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The Effects of Extended Intravenous Nicotine Administration on Body Weight and Meal Patterns in Male Sprague-Dawley Rats

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Abstract

Rationale—Increased appetite and weight gain after cessation is a deterrent for quitting smoking. Attempts to understand the mechanism for these effects using animals have been hampered by the difficulty or inconsistency of modeling the effects seen in humans.

Objective—To examine the effects of extended daily access to intravenous nicotine, via programmed infusions, on body weight and meal patterns in rats.

Methods—IV nicotine infusions (.06 mg/kg/inf) were administered noncontingently, every 30 min throughout the dark cycle and the last three hours of the light cycle, to emulate self-administration. The effect of these infusions on food intake, meal patterns, and weight change were examined relative to a control group during treatment and in a post-nicotine phase.

Results—Nicotine-treated rats gained half the weight that vehicle treated animals gained and ate approximately 20% less food overall than vehicle-treated rats. Whereas a compensatory increase in meal frequency occurred during the dark period to account for smaller meals, no compensation was observed throughout the light period. In a post-nicotine phase, the nicotine group maintained a lower weight for one week and then gained weight back to control levels. The rate of weight gain post-cessation was faster in animals that had received nicotine compared to controls.

Conclusion—Compared to previous studies examining the effects of minipump or i.p. injections of nicotine on food intake, the present study was able to detect previously unknown circadian differences in meal patterns which will be in the development of smoking cessation and weight gain prevention drugs.

Index terms

Intravenous nicotine; meal patterns; nicotine; operant; programmed infusion

Introduction

The prevalence of obesity and overweight in the United States has been steadily increasing and a substantial fraction of overweight may be linked to smoking cessation (Flegal et al. 1995, 2010). Thus, most ex-smokers weigh more than current smokers (Flegal et al. 1995; Klesges et al. 1989; Pistelli et al. 2009; Rodin 1987; Swan and Carmelli 1995). Despite the health risks associated with smoking, many individuals and especially women smoke as part of a weight loss strategy (Camp et al. 1993) and cite a fear of weight gain as a major deterrent to quitting (Jeffery et al. 2000).

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(NOTE: Tables S1–S3 are intended for deposition as on-line supplementary materials)

The mechanism underlying these effects is best approached using animal models, and many studies have shown that nicotine can affect food intake, metabolism, and energy expenditure. In rats, chronic administration of nicotine either via minipump infusion or multiple daily injections results in a dose-dependent decrease in food intake, with varying effects on food intake after treatment is stopped depending on the route of administration (Bellinger et al. 2003b; Grunberg and Bowen 1985; Guan et al. 2004; Miyata et al. 1999 and 2001). In minipump studies that produce steady state plasma nicotine levels, the decrease in food intake often lasts only a few days, and after cessation a significant increase in food intake occurs. In contrast, injection procedures that produce fluctuating nicotine levels often have longer-lasting effects, but fail to show an increase in food consumption after nicotine removal. The procedures in these studies have only partially emulated the human smoker or ex-smoker. One critical difference might be the multiple rapid fluctuations in plasma nicotine that occurs while smoking cigarettes (Benowitz 1983; Henningfield et al. 1993; Henningfield and Keenan 1993; Sanderson et al. 1993), and this could be emulated using intravenous injections of nicotine rather than several intraperitoneal (IP) injections or minipumps (Benowitz 1983). The duration of daily exposure may be a factor as well, since extended daily access to intravenous nicotine is needed to produce somatic withdrawal symptoms in rats (Paterson and Markou 2004). Using a protocol with 23 h/day access, O'Dell et al (2007) reported that rats self-administering nicotine at .06 mg/kg per infusion showed a decrease in food intake across a several week study period. However, these authors did not investigate the effects of intravenous nicotine on food intake and weight gain during withdrawal.

In our experiment, we administered nicotine non-contingently, in a pattern similar to that of self-administering rats. Animals received a nicotine infusion every 30 minutes for 15 hours a day during all of the dark period and the last three hours of the light period. These parameters were chosen to closely approximate self-administration and activity patterns in rats (Buttner and Wollnik 1983; Hanson et al. 1979). We chose to examine the effects of nicotine on food intake using this procedure, rather than the self-administration model, to eliminate any confounds associated with the co-administration of two reinforcers. Pellets were available on a fixed-ratio schedule of reinforcement. Food intake, meal patterns, and changes in body weight were analyzed during nicotine treatment and for 12 days after nicotine treatment ended.

Material and methods

Animals and housing

Male Sprague-Dawley rats initially weighing ~275 g were used. All rats were purchased from Harlan (Indianapolis, IN). The principles described in the Guide for the Care and Use of Laboratory Animals were followed throughout and the project was approved by the UF IACUC. Upon arrival, rats were housed individually in a conventional vivarium that was maintained at 22–26°C and 40–80% relative humidity, on a reverse 12:12 h light:dark cycle (lights off at 10:00). Home cages were polycarbonate, with contact bedding (Sani-Chips, Harlan-Teklad). Purina 5001 Chow pellets were available *ad libitum* for the initial portion of the experiment when animals were housed in their home cages. During the test phase, animals were housed in operant conditioning chambers. Autoclaved tap water was available at all times from a standard bottle and sipper tube.

Surgery and catheter maintenance

Rats were anaesthetized with isoflurane and implanted with an indwelling catheter inserted into the right jugular vein as described more fully elsewhere (Rowland et al. 2008). The catheter was made of Micro-Renathane tubing (.094 cm O.D. x .058 cm I.D., Braintree

Scientific, Braintree, MA). Starting the day after surgery, catheters were flushed with heparinized saline daily. On alternating days for the duration of the study, a broad-spectrum antibiotic enrofloxacin (Baytril, 1.5 mg/day; Sigma Chemicals, St. Louis MO) and streptokinase (200 units/day; Sigma Chemicals, St Louis, MO) were administered in sterile saline via the catheter. Most rats recovered operative weights within 2–3 days. Patency was verified at the end of the experiment by euthanizing the rats with euthanasia pentobarbital (Henry Schein Inc., Melville NY) via the jugular catheter.

Apparatus

Rats were tested in operant conditioning chambers (ENV-008CT; Med Associates, St. Albans, VT) fitted with a single fixed lever next to a food receptacle, and controlled by a computer running MedPC-IV. A single press on the lever caused a feeder to release one 45 mg nutritionally complete food pellet into the receptacle. The experimental chambers were fitted with a 15 W house light providing the same reverse 12:12 cycle as the vivarium and were located in ventilated sound-attenuating cabinets. A tube of autoclaved water was available continuously from a sipper spout on the wall opposite the receptacle. The max/min temperature inside the operant conditioning chambers was recorded periodically and was typically ~1°C above room temperature, between 23–25 °C.

Rats lived in these experimental chambers for 23 h/day, and were removed only for weighing, catheter maintenance, and chamber cleaning. Nicotine or vehicle (sodium phosphate buffer; pH ~ 7.35; 0.1M) were delivered to rats through polyethylene tubing (PE60) protected by a stainless steel spring and attached to a fluid swivel (375/22PS; Instech laboratories, Plymouth Meeting, PA) that, together with a counterbalance, allowed almost unlimited movement within the chamber. The distal end of the swivel was attached via PE60 and an in-line nitrocellulose filter (0.22 µm; Cameo #25ES; Osmonics) to a 30 ml syringe mounted on an infusion pump (PHM-100, 3.33RPM; Med Associates) on a shelf in the cabinet. The timing and duration (1 s) of the infusions was controlled by Med PC IV software.

Nicotine

Nicotine hydrogen tartrate (Sigma Chemicals, St Louis, MO) was dissolved in sodium phosphate buffer (pH~7.35; 0.1M) containing 17 mg heparin per 100 ml. Vehicle was the buffer-heparin solution. The concentrations of the nicotine solution were adjusted to deliver doses of approximately 0.01 or 0.06 mg/kg body weight per injection, calculated as the free base.

Procedure

Two days after catheterization, rats were placed into the experimental chambers for 23 h sessions. No specific lever training for food was conducted and all rats acquired the operant conditioning task within the first night. Baseline measures of body weight and food consumed were measured for 5 days. After the baseline period, rats were divided into three groups matched for body weight. Testing continued with their catheters now attached to the infusion line. For the first 2 days all rats received programmed injections of vehicle. The infusions (0.04 ml) were 1 s in duration every 30 min for the last 3 h of the light phase and for the entire dark phase (12 h). This resulted in a total of 30 injections over a 15 h period within each 23 h test session. This period coincided with maximal feeding and self-administration times of rats as determined in other self-administration studies (unpublished work, 2007). Rats then entered a 12 day treatment phase during which one group of rats received vehicle injections and another group received nicotine. To avoid acute aversive effects of high doses of nicotine, the nicotine group initially received 0.01 mg/kg/injection and then had their drug dose increased over 3 days. The final dose of nicotine, maintained

for the last 9 days of the treatment phase, was 0.06 mg/kg per injection (1.8 mg/kg total daily dose). After the treatment phase, rats entered a cessation phase during which nicotine infusions were replaced by vehicle. The cessation phase lasted 12 days. Body weight and food intake were recorded daily.

Data Analysis

The number of lever presses on the food lever was recorded every 10 min for each 23 h session. Based on Zorilla et al. (2005), meals were defined as 4 or more pellets in any 10 min bin with no more than a 10 min interval between pellets. Daily meal number as well as the number taken during the dark (night) and light (day) phases was determined. Absolute intake was calculated by adding all pellets consumed as meals throughout the 23 h period, the dark period, and the light period; pellets that were not a part of the meal criteria were excluded from analysis. Mean meal size was determined by dividing total pellets by number of meals and was computed separately for dark (12 h) and light (11 h) phases. Data for each rat were subsequently averaged across days to yield seven principal phases: baseline (days 1–5), days 6–7 during which the animal received infusions of the vehicle, treatment days 8–10 during which the dose was incrementing, treatment days 11–14 and 15–19 during which the dose was constant, and cessation days 20–23, 24–27 and 28–31 during which nicotine was replaced with vehicle. Body weights were recorded daily and were reported as change from the initial value at the start of day 1. One-way ANOVAs were used to examine differences in body weight and meal patterns (food intake, meal number and meal size) between animals that received the vehicle or nicotine across groups of days during the treatment and cessation phases. The variables of interest were analyzed throughout the 23 h period as well as the dark and light periods. Detailed results of the ANOVAs are presented in Supplementary on-line materials; significance ($p < .05$) is indicated in the text. Additional analyses using tests for linear regression were conducted on the rate of body weight gain to determine if the slopes differed within groups between the treatment and cessation phases and between groups during the treatment and cessation phases; significance ($p < .05$) is indicated in the text.

Results

Body Weight

During baseline, the groups did not differ in weight gained over the five-day period (Table S1). During the treatment phase, rats that received nicotine gained less weight than those that received the vehicle (Figure 1). This effect was dose-related and was significant on days 8–13. During the cessation phase, the cumulative weight gain for rats that received nicotine continued to be significantly less than controls through day 21, but not thereafter. In order to better understand the relationship between nicotine and weight gain, we compared the slopes between the treatment and cessation phases for the nicotine and control groups and examined whether the two groups differed in their rate of weight gain during cessation. Animals in the control group (Figure 2A) gained weight at a faster rate during treatment than during cessation ($F(1, 238) = 22.764$; $p < .0001$). In contrast, animals in the nicotine group (Figure 2B) gained weight at a faster rate during cessation than treatment ($F(1, 205) = 12.191$; $p < .001$). The two groups were then compared during the treatment (Figure 2C) and cessation (Figure 2D) phases. Rats that received nicotine gained weight at a slower rate during the treatment phase compared to controls ($F(1, 250) = 48.285$; $P < .0001$) and gained weight at a faster rate than those that had received vehicle during the cessation phase ($F(1, 210) = 8.505$; $p < .01$).

Food intake – baseline and treatment phases

Food intake and meal patterns were analyzed in detail for the control and nicotine groups. During both baseline and the first two days of treatment when the two groups received vehicle (data not shown), there were no significant differences in total pellet intake, mean meal size, or meal number during either dark or light periods, or over the entire 23 h.

The treatment phase was separated into three segments: days 8–10 (.01 mg/kg/infusion), days 11–14 and days 15–19 (0.06 mg/kg/infusion; Table S2). Data from controls that received vehicle throughout were segmented in the same way. Animals receiving nicotine ate significantly fewer pellets per 23 h than controls on days 8–10, 11–14, and 15–19 (Figure 3A). When analyzed separately for light and dark periods, nicotine-treated rats ate less during the light on days 11–14 and 15–19 (Figure 3C) but there were not any significant differences between groups during the dark period (Figure 3B).

Examination of meal size revealed that rats given nicotine ate smaller meals than controls on days 11–14 and 15–19. When analyzed separately for light and dark periods, the smaller meal size of the nicotine-treated rats was significant during the light phase on days 11–14, and during the dark phase on days 11–14 and 15–19 (see Supplementary Materials, Figures 1A–C)

Examination of the number of meals revealed no overall effect. However, planned comparisons of day and night phases showed that during the light phase, nicotine-treated animals took slightly but not significantly fewer meals than controls on days 11–14 and 15–19, whereas during the night phase, these rats took significantly more meals than controls on days 15–19 and moderately more on days 11–14 (see Supplementary Materials, Figures 2A–C)

Food intake – cessation phase

The cessation phase was divided into three segments as well: days 20–23, days 24–27, and days 28–31 (Table S3). Animals that received nicotine continued to eat fewer pellets in 23 h compared to those that received the vehicle on days 20–23 (Figure 3A). There were no other significant differences in total food intake between the nicotine-treated and control groups during any of the 4-day segments of the cessation phase (Figures 3B–C). Likewise, the mean meal size and the number of meals did not differ between groups during any of these segments (see Supplementary Materials, Figures 1–C and 2A–C).

Discussion

In the present experiment, we found that noncontingent intravenous nicotine induced changes in body weight coincide with specific changes in meal patterns in both the light and dark periods of the light cycle. As evidenced by decreases in meal size, IV noncontingent nicotine appears to have an effect on satiety mechanisms entrained to the light and dark cycle. Specifically, nicotine reduced the size of meals in both the light and the dark periods while compensating for this decrease in food intake by increasing meal frequency in the dark period only. In addition, the effects of nicotine withdrawal on food intake were also assessed. Though a dramatic rebound in weight gain above control levels was not observed, rats that received nicotine gained weight at a faster rate post-cessation than animals that received the vehicle and no longer differed from controls after approximately one week.

Similar to past studies that administered nicotine to animals via osmotic minipumps (Grunberg and Bowen 1985; Grunberg et al. 1985; Grunberg et al. 1986; Miyata 2001), rats in our experiment showed a slowing of weight gain with nicotine administration. Similar to reports in animals receiving IP injections of nicotine (Bellinger et al. 2003 a and b; Guan et

al. 2004; Kane et al. 2000; Li et al. 2000), this effect persisted throughout the nicotine treatment phase. In contrast to some studies using IP injections (Bellinger et al. 2003b), animals in the present study demonstrated a rebound in weight gain up to control levels that occurred ~1 week after nicotine was removed and was characterized by an increase in the rate of weight gain. These results are in support of studies indicating that individuals who quit smoking show a rebound in weight gain to pre-smoking levels following cessation (Borrelli et al. 1999). Our protocol of noncontingent intravenous nicotine administration therefore mimics the weight loss seen during smoking in humans and the pattern of weight gain after quitting. It would be of interest to examine more prolonged cessation or abstinence periods using this protocol.

In addition to investigating the effects of nicotine on body weight, we also examined changes in meal patterns during and after nicotine treatment. As noted above, whereas decreases in food intake using minipumps are typically transient (Miyata et al 1999 and 2001), the effects we found were sustained over 12 days, a result that complements human studies as well (Klesges et al. 1989). The present study also demonstrated an increase in food intake over days 9–12 after nicotine cessation, compared with controls. This delay in a return to baseline food intake most likely accounts for the persistent body weight difference between groups during this time. We also found that nicotine produced a small decrease in the nocturnal meal size but an increase in meal number, such that total intake was only slightly decreased. Thus, nicotine may speed or amplify satiation, as has been suggested from previous studies using IP injections (Bellinger et al. 2003 a and b; Guan et al. 2004).

In the only other study using intravenous nicotine administration to evaluate changes in meal patterns, O'Dell et al. (2007), found similar decreases in food intake. In their comprehensive analysis of meal patterns, they also reported decreases in total food intake in both the light and dark cycles in rats that received either a low (.03mg/kg/inf) or a high (.06mg/kg/inf) dose of nicotine. We similarly examined the effects of two doses of nicotine on meal patterns (.03 and .06 mg/kg/inf). However, the effects of the lower dose on meal patterns and body weight were not presented because they were intermediate, but tended not to differ from either vehicle or .06 mg/kg/inf groups. The two studies differed in their analysis of total food intake and meal pattern definitions. Whereas O'Dell et al. 2007 normalized their data per Kleiber's law as a function of body weight, we chose not to do this because previous studies indicate that for rats in particular, food intake is stable throughout time and does not increase with age (Keenan et al. 2005). In addition, back transformations of the O'Dell data demonstrate only modest effects of nicotine on total food intake. Our meal pattern analysis enabled us to directly compare our results with previous studies using minipumps and IP injections, whereas O'Dell et al. 2007 analyzed meals in a different manner, making it more difficult to compare their results with prior experiments examining nicotine's effects on meal patterns. While O'Dell et al. 2007 examined some meal parameters that we did not (i.e. meal duration, eating rate, and the circadian regulation of food intake as modeled by cosinor analyses), they did not examine the effects of nicotine on body weight nor did they investigate changes in meal patterns post-cessation.

The purpose of the present study was to examine the effects of nicotine on body weight and meal patterns with a more representative mode of administration, independent of any potential confounds caused by the presence of multiple reinforcers. It is for this reason that we did not choose the self-administration model. One potential problem with our model of noncontingent nicotine administration is the stress response that accompanies experimenter given drugs of abuse. In particular, passive administration of nicotine results in persistent elevated levels of corticosterone compared to response-contingent nicotine (Donny et al. 2000), and there is evidence to indicate that corticotropin releasing factor (CRF) agonists can alter meal structure and decrease food intake (Fekete et al. 2011). However, CRF and

corticosterone levels are also increased during withdrawal (Koob and Volkow 2009), and yet we observed increases in food intake post-cessation rather than decreases. Therefore, changes in stress hormones seem an unlikely cause of the meal pattern changes seen in the present study during nicotine treatment. An additional drawback to using our particular model of noncontingent nicotine administration is that it may not be entirely representative of the pattern of self-administration seen in rats. A possible technique to avoiding confounds associated with self-administration would be the use of yoked controls, which would not only involve the passive administration of nicotine, but would administer the drug in a pattern identical to self-administering rats. Future studies using similar meal pattern analysis should investigate the role of nicotine self-administration or yoked noncontingent nicotine infusions on changes in body weight and meal patterns during treatment and withdrawal.

For similar reasons, we used standard chow pellets instead of a more palatable option. There is mounting evidence to suggest that diet is an important component of nicotine induced weight-loss and weight gain post-cessation. The human literature indicates that ex-smokers who increase their carbohydrate intake and decrease their protein intake seem to be the ex-smokers who are gaining the most weight (Rodin 1987). There seems to be an increase in preference for sweet and high carbohydrate foods during nicotine cessation, whereas smoking cigarettes is associated with a decrease in the consumption of sweet foods (Bowen et al. 1991; Saules et al. 2004; Spring et al. 2003). In addition, animals that receive chronic infusions of nicotine via minipumps eat more sweet high or low calorie foods compared to standard chow, (Grunberg et al. 1985) and the weight attenuating effect for nicotine in rats on a high fat diet is increased compared to those on a standard normal chow diet, an effect that is accompanied by a decrease in abdominal fat (Mangubat et al. 2012). Changes in fat distribution have been seen in human ex-smokers as well, particularly through altered levels of visceral fat (Chiolero et al. 2008; Pisinger and Jorgensen 2007) and these modifications may be influenced by diet. It is possible then, that the current study underestimated the magnitude of nicotine's effects on body weight by using standard chow. However, changes in meal size have been seen in rats given both high- and low-fat diets, though food selection may influence the observed pattern of increases in meal frequency (Wellman et al. 2005). Future studies using intravenous nicotine administration in rats should consider examining changes in food preferences during nicotine treatment and post-cessation for effects on body weight, fat distribution, and changes in meal patterns.

Unique to our study was the discovery of a bigger net effect of nicotine on meal size during the light period compared to the dark, a time at which very little nicotine is being delivered. During this time, the smaller meal size persisted and there was no compensatory increase in meal number. Thus, most of the total decrease in food intake during nicotine came from the light period. To examine this more closely, we subsequently analyzed total food intake, meal number, and average meal size during only the first 8 h of the light when animals received no infusions of nicotine, compared with the last 3 h of the light when the nicotine infusions were occurring. There was no clear evidence that nicotine suppressed intake more during one than the other of these divisions, although it should be noted that total intake was similar in these two periods because food activity in rats is naturally low during the first 8 h of the day, then shows a crepuscular rise. Collectively, these results suggest that either a long half-life metabolite of nicotine (such as cotinine), an effect of nicotine on gene expression, and/or an interaction with a circadian oscillator are important determinants of the action of nicotine on food intake.

The specific light cycle effects observed in this study may be partially mediated by the rat's sleep wake cycle, in that rats naturally eat less during the day because they are asleep much of the time. In our experiment, we did not measure the extent nicotine (taken mostly at night) affected daytime sleep patterns. Nicotine dose dependently decreases REM and slow-

wave sleep and increases wakefulness in rats, effects that are mediated by nAChRs (Salin-Pascual et al. 1999), so it is possible that sleep is increased during the daytime when nicotine administration is low. We note that our programmed infusion protocol in part emulates a human smoker's steady pattern with an 8 h hiatus while they sleep. In addition to direct effects on food intake, nicotine may affect food intake and meal patterns via metabolic changes such as lipolysis that also normally have a circadian cycle (Le Magnen 1985).

Donny and colleagues (2011) have recently proposed that nicotine exerts its effects on food intake in two principal ways: by augmenting satiation and by increasing the reinforcing efficacy of food. The results presented within this paper provide evidence in support of both conclusions. We found a decrease in meal size, which is consistent with augmented satiation and accounts for the decrease in body weight gained throughout the period of nicotine administration. In the cessation period, we found a gradual, yet delayed, increase in food intake and body weight which is consistent with the claim that food motivation is increased over time after nicotine cessation, but may be delayed by a week or two (Bellinger et al. 2003b; Donny et al. 2011).

Insight into the mechanisms involved in nicotine's weight attenuating effects via changes in food motivation through alterations in meal patterns provides direction for those looking to create pharmacotherapies that will aid in smoking cessation but also prevent weight gain. The development of an animal model that includes changes in food intake when drug treatment is stopped, will allow preclinical validation of pharmacotherapies. The mechanisms underlying nicotine's effects on food intake, including its effects on satiation, are complex and involve many different neuropeptides and neurotransmitters. For a comprehensive review of this topic, please consult DiLeone et al. 2012 or Zoli and Picciotto 2012. In summary, nicotinic acetylcholine receptors (nAChRs) are located throughout enteric, sensory, and autonomic neurons, and it is likely that smoking activates receptors in the periphery that are involved in food intake (Jo et al. 2002; Zoli and Picciotto 2012). There are also nAChRs located throughout the hypothalamus, particularly within the arcuate nucleus and the lateral hypothalamus (LH; Jo et al. 2002). Acute nicotine administration reduces food intake and decreases neuropeptide Y (NPY) and NPY mRNA levels in this area (Meguid et al. 2000), and these may serve as possible mechanisms behind nicotine's effects on meal size (Leibowitz and Alexander 1991). Nicotine may also indirectly influence leptin secretion or biosynthesis, (Li et al. 2000), as well as levels of melanin-concentrating hormone (MCH) neurons (Jo et al. 2002). Similarly, there are markers for cholinergic transmission on pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART) neurons in the arcuate nucleus of the hypothalamus (Meister et al. 2006). Nicotine increases the secretion and circulating levels of POMC (Li et al. 2001), effects mRNA levels of CART in the hypothalamus and causes a change in meal size similar to that seen with intraventricular injections of CART near the hypothalamus (Dandekar et al. 2011; Kramer et al. 2007).

In summary, using a protocol that more accurately emulates the episodic and circadian nature of human nicotine administration, we have found effects of nicotine on food intake, specifically via satiety mechanisms, consistent with previous results. Surprisingly, we uncovered some indirect effects of nicotine on food intake, as demonstrated by a decrease in meal size in the light cycle when nicotine levels are lowest. In addition, we were able to successfully model the decreases in weight gain that accompany nicotine administration and the increased rate of weight gain seen post-cessation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Nicotine: body weight change over treatment and cessation. Shown are mean $\pm$ SEM cumulative change in body weight (g) on consecutive days of treatment and cessation phases, in animals that received either vehicle ($n = 9$) or 0.06 mg/kg/inf of nicotine ($n = 8$). $^{\wedge}P = .07$, $^*P < 0.05$, $^{**}P < 0.01$ difference between vehicle and nicotine

Fig. 2.

A. The difference between the rate of body weight gain throughout the treatment phase and the cessation phase in animals given vehicle, expressed as a percentage of the body weight at the end of the previous phase ($n = 9$). B. The difference between the rate of body weight gain throughout the treatment phase and the cessation phase in animals given nicotine, expressed as a percentage of the body weight at the end of the previous phase ($n = 8$). The difference between the rate of body weight gain throughout the treatment phase between animals given vehicle ($n = 9$) and those given nicotine ($n = 8$). D. The difference between the rate of body weight gain throughout the cessation phase between animals given vehicle ($n = 9$) and those given nicotine ($n = 8$).

Fig. 3. Total number of pellets. Shown are mean $\pm$ SEM total lever presses for pellets, averaged over the ranges of days indicated during 23 hours (a), the dark period (b), or the light period (c), in animals that received either vehicle ($n = 9$) or 0.06 mg/kg/inf of nicotine ($n = 8$) during the treatment phase. $^{\wedge}P < .01$, $*P < 0.05$, $**P < 0.01$ difference between vehicle and nicotine