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Meal patterns in male rats during and after intermittent nicotine administration

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Abstract

Continuous administration of nicotine (NIC) reduces food intake (FI) and body weight (BW), whereas rebound eating and BW gain occur after NIC cessation. However, generalizations derived from prior studies on meal patterns in rats using continuous 24-h NIC administration are limited, because human smokers use NIC intermittently during their active period. In the present study, computerized meal pattern analyses (MPA) were conducted for adult male rats treated for 14 days with either saline or 2 or 4 mg/kg/day of NIC spread over five equal amounts during the dark phase. MPA analyses continued for 14 days after cessation of NIC. Only the 4 mg/kg/day NIC dose caused consistent changes in meal patterns and only that dose is reported herein. Dark period FI was reduced, whereas light period FI was unchanged in the NIC-treated group; thus, there was no rebound eating during the 12-h nontreatment phase. MPA analyses revealed the FI reduction on Day 1 of NIC administration was caused by a persistent decrease in dark phase meal size. On Day 5 of NIC, the rats compensated by significantly increasing the number of meals they took, which tended to normalize dark phase FI. Congruently, dark phase intermeal interval was decreased. Importantly, these changes in meal patterns persisted for 2 weeks after termination of NIC. Upon NIC cessation, the NIC group had a transient elevated FI. The NIC-treated group's BW was significantly suppressed by Day 6 of NIC and after stoppage these rats slowly, but incompletely, regained lost BW over the next 14 days. These results document that administration of NIC during the dark phase resulted in a reorganization of the microstructure of FI in male rats and that long-lasting alterations in the microstructure of FI (e.g., meal size and meal number) were noted for up to 2 weeks after cessation of NIC. These results differ from studies in which NIC was given continuously 24-h/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; Hyperphagia; Smoking; Body weight; Water intake

1. Introduction

The personal costs associated with obesity include increased health risks and early mortality (Bray, 1996; Klein, 2001), as well as discrimination in employment and difficulties in personal relationships (Vener and Krupka, 1985). As a consequence, some persons are willing to use risky weight-altering products in order to lose or to maintain their body weight (BW). Smoking, for example, represents a means by which young adults are able to affect some control of their BW (Gerend et al., 1998). Yet, smoking carries a known health risk and once established is refractory to many current behavioral therapies (Perkins, 2001). Treatment difficulties may occur because the motivation to smoke is complex, involving the reinforcing actions of smoking as well as the weight-reducing properties of smoking (Flegal et al., 1995; Kawachi et al., 1996). Indeed, the importance of smoking to weight regulation is evident in studies in which some young adults would refuse to stop smoking should cessation of smoking result in a weight gain of as little as 5 lb (Pomerleau and Kurth, 1996). The utility of smoking for the control of weight may be particularly salient, given the social and cultural pressures to remain thin (Blanck et al., 2001; Vener and Krupka, 1985).

It is well documented that nicotine (NIC) derived from smoking promotes BW loss and that cessation of NIC

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causes BW gain in animal models (Grunberg et al., 1986, 1987; Levin et al., 1987; Wager-Srdar et al., 1984) and humans (Flegal et al., 1995; Kawachi et al., 1996; Perkins et al., 1987). The aforementioned unwillingness to stop smoking because of BW gain is endemic and calls for a better understanding of how NIC prevents BW gain and importantly how NIC withdrawal promotes excessive BW gain. Relatively few studies have examined the changes in meal patterns that are produced by exposure to NIC and by withdrawal from NIC. Blaha et al. (1998) reported that constant NIC infusion (6 mg/kg/day), for 7 days in male and female rats altered food intake (FI) by decreasing meal size, but not meal number. After cessation of NIC, 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 constant NIC infusion (5 mg/kg/day) for 7 days reduced FI for 2 days through a decrease in meal size, without changing meal number. NIC cessation in that study led to a 2-day period of hyperphagia that was caused by an increase in meal size that exceeded a compensatory decrease in meal number. Thereafter, meal size, meal number and FI were normal. In the Miyata et al. (2001) study, BW was significantly reduced during NIC administration and for 1 day thereafter.

Although these important studies document that meal parameters are altered during NIC administration and following cessation of NIC, several key design issues limit the generality of this research. First, in the above (Blaha et al., 1998; Miyata et al., 2001) and in many earlier studies (Faraday et al., 2001; Grunberg et al., 1986, 1987; Levin et al., 1987, 1993), NIC was administered in doses of 6–12 mg/kg/day, which result in plasma NIC levels that are in excess of that obtained by even heavy smokers (Li et al., 2000a; Murrin and Ferrer, 1987). Second, a common method used in these early studies (Faraday et al., 2001; Grunberg et al., 1986, 1987; Levin et al., 1987, 1993 Blaha et al., 1998; Miyata et al., 2001) was to administer NIC chronically 24 h a day in rats using osmotic minipumps. Although this method may generate useful information regarding the impact of NIC delivered via skin patches in persons attempting to quit smoking, this method does not approximate the intermittent use of NIC in human smokers during their awake period. Additionally, use of osmotic minipumps involves induction of anesthesia and surgical manipulation, which in turn can dramatically alter meal patterns in most rats for a period of 3–4 days (Meguid et al., 1992; Varma et al., 1999). Moreover, infusion of a constant supply of NIC may alter sleep patterns and importantly may induce receptor down-regulation (Li et al., 2000a). Additionally, a constant administration regimen does not allow for an assessment of the possibility of compensatory changes in eating that may occur during the "off"-phase of NIC administration. Finally, the meal pattern analysis studies (MPA) (Blaha et al., 1998; Miyata et al., 2001) have administered NIC for a relatively short period of time (i.e., 7 days).

The above design issues raise important questions as to whether comparable changes in FI, meal patterns, and BW would be observed for a more appropriate physiological NIC model (i.e., pulsed administration of NIC during the dark or active phase). Accordingly, the present study determined the effects of intermittent NIC administration (five times a day during the rats' activity period for 14 days) at 2 or 4 mg/kg/day and its withdrawal (14 days) on FI, meal patterns, water intake, and BW loss/gain in male rats. Meal patterns were assessed using computerized feeding monitors and standard MPA (Bellinger and Mendel, 1995; Bellinger et al., 1997; Castonguay et al., 1986).

2. Methods

2.1. Animals

Adult male (250–275 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-h light–dark cycle (lights out at 0800 h). This work was reviewed and approved by the Baylor College of Dentistry's Institutional Animal Care and Use Committee.

2.2. Drugs

The NIC solution was prepared by dissolving 0.4 or 0.8 mg NIC hydrogen tartrate salt (Sigma, St. Louis, MO), calculated as the salt, into 1 ml of 0.9% SAL vehicle. Five daily doses were injected intraperitoneally during the dark phase giving daily doses of NIC of 2 and 4 mg/kg/day. The dose for each rat was based on the highest BW for that rat during the injection period. Murrin and Ferrer (1987) reported that administration of 1.5 mg/kg NIC produced plasma cotine levels in rats that were comparable to that recorded in humans smoking one pack of cigarettes per day. The highest NIC dose used herein (4 mg/kg) would be expected to produce a plasma NIC level comparable to that evident after consumption of 2.7 packs/day.

2.3. Apparatus

Each of 16 MPA feeding modules were equipped with photobeam computer activated pellet feeders (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 photobeam 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. Water intake was measured to the nearest ml using spill-proof water bottles (Bio-Serv).

2.4. Procedure

The rats were handled and adapted to laboratory conditions for 4 days after arrival in the vivarium. The rats were then placed into one of the specially constructed sound-attenuated 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 intraperitoneally five times a day with 0.25 ml of saline to desensitize the animals to the injection procedures.

Groups of rats were given saline (control, $n=10$) or NIC at 2 ($n=11$) or 4 mg/kg/day ($n=10$) in five equal doses every 2 h during the dark phase for 14 days. MPA was conducted during NIC administration and for 14 days after cessation of NIC. Because only 16 feeders were available in this laboratory, rats from each NIC and vehicle group were run in squads until the proper group size was reached. BWs were recorded daily, and were 284.4 ± 7.2 and 280.3 ± 7.1 g for the SAL and NIC groups, respectively, at the start of experimental injections.

2.5. Data analyses

Meal patterns were analyzed using proprietary computer programs (Bellinger and Mendel, 1995; 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., three pellets). The 15 standard components of meal patterns determined in this system were: (1) total 24-h FI, (2) total 24-h number of meals consumed, (3) total 24-h meal size, (4) total 24-h meal duration, (5) total 24-h-intermeal-interval, (6) dark phase total FI, (7) dark phase total number of meals consumed, (8) dark phase meal size, (9) dark phase meal duration, (10) dark phase intermeal interval, (11) light phase total FI, (12) light phase total number of meals consumed, (13) light phase meal size, (14) light phase meal duration, and (15) light phase intermeal interval.

Data were analyzed by one-way and two-way analysis of variance (ANOVA) using the between-group factor of NIC 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. Inasmuch as preliminary analyses using Abstat (Anderson-Bell, Arvada, CO) documented that there was a significant Dose × Day interaction only for 4 mg/kg/day NIC vs. saline control, data from these groups were retained for subsequent analyses. 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 daily FI values (g/24 h) for the NIC group were suppressed significantly, Group × Days, $F(27,486)=4.13$, $P<.001$, relative to the saline control group, starting on Day 1 and extending through the first 9 days of the 14 days of treatment (Fig. 1A). Upon cessation of NIC, the rats increased their 24-h FI, but this reached statistical significance only on Day 19.

The dark phase FI values for the NIC group were suppressed significantly Group × Days, $F(27,486)=3.28$, $P<.001$, relative to saline control group, starting on Day 1 and extending through the first 9 days of the 14 days of treatment (Fig. 1B). Upon cessation of NIC, the rats increased their dark phase FI significantly on Days 15 and 16. In contrast, the light phase FI of the NIC group was similar to that of the saline control group, group effect, $F(1,18)=0.43$, $P>.52$, except on Days 11 and 12, where it was suppressed (Fig. 1C). Upon NIC cessation, the light phase FI of the two groups did not differ significantly. These light–dark phase FI data demonstrate that the reduction of FI induced by 4 mg/kg NIC reflects the impact of NIC administration on FI during the dark phase. Moreover, there was no evidence of compensation in FI in the NIC group during the light phase during the 14 days when NIC was administered only during the dark phase.

3.2. Meal size

The NIC group exhibited an immediate and significant, group effect, $F(1,18)=4.91$, $P<.04$, reduction in 24-h meal size on the first day of treatment and the meal size of the NIC group remained below that of the saline group throughout the treatment period (Fig. 2A). The impact of NIC on reducing meal size was restricted, group effect, $F(1,18)=7.15$, $P<.02$, to the dark phase (Fig. 2B) as there were no differences, $F(1,18)=0.43$, $P>.51$, between the groups during the light phase (Fig. 2C). These outcomes show that limitation of administration of NIC to the dark phase did not result in compensatory increases in meal size or FI during the light phase. The data also show that after cessation of NIC, meal size of the treated group remained less than the saline-treated control group. Again, there was no compensation evident during the light phase.

3.3. Meal number

The total number of meals taken by the NIC group over 24 h was significantly, Group × Days, $F(27,486)=2.24$, $P<.001$, increased from Days 5 through 14 of NIC administration (Fig. 3A). Moreover, the increase in meal number evident in the NIC group persisted for 13 days after cessation of NIC. The number of meals taken by the NIC group during the dark phase was also significantly,

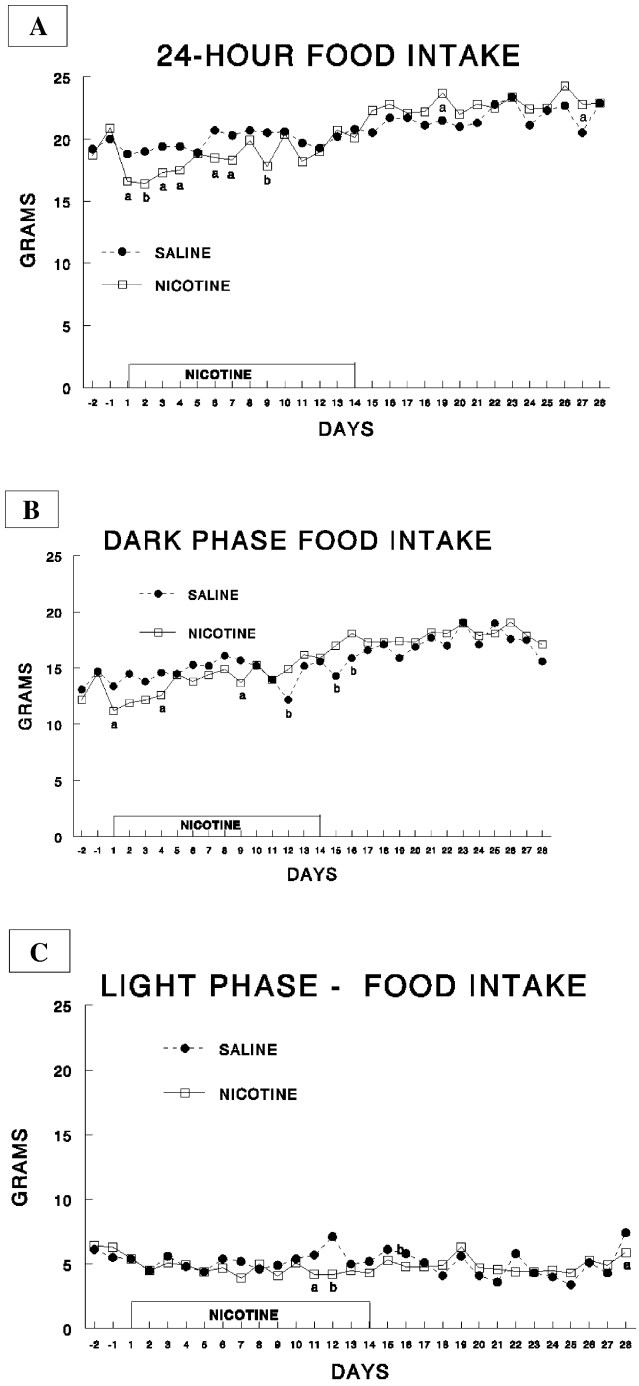

Fig. 1. Panel A: Mean group 24-h food intakes (g) recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 0.4 – 1.3. Panel B: Mean group dark phase intakes recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 0.3 – 1.3. Panel C: Mean group light phase intakes recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 0.3 – 1.0. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

Group × Days, $F(27,486) = 1.52$, $P < .05$, increased on the fifth day of treatment (Fig. 3B). Notably, the NIC group continued to take more meals during the dark phase than the saline-treated group for 14 days after cessation of NIC. In contrast, there were no significant between-group differences, $F(1,18) = 0.06$, $P > .81$, in the number of meals consumed during the light phase by the two groups (Fig. 3C) either during the NIC administration period or after cessation of NIC. Thus, there was no evidence of compensation (i.e., reduced meal number) in the NIC group during the light-phase during the 14 days when NIC was administered

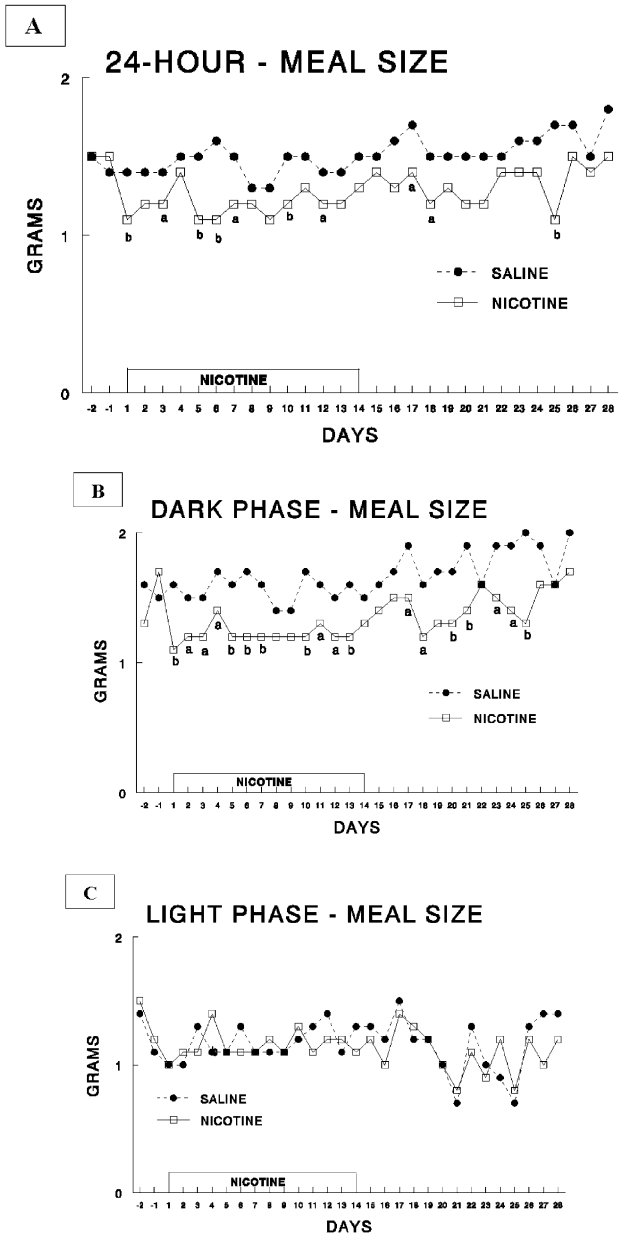

Fig. 2. Panel A: Mean group 24-h meal size (g) recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 0.2 – 0.2. Panel B similarly depicts mean group dark phase meal size. S.E.M. range = 0.1 – 0.4. Panel C depicts mean group meal size during the light phase. S.E.M. range = 0.1 – 0.3. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

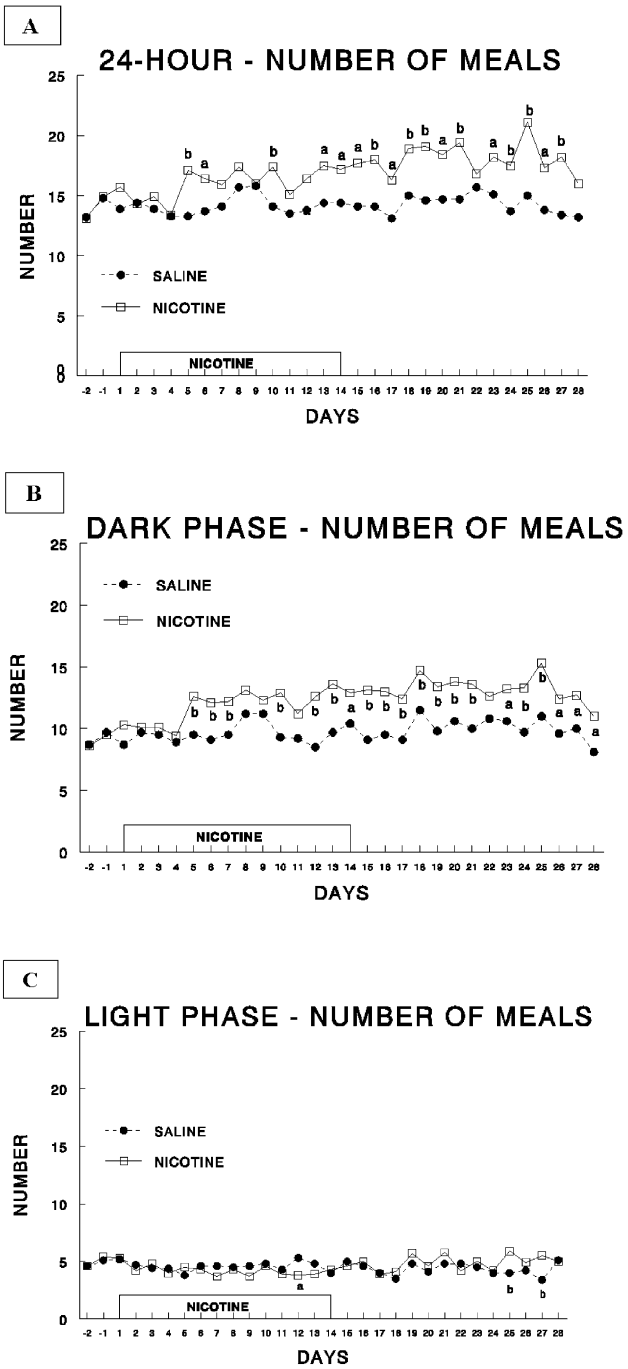

Fig. 3. Panel A: Mean group 24-h meal number recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 0.4–2.0. Panel B: Mean group dark phase meal number. S.E.M. range = 0.4–1.8. Panel C: Mean group light phase meal number. S.E.M. range = 0.2–0.7. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

during the dark phase or after cessation of NIC. The profile of these results indicate that while meal size and FI are suppressed on Day 1 of NIC treatment, a 5-day delay is evident before there is a compensatory increase in meal number and this compensation persists for as long as 2 weeks after cessation of NIC.

3.4. Intermeal interval

Consistent with their increase in 24-h meal number, the 24-h intermeal interval of the NIC group was significantly, group effect, $F(1,18) = 4.88$, $P < .05$, decreased by the fifth day of treatment and remained low even after cessation of

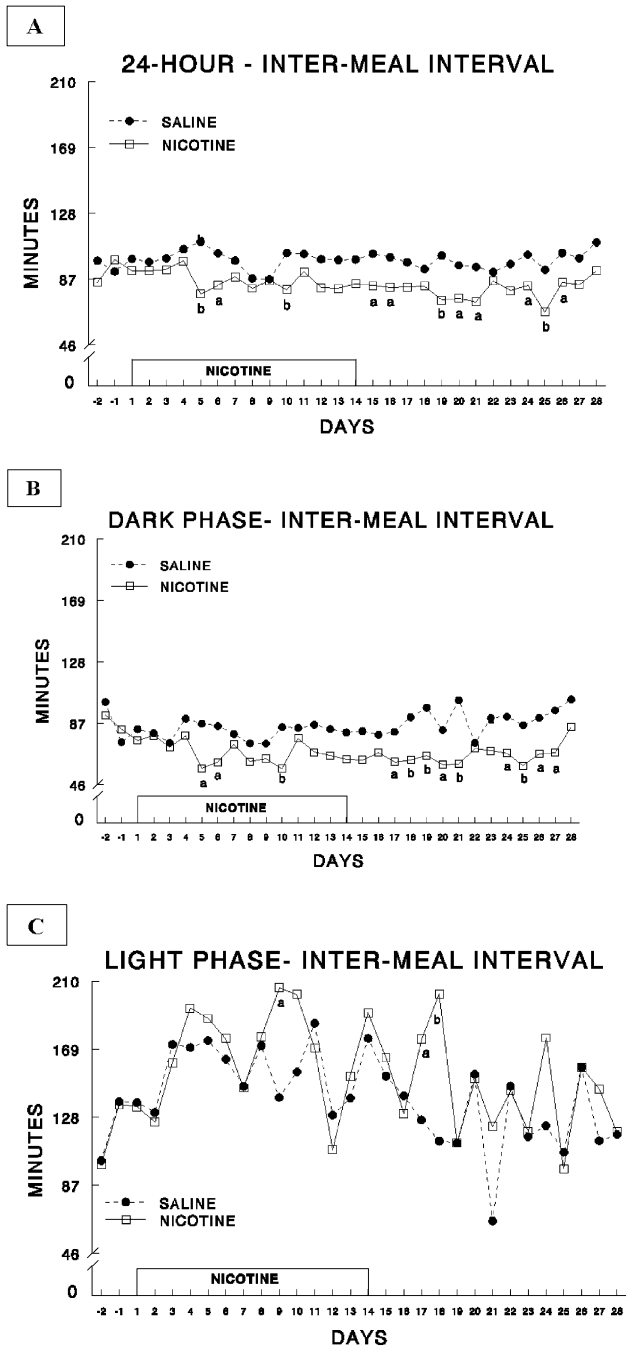

Fig. 4. Panel A: Mean group 24-h intermeal interval (min) recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 3.7–14.6. Panel B similarly depicts mean group dark phase intermeal interval. S.E.M. range = 4.3–11.8. Panel C depicts mean group intermeal interval during the light phase. S.E.M. range = 9.1–44.3. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

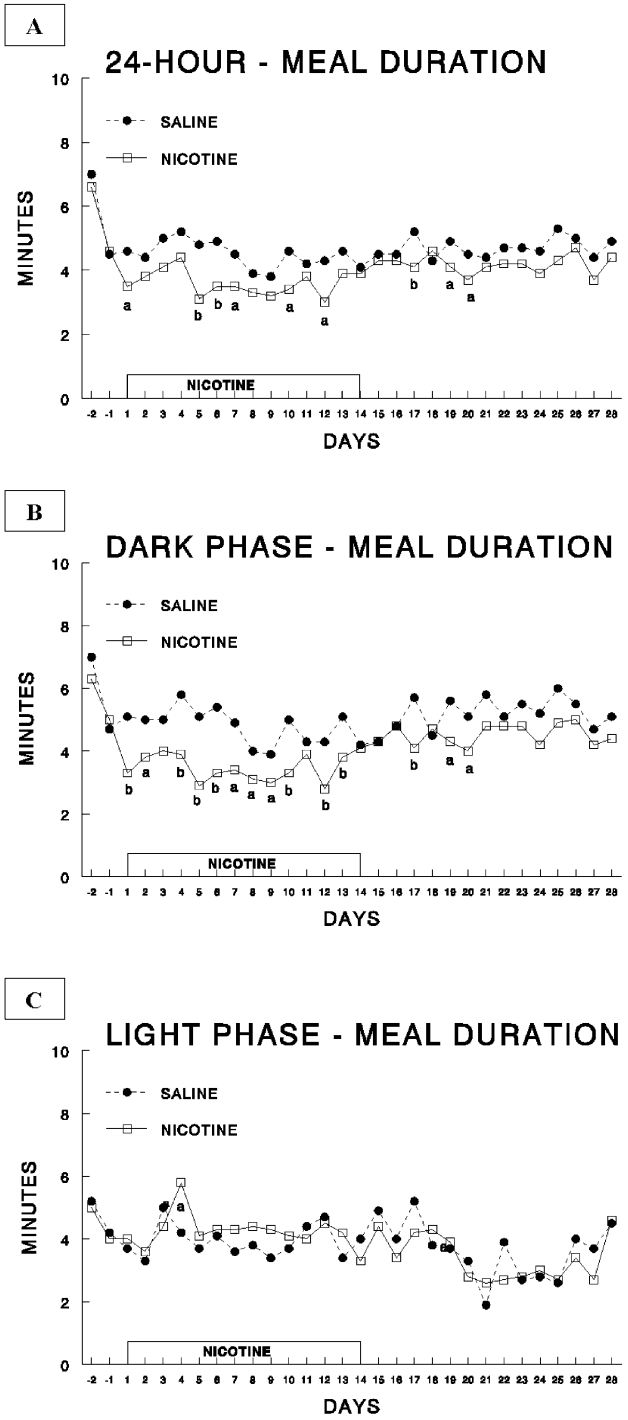

Fig. 5. Panel A: Mean group 24-h meal duration (min) recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 0.2–1.0. Panel B similarly depicts mean group dark phase meal duration. S.E.M. range = 0.2–1.0. Panel C depicts mean group meal duration during the light phase. S.E.M. range = 0.2–1.3. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

NIC (Fig. 4A). The impact of NIC on reducing intermeal interval (Fig. 4B and C) was restricted to the dark phase (dark phase, group effect, $F(1,18) = 6.92$, $P < .02$; light phase, group effect, $F(1,18) = 0.93$, $P > .35$) starting on Day 5 of treatment, during the rest of treatment and throughout the 14 days after termination of NIC.

3.5. Meal duration

Consistent with their reduced meal size, the 24-h meal duration of the NIC-treated group was also reduced significantly, group effect, $F(1,18) = 10.65$, $P < .01$, on Days 1 through 12 of treatment (Fig. 5A). The effect of NIC on reducing meal duration was restricted to the dark phase [dark phase, group effect, $F(1,18) = 18.10$, $P < .001$; light phase, group effect, $F(1,18) = 0.01$, $P > .92$], which is consistent with the decreased meal size shown in that phase (Fig. 5B and C). After cessation of NIC, there were several days when the 24-h meal duration of the NIC group was less than that of the saline-treated group; these times coincided, as expected, with reduced meal size in the NIC-treated group.

3.6. Water intake

NIC treatment or its withdrawal did not affect 24-h water intake, $F(1,18) = 0.14$, $P > .71$, relative to that of the saline control group (Fig. 6). The sharp increase and decrease of water intake evident in both groups on Days 16 and 17, respectively, may be related to the cessation of daily injections for the NIC and saline groups.

3.7. Body weight

The BW gains of the NIC-treated group, relative to that of the control group, were significantly, group effect, $F(1,18) = 8.45$, $P < .01$, suppressed by Day 6 of the NIC administration period (Fig. 7). The weight gains of the NIC group remained significantly suppressed during the rest of the NIC treatment period and for 14 days after cessation of

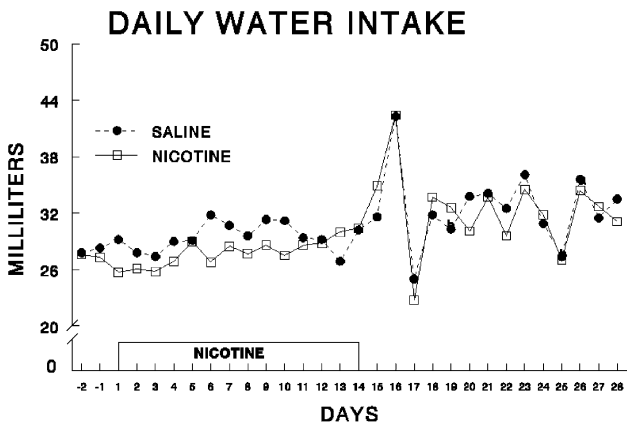

Fig. 6. Mean group daily water intakes (ml) recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. S.E.M. range = 1.2–3.7. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

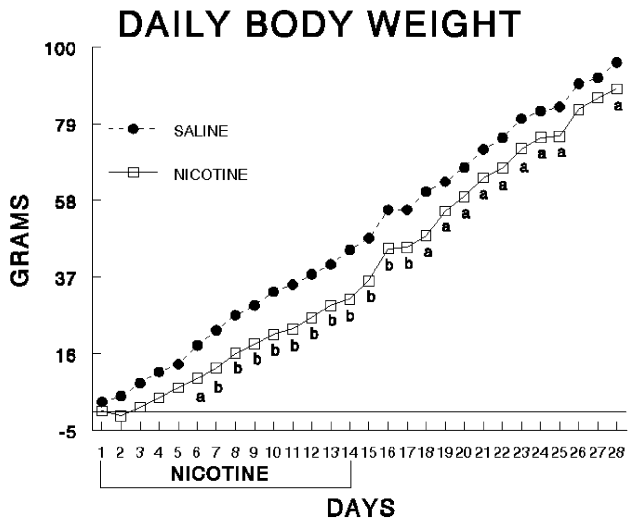

Fig. 7. Mean group daily body weight gain (g) recorded prior to, during, and after daily administration of saline vehicle or 4 mg/kg/day nicotine. At the start of injections, the body weights of saline and nicotine groups were 284.4 ± 7.2 and 280.3 ± 7.1 g, respectively. S.E.M. range = 0.6 – 4.4. Significant differences between saline and nicotine groups are denoted by a letter (a = $P < .05$, b = $P < .01$).

NIC injection. The reduced weight gain noted in the NIC group corresponds to the reduction in 24-h FI noted in Fig. 1A during NIC administration. However, BW remained below the control group even after FI normalized on the 10th day of NIC treatment and during the 14 days of nontreatment when daily FI of the two groups continued to be similar.

4. Discussion

In the present study, male rats exhibited a complicated and dynamic pattern of changes in meal parameters, FI, and BW during intermittent administration of 4 mg/kg/day NIC during the dark phase and for a period of 2 weeks after the cessation of NIC. The immediate effect of this NIC dose and pattern of administration was to reduce FI on the first day of treatment. Analysis of the microstructure of feeding revealed that FI was reduced by an immediate (i.e., Day 1) attenuation of meal size and of meal duration. The difference between NIC and control groups in 24-h meal size noted in the present study can be solely attributed to a decrease in meal size during the dark phase in that meal size of the NIC and saline treatment groups did not vary significantly during the light phase. This suggests that the inhibitory action of dark phase injected NIC on meal size and the resulting suppression of total FI during the dark phase does not result in compensation in meal size during the noninfused light phase.

At Day 5 of treatment, the NIC rats exhibited a significant and persistent increase in meal number. The mechanism(s) that may underlie the delayed increase in meal number in NIC rats is uncertain. However, one may speculate that the rat may be experiencing a homeostatic compensation for the NIC-induced reduction in meal size with resultant decrease in FI. This compensation 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. The decrease in the length of satiety is manifested as a decrease in the intermeal interval, which results in an increase in meal frequency. Using this strategy, the rat tries to maintain a normal daily FI. Alternately, NIC could be directly affecting meal number, but with a different temporal onset pattern from that of the effect of NIC on meal size. If the former is true, it suggests that NIC 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 5 days for this hypothesized compensation to be manifested. BW was reduced significantly on Day 6, so the drive to increase meal number as a means of compensation may be tied to a signal related to the reduced BW.

By Day 9, the total FI of the NIC rats appeared normal, but was in fact disordered in that this apparently normal FI resulted from a persistent decrease in meal size and a persistent increase in meal number.

The complicated patterns of NIC action evident above raise the issue as to the neurochemical substrates that underlie the action of NIC on meal size and meal number. Although NIC elicits the secretion of the classic monoamines norepinephrine, dopamine, and serotonin (Yang et al., 1999; Li et al., 2000b; Miyata et al., 2001), each of which are known to affect overall eating in the rat (Blundell, 1977; Hoebel et al., 1989; Wellman et al., 1993), pharmacological antagonism of these receptors does not alter NIC-induced hypophagia (Zarrindast and Oveisi, 1997). NIC given constantly by osmotic minipumps (5 mg/kg/day) has been suggested (Miyata et al., 1999) to decrease meal number in adult male Fischer-344 rats by increasing the release of lateral hypothalamic dopamine, while preventing a compensatory change in meal number by increasing lateral hypothalamic serotonin. These data contrast other reports by the same laboratory, wherein a constant infusion of NIC at 6 (Blaha et al., 1998) or 5 mg/kg/day (Miyata and Meguid, 2000) caused a decrease in meal size, without changing meal number in adult male Fisher-344 rats. The reason for these differing results is unclear. However, the above studies employed Fischer 344 rats, which may be problematic for studies of meal pattern (Glendinning and Smith, 1994). The present study showed that intermittent NIC administration during the rats' active period immediately decreased meal size. This was followed several days later by a compensatory increase in meal number. These changes remained for some time even after cessation of NIC, which was not observed in the earlier studies in male rats (Blaha et al., 1998; Miyata and Meguid, 2000; Miyata et al., 1999). The reasons for the differences in meal patterns are uncertain, but could relate to rat strain (Glendinning and Smith, 1994),

the fact that a constant infusion of NIC may not evoke a physiological delivery pattern, and that a dose of NIC (i.e., greater than 4 mg/kg) was used that exceeds that taken by heavy smokers (Murrin and Ferrer, 1987) and may elicit stereotypic behavior (Li et al., 2000a).

Additionally, it has been reported that NIC either inhibits (Frankish et al., 1995) or enhances (Li et al., 2000a) the formation of the orexigenic polypeptide neuropeptide Y (NPY) in the arcuate nucleus and its concentration in the paraventricular nucleus. The latter authors (Li et al., 2000b) report that NIC selectively decreases a subpopulation of the Y_1 receptors and it has been reported that NPY increases meal size without substantially altering meal number (Leibowitz and Alexander, 1991). These changes could explain the NIC-induced suppression of meal size in the present study, but cannot account for the delayed compensatory increase in meal frequency we observed. Paradoxically, it has been reported that NIC also increases the expression of orexin and its receptors, but decreases orexin-A binding sites (Kane et al., 2000, 2001), which could account for the decrease in FI observed in the present study. Leptin is unlikely to play a role in the effect of NIC on meal patterns inasmuch as plasma leptin level is not reliably altered by NIC administration in humans or animals (Eliasson and Smith, 1999; Hodge et al., 1997; Li et al 2000b; Nicklas et al., 1999; Oeser et al., 1999). Indeed, Miyata and Meguid (2000) reported that administration of 5 mg/kg/day NIC by pumps in male rats for 7 days reduced FI, BW, and plasma leptin concentration. Therefore, the reduced plasma leptin did not correct the NIC-induced suppression of feeding. In the present study, BW was significantly suppressed on Day 6, yet FI was not corrected until Day 10. This would indirectly support the idea that plasma leptin probably does not have a major role in the NIC suppression of FI or in the recovery of FI during prolonged NIC infusion. The aforementioned studies thus do not offer a complete explanation for the pattern of changes in meal pattern noted in NIC rats in the present study.

Consistent with the NIC-induced decrease in FI, BW gain was attenuated in NIC-treated rats. Cessation of NIC resulted in a short period of hyperphagia, but this change in FI did not correct the NIC group's attenuated BW. These data are consistent with many earlier reports (Grunberg et al., 1987; Levin et al., 1987, 1993; Miyata et al., 1999, 2001; Schwid et al., 1992) in which NIC treatment suppressed BW gain during the treatment period and for several days thereafter. However, only part of the suppression of BW can be attributed to NIC decreasing FI. BW was suppressed during the last 5 days of NIC administration and for 14 days thereafter. During these 19 days, FI of the NIC group was not significantly less than the saline group; thus, other factors may be involved in either weight loss or prevention of regaining the lost BW. 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 BW even when FI is only returned to prerestriction levels. The NIC rats, on cessation of drug administration, had either slightly elevated or normal FI, but did not completely regain their lost BW. These data indicate the NIC rats may in fact have been consuming slightly more food relative to their BW than the control rats. These data further indicate that NIC treatment did not produce the normal decrease in energy expenditure associated with reduced food consumption and actually enhanced energy expenditure during the drug withdrawal period. It is known, for example, that NIC can acutely elevate basal metabolic rate in humans and rats (Perkins 1992; Grunberg et al., 1988) and stimulate brown adipose tissue thermogenesis (Lupien and Bray, 1988). These changes would act to limit gains in BW.

We recognize that the present findings are specific to the gender tested (i.e., males) and to the specific procedures utilized (e.g., pellet diet formulation, NIC dose, dose duration). In order to generate a more physiological approach to NIC administration, the present study used repetitive daily injections. While the NIC administration procedures of this study avoid complications associated with surgical implantation of osmotic minipumps, these repetitive daily injections are likely to produce some degree of stress. The fact that control groups were also injected and the absence of NIC-vehicle differences during the light phase during the NIC treatment period argue against the possibility that injection stress rather than NIC produced the changes in meal patterns of this study.

Another important procedural variable is the diet (e.g., 45 mg Bio-Serv pellets vs. other diets used to examine the impact of NIC treatment on meal patterns; Blaha et al., 1998; Miyata and Meguid, 2000; Miyata et al., 1999). The 45-mg pellets are a grain-based milled diet that is similar in composition to the coarsely ground grain-based Purina 5008 (Ralston Purina, St. Louis, MO), used in the above studies and thus one might expect that diet effects would be minimal. However, one would suspect that different patterns might emerge were NIC-treated animals to be presented with a variety of diets differing in macronutrient composition and other factors such as texture.

Our findings were generated using adult male rats. We note that females are particularly resistant to smoking cessation (Perkins, 2001) are likely to use smoking as a means of weight control and, in many instances, are more sensitive to psychostimulants such as NIC (Benowitz and Hatsukami, 1998). Studies in progress are designed to determine, using parallel procedures, the impact of NIC administration and NIC cessation on BW and meal parameters of female rats.

In summary, these results document that administration of NIC to male rats during the dark phase resulted in a reorganization of the microstructure of FI, i.e., initially NIC 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 NIC 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 FI for up to 2 weeks after cessation of NIC is uncertain and awaits further study. Finally, these results differ from studies in which NIC was given continuously 24-h/day and indicate that dark phase NIC administration in rats may represent an appropriate model to study the impact of NIC on meal patterns.

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