Efficacy of Providing Nicotine in a Liquid Diet to Rats (44407)

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Abstract. **To determine if rats would consume nicotine at psychoactive levels, a nutritionally balanced diet with 0, 20, 60, or 200 mg of nicotine tartrate per kg of diet was provided. Diet consumption and body weight differences were recorded for 14 days after which, following 16 hr of withdrawal, animals were given access to a two-bottle choice of the previously presented diet and a nicotine-free diet. Spontaneous horizontal motor activity was recorded 8, 16, and 24 hr after withdrawal. By Day 14, all animals showed a significant increase in diet consumption and significant weight gain compared to Day 1. Animals consumed an average of 2.1, 6.8, or 19.5 mg/kg/day of nicotine on the low, medium, and high-nicotine diets, respectively. However, animals receiving the high-nicotine diet consumed less diet and gained less weight than the control, low, and medium nicotine groups. During only the first 4 hr of the two-bottle choice (16–20 hr postwithdrawal), the high-nicotine group consumed significantly higher amounts of nicotine base than the other groups, but also consumed more of the control diet during the first 2 hr. In a replicate experiment, animals receiving the medium-nicotine diet showed an increased consumption of the nicotine diet and increased preference for nicotine following a 14-day exposure compared to the control-fed animals and compared to a baseline preference test. Also, this group showed differences in locomotor activity consistent with other studies using an injection regimen or subcutaneuos pumps to induce dependence. Finally, animals in all three groups exhibited high plasma nicotine and cotinine (a major nicotine metabolite) levels. Because animals in all groups tolerated the diet well, gained weight, selected the nicotine diet in a choice test, and showed withdrawal symptoms, we conclude that the liquid diet proved to be a satisfactory method of inducing nicotine dependence in rats.** [P.S.E.B.M. 1999, Vol 221]

Most animal models of physical dependence on nicotine involve intravenous, subcutaneous, or intraperitoneal injections (1–4). Although these methods are useful for determining the chronic effects of nicotine, they may not provide the steady-state nicotine intake necessary to investigate nicotine addiction observed in heavy smokers. An alternative method, the volitional oral intake in animals, has been successful only in certain animal strains (5, 6) and under schedule-controlled situations of polydipsia (7). Murrin *et al.* (8) found that rats tolerated nicotine in their drinking water very poorly. Collins *et al.* (9) progressively increased the levels of alcohol and nicotine in water solutions and was able to produce high amounts of nicotine intake; however, the process took almost 6 months. Furthermore, other studies concluded that free access to solutions of nicotine did not induce preference for nicotine over water (10). Although significant plasma nicotine levels were produced (1), the amount of nicotine ingested by the animals was still minimal. The most effective method heretofore of maintaining high plasma levels of nicotine has been with the osmotic pump (8, 11, 12). Previous studies demonstrated the ability of the osmotic pump to produce withdrawal symptoms when the pump was removed (12) as well as a dose-dependent increase in nicotine

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and cotinine levels in plasma after 2 weeks of nicotine administration (8, 11). Animals showed full recovery of water and food intake 4–5 days after implantation. However, this method of administration cannot been used to evaluate voluntary drug intake.

The experiments presented in this report were designed to determine if physical dependence on nicotine could be established by incorporating nicotine into a nutritionally balanced liquid diet. Nicotine dependence was examined using a two-bottle choice paradigm where the nicotine-containing diet and a control nicotine-free diet were simultaneously presented to the animals. Because nicotine preference in smokers is reportedly increased after an overnight abstinence (13), a 16-hr withdrawal period preceded the choice tests. The aims of the current study were to determine: 1) if rats would ingest and tolerate high levels of nicotine when presented in a liquid diet; 2) if these levels of ingested nicotine would affect body weight or produce signs of physical dependence; and 3) if administration of nicotine in a liquid diet would provide sufficiently elevated plasma nicotine and cotinine levels.

Materials and Methods

Experiment 1. *Animals.* Thirty-two male Long-Evans rats (Charles River, MA) weighing 121–138 g were individually housed in suspended metal cages in a colony room under temperature and humidity control in accordance with the NIH principles of laboratory care. The colony room was maintained on a 12:12-hr light:dark cycle of 0700–1900 light. Rats were divided into four groups. Twenty-four rats were administered a complete and balanced liquid diet (prepared to our specifications by Research Diets, Inc., New Brunswick, NJ) used in previous studies (14). For each kg of diet, 20, 60, or 200 mg of nicotine tartrate salt (Sigma Chemical Co., St. Louis, MO) were added. The remaining eight animals received a control liquid diet devoid of nicotine. Animals were administered nicotine in their diet from Day 1 upon arrival at the facility. Bottles were placed outside the middle of the cage. Diet consumption was recorded by weight every day. Body weight measurements were taken every 3 days, and nicotine intake was calculated as the free base.

Two-bottle choice procedure. At 7 AM on Day 15, bottles were removed from all animals for a period of 16 hr. At 11 PM, 4 hr into the animal's dark or active period, rats were presented a two-bottle choice with one bottle containing their normal nicotine-containing diet and the other bottle containing the same diet without nicotine. The position of the bottles alternated between individual animals in each group so that half were presented the nicotine diet on the left, and half on the right. Animals normally fed the control diet were divided into two groups; four animals were fed the control diet only, the other four were given a choice of the control diet or a diet containing 60 mg of nicotine salt per kg of diet (medium-nicotine-containing diet). After 2, 4, 12, and 24 hr, diet consumption was measured by volume then converted to grams of diet.

Locomotor activity. Following the two-bottle choice procedure, animals were returned to their prechoice diet condition until baseline consumption was resumed, a period of 7 days. On Day 22, the first two animals in each group were put through a second 16-hr withdrawal. Horizontal and vertical motor activity were then measured using an Opto-Varimex-Minor instrument with Vertical Infrared Sensors (Columbus Instruments, Columbus, OH). Animals were placed in a Plexiglass cage inside the activity chamber measuring 42.5 × 40 cm for 10 min plus a 1-min habituation period. Twelve horizontal infrared beams were placed 2.5 cm apart ≈ 1.25 cm from the bottom of the chamber outside the Plexiglass cage. Total horizontal activity was recorded (any intersection of beams on the X or Y-axis were counted). In addition, two vertical monitors consisting of 12 beams, spaced ≈ 33 mm apart were placed 12.5 cm from the bottom of the chamber. On Day 23, three more animals from each group were withdrawn from nicotine and tested, and the procedure was repeated on Day 24 until all 32 animals had been tested for locomotor activity following a 16-hr withdrawal period. Measurements were taken from separate groups of animals on 3 consecutive days so that the activity counts were made at approximately the same time each day for all animals. After the measurement of locomotor activity, animals were placed back on their respective diets.

Determination of plasma nicotine and cotinine levels by gas chromatography. One week following the assessment of locomotor activity, animals were sacrificed, and blood was taken from the trunk of the animal. For the analysis of nicotine and cotinine, a 0.5-ml aliquot of plasma was diluted to 4.0 ml with a saturated solution of potassium carbonate, followed by gentle mixing for 5 min. A 0.5-ml aliquot of methylene chloride was then added, and the mixing was continued for an additional 10 min. Following centrifugation of the mixture (5000*g* for 10 min) to separate the organic and aqueous phases, a 2-ml aliquot of the organic phase was sampled with a gas-tight syringe (Hamilton 1750) and injected (in the splitless mode) into a Hewlett-Packard 5890 gas chromatograph equipped with a computer-controlled Hewlett-Packard 5971 mass-selective detector. The compounds were separated on a DB5, fused silica capillary column (J and W Scientific, Folsom, CA) coated with methyl-phenyl(5%)-silicone(30 m × 0.25 mm, film thickness 0.5 mm) with helium as carrier gas at a velocity of 0.8 ml/min. The oven temperature during injection was held at 80°C for 2 min and then increased at a rate of 50°C/min to 250°C. Injector and detector temperatures were 250°C and 280°C, respectively. Detection was in the multiple ion mode, with nicotine and cotinine monitored at the selected masses of 84 and 98, respectively. Under these conditions, nicotine and cotinine eluted at 5.76 and 7.55 min, respectively. Quantitation of nicotine and cotinine was based on calibration curves obtained by analysis of 0.5-ml

aliquots of plasma from untreated rats spiked with varying known amounts of nicotine and cotinine.

Statistical analysis. Nicotine diet, base consumption, and control diet consumption were all normalized to reflect intake on a per 2-hr time period basis, so that repeated measures ANOVA could be used to detect differences in consumption. A factorial ANOVA detected differences for the 24-hr period during the preference test. For analysis of locomotor activity and plasma nicotine and cotinine levels, a factorial ANOVA was used, and for body weight, diet, and nicotine consumption over the 14-day period for Experiment 1 and Days 11–30 for Experiment 2, a repeated measures ANOVA was used, except to detect differences for the full 24-hr period during the preference test, when an unpaired *t* test was used. All significant *F*-values were reanalyzed using Fisher's PLSD *post hoc* test for all diet conditions.

Results

Diet Consumption. Diet consumption on a g/kg basis for the first 14 days was analyzed using a repeated measures ANOVA with level of nicotine and days of consumption as independent variables and consumption as the dependent variable. There was a significant main effect of level of dietary nicotine (F (3, 325) = 15.15, $P < 0.0001$) as well as a significant effect of time (days) (F (13, 325) = 30.28, $P < 0.0001$) and an interaction (F (39, 325) = 3.07, $P < 0.001$). Fisher's PLSD *post hoc* test revealed significantly lower food consumption for the high-nicotine diet group compared to the control diet over all 14 days. There were no differences in consumption of the control and low-nicotine-diets, except on Day 4, and by the last 2 days prior to the preference test, all groups consumed equal amounts. By Day 14, all rats were consuming the same amount of diet (261.8 ± 5.9 g/kg).

Nicotine Consumption. Nicotine consumption, expressed as mg of nicotine base consumed/kg/day, was also analyzed using a similar repeated measures ANOVA with level of nicotine base/kg body weight and time (days) of consumption serving as variable factors. There was a significant main effect of diet (F (3, 325) = 558.3, $P < 0.0001$), and of time on diet (F (13, 325) = 11.1, $P < 0.0001$). Furthermore, there was a significant interaction between nicotine intake and time on diet (F (39, 325) = 8.8, $P < 0.0001$). Fisher's PLSD test revealed significantly higher nicotine intake by rats on the high-nicotine diet compared to rats on the other nicotine levels, and furthermore, significant differences were detected between the low- and medium-nicotine diets (Fig. 1). Therefore, while the low and medium-nicotine diets did not significantly alter total food consumption, they did produce increases in nicotine consumption.

Body Weight. Further differences were seen in the analysis of body weight gain over these 14 days. A repeated measures ANOVA revealed an overall effect of nicotine level in the diet (F (3, 112) = 27.5, $P < 0.0001$) and days

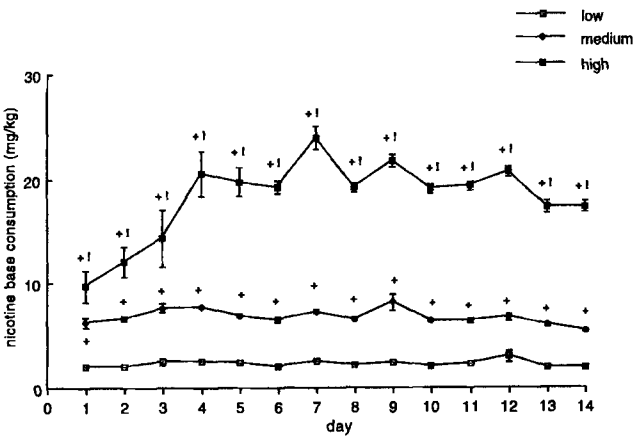

Figure 1. Nicotine (base) consumption for days 1–14 for animals exposed to nicotine during Experiment 1. Data are expressed as mg/kg nicotine (base) consumption. Error bars reflect SEM; + indicates significantly different from low-nicotine diet treatment; ! indicates significantly different from medium-nicotine diet treatment, using Fisher's PLSD, $P < 0.05$.

(F (4, 112) = 1653.436, $P < 0.0001$), with significant increases in body weight detected over the first 14 days. There was also a significant interaction between these two factors (F (12, 112) = 21.2, $P < 0.0001$), indicating that body weight differences were dependent on both level of dietary nicotine and time on diet. Fisher's PLSD *post hoc* analysis revealed significant differences in consumption by rats on the medium- and high-nicotine diets compared to the controls, and to each other between Days 4 and 14. Although the medium-nicotine-containing diet did not affect total food consumption, it did significantly reduce body weight after 4 days compared to animals fed the nicotine-free diet. We have already shown that this control diet produced similar 8-day body weight gains as a standard Chow diet in animals 120–130 g (unpublished observations).

Two-Bottle Choice. During the two-bottle choice procedure, measurements were made of nicotine-diet- and nicotine-free control diet consumption at 2, 4, 12, and 24 hr after presentation of the choice. Consumption of each diet per kg body weight normalized on a per 2 hr basis and the nicotine diet consumption as a fraction of nicotine-containing plus nicotine-free diet were recorded and analyzed for each time period using a repeated measures ANOVA; the full 24-hr period was analyzed using a factorial ANOVA. There were no significant differences between groups in consumption of the nicotine diet; however, there was a significant effect of time (F (3, 69) = 16.76, $P < 0.0001$), where consumption of this diet decreased significantly throughout the choice period in all groups. A 24-hr analysis revealed that the low-nicotine diet group consumed more of the nicotine diet than the control-fed animals, and those exposed to the high-nicotine diet consumed less of the nicotine diet than the low or medium-nicotine diet groups (F (3, 26) = 3.25, $P < 0.04$). On the other hand, analysis of consumption of the control diet during the choice period showed an effect of diet condition (F (3, 69) = 3.16, $P < 0.04$) and time (F (3, 69) = 13.7, $P < 0.0001$). Animals in

the high-nicotine diet condition ate significantly more control diet during the first 2 hr (11 PM–1 AM) than any other group. Animals in the low-nicotine group consumed less control diet than those animals on the control diet. A 24-hr analysis reiterates these differences (F (3, 26) = 4.3, $P <$ 0.01). Finally, with respect to absolute amounts of nicotine intake expressed in mg base/kg body weight, there was a significant effect of diet condition (F (3, 69) = 10.7, $P <$ 0.0001), time (F (3, 69) = 14.8, $P < 0.0001$) and a significant interaction (F (9, 69) = 6.1, $P < 0.0001$. The high-nicotine-diet group consumed more nicotine base during the first 4 hr of the two-bottle choice test than the control-fed or the low-nicotine-diet group (Fig. 2).

The differences in control and nicotine diet consumption can also be expressed as a preference ratio, which is shown in Figure 3. Analysis revealed a significant interaction of diet condition and time (F (3, 23) = 2.2, $P < 0.04$). Animals in the low-nicotine-diet condition consistently showed a significant increase in nicotine preference compared to controls over all time periods, and those in the medium-nicotine-diet group showed a higher preference ratio during the last two periods. Animals in the high-nicotine-diet group, however, showed a preference ratio that was similar to that on the control diet, and significantly lower than that on the other two diet conditions. This effect was also seen in the full 24-hr analysis (F (3, 23) = 3.7, $P <$ 0.02). The failure of the high-nicotine-diet animals to exhibit a preference for the nicotine diet may be explained by the intake of nicotine base on a per kg basis. There was a significant effect of diet (F (3, 39) = 10.7, $P < 0.0001$), time (F (3, 69) = 14.8, $P < 0.0001$), and an interaction (F (9, 69) = 6, $P < 0.0001$) with those in the high-nicotine-diet group consuming more nicotine than the other groups during the first two periods (Fig. 2). One control animal was excluded from the analysis after failing Dixon's test for outliers for all time periods.

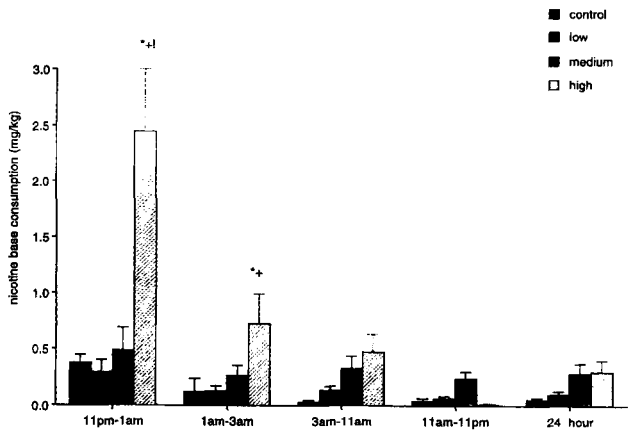

Figure 2. Nicotine (base) consumption expressed as mg/kg body weight during two-bottle choice in Experiment 1. Consumption measurements were analyzed on a per 2-hr time period. Error bars reflect SEM; *indicates significantly different from nicotine (base) consumption of animals placed on the control diet; + indicates significantly different from nicotine (base) consumption of animals placed on the low-nicotine diet; ! indicates significantly different from nicotine (base) consumption of animals placed on the medium-nicotine diet, using Fisher's PLSD, $P < 0.05$.

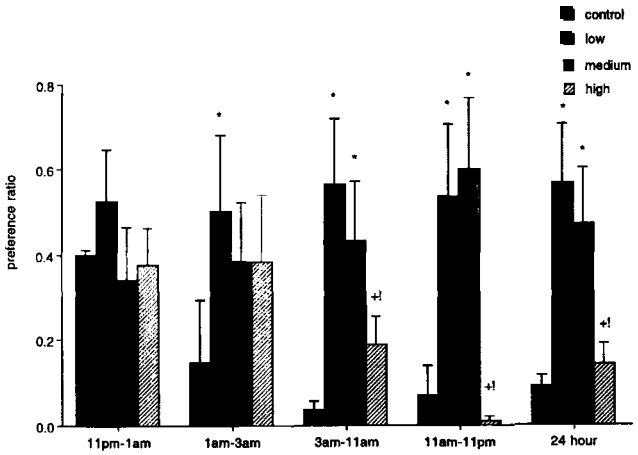

Figure 3. Nicotine preference ratio during the two-bottle choice in Experiment 1. Ratio was computed using the formula:

$$\frac{\textit{nicotine diet consumption}}{\text{total consumption (nicotine diet + control diet)}}$$

A ratio of 0 reflects preference for control diet; 1 reflects preference for nicotine diet; * indicates significantly different from control animals preference; + indicates significantly different from preference of animals on the low-nicotine diet; ! indicates significantly different from preference of animals on the medium-nicotine diet, using Fisher's PLSD, $P < 0.05$.

Locomotor Activity. Analysis of locomotor activity counts using a factorial ANOVA revealed no significant difference in vertical or horizontal motor activity 16 hr after diet removal.

Plasma Nicotine and Cotinine Levels. All three groups of animals on the nicotine diets showed high levels of plasma nicotine (low nicotine diet = 26 ± 3.4, medium nicotine diet = 42.4 ± 3.3, and high nicotine diet = 34 ± 1.1 ng/ml) and cotinine (low nicotine diet = 65.5 ± 8.4, medium nicotine = ± 16.9, and high nicotine diet = 201.9 ± 31.4 ng/ml). The rats on the medium-nicotine diet showed significantly higher levels of both compounds than those on the low-nicotine diet, and those on the high-nicotine diet showed higher levels of cotinine than those on the low-nicotine diet.

Materials and Methods

Experiment 2. Experiment 2 was conducted to examine the preference for the nicotine diet over the control diet more explicitly during the choice period and to characterize the effect of nicotine withdrawal more fully on locomotor activity.

Animals. Twenty-four Long-Evans male rats weighing between 120 and 130 g were fed the control diet for a period of 9 days, at which time they were all subjected to a 16-hr withdrawal period from the diet and given a choice of either the control diet or a medium-nicotine-containing diet (containing 60 mg of nicotine salt/kg of diet). Consumption measurements were made at 2, 4, 12, and 24 hr after diet

presentation, and animals were split into two groups; 12 were fed the control diet, and 12 were fed the medium-nicotine diet for a period of 14 days. The medium-nicotine diet was chosen since, in Experiment 1, plasma nicotine levels peaked in this group, and at the same time consumption patterns were the same as for the controls. Groups were balanced according to their nicotine diet intake during the first preference test. On Day 14, bottles were removed for a second time, and animals were given a second preference test. The position of the nicotine and control diet bottles on the second preference test was alternated from the first. Following the second preference test, animals were returned to their respective diets for 1 week, at which time the bottles were again removed at 7 AM, and locomotor activity measurements were made for a period of 10 min 8, 16, and 24 hr after withdrawal. It should be noted that the 8- and 24-hr times fell within the animal's light cycle, and the 16-hr point was taken during the dark period.

Results

Nicotine Diet Consumption and Body Weight Gain. There was a significant effect of diet consumption (F (1, 378) = 18.4, $P < 0.0003$), day (F (18, 378) = 3.9, $P < 0.0001$), and an interaction (F (18, 378) = 3.9, $P < 0.0001$) on consumption of the nicotine diet in nicotine-fed animals and the control diet in control-fed animals during the days following the first preference test. However, there were only significant differences in consumption during Days 11, 12, 15, 16, and 25. Body weight differences were consistently noted after Day 14 (three days after the first preference test). There was a significant effect of diet (F (1, 154) = 7.4, $P < 0.01$), day (F (7, 154) = 504.2, $P < 0.0001$), and an interaction (F (7, 154) = 7.1, $P < 0.0001$). Both groups showed significant weight gain compared to Day 11.

Two-Bottle Choice. During the first preference test, prior to any nicotine exposure, no significant differences in consumption of the control diet or the nicotine diet were noted between groups. However, there was a significant effect of time period (F (3, 63) = 9.25, $P < 0.0001$) on consumption of the nicotine diet; less of the nicotine diet was consumed by both groups during the last three time periods (on a per 2-hr basis) compared to the first 2-hr period. There were no differences between groups in preference ratio except for a significant effect of time (F (3, 63) = 7.1, $P < 0.0004$), where again, preference ratios were significantly higher for both groups during the first time period compared to later periods (Fig. 4, top).

The second preference test was conducted after one group had been exposed to the nicotine-containing diet for 14 days. When preference ratio was computed, there was a significant effect of diet (F (1, 60) = 5.5, $P < 0.02$). The nicotine diet group displayed a significantly higher preference ratio during the first three time periods (the first 12 hr after presentation) as compared to the control diet group (Fig. 4, bottom). Using a repeated measures ANOVA to

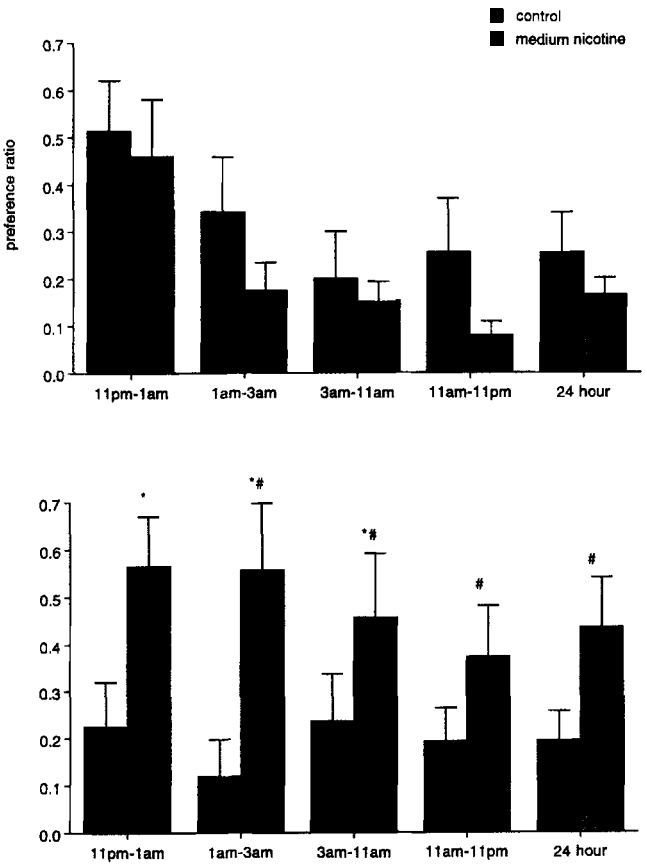

Figure 4. Nicotine preference ratio during the two-bottle choice for Experiment 2. Top: Preference ratio obtained after a 10-day exposure in both groups to the control diet, before animals were split into groups and received the nicotine-containing diet. Bottom: Preference ratio obtained after a 14-day exposure period by the medium-nicotine group to the nicotine-containing diet. Error bars indicate SEM. * indicates significantly different from control animals preference, using Fisher's PLSD, $P < 0.05$. # indicates significantly different from the first preference test.

detect the differences between preference test #1 and preference test #2, it was found that consumption of the nicotine diet in nicotine-fed animals significantly increased during the last three time periods (F (1, 66) = 5.6, $P < 0.02$) as well as for the full 24-hr analysis (t = –2.3, $P < 0.03$). Control animals did not significantly alter their preference ratios between preference test 1 and 2. Additionally, there was a significant effect of diet (F (1, 60) = 5.8, $P < 0.02$), time (F (3, 60) = 5.9, $P < 0.001$) and an interaction (F (3, 60) = 2.9, $P < 0.04$) on consumption of the nicotine-containing diet. Animals exposed to the nicotine diet for 14 days consumed significantly more of that diet during the first two time periods than those exposed to the control diet (Fig. 5, top). Furthermore, analysis of the full 24-hr time period revealed a significant effect of group (t = 2, $P < 0.05$). There was also a significant effect of time on control diet consumption (F (3, 63) = 12.2, $P < 0.0001$) and an interaction (F (3, 63) = 4.8, $P < 0.0001$) where those fed the control diet consumed significantly more of that diet during the second time period (2–4 hr after presentation). Analysis of the 24-hr consumption levels also did not reveal

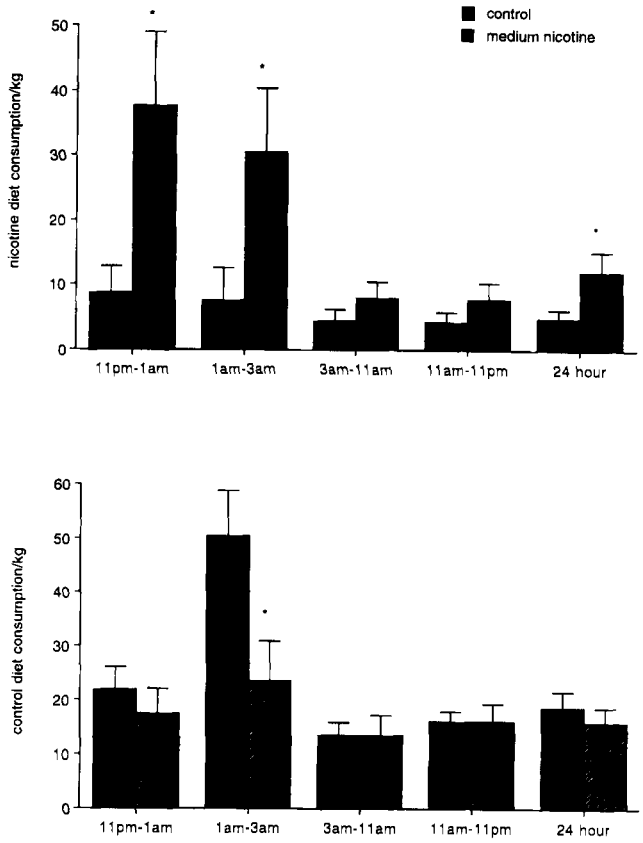

Figure 5. Diet consumption in g/kg during preference test #2 of Experiment 2. Top: Nicotine diet consumption. Bottom: Control diet consumption. Animals were given a choice of their normal control diet or the diet containing the medium level of nicotine. Changes along the *x*-axis are over time, from 11 PM, when diets were first presented, throughout the night. The 11 AM–11 PM displays nicotine consumption for the next 12 hr, and the 24-hr time period is a cumulation of all intervals. Consumption was measured on a per 2-hr time period. Error bars reflect SEM. * Indicates significantly different from nicotine diet consumption of animals placed on the control diet, using Fisher's PLSD, $P < 0.05$.

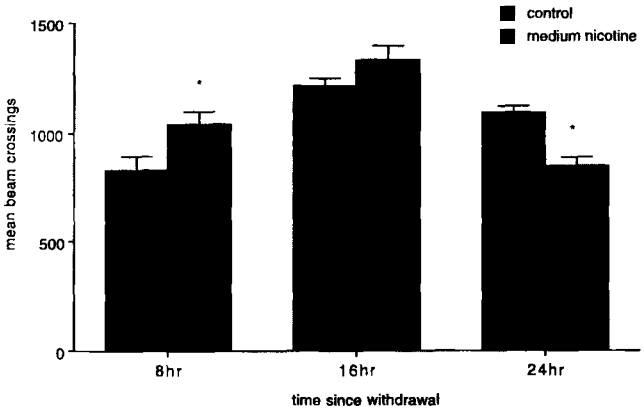

Figure 6. Horizontal motor activity during 10- min period after 8 hr, 16 hr, and 24 hr of withdrawal from diets. The 16-hr time point was taken during the animal's active dark cycle. Error bars reflect SEM. * Indicates significantly different from controls, using Fisher's PLSD, $P < 0.05$.

any differences between groups in consumption of the control diet (Fig. 5, bottom).

Locomotor Activity. There was a significant effect of time (F (2, 44) = 23.8, $P < 0.0001$) as well as an interaction of diet condition and time (F (2, 44) = 9.8, $P < 0.0003$) on locomotor activity following withdrawal. Animals fed the nicotine-containing diet displayed increased horizontal motor activity counts 8 hr after withdrawal and less activity 24 hr after withdrawal. However, 16 hr after withdrawal, during the animals' dark cycle, no differences between groups were observed (Fig. 6).

Discussion

Liquid Diet Consumption. The results of the present study indicate that a liquid diet supplying low, medium and high levels of nicotine is well tolerated by rats and, in fact, animals not only maintained but significantly increased body weight over the course of the experiment. Growth on the liquid diet supplying low and medium levels of nicotine showed similar 8-day weight gains as animals fed a traditional Chow diet. Although animals in the low- and medium-nicotine groups consumed virtually the same amount of diet, their nicotine consumption, expressed in mg/kg, was significantly different from controls and from each other. Although the high-nicotine group consumed less diet than the other groups, nevertheless this group consumed enough food to gain weight. Rats in the medium-nicotine-diet group consumed the same amount of food as those in the control and low-nicotine-diet groups yet exhibited a significant decrease in body weight. Since they consumed the same amount of diet but did not gain as much weight over the exposure period, we would conclude that oral nicotine was exerting a pharmacological effect. This observed effect would be analogous to a proposed nicotine-mediated increase in metabolic rate among smokers (15).

It is interesting to note the inverse relationship between consumption of nicotine and consumption of diet for the high-nicotine-containing diet group. These animals reduced diet consumption as nicotine intake increased. Along these lines, smokers "downwardly titrate" nicotine in the number of cigarettes smoked when nicotine levels in the cigarettes are increased, or similarly when nicotine administration is supplemented with capsules or nicotine skin patches (16, 17). This was not true in the medium-nicotine-diet group. These animals did not differ in their consumption of diet compared to the low-nicotine group although they did consume significantly more nicotine. The intake of nicotine in animals follows other dose-response curves of schedule-controlled self-administration paradigms. Lower doses support self-administration and higher doses decrease response and infusion rate (4, 18). Rats may either reach an intake "plateau," or nicotine at high doses may produce adverse effects (18). In any case, oral nicotine in the present study yielded a dose-response relationship similar to that observed using other paradigms and drugs.

In a previous study using oral administration, nicotine was added to a 10% sucrose solution (10). At this concentration, animals consuming the nicotine plus sucrose solu-

tion consumed the same amount as those receiving only the 10% sucrose. These animals were not subjected to withdrawal, and the amounts of nicotine ingested (1.2 mg/kg/day) were lower than the medium-and high-nicotine diet groups in the present study. Other nonoral self-administration paradigms have yielded nicotine intakes of ≈ 0.18–1.39 mg/kg per day (4, 19). In the present study all animals consumed the liquid diets regardless of level of nicotine. Therefore, this method may provide successful oral administration of nicotine at considerably higher levels than previously administered.

Nicotine Diet Preference. The two-bottle choice procedure was used to evaluate preference for nicotine over the control diet following its oral administration over a period of 14 days. In all groups, animals consumed some amount of each of the diets. Animals fed the low and medium levels of nicotine consumed more of the nicotine diet than the controls or those on the high-nicotine diet, although this difference did not reach statistical significance. Additionally, there were significant effects on consumption of the control diet, as rats on the high-nicotine diet displayed hyperphagia, a previously reported symptom of nicotine withdrawal (20). In a self-administration paradigm, Donny *et al.* (2, 19) demonstrated that increasing nicotine dose through intravenous infusion on a fixed-ratio schedule of reinforcement resulted in a decrease in response and infusion rates, with an increase in total nicotine intake. Likewise, animals exposed to the high-nicotine diet in Experiment 1 consumed higher levels of nicotine despite lesser diet consumption. The nicotine preference ratio for the consumption of diet by the high-nicotine group was lower than that of the other two nicotine groups for the later time periods, perhaps because this group consumed so much of the control diet. This may indicate that, because of the high concentration of nicotine in their diet, these animals were able to consume enough nicotine to alleviate withdrawal, whereas animals in the other groups were forced to consume more diet to ingest the necessary level of nicotine. In fact, for the full 24-hr time period, animals on the low-nicotine-containing diet ate more of the low-nicotine diet than controls, but comparable amounts of nicotine to the control animals who consumed small amounts of the medium-nicotine-containing diet. Animals on the low- and medium-nicotine diets may have reached food satiety levels before reaching nicotine satiety. Along these lines, other studies have questioned the efficacy of nicotine as a positive reinforcer (3), especially in situations of withdrawal (21) and have attributed its ability to produce addiction through its alleviation of withdrawal symptoms.

In Experiment 2, animals did not prefer the nicotine diet during the first choice test. Although all rats sampled both diets, there was a preference for the control diet over the 24-hr period. This was not the case in the second choice test during which rats that were fed the nicotine-containing diet for 14 days now selected the nicotine diet over the control diet more so than those fed the control diet. Those same rats fed the nicotine-containing diet also selected the nicotine diet over the control diet more so during the second choice test compared to the first choice test. Taken together, these data cannot be interpreted as an adaptation to the taste of the nicotine diet that might have developed over the 14 days. These rats had access to a familiar control diet during the second choice period but now selected the nicotine diet more so than the control-fed rats. The rats fed the control diet selected the nicotine diet less in the second choice test. Thus, even if the nicotine-fed rats had adapted to the bitter taste of the diet over the 14 days, one would predict that they would still have selected the control diet when given the choice. The fact that they consumed more of the nicotine diet than the controls and more during the second choice test as compared to the first, indicates that the 16-hr withdrawal period prompted them to select the nicotine diet.

The preference ratio for the nicotine never exceeded a value of 0.6, indicating that rats sampled both diets during the choice period. This may be consequent to the withdrawal-induced hyperphagia. This preference ratio is similar to the maximum attained by Collins and Marks (5) who evaluated nicotine selection in a choice test across mouse strains. It should be noted, however, that it is not necessary that the preference ratio exceed a value of 0.6 as an indicator of nicotine dependence; rather, it is only necessary for the animals to consume sufficient amounts of nicotine to alleviate withdrawal. In the present study, the finding that the nicotine-fed animals increased their preference ratio during the second choice test is consistent with the hypothesis that they adjusted their nicotine intake to offset the nicotine withdrawal. It was also noted that the within-group variability in preference ratios was quite high; that is, it tended to be very high or zero. It is possible that the animals who displayed a ratio of zero were not eating at all as a consequence of nicotine withdrawal. When the preference ratios were recalculated as a ratio of total intake among groups (total nicotine intake/(total control + total nicotine intake) in each group for each time period, the preference ratios in Experiment 2 reached 0.68 for the medium-nicotine-diet-fed animals during hours 1–3. While this did not change the results of Experiment 1, the animals in the medium-nicotine group also did not show increased consumption of the nicotine diet as they did in Experiment 2.

Locomotor Activity. Previous studies have reported a decrease in spontaneous motor activity during nicotine withdrawal (12, 22, 23), although this depression in activity was dependent on dose and withdrawal time. Helton *et al.* (24), using an osmotic mini-pump, did not report any differences in locomotor activity among nicotine-treated rats during withdrawal following a 12-day administration period. In the present study, no significant differences in motor activity were found 16 hr following nicotine withdrawal, taken either during the light or dark cycle, but differences were found 8 and 24 hr after removal of the diets. These behavioral data help support the hypothesis that these animals were dependent on nicotine after a 14-day exposure.

While the effect of nicotine withdrawal on motor activity was dependent on time, the increase in activity at 8 hr is consistent with anxiety and restlessness reported after smoking cessation (12, 24) as well as an increase in the startle response in rats following nicotine withdrawal (25). The decrease in locomotor activity after 24 hr of withdrawal is in agreement with results reported by others (12, 22). Although the amount of nicotine consumed by the medium-nicotine diet groups was considerably more than in the above studies, their method of administration was also by injection rather than oral administration. The increase in motor activity after 8 hr of withdrawal may be a manifestation of anxiety and other "abstinence signs" such as shakes, tremors, gasps, and writhes (12).

Nicotine/Metabolite Levels in Plasma. Verification of the bioavailability of nicotine administered through the liquid diet comes from plasma samples following over a month on the diet. There were significant elevations in plasma nicotine and cotinine levels in animals on all three diets. Not surprisingly, the animals on the medium- and high-nicotine diets had higher levels of these compounds than those on the low-nicotine diet. The levels of nicotine and cotinine found in plasma were consistent with studies that examined these compounds in light, moderate and heavy smokers (26).

Previous studies have demonstrated that smokers eliminate nicotine and cotinine more quickly than nonsmokers (27, 28). Nicotine is metabolized, in part, by some forms of the liver cytochromic P450 enzyme (29, 30), and chronic nicotine administration may induce an upregulation of hepatic cytochrome P450 enzyme activity, resulting in a faster elimination rate for animals exposed to high levels of nicotine. Previous work has also detected induction of P450 in the rat brain after 10 days of systemic nicotine administration (31). Sastry *et al.* (32), furthermore, determined that while plasma nicotine concentrations increased, reaching a peak at 10 min after intravenous injection, then decreased, cotinine levels remained stable. Because of its long half-life, the levels of cotinine in plasma are relatively stable throughout the day. Along similar lines, other investigators have determined that the half-life of cotinine in urine of smokers is lower than that of nonsmokers exposed to environmental tobacco smoke (28). This would support the concept that cotinine elimination is enhanced by prior exposure to nicotine. This, in turn, may explain why the plasma cotinine levels in animals exposed to the high-nicotine diet was almost identical to that of animals on the medium-nicotine diet.

In conclusion, animals exposed to various levels of nicotine tartrate salt, in a nutritionally balanced diet, tolerated the diet well, and appeared healthy with increasing levels of consumption over 30 days with adequate body weight gain. However, the nicotine intake apparently increased metabolic rate as these rats did not gain as much weight as the controls even though they consumed the same amount of diet. The animals on the medium- and high-nicotine diets also displayed nicotine and cotinine levels consistent with those observed in heavy smokers. Furthermore, nicotine-fed animals showed symptoms of withdrawal including hyperphagia, changes in locomotor activity, and an increase in nicotine intake during a choice test. Taken together, these data suggest that this diet provides a novel and effective method of nicotine administration in rats.

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