# Individual Differences in Voluntary Self-Administration of Oral Nicotine in Female Rats

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*Eight adult female Sprague Dawley rats had free access to both a nicotine solution and distilled water. At no time were rats deprived of food, water, or forcefully exposed to nicotine. Individual consumption patterns emerged with some animals drinking little nicotine solution and others preferring it over water. Results suggest that providing a nicotine solution of 1µg/ml nicotine hydrogen tartrate salt (SigmaAldrich) continuously in the home cage is sufficient to establish voluntary self-administration of nicotine in some rats. Further, this method of nicotine self-administration is promising and yields the kind of individual nicotine intake patterns seen in human tobacco users.*

Nicotine is one of few addictive (Stolerman & Jarvis, 1995; US Department of Health and Human Services [USDHHS], 1998) psychoactive agents legally and readily available for human consumption in a variety of relatively inexpensive tobacco and tobacco replacement products. The widespread availability of nicotine-containing products, together with the appetitive (Russell, 1980) and addictive properties of nicotine, create a rich environment for repeated exposure, dependence, and the unfortunate health consequences that have been associated with chronic tobacco use (Heinrich, 2003). It is generally agreed that a better understanding of the factors that contribute to nicotine use is an important scientific and societal goal.

Experiments investigating the impact of nicotine on the behavior and physiology of nonhumans provide an important contribution to the goal of establishing a direct functional relationships between nicotine exposure and the behavior and physiology of a living organism. Although intravenous injection is the most common experimental paradigm for investigating nonhuman nicotine self-administration (Corrigal, Coen, & Adamson, 1989; Valemtine, Hokanson, Matta, & Sharp, 1997), there is renewed interest in rodent oral self-administration procedures (Adriani, Marcri, Pacifici, & Laviola, 2002; Biondolillo & Pearce, 2007; Dadmarz & Vogel, 2003; Klein, Stine, Vandenbergh, Whetzel, & Kamens, 2004; Maehler, Dadmarz, & Vogel, 2000) which were popular prior to the development of intravenous models (Flynn, Webster, & Ksir, 1989; LeHouezec, Martin, Cohen, & Molimart, 1989). Advantages of oral self-administration procedures include a relatively unlimited duration of experimental investigation, complete freedom of movement by an intact subject, measurement of consummatory responses, and the increased likelihood that individual patterns of nicotine self-administration will present themselves (Abreu-Villaca, Queiroz-Gomes Fdo, Dal Monte, Filgueiras & Manhaes, 2006; Flynn, et al., 1989; Perkins, 1995). All of these features are relevant to a model attempting to mimic voluntary nicotine exposure as seen in the human tobacco user.

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Although a voluntary self-administration approach offers many advantages, it does come with methodological challenges. The most critical challenge stems from the very nature of the approach, which allows subjects to voluntarily drink (or not) a solution containing nicotine. Failure to demonstrate preference for the nicotine solution or even substantial levels of consumption of nicotine in rats using the most common oral self-administration situation, the two bottle choice paradigm (Flynn et al., 1989; LeHouzec et al., 1989; Smith & Roberts, 1995) has resulted in researchers resorting to methods of forced exposure (LeHouzec et al.) or sweetening the solution containing nicotine (Meliska, Bartke, McGlacken, & Jensen, 1995; Smith & Roberts). Although these procedures increase the consumption of nicotine, they do little to support the position that nicotine possesses reinforcing qualities for rats. In the absence of substantial evidence that rats will voluntarily consume a nicotine solution, one must question the assumption that nicotine possesses reinforcing properties for rats when administered orally (see Frenk & Dar, 2000, for a review questioning the assumption that the reinforcing properties of nicotine have been established using any method in nonhumans).

Studies using oral nicotine self-administration by rats must address some basic issues of the approach before it can be used to ask more important questions about nicotine use and addiction. For example, if the goal of the research is to determine the effects of nicotine using a voluntarily self-administered model, research subjects must first drink the solution containing nicotine, and they must drink it in sufficient quantities to experience a pharmacological effect. This approach, as attractive as it is in terms of potential applicability, clearly depends on rats' initial reactions to the olfactory and gustatory cues of the nicotine solution itself as well as any post oral impact associated with consumption of the nicotine solution. The problem may be further complicated by the possibility that different rats may respond differently to the gustatory cues associated with a given concentration of nicotine solution as well as the post-oral pharamacological effects of nicotine.

The conclusion that rats will develop a preference for a nicotine solution over water lacks adequate support. Much of the data reported from oral nicotine self-administration studies were generated with group designs (Flynn et al., 1989; Smith & Roberts, 1995) and interpreted through the analyses of group data with no consideration of the intake patterns of individual rats. However, Maehler et al. (2000) reported data indicating that rats develop idiosyncratic patterns of consumption with some preferring and others avoiding the nicotine solution; thus supporting our concern that individual rats may in fact respond quite differently to the taste of a nicotine solution and/or the pharmacological effects of nicotine contained in the solution. More recently, Dadmarz and Vogel (2003) noted that group means may mislead in their conclusions and individual animals responses to nicotine are the determining forces in nicotine self-administration.

The first aim of the this study was to demonstrate voluntary intake of nicotine via an oral solution without depriving rats of water, forcing exposure to nicotine, or relying on a sweetened solution to encourage consumption. The second aim was to describe any idiosyncratic patterns of nicotine self-administration in individual subjects. The general assumption by those who use or critique the oral self-administration model seems to be that all rats exposed to a nicotine solution must respond in the same way to it, showing a clear preference for the nicotine solution over water in order to conclude the model is valid. We disagree with this and assume that individual rats, like humans, may react very differently to nicotine. In fact, we anticipated that some rats would drink little or none of the nicotine solution but that other rats might establish a pattern of consumption with continued and constant exposure to nicotine in the home cage. Our oral self-administration model provides an ideal way to both demonstrate and take advantage of idiosyncratic nicotine self-administration patterns as rats are free to consume nicotine in varying amounts from one day to the next. Demonstrating individual differences in nonhumans would be directly in line with the observation that humans differ greatly in their frequency of tobacco use and propensities to become "dependent" on tobacco products (Shiffman, 1989). Such findings would suggest that under conditions of voluntary access, nicotine availability is sufficient to establish chronic exposure in some rats but not in other rats; findings which would converge with those reported by Maehler, et al. (2000) and would support the position that models of nicotine use and dependence should be equipped to explain variations in individual use and development of dependence (Shiffman, 1991).

## Method

### Animals and Maintenance

Eight female Sprague Dawley rats, approximately 64 days old at the beginning of the study were housed individually in 46 (d) x 30 (h) x 28 (w) cm wire mesh cages equipped with a 28 x 16.5 cm loft accessed by a 28 x 9 cm ramp. Animals were housed in a temperature and humidity controlled colony room under a reverse 12:12 light/dark cycle. Rats were treated in accordance with the ethical standards of the APA;

approval for procedures used was granted by the Institutional Animal Care and Use Committee at Arkansas State University, and all rats remained healthy for the duration of the experiments. Two water bottles (BioServ #9010 100 ml) were mounted on the outside of the cage 23 cm apart. The drinking reservoirs extended into the home cage approximately 4 cm and rested approximately 2 cm from the floor of the loft. Rats accessed liquid from a 2.5 cm diameter opening in the bottle reservoir. Lab Diet 5012 Food pellets were piled in a corner of the cage as needed. Food pellets were discarded and replaced with every cage cleaning. Visual access to adjacent animals was reduced with opaque dividers between cages. Rats had free access to Cell Sorb Plus bedding, food, and water at all times in the home cage. Cages were cleaned and rats were inspected and weighed every 3 days.

**Phase 1**

During Phase 1, both cage bottles were filled with 100 ml of distilled water (DW). The amount consumed from each bottle, rounded to the nearest ml, was recorded at the same time daily (8:00 am, 2 hours into rats' light cycle) prior to rinsing and filling bottles with fresh DW. Phase 1 served to allow rats to acclimate to conditions of the lab and to establish individual baseline drinking patterns across bottles, as well as total volume intake prior to nicotine availability. Further, baseline choice consumption was used to determine the presence of a preferred bottle or bottle position. Phase 1 continued for 30 days. By this time it was clear that rats were consuming consistently from both bottles and did not vary more than 10 ml in total volume consumed each day across 10 consecutive days.

**Phase 2**

Conditions and procedures of Phase 2 were identical to those of Phase 1 with the exception that one of the bottles was filled with a nicotine solution (1μg/ml; Sigma Aldrich nicotine hydrogen tartrate salt dissolved in distilled water). This concentration of nicotine was selected based on evidence that concentrations exceeding 5 μg/ml are avoided, possibly due to bitter taste cues (Flynn et al., 1989).

The position of the bottle containing the nicotine solution (NS) was counterbalanced randomly across rats but bottle positions were always the same

**FIGURE 1**

**Two-bottle choice consumption during the final 15 days of Phase 1 and Phase 2 averaged over eight subjects. A. During Phase 1 both bottles contained distilled water (DW); B. During Phase 2 one bottle contained a nicotine solution (NS) and the other contained DW. Data points reflect mean scores and error bars reflect standard deviations.**

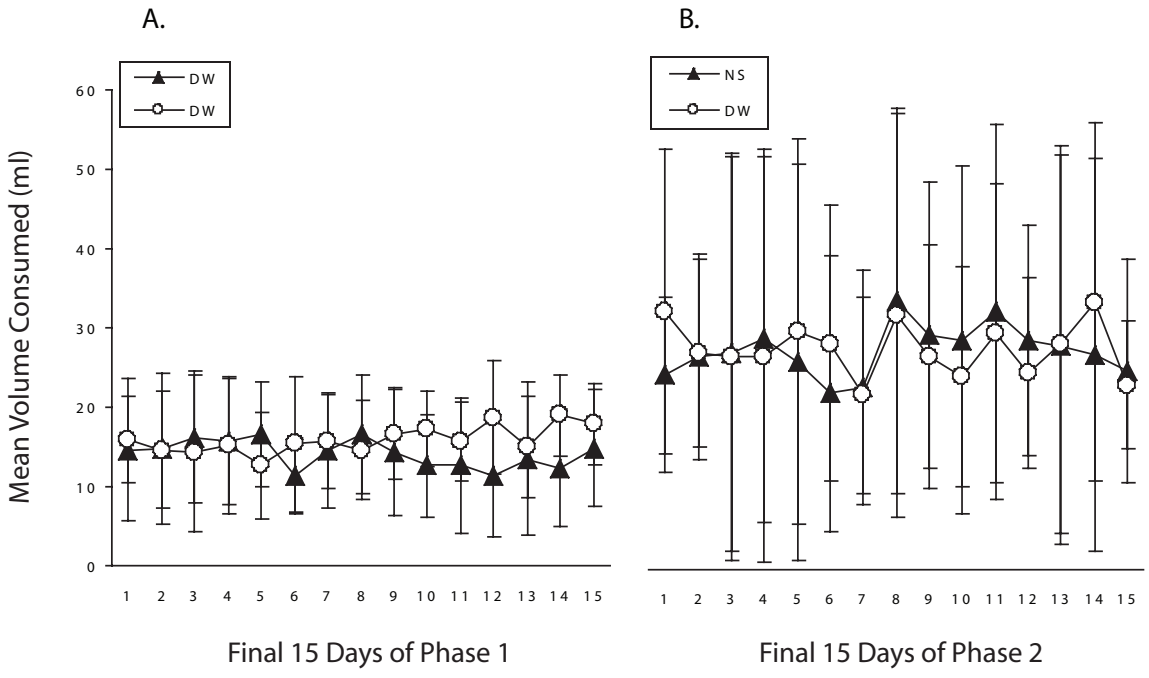


**FIGURE 2**

**Raw two-bottle choice consumption values of individual rats that consumed little NS (R1, R8) or demonstrated no preference for either the NS or DW (R6, R7) during the final 15 days of Phase 2. The right panel presents consumption during a final post test following nicotine deprivation.**

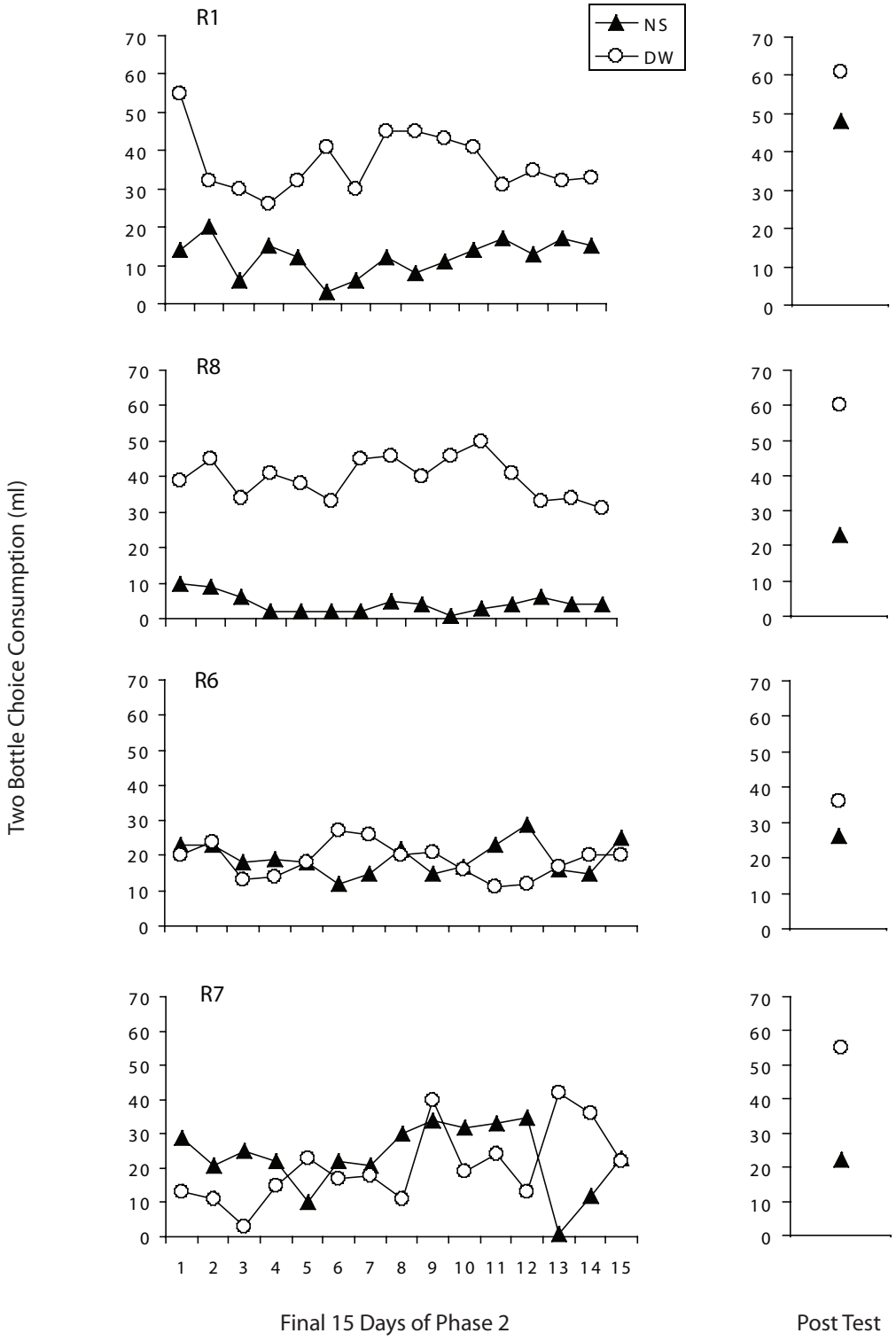
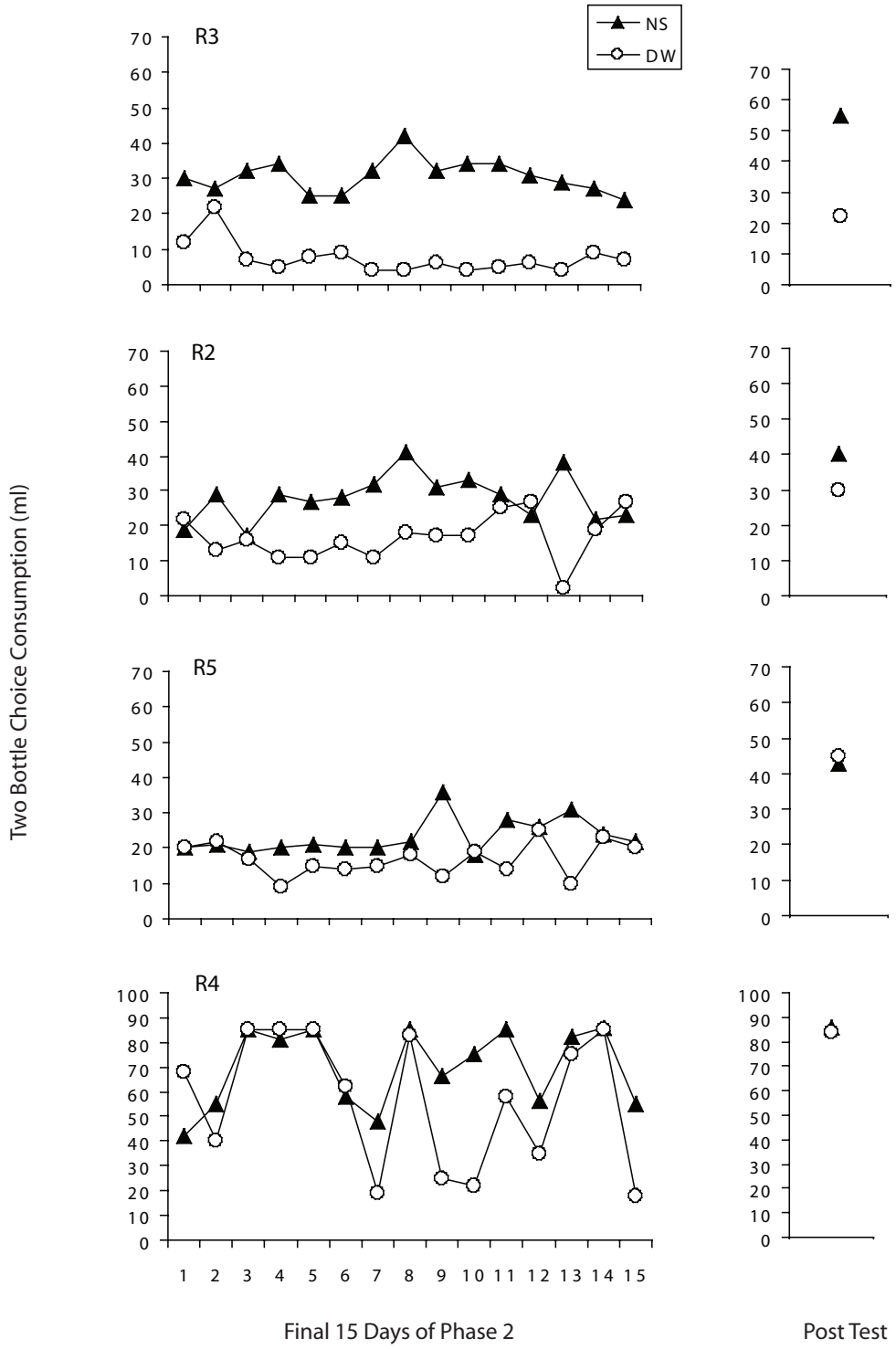


**FIGURE 3**

**Raw two-bottle choice consumption values of individual rats that consumed more NS than DW during the final 15 days of Phase 2. Note the axis change for R4 which consumed considerably more liquid than the other animals. The right panel presents consumption during a final post test following nicotine.**



within subjects. Volume intake from each bottle continued to be recorded at the same time each morning prior to filling tubes with fresh DW or NS. Phase 2 was used to compare intake patterns across days when rats could freely choose between DW and NS to drinking patterns when both bottles contained DW. Together, Phase 1 and 2 allowed us to determine if the NS possessed aversive gustatory features that were avoided immediately and to determine if rats might develop an increased appetite for nicotine with prolonged availability of nicotine in the home cage.

Rats were never deprived of water or food nor were they forced to consume the NS. In fact, NS and DW bottles were consistently placed on the left or right side of the home cage for a given subject to provide a clear location cue. The decision to hold bottle position constant was based on pilot data collected in our lab which suggested that during initial exposure rats did not appear to discriminate between the NS and DW. Specifically, reversing bottle positions daily resulted in all rats' failure to develop consistent patterns. By holding the position of the nicotine bottle constant, position served as a relevant stimulus to be associated with any post-oral effects of nicotine. Given the weak concentration of nicotine in our NS, it is possible that taste cues were minimal or were not aversive and therefore the NS is not avoided based on taste cues alone.

Following Phase 2, rats were prepared for a pilot study to examine the influences of nicotine pre-exposure on conditioned taste aversion, the results of which were not reported here. During this pilot, study rats did not have access to the oral nicotine solution for 12 days. Rats were not water deprived and conditions were identical to that of Phase 1. On day 13 of nicotine deprivation, rats were introduced to a post-test. Subjects were presented with two bottles: one containing DW and one containing NS. Subjects were allowed to sample from either bottle for 24 hours. Bottles were placed in the same positions as in Phase 2.

## Results

Figure 1 illustrates two-bottle choice consumption during the final 15 days of Phases 1 and 2, left and right panels respectively. Data in Figure 1 represent mean volume consumed ($N = 8$) each day, from each bottle. Error bars denote empirical standard deviations. By the end of Phase 1, group means revealed rats were consuming consistently from both bottles (left panel Figure 1) and total consumption was stable with less than 10 ml difference in total volume consumed across the final 15 days of the Phase. By the end of Phase 1, total volume intake was, on average, 32 ml (means calculated on all 8 rats' final 7 sessions of Phase 1 with left bottle volume consumed $M = 15.56$ ml vs. right bottle volume consumed $M = 16.57$ ml).

We used an alpha level of .05 for all statistical tests. A repeated measures ANOVA across days confirmed no statistically significant difference in volume consumed from the left versus right bottle, $F(1, 7) = 1.28$, $p > .05$.

The right panel in Figure 1 illustrates two-bottle choice consumption during Phase 2 when rats had access to both NS and DW. By the end of Phase 2, all rats were drinking from both tubes. However, a notable feature of the data from Phase 2 was an increase in error variance as different rats exhibited individual patterns of consumption of DW and NS. The error variance was especially noteworthy when you compare it to the error associated with Phase 1 behavior (left panel). The second notable feature of these data was the increase in total fluid intake when compared to that of Phase 1. By the end of Phase 2, total volume intake was, on average, 53 ml of liquid (mean calculated on all 8 rats, final 7 sessions). A repeated measures ANOVA applied to total volume consumed in the final sessions of Phase 1 versus Phase 2 revealed a statistically significant main effect of phase, $F(1, 7) = 7.86$, $p < .05$. Further, a breakdown of total volume consumed from bottles containing nicotine versus DW revealed that consumption increased from Phase 2 comparably for both bottles (NS, $M = 18.32$ vs. $M = 27.34$; DW, $M = 19.00$ vs. $M = 26.14$ for initial and final sessions of Phase 2 respectively) although the differences between initial and final ingestion for each solution were not statistically significant, $F(1, 7) = 1.35$, $p > .05$.

Figures 2 and 3 allow examination of intake data for individual rats during the final 15 days of Phase 2 (left panels). These data revealed clear differences in consumption patterns across individuals. Two rats (R1 and R8) consumed little or no NS (Figure 2). Four rats (R4, R5, R6, R7) consumed relatively equal amounts of either solution (Figures 2, 3); and two rats (R3 and R2) typically consumed more NS than DW. Note the change in Y axis scale for subject R4. Although this animal did not show a clear preference for NS over DW, she was drinking considerably more NS than the other rats. Individual consumption trends emerged during the final 15 day of Phase 2 such that 3 rats—R6, R2 and R5—began and ended the phase with relatively equal sampling of NS and DW. Three rats—R1, R8, and R—began and ended the phase with large sampling differences between the two bottles. Two rats, R4 and R7, exhibited erratic sampling patterns of both NS and DW.

The single data points presented in the right panel of Figures 2 and 3 represent consumption of NS and

DW on a single post test day at the end of Phase 2. It is relevant to note that regardless of voluntary consumption levels during Phase 2, following 12 days of nicotine deprivation, 6 of 8 rats consumed more NS than they had during Phase 2. Only two animals, R6 and R7, did not show an increase, nor decrease, in NS consumption during the post-test. Rather, their intake levels of NS were comparable to those observed during the final days of Phase 2.

## Discussion

The two-bottle free-choice method is widely accepted as a valid experimental method in nonhuman studies investigating voluntary intake of, and preference for, oral solutions (Bachmanov, Tordoff, & Beauchamp, 1996; Meliska, et al., 1995). The present study supports evidence reported by Maehler et al. (2000) that female Sprague Dawley rats will voluntarily self-administer nicotine in an oral solution, and that individual differences are clearly evident in consumption patterns (see Dadmarz & Vogel, 2003) with some subjects consuming little nicotine and others consuming more nicotine solution than water during the final days of choice Phase 2. Although discussion of individual differences in vulnerability to nicotine addiction or dependence is premature, these data indicate individual differences in voluntary self-administration. We suggest variability in subjects' reaction to nicotine which makes further investigation with this approach to self-administration even more attractive as a means of answering questions about factors that contribute to the behavior of individuals voluntarily approaching or avoiding nicotine.

Individual tendencies to consume the nicotine solution are clear, though an explanation for them is not readily evident. To interpret our consumption data, one must consider the potential influence of two factors associated with a nicotine solution. The first factor, the stimulus features of the solution (i.e., odor and taste cues) would be present upon a subject's initial exposure to the solution. If these stimulus features were aversive, one would expect rats under conditions of no deprivation to simply avoid the solution. In Phase 2, although Subject R8 drank little of the NS, she didn't avoid it altogether—sometimes drinking as much as 10 ml of the solution in a 24-hour period. Further, all of the other rats reliably consumed some of the NS. Together these data indicate that our NS did not present odor and taste characteristics that female Sprague Dawley's actively avoid. Recent data published from our lab (Biondolillo & Pearce, 2007) using a multiple bottle choice procedure support this assumption.

On the other hand, if the stimulus features of the solution are attractive to rats, one will expect rats to readily consume the solution upon habituating to its novelty. Although this will provide the simplest explanation for our rats that consumed the NS, it also suggests that we are dealing with different populations of rats—those who like the taste of the nicotine solution, those who do not, and those who show no clear preference.

However, several additional points make the appetitive features an unlikely solitary explanation. First, rats did not gradually increase consumption of the NS across days as one might expect if they were in part controlled by mere exposure as in habituation to novelty or experience-induced palatability (Flynn et al., 1989). Rather, they tended to either consume a stable amount (R1, R8, R3, R5) of NS from day-to-day or to behave unpredictably (see R7, R4) from day-to-day. Second, evidence from earlier work in our lab indicated that if the bottle positions were rotated from day-to-day, rats' consumption behavior was even more erratic with rats neither consistently approaching or avoiding the NS (e.g., all rats tended to behave like R6 and R7). Taken together, our data suggest that taste cues alone cannot account for the observed behaviors. Furthermore, previous studies revealed the taste of nicotine alone does not contribute heavily to its voluntary consumption (Glick, Visker, & Maisonneuve, 1996). Evidence from Maehler et al. (2000) showed both male and female rats titrate their nicotine intake despite varying concentrations of the drug in solution; suggesting it was not the taste of the solution, but the amount of nicotine in the solution that determined intake.

It is possible that rats either are unable to discriminate between the NS and DW from taste cues or that they can discriminate between the two but find the solutions to be of equal taste valance/value. If a subject's initial experience with the nicotine solution is one of indifference or inability to discriminate between the nicotine solution and control vehicle, then one will expect, putting bottle and location preferences aside, relatively equal sampling from the bottles containing the nicotine solution and control vehicle. Rats R6, R7, and R5 fit this pattern. Based on these 3 rats' data alone, one can conclude that either the nicotine solution produces little discernable post-oral effects (i.e., pharmacological) or it produces delayed post-oral effects that the subjects are unable to associate with the location cue. Though there is systemic absorption of pharmacologically significant amounts of nicotine when delivered orally, this route relies on the relatively slow process of gastric absorption and first-pass metabolism before becoming avail-

able in the bloodstream. Questions arise concerning both the speed with which nicotine becomes available and the amount of nicotine ultimately available to the central nervous system—important issues for the development of bottle preference learning and dependence (for review see Warnakulasuriya, Sutherland, & Scully, 2005).

We deliberately placed the bottle containing NS in the same location from day-to-day (Lee, Chen, Shih, & Hiroi, 2004) to facilitate any discrimination between the taste and/or post-oral effects of nicotine, and our data suggested that some of our subjects (R1, R8, R3, R5) could discriminate between the two bottles. Post-test data provide indirect evidence that the NS was, at some level, reinforcing to subjects as deprivation from NS resulted in markedly increased consumption for some rats (R1, R8, R3, R5) and levels of consumption for the remaining rats were comparable to that of Phase 2.

In conclusion, our results demonstrate that chronically exposing female Sprague Dawley rats to a weak concentration of oral nicotine results in reliable, voluntary self-administration in some rats. These data add strength to the case that reporting only group means may be insufficient for our understanding of nicotine consumption in a voluntary model. We generalize these data only to female Sprague Dawleys but plan a formal test of males in a future study. It will be interesting to see if environmental availability will exert the same influence on older rats, or if this effect is somehow linked to age of exposure as rats, like humans, have been shown to be more vulnerable to nicotine during adolescence. Although the study is limited in that it did not address sex, strain, development, or differences brought on by duration or concentration of oral nicotine, the strength of this study lies in the method employed which clearly reveals individual differences in nicotine self-administration. Individual differences are also observed in human tobacco users (Benowitz & Jacob, 1997; Shiffman, 1989, 1991) making this method a viable comparative tool to analyze factors contributing to voluntary nicotine self-administration.

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