Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 80 (2005) 437–444

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Meal patterns in female rats during and after intermittent nicotine administration

Larry L. Bellinger[a,*], Paul J. Wellman[b], Antonio Cepeda-Benito[b], Phillip R. Kramer[a], Guoqiang Guan[a], Connie M. Tillberg[a], Priscilla R. Gillaspie[a], E. Gerald Hill[a]

[a]*Department of Biomedical Sciences, Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246, USA*[1]
[b]*Behavioral Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235, USA*

Received 14 April 2004; received in revised form 11 December 2004; accepted 30 December 2004
Available online 2 February 2005

Abstract

Previously we observed in male rats that intermittent administration of nicotine (NIC) during the dark phase reduces food intake (FI) by initially decreasing only dark phase meal size. This was followed several days later by an increase in dark phase meal frequency such that FI returned to normal, while body weight remained suppressed. Termination of NIC treatment resulted in a modest dark phase hyperphagia. Since some human females use NIC as a weight control drug, the present study investigated changes in FI and body weight regulation in adult female rats treated for five estrous cycles with saline or a 1.40 mg/kg/day (free base) dose of NIC, which was given in four equal i.p. doses during the dark phase. The rats were followed for 15 days after cessation of NIC. Initially both dark and light phase FI were reduced and this was caused by an immediate decrease in dark and light phase meal size; the attenuation of meal size continued after cessation of NIC. On day 7 of NIC, the rats compensated by significantly increasing the number of dark, but not light, phase meals they took. This resulted in a normal 24-h FI, which was caused by a dark phase increase in FI coupled with a continued decrease in light phase FI. Importantly, these changes in meal patterns persisted for some time after termination of NIC. Upon NIC cessation, the NIC group showed no hyperphagia even though their body weight was significantly decreased. These results document that administration of NIC during the dark phase resulted in a reorganization of the microstructure of FI in females rats that resembles, but does not exactly duplicate, that observed in male rats. Like males, long lasting alterations in the microstructure of FI (e.g., meal size and meal number), were noted in female rats for up to 2 weeks after cessation of NIC. These results differ from studies in which NIC was given continuously 24-h per day and indicate that dark phase NIC administration in rats may represent an appropriate model to study the impact of NIC on meal patterns.

Keywords: Meal size; Meal number; Hypophagia; Smoking; Body weight; Water intake

1. Introduction

Smoking is a major health risk in the world and in particular with young adults (Giovino, 2002). Obesity is also a major health concern of epidemic proportions in the United States and especially with the younger members of society (Mokdad et al., 1999). Still many young adults are concerned about their body weight (Pomerleau and Kurth, 1996). Nicotine from smoking promotes body weight loss and cessation of nicotine causes body weight gain in animal models (Grunberg et al., 1986; Levin et al., 1987) and humans (Flegal et al., 1995;). Many young adults and in particular females use smoking as an effective means of body weight control (Delnevo et al., 2003; Stice and Shaw, 2003) and in many instances are more sensitive to psychostimulants such as nicotine (Benowitz and Hatsukami, 1998). Disturbingly, 75% of all women smokers said they would not quit smoking if they gained more than 5 lbs. and of even greater concern 60% of women less than 25 years of age said they would not stop smoking if they gained any weight (Pomerleau and Kurth, 1996). This

* Corresponding author. Tel.: +1 214 828 8322; fax: +1 214 828 8951.
E-mail address: lbellinger@bcd.tamhsc.edu (L.L. Bellinger).
[1] A member of the Texas A&M University System Health Science Center, Dallas, TX 75246, USA.

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.12.010

unwillingness to stop smoking (Perkins, 2001) because of body weight gain is endemic and calls for a better understanding of how nicotine lowers body weight and how its withdrawal promotes body weight gain. This is especially true since currently used body weight management programs are not successful in preventing the body weight gain associated with cessation of smoking (Perkins et al., 1997).

Surprisingly, the literature to date has mainly described that nicotine and its withdrawal do affect food intake and body weight, and only a few studies have tried to discern how smoking and withdrawal from nicotine alter meal patterns. Blaha et al. (1998) reported that in male and female rats, a constant 7-day infusion of nicotine (2.1 mg/kg/day, free base) reduced food intake as a result of a decrease in meal size, while meal number remained unchanged. After cessation of nicotine, meal size quickly returned to normal and there was no rebound hyperphagia. In a second study using female rats, Miyata et al. (2001) reported that a constant 7-day infusion of nicotine (1.74 mg/kg/day free base) reduced food intake. The reduced food intake was mainly caused by a short-term decrease in meal size. However, while meal number remained unchanged on the first 2 days of nicotine infusion, it was reduced on day 3. Body weight was significantly reduced by the third day of nicotine infusion and remained significantly less than the controls 1 day after cessation of nicotine. In this study, nicotine cessation led to a 4-day period of hyperphagia that was caused by an increase in meal size that was accompanied by a decrease in meal number. However, even the aforementioned important studies have design flaws. First, the dose of nicotine used exceeded that obtained by heavy smokers, i.e., >1.41 mg/kg/day free base, (Murrin and Ferrer, 1987; Li et al., 2000). Second, nicotine was administered chronically 24-h a day using pumps, whereas humans use it intermittently during their awake period. The latter is especially important, since a constant supply of nicotine into the rat's system may have affected sleep patterns or may down-regulate nicotine receptors (Pauly et al., 1990). Lastly, nicotine was administered for a relatively short period of time in these studies, i.e., 7 days. These concerns were addressed in two recent studies (Bellinger et al., 2003a,b) where male rats were given nicotine (1.4 mg/kg/day, free base) in five equal doses administered only in the dark phase for 14 days. Nicotine-treated male rats decreased their dark phase food intake on the first day of treatment and this was caused by a significant reduction in meal size (Bellinger et al., 2003a). There was no compensation during the light phase, when nicotine was not administered. On day 5, the rats compensated for the nicotine induced decrease in meal size by increasing their dark phase meal number such that 24-h food intake normalized by day 10. Upon cessation of nicotine treatment, the rats experienced a brief period of dark phase hyperphagia. The rats were monitored for 14 days after stopping nicotine treatment and during most of this period, dark phase meal size continued to be reduced, whereas dark phase meal number continued to be increased. Body weight was significantly reduced by day 6 of treatment and was still attenuated 14 days after cessation of nicotine. These differences in meal patterns are more pronounced and different than that found in the male rats of the Blaha et al. (1998) study. The present study, using the earlier (Bellinger et al., 2003a) more physiological paradigm, determined the effect of nicotine administration on meal patterns and body weight of female rats.

2. Methods

2.1. Animals

Adult female (~225 g) Sprague–Dawley out-bred rats (Harlan Industries, Houston, TX) were housed in individual cages in a temperature-controlled (23 °C) room under a 12/12 light–dark cycle (lights out at 0830 h). This work was reviewed and approved by the Baylor College of Dentistry's Institutional Animal Care and Use Committee. Sprague–Dawley rats were chosen for meal pattern analysis because this strain shows consistent day to day meal patterns (Glendinning and Smith, 1994).

2.2. Drugs

The nicotine solution was prepared by dissolving nicotine hydrogen tartrate ([−]-1-methyl-2-(3-pyridyl)pyrrolidine, Sigma, St. Louis, MO) into 0.9% NaCl. Starting at the beginning of the dark phase (0830 h), the rats were injected intraperitoneally (i.p.) with four equally spaced (every 3 h during the dark phase) doses of nicotine (total daily dose of 1.40 mg/kg, free base) or saline (volume ~0.23–27 ml/injection). The dose for each rat was based on the highest body weight for that rat during the injection period (Bellinger et al., 2003a). Our choice of a 1.40 mg/kg nicotine dose was based on a number of considerations. Humans smoking one to three packs of cigarettes per day take a total daily dose of approximately 0.3–0.5 mg/kg/day of nicotine through their lungs (Benowitz and Jacob, 1984; Perez-Stable et al., 1998). When rats self administer nicotine, 0.18–1.38 mg/kg/day of nicotine is injected through jugular cannulas (Valentine et al., 1997) and a somewhat greater portion of the nicotine taken this way would be available to the brain, because it would reach the brain without passing through the liver. It should be noted that 70–75% of nicotine given by the i.p. route used in the present study would be removed by the liver during a single pass (Svensson, 1987) and thus would not reach the brain. Therefore the effective nicotine dose used in the present study that would reach the brain would be about ~0.42 mg/kg/day and is in the range of that used spontaneously by both humans and rats.

2.3. Apparatus

Each of 16 sound attenuated meal pattern analysis feeding modules is equipped with a photo beam computer activated pellet feeder (Med Assoc. East Fairfield, VT). The rats were presented with precision-made 45 mg rodent chow pellets (Product No. FO 165, Bio-Serv, Frenchtown, NJ). This pellet diet consists of 20.9% protein, 4% fat, and 59.5% carbohydrate (3.58 kcal/g). When a rat removed a pellet from the trough of the feeder, a photo beam positioned at the bottom of the trough was no longer blocked, signaling the computer to drop another pellet, and to record the time of each pellet delivery. Analysis of the electronic record of taking pellets from the trough over time was used to determine meal patterns (Bellinger et al, 1994; Castonguay et al., 1986). Spillage of food pellets was not a problem, as the rats dropped less than five pellets a day (<225 mg/day). Water intake was measured to the nearest milliliter using spill-proof water bottles (Bio-Serv, Frenchtown, NJ).

2.4. Procedure

Upon arrival in the vivarium the rats were handled and adapted to laboratory conditions for 12 days. The rats were then placed into one of the feeding modules (for details consult Bellinger and Mendel, 1995; Bellinger et al., 1997) for at least 3 days of additional adaptation. During the last 2 days of adaptation, the rats were injected i.p. four times a day with 0.25 ml of saline to desensitize the animals to the injection procedures.

Prior to experimentation, the rats also received daily vaginal smears at 0800 h using a 200 μl lavage of sterile phosphate-buffered saline. The lavage was placed on a slide, dried and then stained with eosin followed by a methylene blue stain. The stage of the estrous cycle, i.e., diestrus-1, diestrus-2, proestrus, estrus or metestrus, was determined using the criteria of Long and Evans (1922). In the present study, nicotine injections started on the estrus day of the cycle. Food intake and meal patterns of female rats vary with their estrous cycle (Brobeck et al., 1947; Drewett, 1974; Eckel et al., 2000) with meal size being attenuated during estrus.

The rats were given either saline (control, n=8) or nicotine (n=8) starting at 0830 h for five estrous cycles, i.e., 25 days. Meal pattern analysis was conducted during nicotine administration and for 15 days after cessation of nicotine. Body weights were recorded daily and were 234.0±2.9 and 228.9±2.5 g for the control and nicotine groups, respectively, at the start of experimental injections.

2.5. Data analyses

Meal patterns were analyzed using proprietary computer programs (Bellinger et al., 1997) that were updated for this study. In these analyses, a meal was defined (Castonguay et al., 1986) using a 10-min end of meal criterion (i.e., no pellets consumed for 10 min denotes the end of a meal) and the minimum meal size was set at 135 mg [i.e., 3 pellets]. The 15 standard components of meal patterns determined in this system were: 1. Total 24-h food intake, 2. Total 24-h number of meals consumed, 3. Total 24-h meal size, 4. Total 24-h meal duration, 5. Total 24-h-inter-meal interval, 6. Dark phase total food intake, 7. Dark phase total number of meals consumed, 8. Dark phase meal size, 9. Dark phase meal duration, 10. Dark phase inter-meal interval, 11. Light phase total food intake, 12. Light phase total number of meals consumed, 13. Light phase meal size, 14. Light phase meal duration, and 15. Light phase inter-meal interval.

Data were analyzed by one- and two-way analysis of variance (ANOVA) using the between-group factor of nicotine dose and the within-group factor of experiment day. Preliminary analyses indicated that there were no significant differences among the groups in any meal parameter during the 2 days prior to start of experimentation. Significant main effects were further analyzed using Duncan's multiple range test (for k=2 means). Differences in P that were less than 0.05 were deemed to be statistically significant.

3. Results

3.1. Food intake

The average total daily food intake (g/24-h) of the nicotine group was suppressed significantly, group×days interaction, $F(40,560)=2.23$, $P<0.001$, relative to the saline control group, starting on day 1 and extending through the first 5 days of the 25 day treatment period (Fig. 1A). Upon cessation of nicotine, the nicotine-treated rats failed to increase 24-h food intake, despite having significantly lower body weights.

The dark phase food intake values for the nicotine group were suppressed significantly, group×days interaction, $F(40,560)=2.24$, $P<0.001$, relative to the saline control group, starting on day 1 and extending through day 2 (Fig. 1B). Interestingly, on days 9, 11, 15, 18 and 23, the nicotine-injected rats ate significantly more than the saline treated rats. Upon cessation of nicotine treatment, the nicotine group did not increase 24-h food intake. The light phase food intake of the nicotine group was suppressed significantly, group×days interaction, $F(40,560)=1.56$, $P<0.02$, relative to saline control group, starting on day 1 and on various days during nicotine treatment (Fig. 1C). These data show that the nicotine effect carries over into the light phase, when nicotine was not being administered. No compensatory rebound effect was observed. Upon nicotine cessation, the light phase food intake of the nicotine-injected group was significantly lower than that of the control group on days 26, 30, and 31.

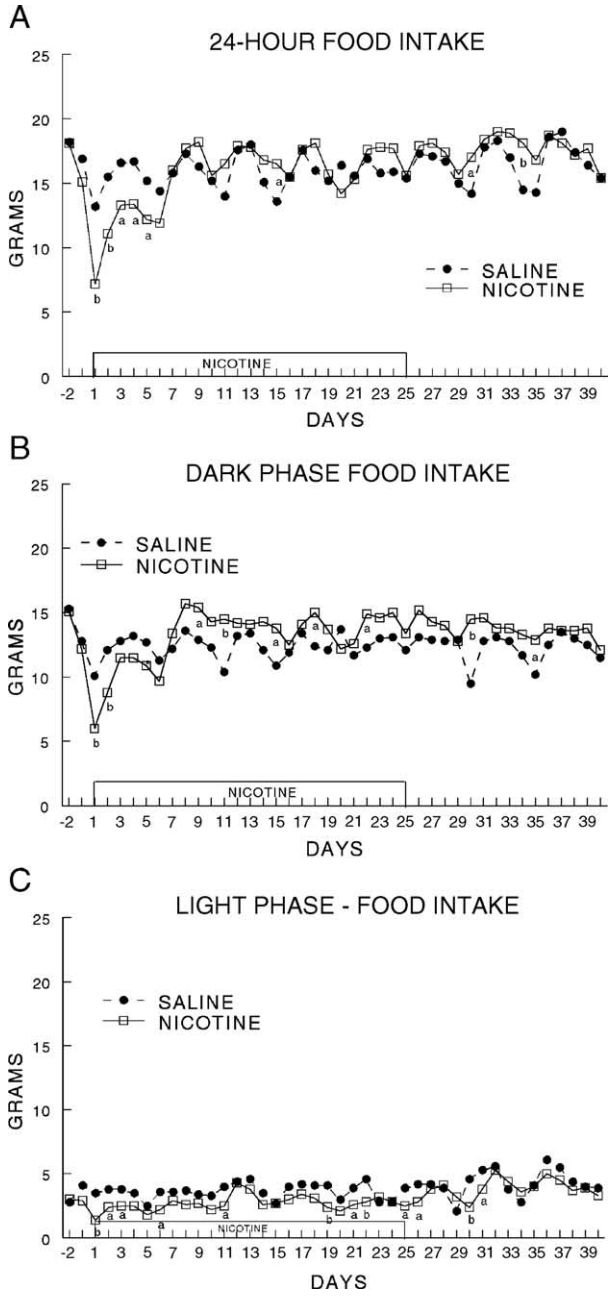

Fig. 1. Panel A: Mean group 24-h food intake (g) recorded prior to, during, and after daily administration of saline vehicle or 1.4 mg/kg/day nicotine (free base). SEM range=0.31–2.61. Panel B: Mean group dark phase food intake recorded prior to, during, and after daily administration of saline vehicle or 1.4 mg/kg/day nicotine. SEM range=0.3–2.57. Panel C: Mean group light phase food intake recorded prior to, during, and after daily administration of saline vehicle or 1.4 mg/kg/day nicotine. SEM range=0.17–1.23. Significant differences between saline and nicotine groups are denoted by a letter (a=p<0.05; b=p<0.01).

3.2. Meal size

The nicotine-treated group reduced 24-h meal size on the first day of treatment and throughout the treatment period, as revealed by a significant [ANOVA group effect, $F(1,14)=6.09$, $P<0.03$], reduction in 24-h meal size on the first day of treatment and it remained below that of the saline group throughout the treatment period (Fig. 2A). Meal size was decreased starting on day 1 in the dark phase, group effect, $F(1,14)=6.58$, $P<0.03$, and continued to be decreased thereafter (Fig. 2B). Meal size was significantly, group effect, $F(1,14)=7.43$, $P<0.02$, reduced

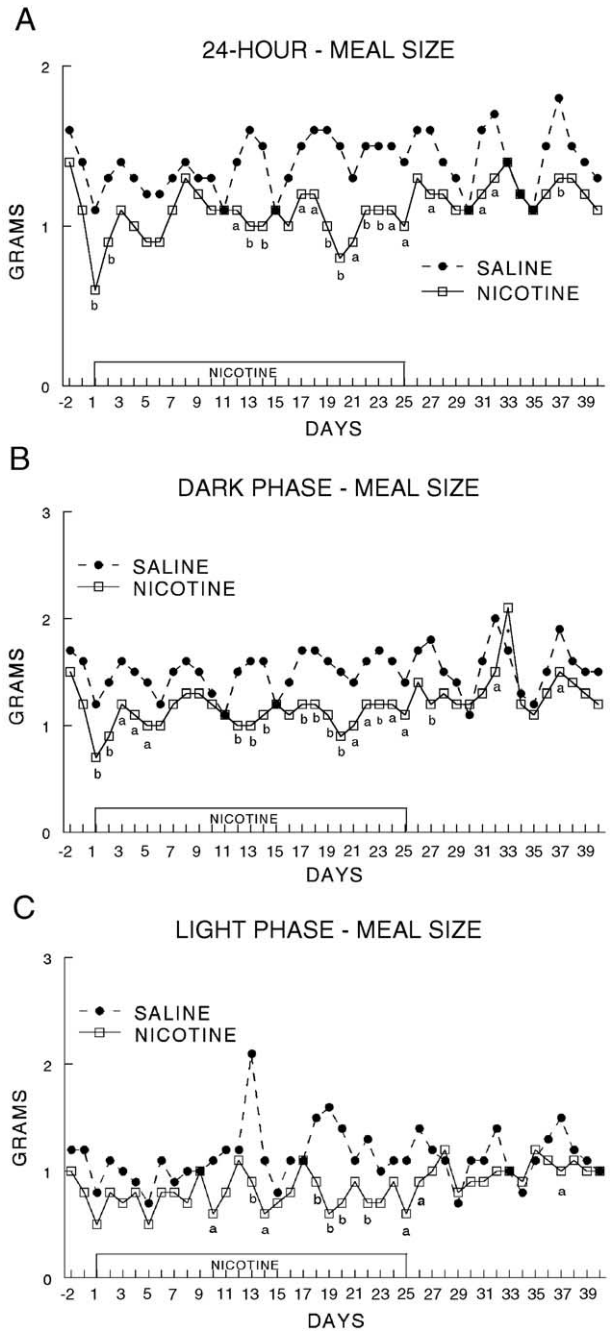

Fig. 2. Panel A: Mean group 24-h meal size (g) recorded prior to, during, and after daily administration of saline vehicle or 1.4 mg/kg/day nicotine. SEM range=0.04–0.22. Panel B similarly depicts mean group dark phase meal size. SEM range=0.07–0.25. Panel C depicts mean group meal size during the light phase. SEM range=0.05–0.58. Significant differences between saline and nicotine groups are denoted by a letter (a=p<0.05; b=p<0.01).

in the light phase starting on day 10 (Fig. 2C) and various days, i.e., 13, 14, 18, 19, 20, 22, 25, 26 and 37, thereafter; even after cessation of nicotine. These data show that the nicotine administered during the dark phase can carry over into the light phase when nicotine is not being administered and demonstrates there is no compensatory rebound effect during the light phase. Following cessation of nicotine, there was no rebound increase in meal size.

3.3. Meal number

The total number of meals taken by the nicotine group over 24-h was significantly, group×days interaction, $F(40,560)=3.03$, $P<0.001$, increased starting on day 13 and continued throughout the remaining treatment period (Fig. 3A). Moreover, this increase in meal number continued after cessation of nicotine treatment. The number of meals taken by the nicotine group during the dark phase was also significantly increased, group×days interaction, $F(40,560)=2.81$, $P<0.001$, the effect started on day 7 of treatment and continuing throughout the remaining treatment period (Fig. 3B). Notably, after cessation of nicotine treatment, the nicotine group continued to take more meals during the dark phase than did the saline treated group on various days over the next 15 days. In contrast, there was no significant between-group differences, $F(1,14)=0.09$, $P>0.76$, in the number of meals consumed during the light phase either during the nicotine administration period or after cessation of nicotine. Thus, there was no evidence of compensation (i.e., reduced meal number) in the nicotine group during the light phase over the 25-day period when nicotine was administered during the dark phase or even after cessation of nicotine. The profile of these results indicate that while meal size and food intake are suppressed on day one of nicotine treatment, a 7-day delay is evident before there is a compensatory increase in meal number and this effect persists for as long as 2 weeks after cessation of nicotine. Complementary to the meal frequency data the 24-h, dark phase and light phase inter-meal intervals following nicotine treatment were significant decreased (data not shown).

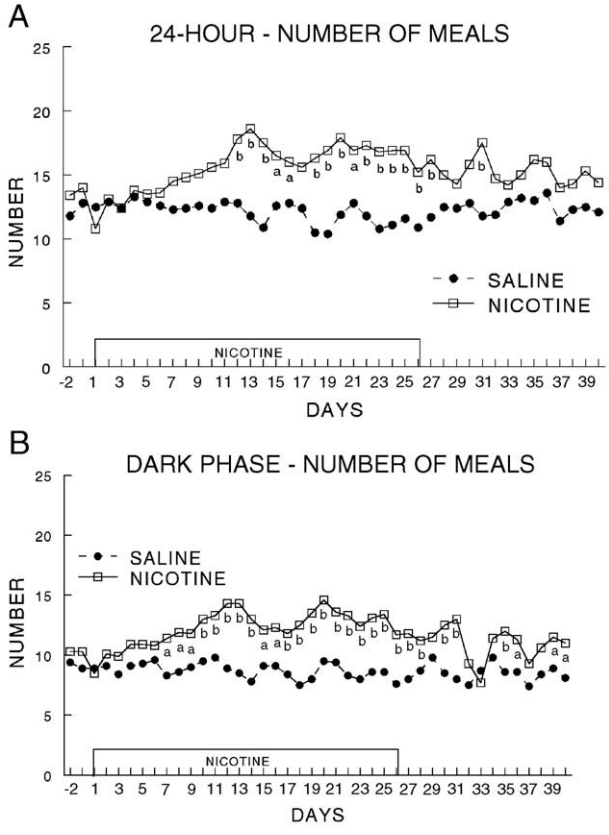

Fig. 3. Panel A: Mean group 24-h meal number recorded prior to, during, and after daily administration of saline vehicle or 1.4 mg/kg/day nicotine. SEM range=0.49–2.22. Panel B: Mean group dark phase meal number. SEM range=0.4–1.96. Significant differences between saline and nicotine groups are denoted by a letter (a=$p<0.05$; b=$p<0.01$).

3.4. Meal duration

In accord with nicotine reducing 24-h, dark phase and light phase meal size, the 24-h [group effect, $F(1,14)=4.91$, $P<0.05$], dark phase [group effect, $F(1,14)=5.65$, $P<0.05$] and light phase [group×days effect, $F(40,560)=1.68$, $P<0.01$] meal duration was reduced in the nicotine-treated group on various days during treatment when compared to the control group.

3.5. Estrous cycle effect

To determine if nicotine interacted with the various stages of the estrous cycle to enhance or reduce its effect, the individual rat data of nicotine group's 24-h and dark phase food intake, 24-h and dark phase meal size and 24-h and dark phase meal number were converted into a percentages of the control data for that day. For each parameter an average value was calculated for each rat across the five estrous cycles and those percentages of control data from diestrus-1, diestrus-2, proestrus, estrus and metestrus were compared using a one-way ANOVA. Nicotine had a similar effect at each stage of the estrous cycle for 24-h [group effect, $F(4,35)=1.82$, ns] and dark phase [group effect, $F(4,35)=0.83$, ns] food intake; 24-h [group effect, $F(4,35)=2.10$, ns] and dark phase [group effect, $F(4,35)=0.34$, ns] meal size; and 24-h [group effect, $F(4,35)=0.46$, ns] and dark phase [group effect, $F(4,35)=1.17$, ns] meal number.

3.6. Water intake

Nicotine treatment or its withdrawal did not significantly affect 24-h water intake, $F(1,14)=0.01$, $P>0.95$, relative to that of the saline control group.

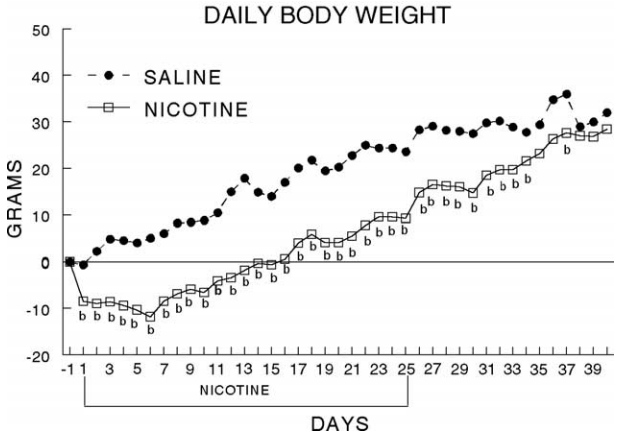

Fig. 4. Mean group daily body weight change (g) recorded from the start of daily administration of saline vehicle or 1.4 mg/kg/day nicotine. At the start of injections, the body weights of saline and nicotine groups were 234.6±2.0 g and 237.4±2.7 g, respectively. SEM range=1.22–3.59. Significant differences between saline and nicotine groups are denoted by a letter (a=p<0.05; b=p<0.01).

3.7. Body weight

The body weight gains of the nicotine-treated group, relative to that of the control group, were significantly, group effect, $F(1,14)=7.52$, $P<0.02$, suppressed after 1 day of nicotine treatment (Fig. 4). The weight of the nicotine group remained significantly suppressed during the rest of the nicotine treatment period and for 11 days after cessation of nicotine. The reduced weight gain noted in the nicotine group corresponds to the reduction in 24-h food intake noted in Fig. 1A during nicotine administration. However, body weight remained significantly below the control group even after food intake normalized on the seventh day of nicotine treatment and this continued for 9 days after cessation of nicotine.

4. Discussion

In the present study, female rats exhibited a complicated and dynamic pattern of changes in meal parameters, food intake, and body weight during intermittent administration of 1.4 mg/kg/day nicotine (free base) in the dark phase (over five estrous cycles, i.e., 25 days) and for 15 days after the cessation of nicotine. The immediate effect of nicotine and the pattern of administration were to reduce 24-h food intake on the first day of treatment. When male rats were given the same dose in the dark phase, they also showed a significant suppression of 24-h food intake starting on the first day of nicotine treatment, but the magnitude of the decrease of intake on the first day of treatment was much less than that observed in the females (Bellinger et al., 2003a). Daily food intake was significantly suppressed for 5 days in the females, whereas in the males their 24-h food intake remained significantly suppressed for 9 days (Bellinger et al., 2003a). Thus, the females were initially more sensitive to the food intake suppressing effects of nicotine than the males (see Grunberg et al., 1986), whereas they recover their normal food intake more rapidly. The latter may be related to the observation that female rats defend their body weight better than do male rats (Nance et al., 1997; Westerterp, 1994). If this were the case, the attempt was not successful as the overall effect of nicotine was to suppress body weight more in females than in males (Grunberg et al., 1986; Bellinger et al., 2003a).

Analysis of the microstructure of feeding of the female rats revealed that their daily food intake was reduced by an immediate (i.e., day 1) attenuation of meal size and of meal duration. Thus, the difference between the female nicotine and control groups' 24-h food intake was mainly attributed to an immediate decrease in meal size during the dark phase that persisted throughout the nicotine treatment and for several days after nicotine treatment stopped. After 9 days of nicotine treatment, meal size was intermittently attenuated during the light phase and this continued for several days post nicotine treatment. This finding of meal size being attenuated in the light phase of female rats contrasts with the finding that light phase meal size was not decreased in similarly treated male rats (Bellinger et al., 2003a). In male rats (Bellinger et al., 2003a), intermittent administration of nicotine during the dark phase only attenuated dark phase total food intake, whereas in female rats nicotine administered in a similar design and dose attenuated both dark and light phase total intake. The data show in female rats that nicotine had a carryover effect during the non-injected light phase on meal size, whereas this did not occur in male rats (Bellinger et al., 2003a). In both genders the food intake suppression during the dark phase did not result in compensatory increase in meal size during the non-infused light phase.

By day 7 of treatment, the nicotine rats exhibited an increase in dark phase meal number, with a complementary decrease in inter-meal interval that also reached significance by day 7. Male rats treated similarly showed a significant increase in dark phase meal number by day 5 (Bellinger et al., 2003a). However, there was a gender difference in the magnitude of this response. In male rats the increase in meal frequency tended to just normalize dark phase food intake (Bellinger et al., 2003a), whereas the female rats of the present study showed a much larger dark phase compensatory effect on meal frequency, i.e., an increase in females of 87 vs 56% in males. This resulted in dark phase food intake being greater in the female nicotine-treated rats than their controls.

The mechanism(s) that may underlie the increase in meal number in nicotine-treated rats is uncertain. However, one may speculate that the rat may be experiencing a homeostatic compensation for the nicotine induced reduction in meal size with resultant decrease in food intake. The compensation to this reduced daily intake is manifested as an increase in meal frequency. Meal frequency would be

increased as the rat experiences a decrease in the length of satiety as a result of reduced size of its preceding meal. Using this strategy, the rat tries to maintain a normal daily food intake.

Alternately, nicotine could be directly affecting meal number, but with a different temporal onset pattern from that which affects meal size. If the former is true, it suggests that nicotine works on pathways involved in determining meal size, without directly affecting pathways that are involved in determination of meal number. An additional question arises as to why it took 7 days in the case of females and 5 days in males for this hypothesized compensation to be manifested (Bellinger et al., 2003a). The body weight of the present females was reduced significantly after the first day of nicotine treatment, whereas it was decreased significantly on day 6 in male rats (Bellinger et al., 2003a). It is possible that the drive to increase meal number as a means of food intake compensation may be tied to a signal related to the reduced body weight in male rats (Bellinger et al., 2003a). However, this does not seem to be a valid possible explanation in female rats whose body weight was significantly reduced after 1 day of nicotine treatment, whereas meal frequency was not increased until day 7 of nicotine treatment. On the other hand, while not significant, inspection of the dark phase meal frequency figure show a trend for meal frequency to be elevated starting on the second day of nicotine treatment in the female rats.

The central mechanism of nicotine's action is unknown, but a recent study by Guan et al. (2004), suggests that nicotine acts at cells near the fourth ventricle. The investigators infused the nicotine receptor antagonist mecamylamine into the fourth ventricle followed by nicotine (1.4 mg/kg/day, free base) treatment of male rats. Administration of the antagonist reversed the normal nicotine induced suppression of dark phase meal size and normalized food intake. This study is complementary to the study by Zarrindast and Oveisi (1997).

In the present study, cessation of nicotine did not result in a period of overeating in the nicotine-treated female rats despite the fact they had reduced body weight compared to the controls. This result contrasts with an earlier study in male rats (Bellinger et al., 2003a) using a similar design that resulted in a short period of hyperphagia following cessation of nicotine treatment. A recent study (Bellinger et al., 2003b) suggested an attenuation of the α-MSH satiety system may be, in part, responsible for the modest hyperphagia observed following nicotine withdrawal in male rats (Bellinger et al., 2003a).

In both the present study and earlier study (Bellinger et al., 2003a) body weight was significantly suppressed for almost 2 weeks after cessation of nicotine. These data are consistent with many earlier reports (Levin et al., 1987, 1993; Miyata et al., 1999, 2001) in which nicotine treatment suppressed body weight gain during the treatment period and for several days thereafter. However, only part of the suppression of body weight can be attributed to nicotine decreasing food intake. In the present study 24-h food intake returned to normal by day 6 in the nicotine-treated group and remained normal for the next 34 days. Body weight was suppressed starting on day 1 and through the next 25 days of nicotine administration and for 11 days thereafter. Therefore, during 31 days the food intake of the nicotine group was not significantly less than the saline group yet during this same time period the nicotine group's body weight remained significantly attenuated. This demonstrates that factors other than food intake must be involved in either weight loss or prevention of regaining the lost body weight. It is well known (Bellinger and Mendel, 1995) that rats subjected to food restriction have a decreased energy expenditure that allows them to quickly regain lost body weight even when food intake is only returned to pre-restriction levels. The present data indicate that nicotine treatment did not produce the normal decrease in energy expenditure associated with reduced food consumption, but may have actually enhanced energy expenditure during administration and even after drug withdrawal. It is known, for example that nicotine can acutely elevate basal metabolic rate in humans and rats (Perkins, 1992; Grunberg et al., 1988), stimulate brown adipose tissue thermogenesis (Lupien and Bray, 1988) and alter fat metabolism (Bishop et al., 2004). These changes would act to limit gains in body weight.

In agreement with early studies (Grunberg et al., 1986; Booze et al., 1999) the nicotine-treated rats appeared to have normal estrous cycles. In addition the present data, i.e., 24-h food intake, 24-h meal size, and 24-h meal frequency, indicate that nicotine's effects and/or the response to nicotine were similar across the five stages of the estrous cycle. Therefore, there was apparently no interaction of the stage of the estrous cycle with nicotine's action on the measured feeding parameters.

In summary, these results document that administration of nicotine to female rats during the dark phase resulted in a reorganization of the microstructure of food intake, i.e., initially nicotine induced a decrease in meal size followed by a possible compensatory increase in meal frequency that finally normalized 24-h intake. These data suggest that the primary effect of nicotine may be in affecting meal size, without compromising the pathways that affect meal frequency. The reason for the long lasting alterations in the microstructure of food intake for up to 2 weeks after cessation of nicotine is uncertain and awaits further study. Finally, these results are complimentary, but not entirely identical to male rats given similar nicotine treatment (Bellinger et al., 2003a). However, the present results differs from studies in which nicotine was given continuously 24-h per day and indicate that dark phase nicotine administration in rats may represent an appropriate model to study the impact of nicotine on meal patterns.

Acknowledgements

Requests for reprints should be directed to the first author at lbellinger@bcd.tamhsc.edu. A preliminary version of this manuscript was presented at the 2004 meeting of Experimental Biology 2004. The Baylor College of Dentistry IACUC approved the procedures of this study. This work was funded (TEF 2000-22) with Tobacco Settlement Proceeds held by TAMUS HSC to LLB.

References

Bellinger LL, Mendel VE. Blood profile and balance study of rats given the putative anorectic agent satietin. Am J Physiol 1995;268:R1–7.

Bellinger LL, Castonguay TW, Bernardis LL. Hormone and somatic changes in rats pair-fed to growth retarded dorsomedial hypothalamic nuclei-lesioned rats. Brain Res Bull 1994;34:117–24.

Bellinger LL, Fabia R, Husberg BS. Meal patterns prior to and following liver transplantation in rats. Physiol Behav 1997;62:525–9.

Bellinger L, Cepeda-Benito A, Wellman PJ. Meal patterns in male rats during and after intermittent nicotine administration. Pharmacol Biochem Behav 2003a;74:495–504.

Bellinger LL, Cepeda-Benito A, Bullard RL, Wellman PJ. Effect of icv infusion of the α-MSH agonist MTII on meal patterns in male rats following nicotine withdrawal. Life Sci 2003b;73:1861–72.

Benowitz NL, Hatsukami D. Gender differences in the pharmacology of nicotine addiction. Addict Biol 1998;3:383–404.

Benowitz NL, Jacob III P. Daily intake of nicotine during cigarette smoking. Clin Pharmacol Ther 1984;35:499–504.

Bishop C, Parker GC, Coscina DV. Systemic nicotine alters whole-body fat utilization in female rats. Physiol Behav 2004;80:563–7.

Blaha V, Yang ZJ, Meguid M, Chai JK, Zadak Z. Systemic nicotine administration suppresses food intake via reduced meal sizes in both male and female rats. Acta Medica (Hradec Kralove) 1998;4:167–73.

Booze RM, Welch MA, Billings KA, Apple SR, Mactutus CF. Behavioral sensitization following repeated intravenous nicotine administration: gender differences and gonadal hormones. Pharmacol Biochem Behav 1999;64:827–39.

Brobeck JR, Wheatland M, Strominger JL. Variations in regulation of energy exchange associated with estrus, diestrus and pseudopregancy in rats. Endocrinology 1947;40:65–72.

Castonguay TW, Kaiser LL, Stern JS. Meal pattern analysis: artifacts, assumptions, and implications. Brain Res Bull 1986;7:439–43.

Delnevo CD, Hrywna M, Abatemarco DJ, Lewis MJ. Relationships between cigarette smoking and weight control in young women. Fam Commun Health 2003;26:140–6.

Drewett RF. The meal patterns of the oestrous cycle and their motivational significance. Q J Exp Psychol 1974;26:489–94.

Eckel LA, Houpt TA, Geary N. Spontaneous meal patterns in female rats with and without access to running wheels. Physiol Behav 2000;70:397–405.

Flegal KM, Troiano RP, Pamuk ER, Kuczmarski RJ, Campbell SM. The influence of smoking cessation on the prevalence of overweight in the United States. N Engl J Med 1995;333:1165–70.

Giovino GA. Epidemiology of tobacco use in the United States. Oncogene 2002;21:7326–40.

Glendinning JI, Smith JC. Consistency of meal patterns in laboratory rats. Physiol Behav 1994;56:7–16.

Grunberg NE, Bowen DJ, Winders SE. Effects of nicotine on body weight and food consumption in female rats. Psychopharmacology 1986;90:101–5.

Grunberg NE, Popp KA, Bowen DJ, Nespor SM, Winders SE, Eury SE. Effect of chronic nicotine administration on insulin, glucose, epinephrine and norepinephrine. Life Sci 1988;42:161–70.

Guan PJ, Bellinger LL, Kramer PR. The fourth ventricle as a site of nicotine's action on feeding behavior. Life Sci 2004;74:2725–37.

Levin ED, Morgan MM, Galvez C, Ellsion GD. Chronic nicotine and withdrawal effects on body weight and food and water consumption in female rats. Physiol Behav 1987;39:441–4.

Levin ED, Briggs SJ, Christopher NC, Rose JE. Sertraline attenuates hyperphagia in rats following nicotine withdrawal. Pharmacol Biochem Behav 1993;44:51–61.

Li MD, Kane JK, Parker SL, McAllen K, Matta SG, Sharp BM. Nicotine administration enhances NPY expression in the rat hypothalamus. Brain Res 2000;867:157–64.

Long JA, Evans HM. The oestrous cycle in the rat and its associated phenomena. Memoirs of the University of California, vol. 6. Berkeley: University of California Press; 1922. p. 1–148.

Lupien JR, Bray GA. Nicotine increases thermogenesis in brown adipose tissue in rats. Pharmacol Biochem Behav 1988;29:33–7.

Miyata G, Meguid MM, Fetissov SO, Torelli G, Kim H-J. Nicotine's effect on hypothalamic neurotransmitters and appetite regulation. Surgery 1999;126:255–63.

Miyata G, Meguid MM, Varma M, Fetissov SO, Kim H-J. Nicotine alters the usual reciprocity between meal size and meal number in female rats. Physiol Behav 2001;74:169–76.

Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP. The spread of the obesity epidemic in the United States, 1991–1998. JAMA 1999;282:1519–22.

Murrin LC, Ferrer JR. Nicotine administration to rats: methodological considerations. Life Sci 1987;40:1699–708.

Nance DM, Bromley B, Barnard RJ, Gorski RA. Sexual dimorphic effects of forced exercise on food intake and body weight in the rat. Physiol Behav 1977;19:155–8.

Pauly JR, Grun EU, Collins AC. Chronic corticosterone administrations modulate nicotine sensitivity and brain nicotine receptor binding in C3H mice. Psychopharmacology 1990;101:310–6.

Perez-Stable EJ, Herrera B, Jacob III P, Benowitz NL. Nicotine metabolism and intake in black and white smokers. JAMA 1998;280:152–6.

Perkins KA. Metabolic effects of cigarette smoking. J Appl Physiol 1992;72:401–9.

Perkins KA. Smoking cessation in women Special considerations. CNS Drugs 2001;15:391–411.

Perkins KA, Levine MD, Marcus MD, Shiffman S. Addressing women's concerns about weight gain due to smoking cessation. J Subst Abuse Treat 1997;14:173–82.

Pomerleau CS, Kurth CL. Willingness of female smokers to tolerate postcessation weight gain. J Subst Abuse 1996;8:371–8.

Stice E, Shaw H. Prospective relation of body image, eating, and affective disturbances to smoking onset in adolescent girls; how Virginia slims. J Consult Clin Psychol 2003;71:129–35.

Svensson CK. Clinical pharmacokinetics of nicotine. Clin Pharmacokinet 1987;12:30–40.

Valentine JD, Hokanson JS, Matta SG, Sharp BM. Self-administration in rats allowed unlimited access to nicotine. Psychopharmacology 1997;133:300–4.

Westerterp KR. Body composition. In: Westerterp-Plantenga MS, Fredrix AB, Steffens AB, editors. Food intake and energy expenditure. Boca Raton, (FL): CRC Press; 1994. p. 259–77.

Zarrindast MR, Oveisi MR. Effects of monoamine receptor antagonists on nicotine-induced hypophagia in the rat. Eur J Pharm 1997;321:157–62.