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The effects of chronic nicotine on meal patterns, food intake, metabolism and body weight of male rats

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ABSTRACT

It is unclear what contribution food intake and metabolism have in causing weight loss after administering a dose of nicotine equivalent to smoking one to three packs of cigarettes per day because previous studies have been of a very short duration. To address this question, male Sprague Dawley rats were housed in computerized food intake modules and fed 45 mg pellets: Group 1 [nicotine injected with 1.4 mg/kg/day (free base), fed ad libitum]; and Group 2 [saline injected and pair-fed by computer with Group 2]; and Group 3 [saline injected (i.p.), fed ad libitum]. The rats received 4 equally spaced injections over the dark phase. Treatment consisted of: Phase 1 (nicotine or saline for 14 days), Phase 2 (all rats saline for 8 days and Phase 3 (pair-fed group "unyoked" for 6 days)). Nicotine inhibited food intake over the first 6 days. On termination of nicotine, there was no compensatory hyperphagia in either Groups 1 or 2; and their body weight was reduced starting on day 5 until day 28. In another study, rats were housed in an indirect calorimetry system. Saline or nicotine was injected for 14 days, as noted above; then all rats were injected with saline for 4 days and then no injections for 10 days to follow changes in body weight. Energy expenditure ($Kcal/Kg^{0.75}$) was measured for 18 days. Nicotine significantly reduced food intake on 7 of 14 days of nicotine injections. The body weight of the nicotine injected rats was significantly reduced starting on day 3 until day 25. There were no differences in energy expenditures of the groups, which suggested that a decrease in food intake and not an increase in metabolism was the reason the rats lost weight after administering nicotine.

1. Introduction

The dangers posed by obesity include increased health risks and early mortality (Bray, 1996; Klein, 2001). In an effort to control their weight, some people are willing to try risky weight-altering products in order to lose or to maintain their body weight. Smoking is used by young adults to maintain a lower body weight (Gerend et al., 1998) even though smoking carries known health risks and is addictive (Perkins, 2001). Smoking is particularly endemic to women, because many of them choose smoking as an effective means of weight control (Grunberg, 1985; Perkins, 1992a; Filozof et al., 2004). Disturbingly, 75% of all women smokers said they would not quit smoking if they gained more than 5 lb, and nearly 60% of women less than 25 years of age said they would not stop smoking if they gained any body weight (Pomerleau and Kurth, 1996). These attitudes are unfortunate since a decrease in the numbers of individuals utilizing smoking as a weight reduction agent would lead to improved health of these individuals and would lower overall health care spending.

In several previous studies testing male and female rats, we observed that nicotine administration during the dark phase initially decreased food intake by attenuating meal size (Bellinger et al., 2003a, 2005; Kramer et al., 2007a). Several days afterwards, the meal size was still reduced, but the food intake of the animals had returned to normal through an increase in meal frequency. It was thought that the body weight of the nicotine injected animals was initially reduced as a result of the rats' lowered food intake. Interestingly, the body weight of the nicotine injected animals remained significantly lower than that of the saline injected animals even after the food intake had returned to normal (Levin et al., 1987; Arai et al., 2001; Bellinger et al., 2003a; Guan et al., 2004; Bellinger et al., 2005; Kramer et al., 2007a). The body weight was also significantly attenuated for several days after the nicotine injections stopped. This finding suggested that, in addition to its effects on lowering food intake, nicotine might also affect the rats' metabolic rate or alternately lower the hypothesized "body weight set point".

The literature is unsettled on the effects of nicotine on basal metabolism (Perkins, 1992b). Various studies have shown that nicotine either increases, has no effect on, or in a few subjects, decreases basal metabolic rate (Dill et al., 1934; Hiestand et al., 1940; Hadley, 1941; Goddard and Voss, 1941; Evans and Stewart, 1943; Ilebekk et al., 1975;

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Wager-Srdar et al., 1984; Robinson and York, 1986; Stamford et al., 1986; Perkins et al., 1990; Schwid et al., 1992; Collins et al., 1994; Bishop et al., 2004). Other studies suggest that nicotine withdrawal either lowers or has no effect on basal metabolism (Glauser et al., 1970; Burse et al., 1975; Dallosso and James, 1984; Perkins et al., 1990; Moffatt and Owens, 1991; Jensen et al., 1995; Filozof et al., 2004). Thus, how nicotine alters body weight gain is unclear.

The above investigations used a variety of methods to measure metabolic rate and most, but not all (Hofstetter et al., 1986; Schwid et al., 1992; Bishop et al., 2004), of the studies measured the changes in metabolism due to nicotine for less than an hour. A possible reason for the different outcomes of the studies described above is that nicotine stimulates energy expenditure for a short period of time, i.e., minutes, but not for prolonged periods of time or that after an initial stimulation of metabolic rate there is a subsequent fall so that there is no net effect of nicotine on energy expenditure. Therefore, even if nicotine increases metabolic rate this increase could only contribute to weight loss if it lasts for days or weeks and metabolism is not compensated, i.e., decreased, following nicotine exposure. The other variables that could contribute to different outcomes in previous studies include the dose of nicotine used (which in some cases, exceeded normal nicotine intake during smoking); giving nicotine constantly instead of intermittently as it is normally used; whether food was present; and giving nicotine in the light phase to rodents when they are not normally active (Bellinger et al., 2003a; Kramer et al., 2007a).

In the present investigation, the first study used computerized pair-feeding, to test whether the weight loss after dark phase nicotine injections is solely due to food intake reduction, an increase in metabolic rate or a combination of the two. This first study also explored if the reduced body weight caused by nicotine injections could be attributed, in part, to the altered meal patterns caused by nicotine. In the second study, an indirect calorimetry system was used to measure energy expenditure for an 18-day period to determine if nicotine exposure and subsequent removal affected the metabolic rate, and, in this manner, altered body weight gain.

2. Methods

2.1. General methods

Male Sprague Dawley rats (Harlan Industries) were caged individually in a light-controlled (12:12 h light/dark; lights off at 0800 h) and temperature-controlled room (23 °C). The rats were given laboratory chow (Harlan) and tap water (in calibrated bottles) ad libitum. The rats had eight days to acclimate to the new surroundings before being placed in the modules or chambers. In the first study, they were then placed into computerized feeding modules, while in the second study the rats were placed into metabolic chambers. In both experiments the body weight of the rats was measured at the beginning of the dark phase on a top loading balance with a tenth of a gram precision.

2.2. Experiment 1: Analysis of feeding behavior and body weight

This experiment was approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee. Feeding behavior was measured using 32 sound-attenuated feeding modules equipped with photobeam computer-activated pellet feeders (Med Assoc. Inc., East Fairfield, VT) that dispensed precision-made 45 mg grain-based rodent chow pellets (Product No. FO 165, BioServ, Frenchtown, NJ). The cages (ENV-008) are made of polycarbonate with aluminum strips at the edges (12.0 in. long, 8.25 in. wide and 8.25 in. tall) with smooth round rods forming a floor for the animals to stand on. The rods were far enough apart to allow for a pellet to fall into a stainless steel removable pan if the pellet was not eaten. Spillage was not a problem as the rats typically dropped fewer than five pellets per day. The chow pellets consisted by weight of 55.3% carbohydrate, 20% protein, 4% fat, 4% fiber, 4% ash and <5% moisture.

The feeding modules have a photobeam that is blocked by a chow pellet within the feeding trough; removal of the pellet caused the photobeam to signal the computer that a pellet had been removed. The computer noted the time and then dispensed another pellet. The number and the time when the pellets were dispensed were recorded. From this information, meal patterns (i.e., meal size, number, and duration) were calculated on a computer using proprietary software. A meal was defined using a ten-minute end of meal definition (i.e., no pellets consumed for ten minutes denoted the end of a meal), and the minimum meal size was set at 135 mg [i.e., 3 pellets].

During a three-day acclimation period in the feeding modules, all the rats were injected i.p. with 0.9% saline (1 ml/kg), starting at the beginning of the dark phase, using red lights, and every 3 h thereafter for a total of four injections.

Following this series of injections, nicotine was prepared by dissolving nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) in 0.9% NaCl at a concentration of 0.35 mg/ml (free base). The rats were divided into the three groups noted above and injected i.p. with nicotine (total daily dose of 1.4 mg/kg, free base) or with saline in four equal volumes over the dark phase. The dose was based on the highest body weight for each rat during the injection period.

In a previously published study from our laboratory, we carried out a dose response study using saline (0), 0.75 mg/kg/day (nicotine free base) and 1.40 mg/kg/day (nicotine free base). There was a significant dose × day interaction only for the 1.4 mg/kg/day (free base) dose vs. saline. Our choice of the 1.4 mg/kg (free base) nicotine dose was also based on several other considerations. Humans smoking one to three packs of cigarettes per day take in a total daily dose of approximately 0.3–0.5 mg/kg/day of nicotine (free base) through their lungs (Benowitz and Jacob, 1984; Perez-Stable et al., 1998). When rats self-administer nicotine, they inject 0.18–1.38 mg/kg/day of nicotine (free base) through the jugular cannulas (Valentine et al., 1997). It is important to note that 70–75% of nicotine given by the i.p. route, as used in the present study, is removed by the liver during a single pass and thus would not reach the brain (Svensson, 1987). In the present study, therefore, the maximal effective nicotine dose reaching the brain would be ~0.42 mg/kg/day (free base), which is in the range of that used spontaneously by both humans and rats. Doses in excess of 1.40 mg/kg (free base) would in fact exceed the nicotine levels taken in by heavy smokers (Murrin et al., 1987) and have been reported to elicit stereotypic behavior (Li et al., 2000).

During days 1–14, Group 1 ($n=8$) was injected with nicotine and fed ad libitum. During this time Group 2 ($n=8$) was injected with saline and pair-fed by computer with the nicotine injected group. The Group 2 "slave" rats received a pellet only when their one-on-one paired nicotine injected master rat took a pellet. Thus, the saline injected animal in Group 2 was given the same number of pellets at the exact same time as when the pellets were consumed by the nicotine injected animal in Group 1. Because the Group 2 animals are given less food than they would eat ad libitum they are hungry and readily eat the pellets when presented. Group 3 ($n=8$) was also injected with saline, but was fed ad libitum. Body weight was recorded daily for all groups to the nearest gram.

On days 15–22, Group 1 received saline injections and was fed ad libitum; Group 2 received saline injections and each rat was pair-fed with a rat in Group 1; and Group 3 received saline injections and was fed ad libitum.

On days 23–28, all groups were placed in hanging cages, received saline injections and were fed ad libitum, i.e., Group 2 was "unyoked". We needed the feeders for another study so we transferred the rats to hanging cages so we could measure food intake and spillage. We continued to inject after unyoking the animals for a few days to see if the unyoking, without removing the stress of the stick, affected feeding and body weight gain. We also wanted to see if body weight would normalize from day 23–28.

2.3. Experiment 2: Measuring metabolic rate

This experiment was approved by the University of Georgia, Institutional Animal Care and Use Committee. Sixteen naïve male Sprague Dawley rats were housed individually in an indirect calorimetry system described previously in detail (Loh et al., 1998; Wang et al., 1999). The cages are made of Perspex (12.5 in. long, 7.5 in. wide and 7.25 tall) with a raised grid floor that allowed spillage to be determined. Briefly, the system measured O_2 consumption and CO_2 production from each cage once every 25 min for 24 h a day except for 30 min during cage cleaning. The precision of measurement by gas analyzers is 0.002%. The difference in oxygen consumption or carbon dioxide production that can be detected between two groups is 0.005%. Heat can conservatively be calculated to within 0.03 kcal/h. The temperature, light cycle and daily injection schedule were the same as in Experiment 1. The rats were fed rat chow from Purina Mills, Richmond, Indiana. During a two-day acclimation period in the chambers, all the rats were injected i.p. with 0.9% saline (1 ml/kg) starting at the beginning of the dark phase and every 3 h thereafter, for a total of four injections. Next the rats were given nicotine or saline injections (as described in Experiment 1) for 14 days, then saline only for four days; and finally no injections for 11 days. Energy expenditure was recorded for 18 days. Food intakes and body weights were recorded daily for 29 days.

2.4. Data analyses

The data of the two experiments were analyzed by two-way analysis of variance (ANOVA) using the between-group factors of nicotine or saline and the within-group factor of experiment day. The significant effects were further analyzed using Duncan's multiple range test. Differences in P equal to or less than 0.05 (two-tailed tests) were deemed to be statistically significant.

3. Results

3.1. Experiment 1

Food intake (Fig. 1A) was significantly attenuated during the 14 days of nicotine injections compared to the saline control group [group effect, $F(1,14)=7.74$, $P<0.01$]. During the next eight days when all the groups received saline, the food intake of the nicotine injected group returned to that of the saline injected control group [group effect, $F(1,14)=0.01$, n.s.]. Interestingly, the nicotine injected group did not show a rebound hyperphagia during this time period despite their significantly lower body weight (see below). During the next six days, all three groups continued to receive saline injections, but pair-feeding was stopped. The food intake of the three groups showed no observable difference [group effect, $F(2,21)=1.02$, n.s.]; interestingly, the pair-fed group did not show a rebound hyperphagia even though the group's body weights were significantly below those of the saline injected control group (see below).

During the nicotine injection period, the meal size (data not shown) of the nicotine injected animals was attenuated compared to the saline control group, but significance was not reached ($P<0.1$). During the second period and the third period meal size of the groups did not differ significantly (data not shown). Meal frequency of the nicotine-treated group was similar to that of the control group throughout three measurement periods.

At the start of injections the body weights of the three groups did not differ significantly (saline, 217.8 ± 9.5 g; nicotine, 232.9 ± 9.0 g; and pair-fed, 222.9 ± 9.0 g). The body weight gain (Fig. 2A) of the nicotine injected and pair-fed groups was significantly reduced compared to the saline injected group starting on day five of nicotine treatment [group effect, $F(2,21)=13.2$, $P<0.001$]. The body weight gain of the pair-fed group was the same as that of the nicotine injected group. When food intake required for body weight gain over the 14 days of nicotine and saline injections was calculated there was a significant difference among the groups, $F(2,20)=5.25$, $P<0.01$. Upon further analysis both the nicotine injected (6.33 ± 0.41 g food per g body weight gain) and pair-fed (6.94 ± 0.82 g food per g body weight gain) groups required a similar amount of food per weight gain, but both groups were significantly ($P<0.01$) different than the saline injected group (4.65 ± 0.24 g food per g body weight gain). The similar food intake per weight gain values of the nicotine and pair-fed groups demonstrates the body weight loss was due to lower food intake and not a difference in metabolism.

During the second period (days 15–22) when the nicotine injections were stopped, the body weight gain of both the nicotine and pair-fed groups remained significantly less than that of the saline injected control group [group effect, $F(2,21)=11.98$, $P<0.001$]. At the end of this 8-day period, these significant differences in body weight remained, although the food intake of the two groups was similar to that of the saline injected control group.

During the final time period (days 23–28), the pair-fed group was allowed to eat ad libitum. The nicotine injected and the pair-fed groups weight gain still remained similar and significantly less than that of the control group through day 28, group effect, $F(2,21)=8.76$, $P<0.01$.

3.2. Experiment 2

The 24-hour food intake (Fig. 1B) of the nicotine injected group was significantly less when comparing the nicotine injected to the saline injected rats on seven of the 14 days [group effect, $F(1,14)=16.46$, $P<0.001$] and the pattern of food intake was similar to that found in Experiment 1. During days 15–18 when the groups received saline, the food intake of the nicotine injected group returned to that of the saline injected control group [group effect, $F(1,14)=1.34$, n.s.]. As in Experiment 1, the nicotine injected rats did not show a rebound hyperphagia during this time period despite their significantly lower body weight (see below). During days 19–28 when the rats were receiving no injections, the food intake of the groups remained similar [group effect, $F(1,14)=0.24$].

At the start of injections the body weights of the two groups did not differ significantly (saline, 294.4 ± 3.4 g and nicotine, 295.9 ± 4.2 g). The body weight gain (Fig. 2B) of the nicotine injected group was significantly reduced compared to the saline injected group starting on the third day of nicotine treatment [group effect, $F(1,14)=13.61$, $P<0.01$]. The food intake of the two groups was similar from day 14 through 28, yet the nicotine-treated group's body weight gain was significantly less than that of the saline injected group through day 25. Thus, it took 11 days after the termination of nicotine treatment for the body weight of the nicotine-treated group to reach the body weight gain of the saline injected group.

During the nicotine and saline injections, the dark phase RQ (Fig. 3A) of the nicotine injected group was significantly less [$F(1,14)=22.77$, $P<0.001$] than that of the saline injected group on days one through seven of nicotine treatment and on day 10, whereas the light phase RQ (Fig. 3B) was significantly less [$F(1,14)=12.61$, $P<0.01$] in the nicotine-treated rats on days one, two and seven. This measurement corresponded to the period when the food intake of the nicotine group was suppressed. Upon termination of nicotine treatment, the RQ of the nicotine-treated group increased for two days, which corresponded to a slight increase in the food intake of this group after termination of nicotine treatment.

Energy expenditure (Kcal/$Kg^{0.75}$) over 24 h (Fig. 4) did not differ significantly between the nicotine injected group and the saline injected group [$F(1,14)=0.2$, n.s.]. When the energy expenditure during the nicotine and saline injection was broken down by dark phase (Fig. 5A) and light phase (Fig. 5B) the findings were similar to the 24-hour data [$F(1,14)=0.38$, n.s. and $F(1,14)=0.23$, n.s.].

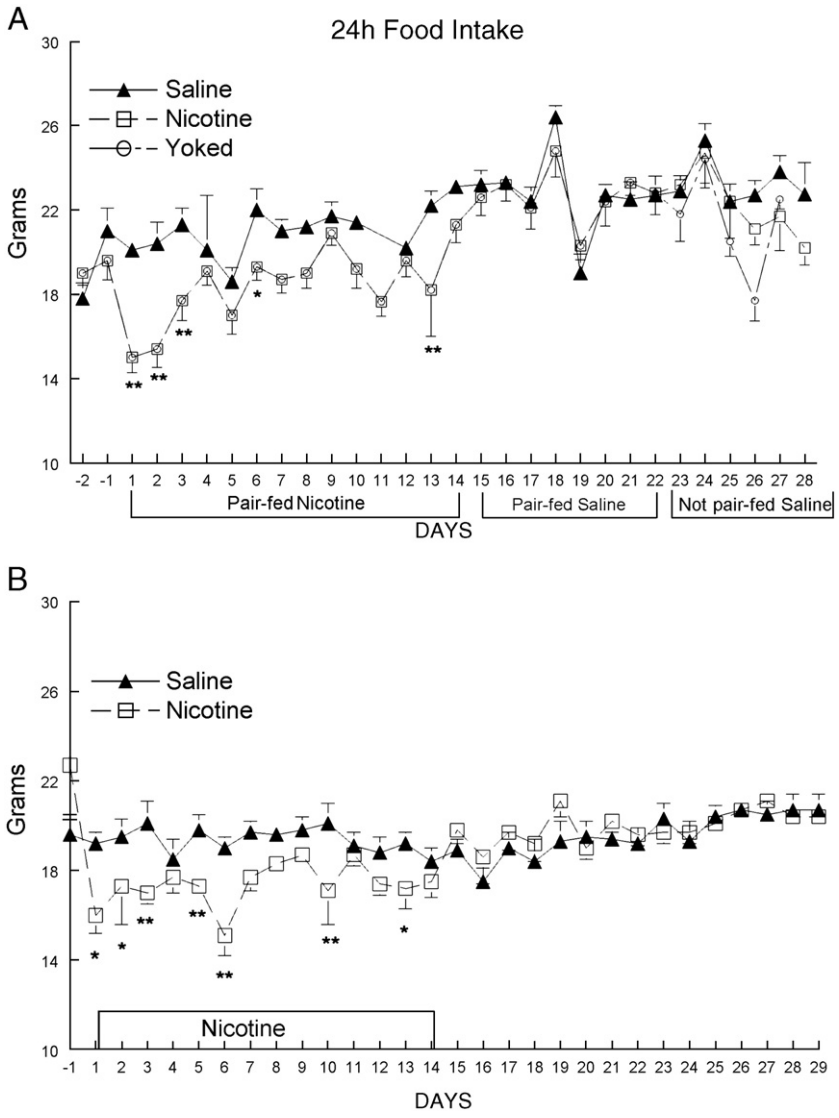

Fig. 1. A: Experiment 1, 24-hour food intake of the rats starting two days prior (−2) to the start of experimentation and continuing for 28 more days. On days 1–14, the rats were injected i.p. four times over the course of the dark phase with 1.40 mg/kg/day of nicotine (free base) and fed ad libitum (Group 1, $n=8$). Group 2 ($n=8$) was injected with saline, and their food intake was pair-fed to Group 1 by computer "yoking". Group 3 ($n=8$) was injected with saline and fed ad libitum. On days 15–22, Group 1 was given saline injections and continued to be fed ad libitum. Group 2 continued to receive saline injections and was still pair-fed to Group 1, and Group 3 continued to receive saline injections and was still fed ad libitum. On days 23–28 all groups were injected with saline and Group 2 was then "unyoked" so all groups were fed ad libitum. Nicotine and saline injected "yoked" group compared to saline control group, $^{*}=P<0.05$, $^{**}=P<0.01$. B: Experiment 2, 24-hour food intake of the rats starting one day prior (−1) to the start of experimentation and continuing for 29 more days. The rats were in metabolic chambers from day −2 to day 18 when they were transferred to hanging cages. On days 1–14, the nicotine injected Group 1 ($n=8$) and the saline injected Group 2 ($n=8$) rats were injected i.p. as described above and fed ad libitum. On days 15–18, both groups were given saline injections. On days 19–29, the rats were placed in hanging cages and not injected. Data are the means ± SEM from the pooled data of eight rats per treatment group. Nicotine group compared to saline control group, $^{*}=P<0.05$, $^{**}=P<0.01$.

Body weight of the two groups began to diverge on day two but during days 2, 3 and 4 there were no differences between the nicotine and saline injected groups in energy expenditure when analyzed in 25-minute increments (Fig. 6), $F(1,14)=0.5$, non-significant. Upon termination of nicotine treatment, 24 h (Fig. 4), dark phase (Fig. 5A) and light phase (Fig. 5B) energy expenditure of the saline and nicotine injected groups was similar.

4. Discussion

In the present study nicotine reduced food intake, consistent with our previously published findings (Bellinger et al., 2003a, b, 2005; Wellman et al., 2005; Kramer et al., 2007a). Nicotine also reduced body weight gain, in Experiment 1 this reduction started on day 5 and continued through day 28 and in Experiment 2 the reduction started on day 3 and continued until day 25. In Experiment 1 the body weight of pair-fed, non-nicotine injected rats showed the same reduction in body weight gain as the nicotine injected rats. If nicotine caused body weight loss by increasing energy expenditure, we would have expected the body weight gain of the nicotine injected group to be less than that of the pair-fed group, but this did not happen consistent with the observation that nicotine reduced body weight solely by decreasing food intake. Also, food intake per weight gain was similar between the nicotine and pair-fed rats suggesting weight loss was not due to an effect on metabolism. In Experiment 2, during the period of food intake suppression, the daily dark and light phase RQ of the nicotine injected rats was significantly reduced. This finding suggests that the nicotine reduced food intake and the animals responded by catabolizing internal energy stores, which most likely contributed to their weight loss (Bizzi et al., 1972). Also nicotine has been reported to increase plasma free fatty acids in rats and this is dependent on the adrenal medullas (Bizzi et al., 1972). Nicotine administered during the

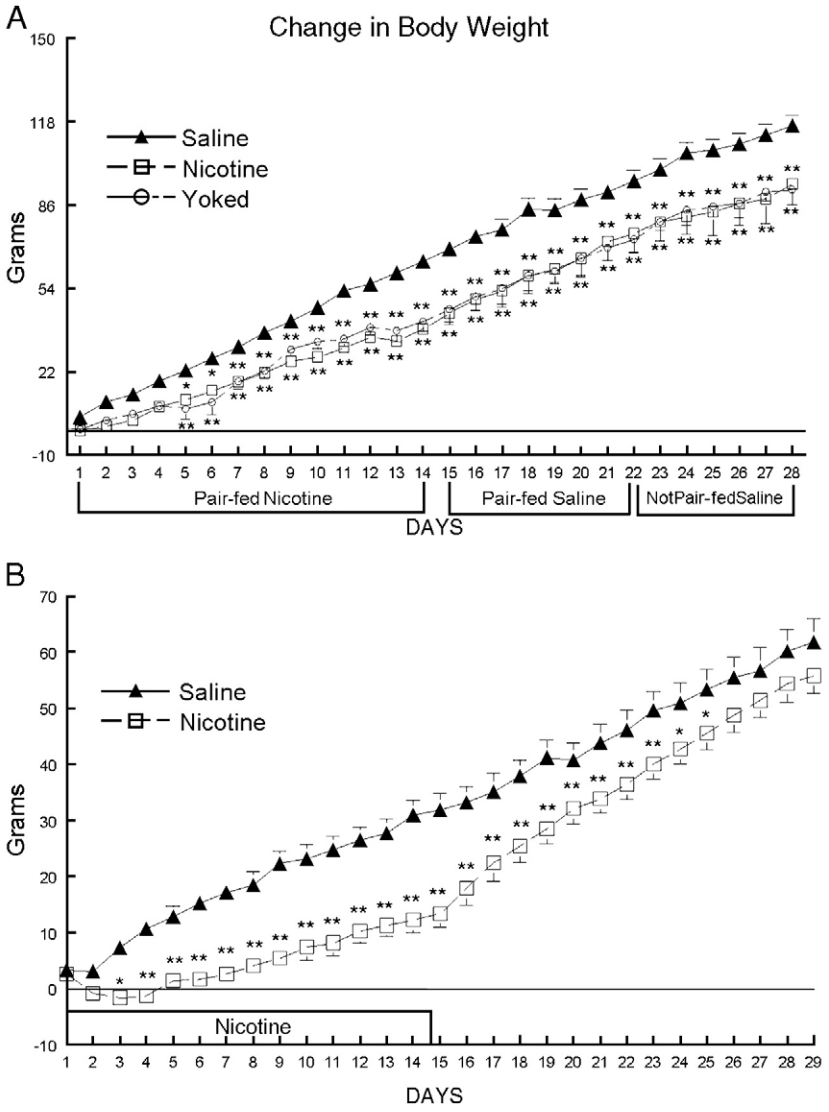

Fig. 2. A: Experiment 1, cumulative change in body weight gain. $^{*}=P<0.05$; $^{**}=P<0.01$. Top asterisk nicotine injected group vs. saline injected control group. Bottom asterisk = saline injected "yoked" group vs. saline injected control group. B: Experiment 2, cumulative change in body weight gain. Nicotine injected group vs. saline injected control group, $^{*}=P<0.05$; $^{**}=P<0.01$. For both experiments data are the means ± SEM from the pooled data of eight rats per treatment group.

dark phase for 14 days did not significantly affect the 24-hour energy expenditure (total of 329 h of measurement) compared to the saline injected animals. Additionally, nicotine administration did not significantly affect the dark phase or light phase energy expenditures compared to the saline injected animals. We also saw no significant effect of nicotine when energy was measured and analyzed in 25-minute increments for the three days when the rats were losing weight. Together, these results strongly suggest that the significant weight loss experienced by the nicotine injected rats can be attributed to the suppression of food intake by nicotine and not by a significant increase in metabolic rate.

4.1. Attenuated body weight gain was persistent after cessation of nicotine

Upon cessation of nicotine the rats did not show an increase in food intake despite their significantly lower than normal body weight. Previous studies from our laboratory have shown either a brief mild hyperphagia following cessation of nicotine (Bellinger et al., 2003a) or no hypophagia (Bellinger et al., 2003b), which was followed by a prolonged attenuation of body weight gain despite normal food intake. This pattern has been reported previously (Levin et al., 1987; Grunberg et al., 1987; Bellinger et al., 2003a), but still is of interest considering the number of reports in the literature suggesting that rats defend their body weight. For example, when normal rats were subjected to severe food restriction for 20 days and then fed ad libitum, the rats showed an immediate and prolonged hyperphagia as they regained lost body weight (Bellinger et al., 1979). If rats are deprived of food for only 24 h and are then re-fed, they also show an immediate hyperphagia that lasts at least two days as they recover lost body weight (Bellinger, 1987). The reason why the nicotine injected rats do not demonstrate an increase in food intake in order to cause a rapid recovery of body weight is unknown, but it is not a unique situation (Bellinger and Mendel, 1995). We previously suggested that the lack of rapid weight regain may be either the prolonged residual effect of nicotine on meal patterns or a nicotine effect on the metabolic rate (Bellinger et al., 2003a, b) but the results of the present study indicate that neither of these possible explanations is correct.

Even more surprising is the observation that the pair-fed rats in the present study also did not demonstrate an immediate and prolonged hyperphagia to regain their lost body weight. Under the ad libitum conditions, they were free to resume any meal patterns they wished to regain weight lost during the "yoked" period. Animals

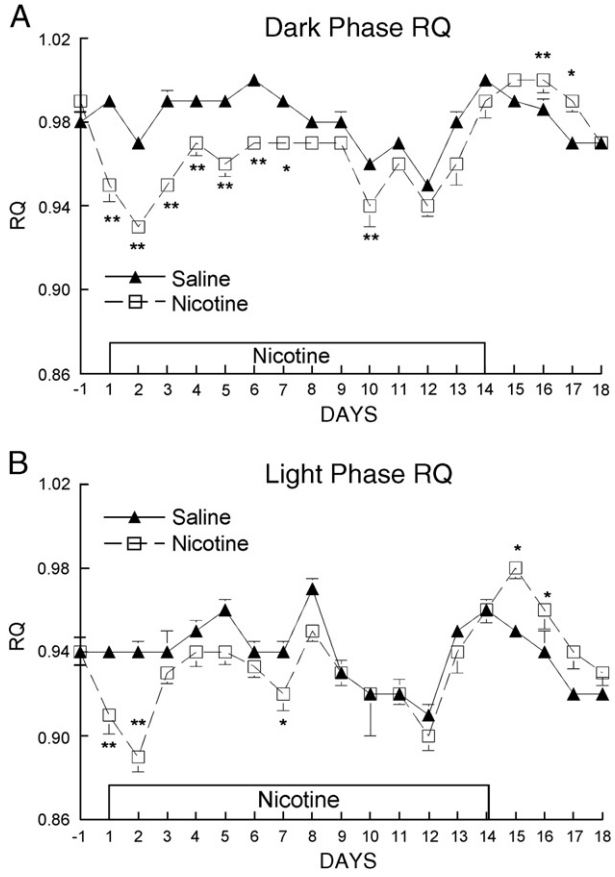

Fig. 3. Experiment 2, dark (A) and light (B) phase respiratory quotients (RQ). Data are the means ± SEM from the pooled data of eight rats per treatment group. Nicotine injected group vs. saline injected control group, * = $P<0.05$; ** = $P<0.01$.

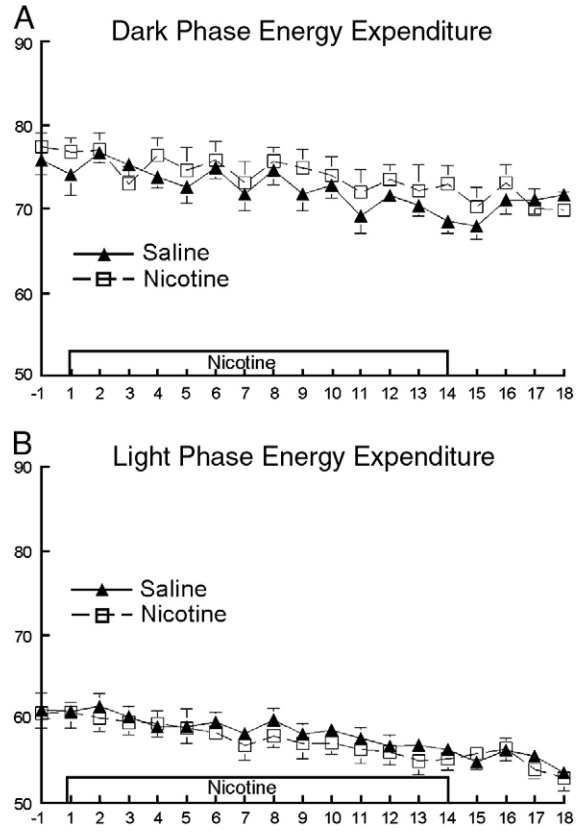

Fig. 5. A. Experiment 2, dark phase energy expenditure (Kcal/$Kg^{0.75}$). Data are the means ± SEM from the pooled data of eight rats per treatment group. B. Experiment 2, light phase energy expenditure (Kcal/$Kg^{0.75}$). Data are the means ± SEM from the pooled data of eight rats per treatment group.

normally defend their body weight (Keesey and Corbett, 1984; Keesey and Powley, 1986; Keesey and Hirvonen, 1997) by altering feeding efficiency, activity, hormones (such as thyroid hormones) or metabolism to attenuate the loss of stored energy or even by gaining weight more efficiently (Levitsky et al., 1976; Boyle et al., 1978; Hill et al., 1984; Bernardis et al., 1988; Munch et al., 1993). Of the above-mentioned physiological changes occurring during food restriction, the one strategy our pair-fed rat could not alter was food availability, as this was being driven by its paired nicotine injected rat. It is possible that if the pair-fed rats assumed the slightly altered meal patterns of the nicotine injected rats, these slight changes somehow prevented them from using the other strategies to gain more weight compared to the nicotine injected animals. It is also possible that nicotine lowered the body weight set point of the rats (Keesey and Corbett, 1984; Keesey and Powley, 1986; Keesey and Hirvonen, 1997; Frankham and Cabanac, 2003). Still this idea does not explain why the pair-fed rats in the present study also showed a prolonged suppression of body weight after returning to ad libitum feeding. The rat handling and injection schedule cannot be responsible for the prolonged weight loss of the pair-fed rats after they resumed ad libitum feeding, because the ad libitum saline control rats were identically manipulated. Under the ad libitum conditions, they should have been able to use the above-mentioned strategies that rats employ to regain weight after deprivation (Levitsky et al., 1976; Boyle et al., 1978; Hill et al., 1984; Bernardis et al., 1988; Munch et al., 1993). It is evident from the data that they did not readily employ these measures.

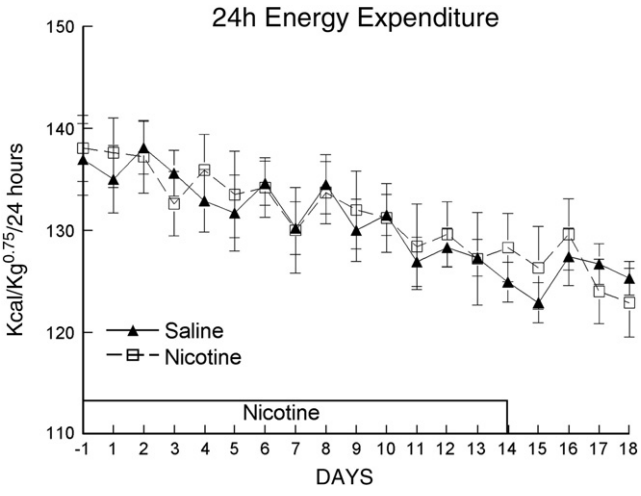

Fig. 4. Experiment 2, 24 h energy expenditure (Kcal/$Kg^{0.75}$). Data are the means ± SEM from the pooled data of eight rats per treatment group.

When nicotine administration was halted the food intake of the nicotine- injected rats increased slightly. While this increase was not significant, there was a significant rise in the 24-hour dark phase and light phase RQ, suggesting that the rats became anabolic for at least two days. Nevertheless, it still took almost two weeks for the significant differences in the body weight of the two groups to be reversed. This slow return of body weight was observed in the first experiment of this study and in previous studies (Levin et al., 1987; Arai et al., 2001; Bellinger et al., 2003a; Guan et al., 2004; Bellinger et al., 2005; Kramer et al., 2007a) that demonstrated the prolonged residual effect of nicotine on body weight compared to normal food intake.

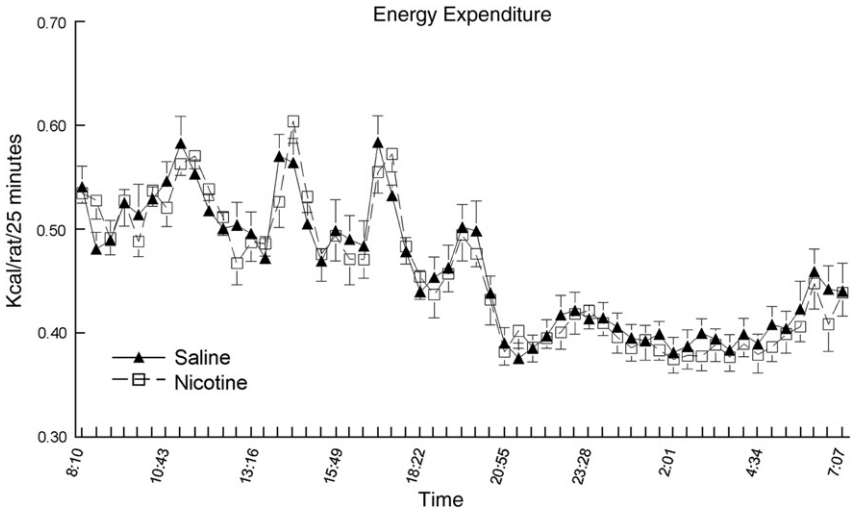

Fig. 6. Experiment 2. Energy expenditure (Kcal/rat) every 25 min and the values at each point included the pooled data from days 2, 3 and 4. Data are the means ± SEM from the pooled data of eight rats per treatment group for the three days.

4.2. How can nicotine effect feeding

In previous studies, nicotine suppression of food intake occurred solely as a result of a reduction of meal size (Bellinger et al., 2003a, b, 2005; Wellman et al., 2005; Kramer et al., 2007a). Food intake and meal size trended smaller as a result of nicotine administration but the change in meal size was not significant ($P<0.1$). However, since meal frequency was not changed when nicotine was administered the significant decreased in food intake after nicotine injection was the result of an attenuation of the meal size. We have previously shown that nicotine significantly attenuates food intake by a reduction in meal size (Bellinger et al., 2003a, b; Guan et al., 2004; Wellman et al., 2005; Kramer et al., 2007a, b), we expect the same result in this study with increased power.

There are a few studies that have focused on mechanisms of how nicotine affects feeding behavior. One series of studies by our laboratory suggested that nicotine may be detected in the fourth ventricle and from there, signals are sent to the perifornical hypothalamus where food intake is attenuated by a reduction in meal size (Guan et al., 2004; Kramer et al., 2007a). Other investigators have suggested that nicotine influences the release of dopamine and serotonin in the ventromedial nucleus and lateral hypothalamic area to affect feeding behavior (Yang et al., 1999; Meguid et al., 2000; Ramos et al., 2004). Lastly, neuropeptide Y has also been proposed as a target of nicotine action (Frankish et al., 1995; Li et al., 2000), whereas our laboratory first reported that nicotine influences agouti-related protein, and this alters meal size and meal frequency (Bellinger et al., 2003b; Fornari et al., 2007).

4.3. Nicotine caused no change in metabolism

Nicotine has been shown to affect the metabolic rate by altering the activity of the sympathetic nervous system through the release of adrenal medulla catecholamines (Ilebekk et al., 1975; Grunberg et al., 1988). If part of the weight loss in rodents is due to nicotine increasing metabolism, it must be demonstrated that any nicotine-induced increase in metabolic rate is of such a magnitude and of such a prolonged nature that it significantly contributes to lowering the body weight. Thus, nicotine could increase metabolism for a few minutes, but if the subsequent metabolic rate returns to below normal for a period of time, then there is no net effect on metabolic rate over time. Upon cessation of nicotine administration, the 24-hour dark phase and light phase energy expenditure was not significantly different from the saline injected controls. Our study measured nicotine effects on metabolism continuously over several weeks and consistent with our study, other short term studies showed that the resting (or basal) energy expenditure was not affected by prior administration of nicotine (Schwid et al., 1992) to food-deprived female rats. Another short term study measured metabolism intermittently on two separate days and the results showed either no effect on resting metabolism or an increase when measures were taken on only a single day (Wager-Srdar et al., 1984). Bishop et al., 2004 measured metabolism intermittently (1-hour recordings) before the rats active phase and gave a very large dose (6 mg/kg/day free base) of nicotine using Alzet pumps. They also found energy expenditure was similar in the nicotine and vehicle groups. Importantly, energy expenditure values from this present study are expected to be more accurate because previous short term studies took intermittent measurements during the rat's inactive phase but this study took measurements every 25 min 24 h a day. In summary, both our long term study and several short term studies found energy expenditure was similar in the nicotine and vehicle groups.

In conclusion, continuous monitoring of meal patterns and metabolism over a 14 day period shows that administration of nicotine to rats induced a decrease in body weight that was not the result of nicotine increasing metabolic rate but by causing a reduction in food intake.

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