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'Nicotine deprivation effect' in rats with intermittent 23-hour access to intravenous nicotine self-administration

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Abstract

Our previous work demonstrates that rats allowed extended 23-hour access to intravenous nicotine self-administration (IVSA) display voluntary, dose-related levels of nicotine intake (i.e., higher doses result in higher intake) that remain stable across 40 days. This study examined whether an escalating dose regimen with intermittent abstinence periods produces different levels of nicotine intake relative to those observed during continuous access to a fixed unit dose. Rats were trained to nose-poke for food and water in 23-hour sessions prior to and after recovery from surgical implantation of jugular catheters. Animals ($n=12$) then were given access to nicotine IVSA in 4-day cycles, each separated by three intervening days of abstinence in their home cage. The unit dose available for nicotine IVSA was increased between cycles as follows: 0.015, 0.03, 0.06, 0.09 mg/kg/0.1 ml infusion/1 s, fixed ratio 1. Control rats ($n=6$) were given access to saline for five 4-day IVSA periods. Nicotine dependence was assessed by examining physical signs of withdrawal following an injection of the nicotinic antagonist mecamylamine (1.5 mg/kg, i.p.). Nicotine intake dose-dependently increased between cycles. Within each cycle, nicotine intake was highest on the first day after abstinence and decreased over the next 3 days of continuous access. Mecamylamine produced a significant increase in overt signs of withdrawal in the 23-hour access animals comparable to that observed in previous studies of nicotine dependence. Our findings suggest that abstinence from nicotine may produce a "deprivation effect" in nicotine-dependent rats. In addition, intermittent access to increasing unit doses appears to produce higher levels of nicotine intake than continuous access to a constant unit nicotine dose.
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Keywords: Intermittent; Intravenous self-administration; Rat; Nicotine

1. Introduction

Much evidence indicates that individuals use tobacco primarily to experience the psychopharmacological properties of nicotine, and that a large proportion of smokers eventually become dependent upon nicotine (Balfour, 1994; Stolerman, 1991). An estimated 23% of the U.S. population age 18 and over smoked every day in the past month (MMWR, 2004), suggesting the continuing high addictive potential of nicotine. Tobacco smoking is the leading, *avoidable* cause of disease and premature death in the U.S., responsible for over 440,000 deaths annually (Fellows et al., 2002). The pervasiveness of tobacco use and the extensive costs to smokers and society provides a compelling basis for elucidating the actions of nicotine within the central nervous system that lead to potential neuroadaptations in the motivational systems which mediate the development of dependence and withdrawal symptoms.

Nicotine acts as a reinforcer and will support intravenous self-administration (IVSA) in various species, including humans, non-human primates, and rodents (Corrigall and Coen, 1989; Donny et al., 1995; Goldberg et al., 1981, 1983; Goldberg and Spealman, 1982; Goldberg and Henningfield, 1988; Watkins et al., 1999). Nicotine IVSA has been demonstrated reliably in the rat in numerous strains and at numerous laboratories (Donny et al., 1995; Corrigall 1999; Rose and Corrigall, 1997; Watkins et al., 1999). The acute positive reinforcing effects of drugs are critically important in establishing self-administration behavior, but other mechanisms have been hypothesized to underlie the transition from initial drug use to drug dependence and involve neuroadaptations

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within brain circuitries and neuroadaptations in the brain stress systems (Koob and Le Moal, 2005) that produce negative reinforcement (Koob and Bloom, 1988). These neuroadaptations may contribute to a negative affective state upon drug termination. Thus, continued drug use to avoid a negative affective state through negative reinforcement processes may at a minimum add to the positive reinforcing effect described by nondependent tobacco users (Koob, 1996; Koob and Le Moal, 2001).

More recently, extensive work has been performed in rats with unlimited access to nicotine, and these studies have explored the relationship between the dose of nicotine self-administered and the patterns of intake that develop or their relationship to the manifestation of a withdrawal syndrome. In general, chronic nicotine IVSA results in dose-dependent increases in nicotine intake (Valentine et al., 1997) and induces physical signs of dependence as manifested by nicotine antagonist-precipitated withdrawal (Paterson and Markou, 2004; O'Dell et al., 2007). However, the transition to dependence in humans follows a number of different trajectories and includes individuals who limit their access to tobacco and then ultimately escalate intake and become dependent and individuals who limit their intake and never develop dependence (referred to as "chippers") and individuals who move back and forth between dependent and nondependent use (Shiffman et al., 1994).

The purpose of the present study was to explore the hypothesis that intermittent exposure to unlimited access to nicotine IVSA would produce an abstinence effect similar to that observed with other drugs of dependence, such as alcohol. With alcohol self-administration, abstinence leads to a dramatic increase in alcohol consumption when access is again available and is known as the alcohol deprivation effect (Sinclair and Senter, 1967). The present study was designed to explore the possibility that similar deprivation-induced increases in nicotine consumption would be observed by allowing rats unlimited access to nicotine IVSA at intermittent intervals (days).

2. Methods

2.1. Subjects

18 male Wistar rats (Charles River, New York) weighing 200–250 g at the beginning of the experiment were housed in groups of three per cage in a humidity- and temperature-controlled (22 °C) vivarium on a regular light/dark cycle (lights on 6 AM–6 PM). Rats were handled daily during an initial 5-day acclimation period where they had *ad libitum* access to food and water. All procedures were conducted in strict adherence to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals.*

2.2. Drugs

The drugs used in these experiments were: (−)nicotine hydrogen tartrate salt and mecamylamine. Both compounds were purchased from Research Biochemicals International (Natick, MA). The dose of mecamylamine refers to the salt form, and the doses of nicotine refer to the free base form. All drugs were dissolved in 0.9% sterile saline, and mecamylamine was administered in a volume of 1 ml/kg. The drug doses were selected carefully based on previous work in our laboratory (O'Dell et al., 2007) and that of other laboratories using extended access to nicotine IVSA (Fu et al., 2001; LeSage et al., 2002, 2003; Valentine et al., 1997).

2.3. Operant chambers

The rats were tested in operant IVSA chambers (Med Associates, St. Albans, VT) that were kept on a regular light/dark cycle (lights on 6 AM–6 PM) inside sound-attenuated chambers with continuous white noise. Each day during testing the rats were removed at 10 AM from the operant chambers and placed into their home cage (n=2–3 per cage) for 1 h so the chambers could be cleaned and the water and food could be replenished. On days when the rats were not tested for nicotine IVSA, they were group-housed (2–3 per cage) in their home cages with free access to food and water using the same lighting conditions as during testing. The exit port of the catheter fittings were connected to polyethylene tubing contained inside a protective metal spring which was suspended into the operant chamber from a liquid swivel attached to a balance arm. The nicotine was delivered via a syringe pump (Razel Scientific Research Instruments, St. Albans, VT) as described in Caine et al. (1993). Operant sessions were conducted using two retractable levers (i.e., active and inactive lever) that extended approximately 1 in. into the chamber. Each response on the active lever resulted in the delivery of nicotine in a volume of 0.1 ml over 1 s. A 28 V white cue light was illuminated above the active lever at the onset of the 1-s infusion and was terminated after a 20-s time-out, during which responses were recorded but had no scheduled consequences.

2.4. Food and water training

During the first 3 days, the animals were allowed to perform nose-poke responses on a fixed ratio 1 (FR1) schedule of reinforcement to obtain palatable chow pellets (45 mg precision food pellets, Formula A/I from Research Diets, Lancaster, NH) from a pellet dispenser with a swing door mounted between two levers on the front wall of the chamber. A nose-poke response was also required in a separate hole positioned on the back of the chamber for administration of 0.1 ml aliquots of water into an adjacent metal dipper cup. By the third day of training all rats had acquired stable levels of food and water responding. The animals were returned to their home cages (n=2–3 per cage) with *ad libitum* access to food and water for one day before catheter implantation surgery.

2.5. Intravenous catheter implantation

Rats were anesthetized with an isoflurane/oxygen vapor mixture (1.0–1.5%) and prepared with chronic indwelling intravenous catheters into the jugular vein as described in Caine et al. (1993). Briefly, the catheters consisted of a 14 cm length of silastic tubing fitted to a guide cannula (Plastics One, Roanoke, VA) bent at a right angle. The skull was exposed and cleaned, and four skull screws were implanted, one in each

quadrant. The bent guide cannula was secured rostral-caudally to the center of the skull using cranioplastic cement. The catheter tubing was passed subcutaneously from the animal's skull to the right jugular vein that was punctured with a 26-gauge needle. Then, 3.7 cm of the silastic tubing were inserted into the vein and tied gently with suture thread. All animals were allowed to recover for 5 days. Catheters were flushed daily with 0.2 ml of sterile physiological saline containing heparin (30 USP units/ml) and the antibiotic Timentin (SmithKline Beecham Pharmaceuticals, Philadelphia, PA). If at any point during the experiment we observed catheter leaks or abnormal shifts in IVSA behavior, then rats received 0.1 ml of the ultra short-acting barbiturate anesthetic Brevital® sodium (1% methohexital sodium, Eli Lilly, Indianapolis, IN) through the catheter. Animals with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 s of the intravenous injection. Data collected from animals with non-patent catheters were excluded from the data analyses.

2.6. Food and water reinstatement

Following a 5-day recovery period, the exit port of the catheter fittings was connected to the metal spring that was attached to the swivel and balance arm. This was done to reestablish stable levels of food and water intake prior to the introduction of the nicotine lever in the next phase of the study. Rats performed nose-poke responses for food and water in 23-hour sessions for 4 days from Monday at 10 AM until Friday at 11 AM in the absence of any levers. All subsequent tests were run for the same 4 days after which time they were group-housed over the weekend in their home cages with free access to food and water. Monday through Thursday, the rats were removed from the cages from 10–11 AM to clean the cages and flush the catheters.

2.7. IVSA behavior

One group of rats ($n=12$) then was allowed to respond on a lever for nicotine IVSA in 4-day cycles, each separated by three intervening days of abstinence in their home cage. During each 4-day cycle, rats were given access to an increasing unit dose of nicotine on an FR1 schedule of reinforcement. The unit dose available for nicotine IVSA was increased between cycles as follows: 0.015, 0.03, 0.06, 0.09 mg/kg/0.1 ml infusion/1 s. This cycle was repeated again for three 4-day cycles using the following doses: 0.03, 0.06, 0.09 mg/kg/0.1 ml infusion/s. The cycle of nicotine IVSA was repeated to examine whether responding on a previously self-administered dose would be similar upon the second exposure to the same dose of nicotine. A separate group of control rats ($n=6$) were treated in the same

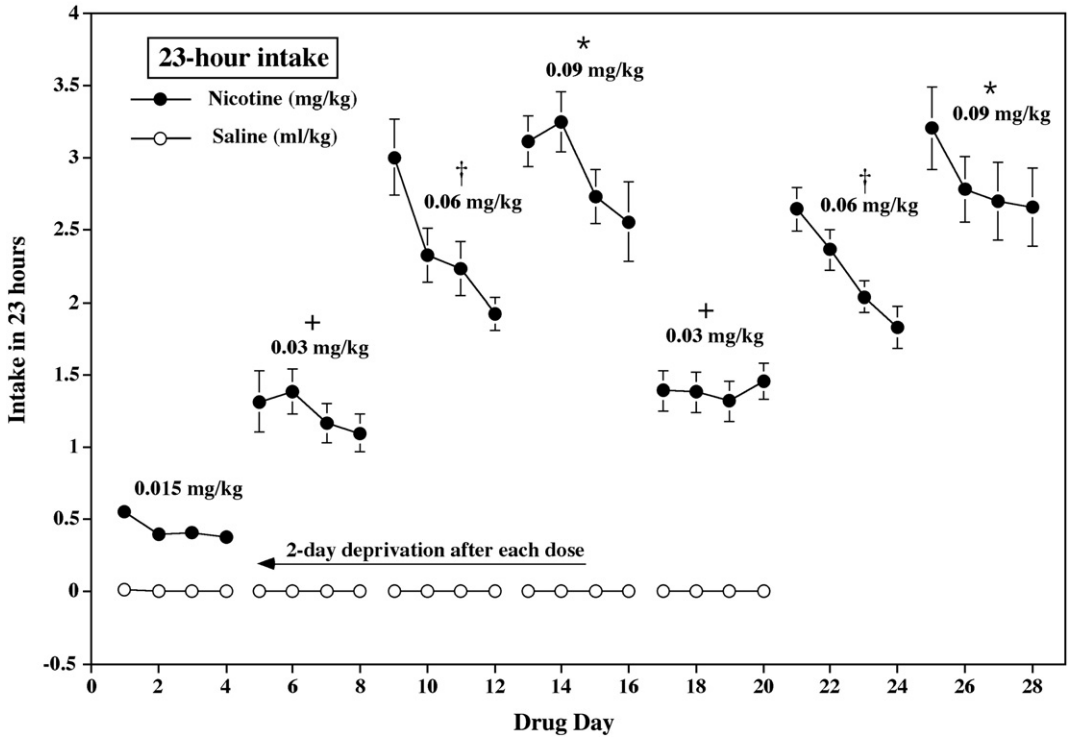

Fig. 1. Nicotine intake (solid circles; mean mg/kg ± SEM) and saline intake (open circles; mean ml/kg ± SEM) during the entire 23-hour session in rats allowed 23-hour access to saline or escalating doses of nicotine (0.015, 0.03, 0.06, or 0.09 mg/kg/0.1 ml infusion) for 4-day repeated cycles of access. Rats receiving intermittent access to nicotine displayed a dose-dependent increase in nicotine intake (main effect of dose; $F_{2,22}=117.1$, $p<0.001$). Asterisks (*) denote that higher levels of nicotine intake were observed across time in rats receiving the 0.09 dose relative to the 0.06 and 0.03 doses of nicotine ($p<0.001$). Daggers (†) denote that higher levels of nicotine intake were observed in rats receiving the 0.06 dose relative to the 0.03 dose of nicotine ($p<0.001$). Plus signs (+) denote that lower levels of nicotine intake were observed in rats receiving the 0.03 dose relative to the 0.06 and 0.09 doses of nicotine ($p<0.001$). The pattern of intake was the same the first and second time the rats received the same dose of nicotine. There were no changes in saline intake across time.

Table 1
Mean intake (mg/kg±SEM) of nicotine is higher in rats receiving 23-hour access to intermittent *vs.* continuous nicotine

Dose of nicotine (mg/kg)	Day 1 intake intermittent access	Average intake continuous access[a]
0.015	0.55±0.06	0.44±0.003
0.03	1.31±0.21	0.8±0.010
0.06	3.00±0.26	1.36±0.006
0.09	3.11±0.18	–

[a] Data from rats receiving continuos access to nicotine are from O'Dell et al. (2007).

manner except they were given access to saline IVSA for five 4-day periods.

2.8. Assessment of nicotine dependence

After the last 4-day cycle of nicotine IVSA using the 0.09 mg/kg dose, the rats were re-introduced to nicotine IVSA using the 0.09 mg/kg dose. On the first day of nicotine IVSA, rats were removed from the operant cages at 6 AM and acclimated to our plastic opaque cylindrical (30×29 cm) observation cages for 30 min. Rats were tested at the end of the dark phase (6 AM) of the light/dark cycle to observe signs following a period of high nicotine intake. During the next 2 days, the rats first received saline at 6 AM, and baseline withdrawal signs were examined; the following day they received mecamylamine (1.5 mg/kg, i.p.) to examine precipitated physical signs of withdrawal. After the injection, the rats were placed into the observation cages and 20 min later were observed for 10 min for somatic signs of nicotine withdrawal according to the method developed by Malin and colleagues (Malin et al., 1992). The signs recorded were blinks, gasps, writhes, head shakes, ptosis, teeth chattering, and yawns. Multiple successive counts of any sign required a distinct pause between episodes. Total number of somatic signs in the 10-min observation period was defined as the sum of the number of occurrences of all of the aforementioned signs. The same observer scored all of the withdrawal signs, and was blind to the animals' drug treatment.

2.9. Data analysis

Repeated-measures analysis of variance (ANOVA) was performed with day, dose, and time (repetition of the same dose) as within-subjects factors on nicotine, saline, or food intake. Linear trend contrasts were conducted to compare the pattern of intake within each 4-day cycle. Pairwise comparisons were used to make individual group comparisons between doses. One-way ANOVAs were used to compare baseline and mecamylamine-induced withdrawal signs, and *post hoc* comparisons were conducted using Fisher's protected least significant difference (PLSD) test. Significance was set at $p<0.05$.

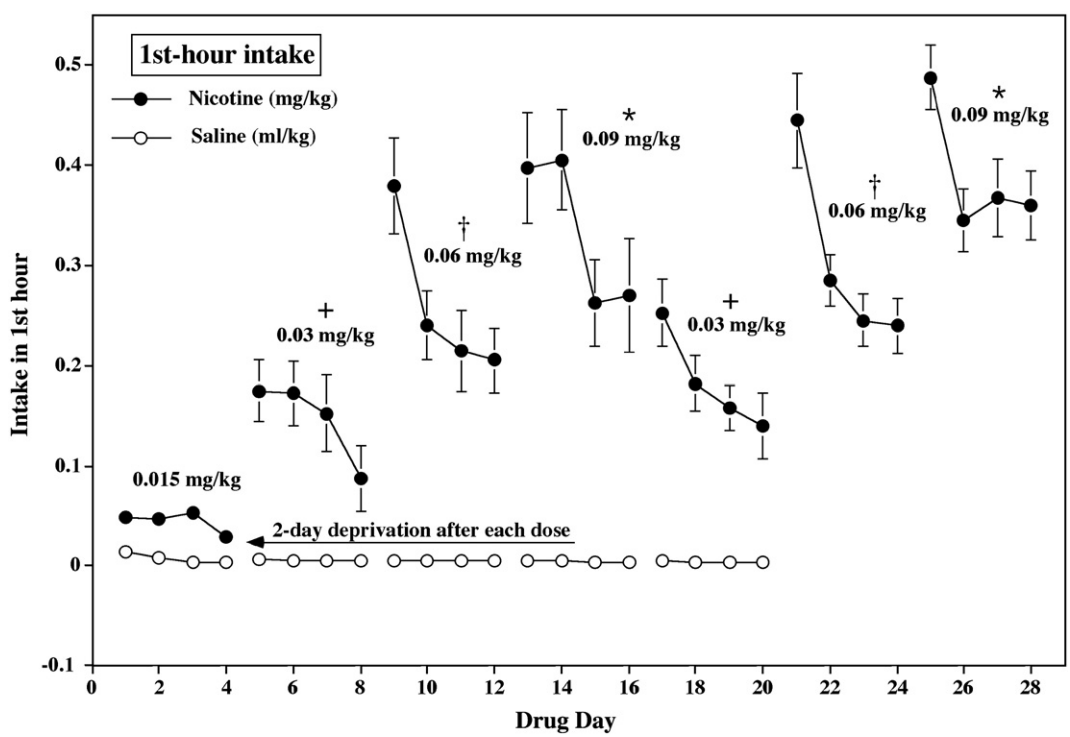

Fig. 2. Nicotine intake (solid circles; mean mg/kg±SEM) and saline intake (open circles; mean ml/kg±SEM) during the first hour of nicotine access (11 AM–12 PM) in rats allowed 23-hour access to saline or escalating doses of nicotine (0.015, 0.03, 0.06, or 0.09 mg/kg/0.1 ml infusion) for repeated 4-day cycles of access. The pattern of nicotine intake in the first hour of access was similar to the pattern of intake observed across 23 h. A dose-dependent increase in nicotine intake was observed (main effect of dose; $F_{2,22}=75.4$, $p<0.001$). Asterisks (*) denote that higher levels of nicotine intake were observed across time in rats receiving the 0.09 dose relative to the 0.06 and 0.03 doses of nicotine ($p<0.001$). Daggers (†) denote that higher levels of nicotine intake were observed in rats receiving the 0.06 dose relative to the 0.03 dose of nicotine ($p<0.001$). Plus signs (+) denote that lower levels of nicotine intake were observed in rats receiving the 0.03 dose relative to the 0.06 and 0.09 doses of nicotine ($p<0.001$).

3. Results

3.1. Nicotine intake

Fig. 1 displays nicotine intake in rats receiving 23-hour access to increasing doses of nicotine in repeated 4-day cycles. Our analysis involved a time (2 levels) × dose (3 levels) × day (4 days) repeated-measure ANOVA. The results revealed a main effect of dose, with rats displaying a dose-dependent increase in nicotine intake ($F_{2,22}=117.1, p<0.001$). Subsequent planned comparisons revealed that higher levels of nicotine intake were observed in rats receiving the 0.09 dose compared to the 0.06 ($F_{1,11}=27.3$, $p<0.001$) and 0.03 ($F_{1,11}=193.5$, $p<0.001$) doses of nicotine. Additionally, significantly higher levels of nicotine intake were observed in rats receiving the 0.06 dose compared to the 0.03 dose ($F_{1,11}=136.6, p<0.001$), as shown in Table 1. The mean level of nicotine intake (mg/kg) across the 4 days of IVSA for the first and second time they received that dose was as follows: 0.03 dose = 1.24 and then 1.39; 0.06 dose = 2.37 and then 2.22; 0.09 dose = 2.11 and then 2.84. The dose-dependent effects of nicotine were not altered across time, as there was no significant *Dose* × *Time* interaction ($F_{2,22}=1.89$, $p=0.15$). There was no difference in nicotine intake the first or second time the rats experienced the same dose of nicotine ($F_{1,11}=0.104, p=0.75$). However, the level of nicotine intake was significantly different across the 4 days of each cycle of nicotine access ($F_{3,33}=18.3$, $p<0.001$). A linear trend analysis revealed that there was a decrease in nicotine intake across the 4 days of drug access ($F_{1,11}=44.2, p<0.001$). The drop in nicotine intake was sharper at the 0.09 and 0.06 doses than the 0.03 dose of nicotine ($F_{1,11}=24.9$, $p<0.001$). The effect of nicotine within the cycles did not change across time when the rats received the same dose of nicotine for the first or second time ($F_{3,33}=0.81, p=0.5$). A direct comparison of the slope of nicotine and saline intake within the three corresponding 4-day cycles revealed a significant difference in the slope across the saline and nicotine groups ($F_{3,48}=4.9$, $p<0.004$) with nearly all of the variability being explained by the strong downward linear trend in the nicotine rats receiving the 0.06 and 0.09 doses of nicotine ($F_{1,16}=10.2, p<0.006$).

3.2. Nicotine intake in the first hour of access

Fig. 2 displays nicotine intake in the first hour of nicotine access in rats receiving 23-hour access to saline or increasing doses of nicotine in 4-day cycles. The pattern of nicotine intake in the first hour of access was similar to the pattern observed

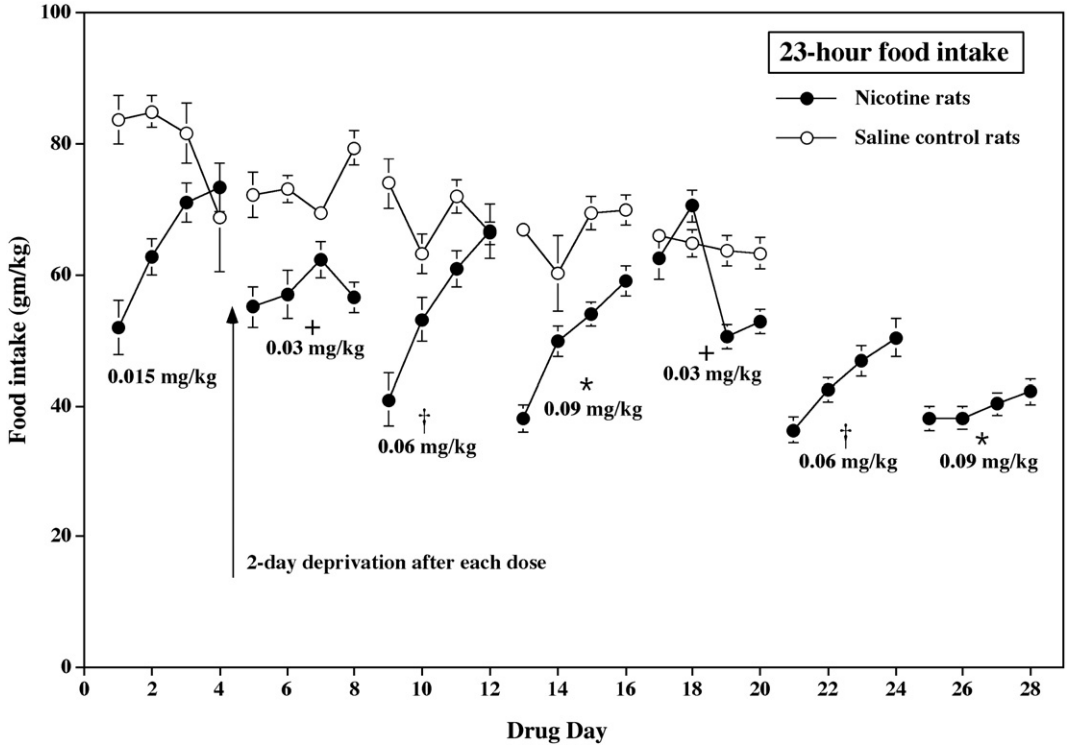

Fig. 3. Total food intake in rats allowed 23-hour access to escalating doses of nicotine (0.015, 0.03, 0.06, or 0.09 mg/kg/0.1 ml infusion) for seven 4-day cycles of nicotine IVSA (solid circles) or in saline control rats (open circles) given five 4-day cycles of saline IVSA. A dose-dependent decrease in food intake was observed (main effect of dose; $F_{2,22}=127.5, p<0.001$). Asterisks (*) denote that lower levels of food intake were observed across time in rats receiving the 0.09 dose of nicotine relative to the 0.06 and 0.03 doses ($p<0.001$). Daggers (†) denote that lower levels of nicotine intake were observed in rats receiving the 0.06 dose relative to the 0.03 dose of nicotine ($p<0.001$). Plus signs (+) denote that higher levels of nicotine intake were observed in rats receiving the 0.03 dose relative to the 0.06 and 0.09 doses of nicotine ($p<0.001$). The pattern of food intake was the same across all doses of nicotine, with rats responding less for food on the first day of nicotine access and then stabilizing their intake across the next 3 days. This likely reflects the fact that on the first day the rats had just received 2 days of free access to food and water in their home cages. This pattern was different in saline control rats that displayed higher levels of food intake overall and did not show an initial decrease in food intake on the first day of the 4-day cycle.

Table 2
Mean (±SEM) precipitated signs of withdrawal during baseline and following a challenge injection of mecamylamine (1.5 mg/kg, i.p.) in rats allowed 23-hour access to nicotine IVSA

	Total	Blink	Gasp	Writhe	Teeth chatter	Yawn	Head shake	Ptosis
Baseline	3.0±0.6	2.2±0.4	0.2±0.1	0.3±0.1	0	0.2±0.2	0	0.1±0.1
Withdrawal	*14.6±1.5**	*6.9±1.4	*2.2±0.3	*3.4±0.6	1.0±0.7	0.8±0.3	0.1±0.1	0.2±0.1

Asterisks reflect a significant difference relative to baseline values ($p<0.05$).

over 23 h. Specifically, a dose-dependent increase in nicotine intake was observed ($F_{2,22}=75.4$, $p<0.001$). An analysis of the first hour of the first days of nicotine access revealed a main effect of time ($F_{1,33}=7.2$, $p<0.01$) with higher levels of intake being observed the second time rats received the same dose of nicotine. The level of nicotine intake decreased across the 4 days of drug access ($F_{3,33}=11.6$, $p<0.001$), consistent with our linear trend analysis ($F_{1,11}=26.9$, $p<0.001$). A direct comparison of the slope of nicotine and saline intake within the three corresponding 4-day cycles revealed a significant difference in the slope across the saline and nicotine groups ($F_{3,48}=3.2$, $p<0.03$) with nearly all of the variability being explained by the strong downward linear trend in the nicotine rats receiving the 0.06 and 0.09 doses of nicotine ($F_{1,16}=7.6$, $p<0.01$).

3.3. Food intake

Fig. 3 displays food intake in rats receiving 23-hour access to increasing doses of nicotine (solid circles) or saline (open circles) in repeated 4-day cycles. Our analysis involved a time (2 levels)×dose (3 levels)×day (4 days) repeated-measure ANOVA. The results revealed a main effect of dose, with nicotine producing a dose-dependent decrease in food intake ($F_{2,22}=127.5$, $p<0.001$). Subsequent planned comparisons revealed that lower levels of food intake were observed in rats receiving the 0.09 dose compared to the 0.06 ($F_{1,11}=25.2$, $p<0.001$) and 0.03 ($F_{1,11}=362.1$, $p<0.001$) doses of nicotine. Additionally, lower levels of food intake were observed in rats receiving the 0.06 dose compared to the 0.03 dose ($F_{1,11}=95.2$, $p<0.001$). The mean level of food intake (g/kg) across the 4 days of IVSA for the first and second time they received that dose was as follows: 0.03 dose=57.7 and then 59.0; 0.06 dose=55.3 and then 44.0; 0.09 dose=50.2 and then 39.6. The dose-dependent effect of nicotine on food intake did change across time ($F_{2,22}=20.6$, $p<0.001$), due to a larger dose-effect the second time the rats received nicotine. Nicotine-treated rats exhibited lower levels of food intake across the 4-day cycles of nicotine intake ($F_{3,33}=16.4$, $p<0.001$), consistent with a linear trend analysis ($F_{1,11}=20.1$, $p<0.001$). However, saline-treated rats did not exhibit any changes in food intake across the 4-day cycles of saline IVSA ($F_{3,15}=1.5$, $p=0.25$). A comparison between saline and nicotine rats revealed that the level of food intake was lower in nicotine rats compared to saline rats ($F_{1,16}=26.7$, $p<0.001$). In addition, the slope of food intake within the three corresponding 4-day cycles revealed that nicotine-treated rats exhibited steeper decreases in food intake relative to saline controls ($F_{1,16}=9.27$, $p<0.008$).

3.4. Mecamylamine-precipitated withdrawal

Table 2 reflects the individual physical signs of mecamylamine-precipitated withdrawal in rats that received extended access to escalating doses of nicotine. Mecamylamine produced an increase in total overt signs of withdrawal relative to baseline measures ($F_{1,22}=46.0$, $p<0.0001$) in rats allowed 23-hour nicotine access. An analysis of individual withdrawal signs revealed a significant increase in precipitated physical signs of blinking ($F_{1,22}=9.9$, $p<0.005$), writhing ($F_{1,22}=5.5$, $p<0.03$), and gasping ($F_{1,22}=28.7$, $p<0.0001$) relative to baseline measures.

4. Discussion

The present results demonstrate that nicotine intake changes dramatically with the imposition of cycles of 4-day access to 23 h of nicotine with three intervening days of no access to the drug. Within each cycle, nicotine intake was highest on the first day after abstinence and decreased over the next 3 days of continuous access. Nicotine intake increased dose-dependently, with levels of intake reaching values as high as 3.0 mg/kg/23 h in rats receiving the 0.06 and 0.09 mg/kg/infusion doses of nicotine. The highest level of nicotine intake was observed in the first hour of drug access, and first hour intake increased with dose and cycles of abstinence. Our findings suggest that abstinence from nicotine may produce a "deprivation effect" in nicotine-dependent rats. In addition, intermittent access to increasing unit doses appears to produce higher levels of nicotine intake than continuous access to a constant unit nicotine dose. Previous work has shown that continuous access to IVSA nicotine produces a dose-dependent intake of nicotine to the point of dependence. Rats were trained to lever press to intravenously self-administer nicotine and to nose-poke for food and water in 23-hour sessions for 40 days of continuous access (O'Dell et al., 2006, 2007). Separate groups of animals then were given access to nicotine IVSA (0.01, 0.03, 0.06 mg/kg/0.1 ml infusion/s; FR1) for 40 consecutive days. The results showed a dose-dependent increase in nicotine intake, but a dose-dependent decrease in lever responding such that the highest dose of nicotine produced the lowest amount of lever responding and the highest amount of nicotine intake. The pattern of nicotine IVSA remained stable across the 40 days. Mecamylamine precipitated robust withdrawal signs following the last nicotine IVSA session, and the magnitude of this effect was positively correlated with the total amount of nicotine that was self-administered.

An additional explanation of the data in the present study is that the increase in nicotine intake after the period of abstinence could be due to the period of abstinence, the change in nicotine

dose, or both. In this study, the abstinence effect is only observed when the rats are switched to a higher dose. The abstinence effect was not observed when the rats were switched from 0.09 mg/kg/infusion to 0.03 mg/kg/infusion. A question for future work is whether there would be an abstinence effect if the rats had been maintained on the same dose of nicotine, and whether the length of deprivation is critical.

Previous studies have shown that the amount of nicotine self-administered is dependent on the dose of nicotine per injection in IVSA. Shoaib and Stolerman (1999) showed that plasma nicotine and cotinine levels are almost twice as high in rats self-administering 0.06 mg/kg/infusion compared to rats that self-administer 0.03 mg/kg/infusion. The present study also confirms that chronic exposure to nicotine across a 23-hour period, even on an intermittent 4 days on/4 days off schedule, produces brain levels sufficient to induce dependence as defined by the precipitation of physical withdrawal signs with the administration of the competitive nicotinic antagonist mecamylamine. The number of withdrawal signs (14.6 ± 1.5) observed in the intermittent exposed animals is comparable to previous studies of utilizing passive (Hildebrand, et al., 1999; Malin et al., 1992; Markou and Paterson, 2001; Skjei and Markou, 2003; O'Dell et al., 2006; Watkins et al., 1999) and active (Paterson and Markou, 2004; O'Dell et al., 2007) administration of nicotine. However, note that the animals in the present study had far fewer days of exposure to 23-hour access to nicotine IVSA (28 days) than in O'Dell et al., 2007 (40 days) suggesting that again the intermittent exposure produces robust nicotine dependence. Additionally, intake levels of 3.0 mg/kg/day in the present study are equivalent to approximately 3× the nicotine intake observed in human smokers of 0.14–1.14 mg/kg/day (Benowitz and Jacob, 1984).

A novel feature of the present experiment was the introduction of 3 days of abstinence between each 4 days of access to the drug. This aspect of our model has some face validity as a procedure for induction of dependence in that there is evidence that undergraduate students largely smoke and drink on weekends during academic years (Dierker et al., 2007), and this may be a pattern of nicotine intake in young adults that leads to dependence in some young smokers. The data in the present study compared to historical data from our laboratories indicate that intermittent access leads to higher levels of drug intake during the first 24 h after abstinence. Such peaks in intake may more readily trigger neuroadaptive mechanisms that contribute to addiction (Becker and Baros, 2006; Lopez and Becker 2005; O'Dell et al., 2004).

Nicotine intake declined back to baseline values in the 23-hour measures by the fourth day of access at each dose. This change presumably reflects some neuroadaptive change, possibly a form of reverse tolerance. It is well documented that chronic nicotine exposure can induce an increase in nicotinic receptors (Benwell et al., 1988), and this may be one explanation of the "satiation-like" effect. Switching from a lower to a higher dose of nicotine also could have potentiated the abstinence effect. The rats may have self-administered a larger amount of nicotine than intended because the dose of nicotine was increased. This might have led to aversive effects (Jorenby et al., 1990), which could have contributed to the rapid decline in nicotine intake on the second and third day that the rats were exposed to a new, high dose of nicotine.

Another notable finding in the present study is that nicotine intake in the first hour of each session after deprivation showed a significant increase over time. The latter observation is consistent with that observed in the first 5 days of continuous access to nicotine IVSA (O'Dell et al., 2007). However, what is particularly notable in the present study is that this increase persisted after each nicotine abstinence period and became significantly larger in the replication of the abstinence dose cycle. These results suggest that the original escalation observed in rats given continuous access to nicotine may be due to neuroadaptive mechanisms upon initial exposure to nicotine. These results have important implications for understanding the patterns of nicotine intake in the human population that lead to vulnerability to dependence.

The present findings also revealed that rats given intermittent access to nicotine IVSA display a decrease in food intake compared to control rats with access to saline IVSA. This effect is particularly notable on the first day after the abstinence period, and the decreased food intake returned to control levels by the third and fourth day of nicotine access. However, the decreases in food intake in the nicotine IVSA rats were re-expressed after each nicotine abstinence period suggesting a rapid tolerance to the anorectic effects of nicotine and a rapid reversal of that tolerance. Whether the same neuroadaptive mechanism is responsible for the change in food intake as observed with the change in nicotine intake remains to be determined. These results are consistent with our previous data in rats receiving continuous access to nicotine IVSA for 40 days where decreases in mean daily food intake remained constant with constant level of nicotine intake (O'Dell et al., 2007). Again, extrapolation to the human condition suggests that the appetite suppressant effects of nicotine are short-lived and require the presence of nicotine.

In summary, the present study shows that intermittent exposure to 23-hour nicotine IVSA can lead to large and rapid intakes of nicotine and that nicotine intake is highest immediately after a nicotine abstinence period. Escalation in nicotine intake is observed during the first hour and increases with repeated cycles of nicotine abstinence. Repeated cycles of nicotine access with ascending doses produced dependence as measured by mecamylamine-precipitated withdrawal with 28 days of access. The results suggest that intermittent access to nicotine IVSA may lead to rapid escalation in intake that could have important implications for adolescent and young adult vulnerability to nicotine dependence.

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