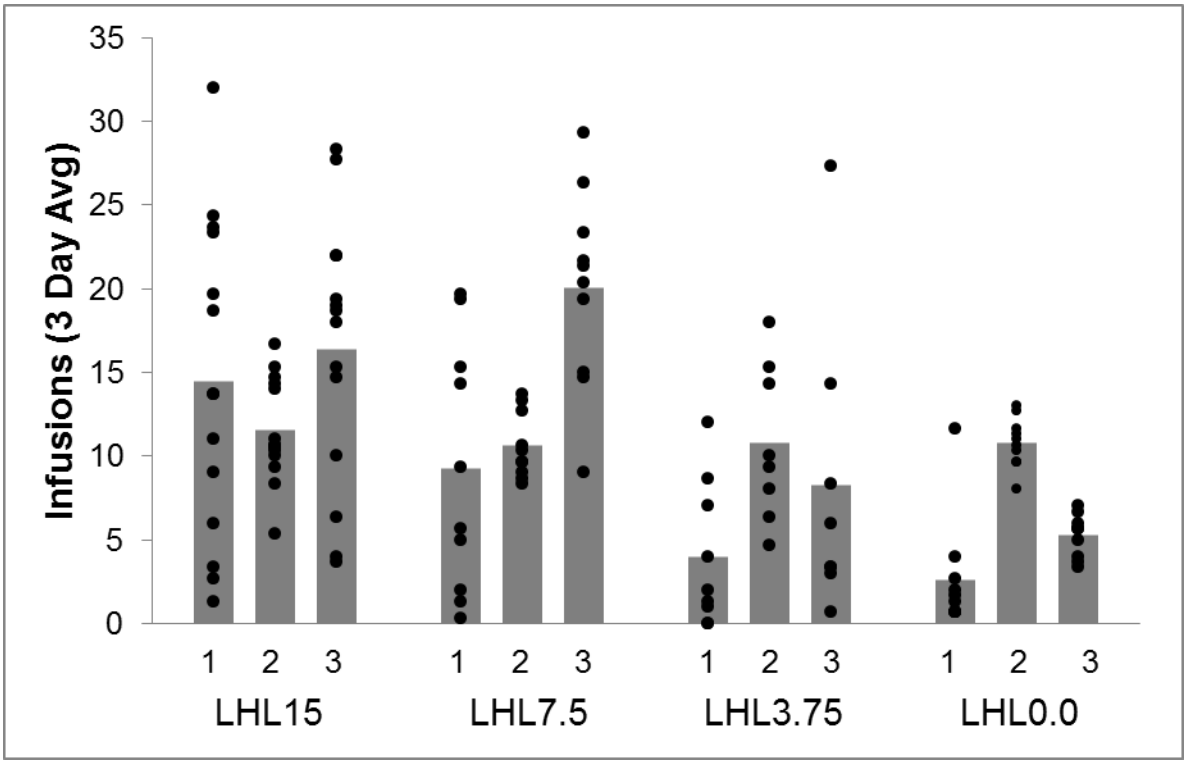

Figure 3. Earned infusions for each LHL group. The three bars for each group represent earned infusions during a different phase for the same group of rats. All groups experienced the indicated dose in Phases 1 and 3. During Phase 2 all rats were switched to 60 μg/kg/infusion of nicotine. Bars represent group averages, and points represent data from individual rats.

4.0 DISCUSSION

The present study found that more than half of the rats given the opportunity to acquire nicotine self-administration did so when the nicotine dose was 7.5 μg/kg/infusion or greater. Rats self-administered low doses of nicotine at a higher rate after experience self-administering a high nicotine dose. Rats also self-administered a vehicle of other cigarette constituents paired with a nicotine associated stimulus at a higher rate after having a history of high nicotine dose self-administration. A history of high dose nicotine self-administration had the greatest impact at a middle dose (7.5 μg/kg/infusion). Additionally, the rate of self-administering low doses of nicotine (and vehicle) was not affected by whether rats acquired self-administration at a low dose before having their dose changed to the high nicotine dose.

In the current study, an increase in self-administration of vehicle was observed following the Phase 2 experience of self-administering a higher nicotine dose, indicating that, for an unknown period of time, even if nicotine were removed from cigarettes completely, current smokers could continue to smoke at a higher rate than individuals who would be starting to smoke. Following nicotine reduction, 50-80% of rats self-administering vehicle continued to meet our criterion for self-administration. The increase in responding for vehicle is indicative of an overall upward shift in the dose-response curve. The peak of the curve as well as the lowest level of responding may be increased following experience with a high dose of nicotine.

Increased responding for vehicle following self-administration of 60 μg/kg/infusion could be related to several mechanisms. Even after nicotine was removed, the operant chamber and active nosepoke will continue to function as discriminative stimuli, signaling the availability of nicotine, until extinction is complete (Skinner, 1953). Also, the presentation of the light cue (which was paired with nicotine in Phase 2) likely maintains responding as a conditioned reinforcer. After many presentations of the light without nicotine, rats will learn that the light and nicotine are no longer associated. For smokers, the contexts in which they engage in smoking behavior and conditioned reinforcers (e.g., taste of a cigarette) will continue to maintain behavior after nicotine reduction for some time. In the present study, behavior was maintained over 16 1-hour sessions. Another study showed that responding was maintained after nicotine removal over 60 sessions until cues were finally removed (Cohen, Perrault, Griebel, & Soubrie, 2005). These data suggest that the process of extinction could be very prolonged. Furthermore, rat self-administration models may overestimate the speed of extinction because learning and extinction occur in one context, the operant chamber. For human smokers, nicotine has been paired with cues in many contexts, and extinction would likely need to take place in many or all of those contexts, increasing the time until behavior is fully extinguished (Wing & Shoaib, 2008). The present study illustrates the need for research investigating methods that may facilitate extinction. For example, a novel cue could be presented during extinction (e.g., a cigarette flavoring), signaling that nicotine is no longer available (Donny et al., 2012).

Another possibility is that a history of exposure to nicotine may increase the reinforcing value of the cocktail constituents. Thus far, there is nothing in the literature to suggest that nicotine may shift the reinforcing value of the constituents used here. However, research on these constituents is sparse, and experiments have not been designed to test this possibility. The five

minor alkaloids employed here (cotinine, myosmine, anatabine, anabasine, and nornicotine) have a similar chemical structure to nicotine (Huang & Hsieh, 2007), and it is possible that a history of nicotine exposure could increase sensitivity to these constituents.

In the present study, rats self-administered nicotine at a higher rate following experience self-administering a high dose of nicotine. Much of this increase may be due to the upward shift in the dose-response curve caused by at least one of the mechanisms discussed above. However, a larger effect of high nicotine dose self-administration took place at a middle dose (7.5 μg/kg/infusion). During acquisition, this dose appeared to be on the ascending limb of the dose-response curve. The larger increase in self-administration at this dose may be indicative of a leftward shift in the dose-response curve. If the ascending limb of the dose-response curve is sharp, then a leftward shift in the dose-response curve would be expected to produce the greatest increase in self-administration on the ascending limb. A leftward shift in the dose-response curve would indicate that experience self-administering a high nicotine dose caused rats to be more sensitive to nicotine than they were prior to that experience.

Another interpretation of the data presented here is that the 7.5 μg/kg/infusion dose in Phase 3 increased the value of the light cue as a reinforcer through reinforcement enhancement (Donny et al., 2003; Palmatier et al., 2006). Nicotine has been shown to increase responding for a moderately reinforcing stimulus, regardless of whether or not the nicotine is delivered contingent on any response. (Donny et al., 2003), a process called reinforcement enhancement. This process is separate from the primary reinforcing effects of nicotine. In Phase 3, the light cue became a reinforcer through its pairing with nicotine. It may be that in Phase 1, the light cue did not function as a reinforcer for rats not meeting the criterion for self-administration (as was true for many rats in the LHL7.5 group). After exposure to a higher nicotine dose, the light cue is

now functioning as a reinforcer, and the nicotine dose is sufficient for producing enhancement. At lower doses (at or below 3.75 μg/kg/infusion), there may not be enough nicotine received to produce enhancement of the light cue. At higher doses, nicotine may have been enhancing the value of the light cue in Phase 1, so no additional enhancement was seen in Phase 3.

The distributions of earned infusions presented in Figure 3 highlight inter-individual variability even within a single history of nicotine exposure. Doses above the threshold for reinforcement but which have historically been on the ascending limb of the dose-response curve (7.5 and 15 μg/kg/infusion) seemed to produce the most variability, with some rats having very high rates of behavior and some rats not meeting the criterion for self-administration. At the highest dose tested (60 μg/kg/infusion), rats that met the criterion for reinforcement self-administered with very little variability. At the lowest end of the dose range, rates of self-administration were generally low. Research investigating the sources of variability in response to nicotine reduction may be useful in identifying at-risk groups in humans.

In the present study, self-administration rates were the same in Phase 3 for LHL and HL rats. Rats in the HL groups experienced 33 more total sessions at the 60 μg/kg/infusion dose than the LHL rats. Despite the difference in exposure to the higher nicotine dose, rates of self-administration following reduction were strikingly similar. These data indicate that the effect of high nicotine dose self-administration is not increased by the duration of self-administration. These data may indicate that the length of time an individual has been smoking will not affect their rate of smoking following nicotine reduction. However, in the present study, rats were exposed to nicotine using a limited-access model (1-h of possible self-administration per day). The duration of high nicotine self-administration may have a greater impact using an extended access model.

These data are reassuring from a policy perspective. If the nicotine content in cigarettes were reduced, it is unlikely that there will be much data examining how a nicotine reduction policy will affect individuals who may start smoking for the first time at a reduced level. However, the data in the present study suggest that a dose of nicotine that reduced smoking in current smokers is likely to have an even greater impact on new smokers. Rates of smoking are likely to be even lower for individuals who never smoked cigarettes with a higher nicotine content. If clinical data support nicotine reduction as an effective strategy for reducing public harm, data like these will be important in determining the potential impact on individuals who have not yet started smoking. Future self-administration research should continue to address concerns about acquisition. Because most smokers begin smoking in adolescents, research addressing nicotine reduction in adolescent animals would be especially beneficial.

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