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Effects of nicotine withdrawal on performance under a progressive-ratio schedule of sucrose pellet delivery in rats

Mark G. LeSage [a,b,*], Danielle Burroughs [a], Paul R. Pentel [a,b,c]

[a] Minneapolis Medical Research Foundation, 914 South 8th Street, D3-860, Minneapolis, MN 55404, United States
[b] Department of Medicine, University of Minnesota, Minneapolis, MN 55455, United States
[c] Department of Medicine, Hennepin County Medical Center, Minneapolis, MN 55404, United States

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Abstract

Although numerous studies have examined the motivational effects of nicotine withdrawal using intracranial self-stimulation (ICSS) threshold assays, relatively few have employed other methods for assessing motivation that use naturally reinforcing stimuli (e.g., food). The objective of the present study was to determine the effects of nicotine withdrawal on motivation using a progressive-ratio (PR) schedule of sucrose pellet delivery. Rats were trained to respond for sucrose pellets under a PR schedule. When stable breaking points and response rates were achieved, PR sessions were suspended and rats were exposed to a continuous infusion of saline or nicotine (3.2 or 8.0 mg/kg/day of the base) via subcutaneous osmotic minipump for nine days. On day nine, pumps were removed. PR sessions resumed 22 h later and continued daily for five consecutive days. Only rats exposed to 8.0 mg/kg/day nicotine exhibited a significant decrease in breaking point and overall response rate compared to saline-exposed rats on day one of nicotine withdrawal. These rats also showed an increasing trend in breaking point and overall response rate over the course of withdrawal, such that these measures were significantly increased on day five of withdrawal compared to baseline. Response rates under each ratio in the PR progression in rats exposed to 8.0 mg/kg/day did not differ from baseline or from those in saline-treated rats, suggesting suppression of breaking points and overall response rates were not attributable to nonspecific motor impairment. In addition, changes in performance throughout the protocol were not associated with changes in body weight. Consistent with findings from ICSS studies, the present study demonstrates that nicotine withdrawal can produce a motivational deficit as indexed under a PR schedule. However, in contrast to ICSS, PR performance appears to be sensitive to increases in motivation late in the withdrawal period. Therefore, PR schedules of natural reinforcement may provide information on the motivational effects of nicotine withdrawal complimentary to that obtained from ICSS threshold studies.

Keywords: Nicotine; Progressive-ratio schedule; Sucrose; Rats; Withdrawal; Motivation

1. Introduction

There is substantial evidence that abstinence from tobacco use produces a withdrawal syndrome in humans. This withdrawal syndrome is characterized by a number of somatic and affective signs and symptoms, including bradycardia, gastrointestinal discomfort, depressed mood, irritability, anxiety, restlessness, difficulty concentrating, sleep disturbance, increased food intake, and craving for tobacco (Hall et al., 1989; Hatsukami et al., 1993, 1984; Hughes et al., 1991; Shiffman and Jarvik, 1976). Some symptoms (irritability, difficulty concentrating) peak within two days of abstinence and decline to pre-abstinence levels in two to three weeks, while other symptoms (increased appetite, craving) peak within two days and remain elevated for up to four weeks (Vandrey et al., 2005; West et al., 1989). There is also substantial variability in the time course of withdrawal symptoms across smokers (Piasecki et al., 1998). Tobacco withdrawal is important because many studies have shown it is a potential factor that motivates relapse and undermines cessation success (Shiffman et al., 2004; Watkins et al., 2000). Therefore, a better understanding of the

* Corresponding author. Minneapolis Medical Research Foundation, 914 South 8th Street, D3-860, Minneapolis, MN 55404, United States. Tel./fax: +1 612 347 5118.
E-mail address: mlesage@mmrf.org (M.G. LeSage).

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neuropharmacological and behavioral mechanisms mediating tobacco withdrawal may be helpful in the design of treatments for tobacco dependence. Animal models are potentially useful for this purpose, since numerous studies have demonstrated various signs of nicotine withdrawal in rodents (Kenny and Markou, 2001).

Distinct somatic signs of nicotine withdrawal in rodents have been identified and characterized (Malin, 2001), which include gasping, writhing, teeth chattering, cheek tremors, head shakes, and body shakes, among other less frequent signs. Another behavioral sign of nicotine withdrawal is an increase in brain-stimulation reward threshold. For example, Epping-Jordan et al. (1998) demonstrated that the threshold level of electrical current that maintains intracranial self-stimulation (ICSS) behavior is increased during nicotine withdrawal. This effect is considered to model the motivational and affective changes that occur during tobacco withdrawal in humans, such as the loss of interest or pleasure in normally rewarding activities (i.e., "anhedonia") that is associated with depressed mood. The somatic-signs and ICSS models have been used increasingly as tools to study nicotine dependence and have been very useful in elucidating potential neuropharmacological mechanisms underlying nicotine withdrawal (Kenny and Markou, 2001; Malin, 2001).

Relatively few studies have examined other behavioral effects of nicotine withdrawal, such as changes in operant behavior maintained by natural reinforcers (e.g., food). Such work is important to determine the relevance of withdrawal-induced changes in brain-reward threshold to motivation for natural reinforcers. To the extent that elevations in ICSS threshold reflect changes in fundamental motivation and reinforcement processes (Markou and Koob, 1993; Watkins et al., 2000), nicotine withdrawal should decrease motivation for natural reinforcers. Indeed, significant suppression of response rates under operant schedules of food delivery have been reported in rats (Carroll et al., 1989; Corrigall et al., 1989) and mice (Rosecrans et al., 1989). However, some studies have failed to show any disruptive effect of nicotine withdrawal on food-maintained operant behavior in rats (Helton et al., 1993; Rosecrans et al., 1989). Differences in the types of operant schedules employed, daily dose of nicotine administered, duration and route of nicotine administration, strain of the animal, and housing conditions (e.g., light/dark cycle) may account for the discrepancies across studies. For example, significant suppression of responding for a sweet-tasting glucose+saccharin solution under a fixed-ratio (FR) schedule and food pellets under a multiple fixed-interval (FI) schedule were observed during nicotine withdrawal in the studies by Carroll et al. (1989) and Corrigall et al. (1989), respectively. However, Helton et al. (1993) observed no effects on food-maintained responding under a light/dark discrimination task. These findings suggest that the degree of disruption of operant behavior during nicotine withdrawal may be dependent upon the reinforcement schedule maintaining behavior.

Studies of the effects of nicotine withdrawal on food-maintained behavior are also important insofar as they may provide insight into the mechanisms underlying the increase in food consumption and body weight during smoking cessation in humans that have been reported in numerous studies (Hall et al., 1989; Hatsukami et al., 1993; Lerman et al., 2004). Several animal studies have shown that body weight and intake of sweet-tasting food increase during nicotine withdrawal in rats that have free access to food, while intake of standard lab chow does not change (Grunberg, 1982; Grunberg et al., 1985, 1986, 1988a; Winders and Grunberg, 1990). These studies contrast with those demonstrating a general disruption of food-maintained schedule-controlled behavior discussed above, at least to the extent that they suggest the motivation to consume sweet-tasting food is increased during nicotine withdrawal, while motivation to consume regular food is unaffected. Thus, whether the motivation to consume food is increased or decreased during nicotine withdrawal may depend upon the conditions of access to food.

The purpose of the present study was to examine the motivational effects of nicotine withdrawal (e.g., anhedonia) using a progressive-ratio (PR) schedule of sucrose pellet delivery. PR schedules are commonly used to assess the reinforcing efficacy or motivational strength of a stimulus. Therefore, some have proposed that withdrawal-induced decreases in PR-maintained behavior reflect the reduced motivation or anhedonia associated with drug withdrawal (Barr and Phillips, 1999; Willner, 1991). PR schedules may therefore be useful in the development of animal models for understanding the factors mediating the anhedonia associated with smoking cessation. Under a PR schedule, an increasing number of responses is required to produce successive reinforcers within a session. The breaking point, defined as either the largest response requirement completed or the number of reinforcers earned in a session, is the primary dependent measure. Because the breaking point varies as a function of either deprivation level or reinforcer magnitude, it is thought to reflect the efficacy or motivational strength of the reinforcer (Hodos and Kalman, 1963; Kennedy and Baldwin, 1972). Procedures used in the present study were similar to those employed in a study by Barr and Phillips (1999) in which significant decreases in breaking point under a PR schedule of sucrose delivery were observed during amphetamine withdrawal.

2. Materials and methods

2.1. Animals

Experimentally-naive male Holtzman rats weighing 300–400 g were maintained under a restricted feeding regimen (approx. 20 g/day rat chow) to maintain stable body weight during the experiment. Each rat was individually housed in a temperature- and humidity-controlled colony room with unlimited access to water under a reversed 12 h light/dark cycle (lights off at 10:00 am). Animal husbandry and experimental protocols were approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation and were in accordance with the 1996 NIH Guide for the Care and Use of Laboratory Animals.

2.2. Apparatus

Subjects were tested in operant-conditioning chambers (Coulbourn Instruments, Allentown, PA), measuring 29 cm long, 33 cm

high, and 26 cm wide. Two response levers were located on the front wall 10 cm above the chamber floor on either side of a food aperture located 2 cm above the floor. Stimulus lights were located 2 cm above each response lever. Each chamber was placed inside a sound-attenuating cubicle equipped with an exhaust fan that provided masking noise. A computer with MED-PC IV software (Med Associates, Inc., St. Albans, VT) was used for operating the apparatus and recording data.

2.3. Drugs

Nicotine bitartrate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline. The pH of the solution was adjusted to 7.4 with dilute NaOH. Nicotine doses are expressed as the base.

2.4. Progressive-ratio training

Twenty-four rats were initially exposed to a conjoint variable-time (VT) 60 s FR 1 schedule of sucrose pellet delivery for magazine and lever-press training. Under this schedule, a single 45 mg sucrose pellet (Research Diets, New Brunswick, NJ) was delivered on average every 60 s, and each lever press on the active lever also produced a sucrose pellet. Responses on the inactive lever were recorded but had no programmed consequence. All rats learned to procure sucrose pellets and learned to lever press within 4 sessions. Once lever pressing occurred reliably, the VT 60 component was terminated and the FR value was gradually increased to FR 10 over several sessions. After response rates were stable under the FR 10 schedule (i.e., no discernible trend across five consecutive sessions), rats were placed on a PR schedule similar to that used by Barr and Phillips (1999) to study the motivational effects of amphetamine withdrawal. Under this schedule, the ratio requirement for sucrose pellet delivery began at two in each session and increased each time a sucrose pellet was earned according to the following progression: 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 179, 219, 268, 328, 402. A session terminated when a rat failed to complete a ratio within 1 h. The number of reinforcers earned per session defined the breaking point. Sessions were conducted five days per week at about 9:00 am.

2.5. Nicotine delivery and withdrawal

After breaking points and overall response rates stabilized under the PR schedule (no significant trend across five consecutive sessions), PR sessions were terminated and rats were implanted with a subcutaneous osmotic minipump (Alzet model 2ML2, Durect Corp, Cupertino, CA) that delivered saline, 3.2, or 8.0 mg/kg/day nicotine for nine consecutive days. Eight rats were used for saline and each nicotine dose. These nicotine infusion rates were selected because they are within the range of those typically used in studies of nicotine withdrawal (Kenny and Markou, 2001; Malin, 2001) and have been shown to reduce nicotine self-administration in rats without producing toxic side effects (LeSage et al., 2003, 2002). Rats were not given the opportunity to perform under the PR schedule during the nicotine infusion period in order to avoid the development of behavioral dependence, resulting from behavioral adaptations (i.e., conditioning) that could occur during nicotine exposure (see Goudie and Demellweek, 1986). Therefore, any changes in performance during the withdrawal period would be an expression of pharmacological dependence, resulting from pharmacological adaptations during nicotine exposure. Pumps were implanted on a Friday after the last baseline PR session. Thus, performance had to be stable across the entire week before pumps could be implanted. On the second Sunday after pump implantation (infusion day nine), pumps were removed and PR sessions resumed 22 h later on Monday morning. This withdrawal time-point was chosen because it is associated with a significant elevation in ICSS threshold during nicotine withdrawal in rats (e.g., Epping-Jordan et al., 1998). PR sessions continued daily for five consecutive days.

2.6. Surgery

Osmotic minipumps were implanted s.c. under isoflurane anesthesia and placed in the intra-scapular area. The pumps delivered a volume of 5 μl/h. The concentration of nicotine was adjusted for differences in body weight. Following surgery, rats were returned to their home cages for nine days. Pumps were removed under brief isoflurane anesthesia on infusion day nine.

2.7. Data analysis

An alpha level of 0.05 was used to determine significance in all statistical analyses. The baseline breaking point and overall response rate were calculated for each rat as the mean number of reinforcers earned and active-lever responses per minute, respectively, during the last five sessions prior to nicotine administration. Breaking point and overall response rate during each day of nicotine withdrawal were calculated as a percentage of baseline. In addition, the response rate under each ratio in the PR progression was calculated in order to examine within-session patterns of responding. The effect of nicotine withdrawal on breaking point and overall response rate was analyzed via two-factor ANOVA, with nicotine infusion rate as a non-repeated factor and withdrawal day as a repeated factor. A significant main effect or interaction was followed by Bonferroni post hoc tests to compare differences between groups at each withdrawal day. Within group changes in breaking point and overall response rate between baseline and withdrawal sessions were analyzed by repeated measures ANOVA with Bonferroni post hoc tests. In addition, significant repeated measures ANOVA was followed by a post hoc test for linear trend to examine the increasing trends in breaking point and response rate that were apparent over the course of withdrawal. Within each group, response rates under each ratio in the PR progression on withdrawal day one were compared to those during baseline by a two-factor ANOVA with phase (baseline and withdrawal) and ratio as factors, followed by Bonferroni post tests. In addition, response rates under each ratio in the PR progression on withdrawal day 1 were compared between groups by two-factor ANOVA with dose and ratio as factors, followed by Bonferroni post tests.

3. Results

3.1. Breaking points

Baseline breaking points were 11.6±0.50, 12.1±0.48, and 12.4±0.69 SEM reinforcers per session for rats exposed to saline, 3.2, and 8.0 mg/kg/day, respectively. No statistically significant difference in baseline breaking points between groups was observed. Fig. 1 shows mean breaking points expressed as a percentage of baseline during each day of withdrawal in rats exposed to each continuous infusion condition. Two-factor ANOVA indicated a non-significant main effect of nicotine infusion rate (i.e., no differences between groups pooled across all withdrawal days, $F=0.60$, $p=0.56$), but a significant infusion rate × withdrawal day interaction ($F=3.8$, $p<0.001$). No statistically significant differences between rats exposed to 3.2 mg/kg/day nicotine and those exposed to saline were observed. In addition, although breaking points were apparently lower on withdrawal day one and higher on withdrawal day four relative to baseline in rats exposed to 3.2 mg/kg/day, these differences were not statistically significant. In contrast, breaking points were significantly lower on the first day of nicotine withdrawal in rats exposed to 8.0 mg/kg/day than those exposed to saline ($t=3.12$, $p<0.05$). In addition, post hoc tests following a significant repeated measures ANOVA ($F=7.47$, $p<0.001$) showed that breaking points in this group were significantly decreased relative to baseline on withdrawal day one ($t=3.02$, $p<0.01$) and increased on withdrawal day five ($t=3.02$, $p<0.01$). Finally, linear trend analysis indicated a significant increasing trend in breaking point across withdrawal days for rats exposed to 8.0 mg/kg/day ($r=0.56$, $p<0.001$).

3.2. Response rates

Baseline overall response rates were 5.4±0.42, 5.3±0.36, and 6.3±0.91 SEM responses per minute for rats exposed to saline, 3.2, and 8.0 mg/kg/day, respectively. No statistically significant difference in baseline response rate between groups was observed. Fig. 2 shows mean overall response rates expressed as a percentage of baseline during each day of withdrawal in rats exposed to each continuous infusion condition. Two-factor ANOVA indicated a non-significant main effect of nicotine infusion rate ($F=0.18$, $p=0.83$), but a significant infusion rate × withdrawal day interaction ($F=3.82$,

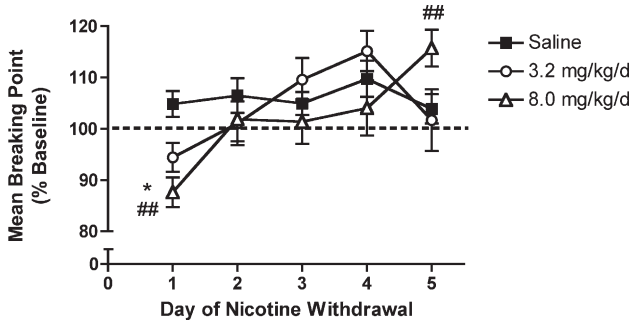

Fig. 1. Breaking points during the five days of withdrawal in rats exposed to saline or the indicated infusion rate of nicotine. Data are expressed as a percentage of baseline performance (mean of the last five sessions) prior to saline or nicotine administration. Each point represents the mean (±SEM) of eight rats. The horizontal dashed line indicates baseline level of performance. *Significantly different from saline group, $p<0.05$. ##Significantly different from the respective group's baseline, $p<0.01$.

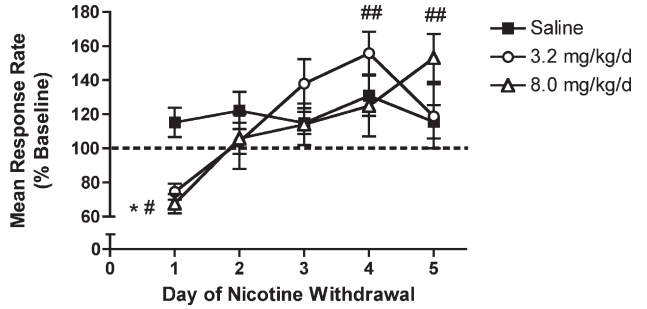

Fig. 2. Overall response rates during the five days of nicotine withdrawal. #Significantly different from the respective group's baseline, $p<0.05$. See Fig. 1 for further details.

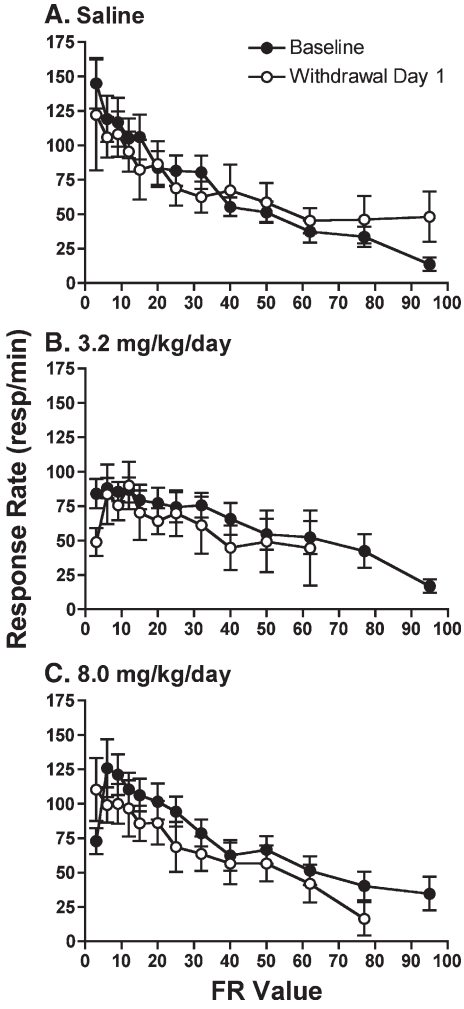

Fig. 3. Mean (±SEM) response rate at each ratio under the PR schedule during the last five days of baseline (closed circles) and the first day of nicotine withdrawal (open circles). Panel A represents data from rats exposed to saline, Panel B rats exposed to 3.2 mg/kg/day nicotine, and Panel C those exposed to 8.0 mg/kg/day. Data are not shown for ratios completed by fewer than four rats.

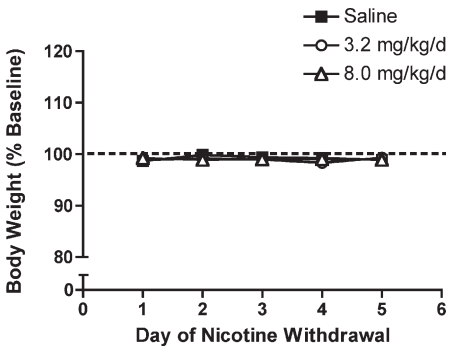

Fig. 4. Mean body weights during the five days of withdrawal in rats exposed to saline or the indicated infusion rate of nicotine. See Fig. 1 for further details.

$p<0.001$). No statistically significant differences between the group exposed to 3.2 mg/kg/day nicotine and that exposed to saline were observed on any day of withdrawal. Mean response rates were decreased on withdrawal day one in rats exposed to 3.2 mg/kg/day relative to baseline, but this difference was not statistically significant. However, this group exhibited a significantly higher response rate on withdrawal day 4 compared to baseline ($t=3.62$, $p<0.01$). In rats exposed to 8.0 mg/kg/day, response rates were significantly lower on the first day of nicotine withdrawal compared to rats exposed to saline ($t=3.12$, $p<0.05$). In addition, post hoc tests following a significant repeated measures ANOVA ($F=7.47$, $p<0.001$) showed that response rates in this group were significantly decreased relative to baseline on withdrawal day one ($t=2.35$, $p<0.05$) and increased on withdrawal day five ($t=4.27$, $p<0.001$). Finally, linear trend analysis indicated a significant increasing trend in response rate across withdrawal days for rats exposed to 8.0 mg/kg/day ($r=0.60$, $p<0.001$).

Fig. 3 shows mean responses rates under each completed ratio in the PR progression during baseline sessions and on the first day of nicotine withdrawal in each group of rats. No significant differences between baseline and withdrawal day one were observed in any group. Moreover, no significant differences in response rate under each ratio were observed between groups on withdrawal day one.

Fig. 4 shows the mean body weight of rats during the withdrawal phase relative to their baseline body weight prior to saline or nicotine administration. No significant changes in body weight were observed either within or between groups. The amount of food needed to maintain stable body weights did not change during or after nicotine administration (data not shown).

4. Discussion

The main findings of the present study are that (a) breaking point and overall response rate under a PR schedule of sucrose pellet delivery were significantly reduced on the first day of nicotine withdrawal following exposure to 8.0 mg/kg/day nicotine, (b) an increasing trend in breaking points and overall response rates was observed over the course of withdrawal from 8.0 mg/kg/day nicotine, such that these measures returned to baseline levels by the second day of withdrawal and were increased compared to baseline on the fifth day of withdrawal, and (c) response rates under each ratio in the PR progression on the first day of withdrawal were not significantly different from baseline or between groups, suggesting that the decrease in overall response rate on this day was not due to a nonspecific disruption of the ability to perform the lever-press response. Rather, it was due to the completion of fewer ratios during the session, while the session duration was similar to that during baseline (Fig. 3).

The present data are consistent with prior studies in rats showing that nicotine withdrawal disrupts responding under other operant schedules of food delivery (Corrigall et al., 1989) and delivery of a glucose+saccharin solution (Carroll et al., 1989). In these previous studies, behavioral sessions continued during the course of nicotine administration and marked tolerance to the rate-suppressant effects of nicotine were observed. Thus, the behavioral disruption that occurred during nicotine withdrawal in those studies could have been due to the development of behavioral dependence (i.e., behavioral adaptations during nicotine exposure) in addition to pharmacological dependence (i.e., pharmacological adaptations during nicotine exposure). Because PR sessions were suspended during nicotine administration in the present study, the disruption in PR performance during withdrawal can only be attributed to development of pharmacological dependence during nicotine exposure. Thus the present findings demonstrate that withdrawal-induced disruption of operant behavior in rats does not require the development of behavioral tolerance to nicotine exposure. Rosecrans et al. (1989) have also demonstrated that disruption of schedule-controlled behavior in mice during nicotine withdrawal is primarily attributable to pharmacological dependence.

To the extent that PR performance provides a measure of motivation (Hodos and Kalman, 1963; Kennedy and Baldwin, 1972), the present findings demonstrate that nicotine withdrawal induces a transient decrease in the reinforcing efficacy of sucrose pellets and, hence, the motivation to work for sucrose pellets. This finding is consistent with several prior studies demonstrating that nicotine withdrawal causes a transient elevation in the threshold level of electrical brain stimulation that can maintain ICSS behavior (Kenny and Markou, 2001), which is considered to reflect a disruption of fundamental motivational and reinforcement processes (Markou and Koob, 1993). However, the present findings differ somewhat from these studies in three ways. First, the duration of disruption in performance was much shorter (one day) than the effect on ICSS thresholds reported in previous studies (four days, Epping-Jordan et al., 1998). Second, the nicotine infusion rate required to observe withdrawal effects (8.0 mg/kg/day) was higher than that typically needed to produce withdrawal effects on ICSS thresholds (3.2 mg/kg/day). Third, the percent change in performance on the first day of withdrawal in the present study (12%) was smaller than that typically reported for ICSS thresholds (approximately 25% averaged across studies, (Epping-Jordan et al., 1998; Skjei and Markou, 2003). Taken together, these differences suggest that PR schedules of sucrose reinforcement appear to be less sensitive than ICSS threshold assays. Differences in the relative sensitivity of ICSS and PR schedules of natural reinforcement to

amphetamine withdrawal are also evident in the literature (Barr and Phillips, 1999; Orsini et al., 2001; Russig et al., 2003).

On the other hand, PR schedules of natural reinforcement (e.g., food delivery in particular) may provide additional information not apparent in ICSS threshold studies. In humans and nonhumans, studies have shown an increase in consumption of sweet-tasting, high-calorie food during nicotine withdrawal (Grunberg, 1982; Grunberg et al., 1985, 1988a,b; Hatsukami et al., 1984, 1993; Hall et al., 1989), suggesting that nicotine withdrawal increases the reinforcing efficacy and motivation to consume that type of food. Because decreases in ICSS threshold are thought to reflect an enhancement of fundamental motivation and reinforcement processes, one might predict from the food-intake studies that ICSS thresholds would be decreased at some point during nicotine withdrawal. However, to our knowledge no studies have shown such an effect. In contrast, the increase in sucrose-maintained PR performance observed late in the withdrawal period in the present study is consistent with the animal and human studies showing increased consumption of sweet foods during nicotine withdrawal. The differences in effects of nicotine withdrawal on ICSS thresholds and food-maintained PR performance suggest there may be differences between the mechanisms underlying the withdrawal-induced changes in motivation for unnatural and natural reinforcers. However, direct comparison of the effects of nicotine withdrawal on PR performance maintained by food versus electrical brain stimulation is needed to confirm this possibility.

The continuous nicotine infusion rate required to produce withdrawal effects on PR performance in the present study (8 mg/kg/day) is within the range of that needed to alter intake of freely-available food in previous studies (6–12 mg/kg/day; Grunberg, 1982; Grunberg et al., 1985, 1988a; Carroll et al., 1989). These infusion rates are considerably higher than those needed to induce somatic signs of nicotine withdrawal (1–3 mg/kg/day; Malin, 2001), suggesting that measures of food intake may be less sensitive to nicotine withdrawal compared to somatic signs. However, the time course of withdrawal-induced suppression of PR responding for sucrose in the present study and intake of freely-available food (Carroll et al., 1989) is comparable to that for somatic signs of withdrawal, which can dissipate within 48 h after cessation of nicotine administration (Malin, 2001).

The decrease in PR performance on withdrawal day one in the present study contrasts with previous studies that showed only increases in intake of sweet-tasting, freely-available food during nicotine withdrawal (Grunberg, 1982; Grunberg et al., 1985, 1986, 1988a,b; Winders and Grunberg, 1990), but is consistent with studies showing only decreases in responding under operant schedules of food delivery (Corrigall et al., 1989; Rosecrans et al., 1989). These findings suggest that the degree of effort needed to procure food may modulate the effects of nicotine withdrawal on food intake. However, it is important to note that in the studies reporting increases in food intake, the level of food intake was averaged across several days of withdrawal. When daily measures have been reported, decreases in food intake have been observed on the first day of nicotine withdrawal followed by increases in intake after three to six days of withdrawal (Carroll et al., 1989). The increase in PR performance on the fifth day of nicotine withdrawal in the present study is consistent with the prior studies showing nicotine withdrawal-induced increases in intake of sweet-tasting food. In contrast, studies using operant schedules of food pellet delivery have not reported increases in responding during nicotine withdrawal, suggesting that nicotine withdrawal may selectively increase the reinforcing efficacy of sweet foods compared to relatively bland food. Thus taken together with prior studies, the biphasic change in PR performance during the course of nicotine withdrawal in the present study suggests the effect of nicotine withdrawal on food intake may depend upon a) the conditions of access to food (i.e., free-access versus access under operant schedules), b) the type of operant schedule of food delivery maintaining responding, c) the time-point during nicotine withdrawal when food intake is measured, and d) the type of reinforcer maintaining responding.

The mechanisms mediating the effects of nicotine withdrawal in the present study are unclear, but withdrawal-induced changes in metabolism may play a role. Continuous nicotine infusion at doses comparable to that used in the present study results in a decrease in plasma insulin levels (Grunberg et al., 1988b). It has been suggested that nicotine withdrawal may lead to an increase in insulin levels, thereby increasing hunger for sweet foods (Grunberg et al., 1985, 1988b). This claim is supported by a previous finding that insulin treatment increases intake of sucrose in rats (Lucas and Sclafani, 1988), as well as the present finding that the breaking point for sucrose was increased on withdrawal day five. However, the decrease in breaking point on day one is inconsistent with this interpretation, suggesting a more complex mechanism is involved. Studies examining the daily time course of changes in biomarkers of metabolism during nicotine withdrawal in rats would help clarify this issue.

The present findings are relevant to studies examining the effects of continuous nicotine infusion on nicotine self-administration. The continuous nicotine infusions rates used in the present study have been shown to decrease nicotine self-administration in rats, which remains suppressed for up to five days after the continuous infusion is terminated (LeSage et al., 2002, 2003). The mechanism for this persistent suppression of nicotine self-administration is not entirely clear. Findings from ICSS threshold studies suggest that it may reflect a disruption of motivational processes, since the duration of elevation in ICSS thresholds during withdrawal from similar nicotine infusion rates is comparable to the duration of suppression of nicotine self-administration. However, the absence of any motivational deficit for sucrose reinforcement beyond the first day of withdrawal in the present study raises some doubt about this interpretation. This issue further suggests that ICSS thresholds and PR performance may provide different information about the motivational effects of nicotine withdrawal.

In conclusion, the present study demonstrates that performance under a PR schedule of sucrose pellet delivery is sensitive to the motivational effects of nicotine withdrawal, thus extending the generality of previous findings with ICSS threshold assays. The evidence of increased motivation after five days of nicotine withdrawal in the present study contrasts with findings

from ICSS threshold studies, suggesting that PR schedules that employ natural reinforcers may provide unique information complementary to that obtained with ICSS threshold procedures. Therefore, PR schedules may be a useful tool for studying the behavioral and neuropharmacological mechanisms of nicotine withdrawal. Given the limited understanding of mechanisms underlying nicotine withdrawal, the availability of multiple approaches to measure its effects may be useful.

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