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Continuous nicotine infusion reduces nicotine self-administration in rats with 23-h/day access to nicotine

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Abstract

The effects of continuous nicotine infusion on nicotine self-administration (NSA) were studied in rats as a model of nicotine replacement therapy (NRT) in humans. A NSA model in which rats had 23-h/day access to nicotine was used to approximate nicotine access conditions in cigarette smokers. In order to estimate serum nicotine concentrations associated with NSA, arterial and venous serum nicotine concentrations were measured during a simulation of NSA. Nicotine was noncontingently administered as 30 doses/12 h of 0.03 mg/kg/inf or 60 doses/12 h of 0.01 mg/kg/inf daily. Venous serum nicotine concentrations were measured after the first nicotine dose of the day, and arterial and venous concentrations were measured after doses in the middle of the day. The range of mean concentrations measured was similar to those reported in cigarette smokers (venous concentrations 6–59 ng/ml, arterial concentrations 42–96 ng/ml). The effects of continuous nicotine infusion on NSA were studied by noncontingently administering nicotine at various rates via osmotic pump to animals self-administering nicotine (0.01 or 0.03 mg/kg/inf) during 23-h/day sessions. Continuous nicotine infusion at all infusion rates substantially suppressed NSA, but suppression was rate-related only for the 0.01-mg/kg/inf NSA unit dose. Nicotine infusion rates producing venous serum nicotine concentrations equaling or exceeding the peak venous levels associated with simulated NSA were more effective than lower infusion rates only at the lower NSA unit dose. The highest nicotine infusion rate had no sustained effect on food-maintained responding, demonstrating its specificity for suppression of NSA. These data provide a model for studying NRT in the rat.

Keywords: Nicotine; Self-administration; Nicotine concentrations; Nicotine replacement therapy

1. Introduction

Nicotine replacement therapy (NRT) in the form of nicotine patches, gum, inhaler or nasal spray is the most commonly used medication for smoking cessation. NRT approximately doubles the cessation rates achieved with counseling alone and has proven to be a valuable adjunct to smoking cessation efforts (Fiore et al., 1994). Nevertheless, the majority of smokers who use NRT even with intensive counseling fail to quit (Fiore et al., 2000). One potential reason for NRT's limited efficacy is that the venous serum nicotine concentrations provided by NRT are typically only 50–75% of those associated with smoking (Benowitz et al., 1987; Hurt et al., 1994; Lawson et al., 1998). These figures may actually underestimate the differences in nicotine concentrations between NRT and smoking. Because nicotine from cigarettes is inhaled and presented directly to the pulmonary circulation and then to the left side of the heart, arterial nicotine concentrations immediately after a puff are 2–10 times higher than concurrent venous concentrations (Gourlay and Benowitz, 1997; Henningfield et al., 1993; Rose et al., 1999a). Thus, smoking delivers nicotine to the brain at concentrations substantially higher than those typically provided by NRT.

Findings from clinical trials using higher doses of NRT for smoking cessation have been equivocal. Combining two forms of NRT (e.g., patch plus gum) is more effective for smoking cessation than one form alone, but it is not clear if the increased efficacy is due to higher serum nicotine

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concentrations or to other factors such as sensory cues or the ability to regulate nicotine levels over the day (Blondal et al., 1999; Fagerstrom et al., 1993). Some studies using two nicotine patches at a time suggest a marginal benefit compared to one patch, but others do not (Dale et al., 1995; Hughes et al., 1999; Jorenby et al., 1995). However, even two nicotine patches do not match pretreatment venous nicotine concentrations for heavy smokers and would be unlikely to match their pretreatment arterial nicotine concentrations (Henningfield et al., 1993; Lawson et al., 1998).

In addition to its use in maintaining abstinence from smoking, NRT has been shown to reduce ad lib cigarette smoking when given to smokers who are not trying to quit. This use of NRT could potentially be of benefit for smokers as a transitional goal toward complete cessation, or for smoking reduction in patients who are otherwise unable or unwilling to quit (Stratton et al., 2001). The magnitude of smoking reduction produced by NRT at usual therapeutic doses is modest (25–50%; Benowitz and Jacob 1990; Foulds et al., 1992b; Lucchesi et al., 1967; Perkins et al., 1992). One study comparing one, two or three nicotine patches during ad lib smoking found a dose-related suppression of smoking, with three patches providing substantially more suppression (40%) than two patches (10%; Benowitz et al., 1998). Venous nicotine concentrations with three patches averaged 45–60 ng/ml, which is higher than the 10–30 ng/ml measured during smoking alone and closer to the expected arterial concentrations. These data suggest that higher NRT doses might provide a therapeutic advantage for smoking reduction but that the doses required may be substantially higher than those currently used.

Despite the extensive use of NRT for smoking cessation therapy, the development of an animal model of NRT has received little attention. Nicotine administered subcutaneously just prior to a 1-h nicotine self-administration (NSA) session reduces NSA in a dose-related manner (Corrigall and Coen, 1989). However, a 1-h NSA session differs from cigarette smoking in that daily nicotine intake is compressed into a short period. An alternative model is NSA with continuous availability of nicotine. With unlimited availability of nicotine at unit doses of 0.0075–0.03 mg/kg/inf, rats self-administer total daily doses of 0.25–1.1 mg/kg, equivalent to the nicotine absorbed from one to four packs of cigarettes by a smoker (Benowitz and Jacob 1984; Valentine et al., 1997). Most NSAs occur during the rat's active (12 h dark) cycle. This model of NSA therefore provides a nicotine dosing regimen with both quantitative and temporal similarities to smoking behavior in humans.

The purpose of the current study was to establish a model of NRT in rats using the continuous access NSA model and to test the hypothesis that NRT doses that match or exceed the venous nicotine concentrations associated with NSA are more effective in suppressing NSA than lower doses. Venous and arterial nicotine concentrations associated with this model were estimated using scheduled nicotine bolus dosing in a pattern simulating NSA, since arterial concentrations more closely represent the actual concentration delivered to the brain. Pilot studies then established continuous subcutaneous (sc) nicotine infusion rates (to simulate NRT) that would equal or exceed the peak venous nicotine concentrations associated with NSA The effects of continuous subcutaneous nicotine infusion on NSA were then studied. We chose to study the effects of nicotine infusion on the maintenance of ongoing NSA, rather than on reinstatement or reacquisition of NSA (which more closely models the typical use of NRT as an aid to smoking cessation), because this is a convenient first step and because reduction of smoking has been suggested as an alternative strategy for smokers who cannot or will not quit (Stratton et al., 2001). The effect of continuous subcutaneous nicotine infusion on food-maintained responding was also studied to determine whether the behavioral effects of continuous nicotine infusion are specific to NSA.

2. Methods

2.1. Subjects

Experimentally naive male Holtzman rats weighing 250–400 g were maintained under a restricted feeding regimen (20 g/day rat chow). Each rat was individually housed in an operant chamber (see below) with unlimited access to water under a reversed 12-h light/dark cycle (lights off at 10:00 AM).

2.2. Surgical procedure

Each rat was implanted with a chronic indwelling jugular catheter under intramuscular (im) droperidol and fentanyl anesthesia. A silicon catheter (0.51 mm i.d. × 0.94 mm o.d.) was inserted into the right jugular vein and advanced to the junction of the vena cava and the right atrium, sutured to tissue surrounding the vein and tunneled subcutaneously to the back where it exited between the scapulae and attached to a guide cannula. The guide cannula was mounted in a harness assembly, which allowed connection to an infusion line for nicotine administration. Some rats were also implanted with a similar catheter in the left femoral vein for blood sampling (see below). For 3 days after surgery, each rat received hourly intravenous infusions of 50 μl of heparinized saline (25 units/ml) and daily intravenous infusions of antibiotic (enrofloxacin, 1.1 mg). Osmotic minipumps (Alzet 2ML2 or 2ML4, Durect Cupertino, CA) were implanted subcutaneously under intramuscular droperidol and fentanyl anesthesia and placed in the intrascapular area. The 2ML2 pumps delivered 5 μl/day for 2 weeks and the 2ML4 pumps delivered 2.5 μl/day for 4 weeks. Following surgery, rats were returned to their operant cages, and NSA continued for an additional 9 days. Data from the first 2 days after pump implantation were not analyzed to allow time for recovery from surgery.

2.3. 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. Three stimulus lights were located 2 cm above each response lever. A water spout mounted in the lower-right corner of the back wall of the chamber provided access to water. Each chamber was placed inside a sound-attenuating cubicle equipped with a fluorescent light that provided ambient illumination during the light-on phase of the light cycle. Infusion pumps (Model RHSY, Fluid Metering, Syosset, NY) were placed outside each cubicle and delivered infusions through PE90 tubing connected to a fluid swivel mounted above the chamber, and from the swivel through a spring leash to the guide cannula mounted on the back of the rat. A computer with L2T2 software (Coulbourn Instruments) was used for operating the apparatus and recording data.

2.4. Drugs

Nicotine bitartrate (Sigma, St. Louis, MO) was dissolved in sterile saline containing 25 units/ml heparin. The pH of the solution was adjusted to 7.4 with dilute NaOH and HCl. Doses were calculated as that of the base.

2.5. Analytical methods

Nicotine and cotinine serum concentrations in serum and brain were measured by gas chromatography with nitrogen phosphorus detection (Hieda et al., 1999; Jacob et al., 1981).

2.6. Experiment 1. Simulation of NSA: venous nicotine concentrations

The purpose of this experiment was to determine the *venous* serum nicotine concentrations associated with simulated NSA under the 23-h/day access model used in this study. Nicotine concentrations were determined using two different unit nicotine doses (0.01 and 0.03 mg/kg/inf). These concentrations were also compared to those associated with simulated NSA under the 1-h/day access model extensively reported in the literature. NSA was simulated for this experiment because the amount and pattern of nicotine intake during a NSA session varies considerably among individual animals. NSA dosing during the 1-h/day access model was simulated using typical daily nicotine intake estimates from published data (Corrigall and Coen 1989; Donny et al., 1995). Dosing during the 23-h/day access model was based on pilot data from our laboratory.

For the 1-h/day access model, eight rats received 15 doses/day of 0.03 mg/kg nicotine iv at 4-min intervals. Venous serum nicotine concentrations were measured 3 min after the first dose of the session and 3 min after the last dose of the session from blood obtained via the femoral venous catheter. For the 23-h/day access model, one group of eight rats received 30 doses/day of 0.03 mg/kg nicotine iv at 25-min intervals during the 12-h dark phase of the light cycle, which is the period when the majority of intake occurs with this model (Valentine et al., 1997). Venous serum nicotine concentrations were measured 3 min after the first and 13th dose of the day. Because nicotine has an elimination half-life of 50 min in the rat (Keyler et al., 1999), the 13th dose occurs after seven half-lives and represents the steady-state postdose venous serum nicotine concentration associated with this dosing regimen. A second group of eight rats received 60 doses/day of 0.01 mg/kg nicotine iv at 12-min intervals during the 12-h dark phase. Venous serum nicotine concentrations were measured 3 min after the 1st and 26th dose of the day. Concentrations after the 26th dose again represented steady-state postdose venous concentrations. In summary, rats in all three NSA groups had blood samples obtained 3 min after the first dose of the day to estimate peak venous nicotine concentrations after that initial dose. Rats in the 23-h/day NSA groups had a second sample after 6.5 h to estimate maximum steady-state concentrations. In the 1-h/day group, a sample was taken at the end of the session to represent the maximum nicotine concentration attained, since steady state is not reached in 1 h.

2.7. Experiment 2. Simulation of NSA: arterial and venous nicotine concentrations

The purpose of this experiment was to determine the *arterial* serum nicotine concentrations associated with simulation of the 23-h/day access NSA model. Rats (10 per group) were prepared as described above for measuring venous serum nicotine concentrations except that only one (jugular venous) cannula was implanted, and nicotine was administered as above. On the third day of dosing, rats were anesthetized while nicotine dosing continued as scheduled, and femoral venous and arterial catheters were implanted. Venous and arterial blood samples were taken 1 min prior to the 14th dose for the 0.03-mg/kg unit dose and 1 min prior to the 27th dose for the 0.01-mg/kg unit dose. A second arterial sample was taken 10 s after each of these doses, and a second venous sample was taken 30 s after each dose. These sampling times after the unit dose were chosen to represent reported peak arterial and venous concentrations with this mode of nicotine dosing (Hieda et al., 1999).

2.8. Experiment 3. Extinction and reacquisition of NSA

The purpose of this experiment was to characterize further NSA in rats under the 23-h/day access model. Ten rats were given access to 0.01 mg/kg/inf nicotine under a fixed ratio (FR) 1 schedule. Under this schedule, each response on one (active) lever produced a nicotine infusion

of 50 μl/dose delivered over 1 s. Responses on the other (inactive) lever were recorded but had no programmed consequence. A 7-s timeout period followed each infusion during which the light above the active lever was extinguished and responses had no programmed consequence. Sessions started at the onset of the dark phase (10:00 AM). During the 1 h period between sessions, chambers were serviced, and rats were fed. After substantial responding developed under the FR 1 schedule (at least 25 infusions per session), the response requirement was increased to FR 2 and then FR 3, with each FR value in effect for 3–12 sessions. Rats were considered to have acquired NSA when, under the FR 3 schedule, at least 25 infusions were earned per session and the ratio of active to inactive lever presses was at least 2:1 for three consecutive sessions. Once a rat met the acquisition criterion, extinction of NSA was arranged. During this phase, saline was substituted for the nicotine unit dose under the FR 3 schedule for seven consecutive sessions. The volume and duration of saline infusions was equivalent to nicotine unit dose infusions. After the extinction phase, the FR 3 schedule of nicotine delivery was resumed and reacquisition of NSA was observed over five consecutive sessions.

2.9. Experiment 4. Effects of continuous nicotine infusion on NSA

The purpose of this experiment was to determine if continuous infusion of nicotine at doses that match or exceed the venous and arterial nicotine concentrations associated with the simulated NSA in Experiments 1 and 2 are more effective than lower doses in suppressing NSA. Four groups of six rats each were allowed to acquire NSA using the procedures described in Experiment 3 with a unit nicotine dose of 0.01 mg/kg/inf, while four other groups were trained with a unit dose of 0.03 mg/kg/inf. The 0.01-mg/kg NSA dose represented the lowest unit dose, which maintained robust NSA while the 0.03-mg/kg dose produced typical serum nicotine concentrations achieved by a smoker (Benowitz and Jacob, 1984). After acquiring NSA under a FR 3 schedule of nicotine delivery, osmotic pumps were implanted. Each pump contained appropriate concentrations of nicotine to provide either 1.0, 2.1 or 3.2 mg/kg/day. Pump model 2ML2 was used in rats responding for 0.01 mg/kg/inf nicotine per infusion while pump model 2ML4 was subsequently used in rats responding for 0.03 mg/kg/inf nicotine because the 2ML4 pumps were found to provide more reliable nicotine infusion rates. In pilot experiments, serum nicotine concentrations at a given calculated infusion rate were consistently higher with the 2ML4 pumps than with the 2ML2 pumps. Therefore, the same calculated infusion rates of 1, 2.1 and 3.2 mg/kg/day were used for both experiments, but the 2ML4 pumps produced the higher serum nicotine concentrations desired for the NSA experiment using the 0.03-mg/kg NSA dose. That is, the differing infusion rates from the two osmotic pumps served as the means to achieve the different serum nicotine concentrations required for the two different NSA unit dose sizes. The nicotine infusion rates were chosen on the basis of pilot studies to produce venous serum nicotine concentrations lower than, equal to or in excess of venous serum nicotine concentrations associated with simulated NSA. To determine serum nicotine levels being provided by the osmotic pumps, NSA was terminated on Day 9, and further responding on operant levers had no consequences. Two days later, rats were anesthetized, and arterial and venous femoral cannulas were implanted. Simultaneous samples of arterial and venous blood were obtained, representing nicotine levels provided by the osmotic pumps alone.

2.10. Experiment 5. Effects of continuous nicotine infusion on food-maintained behavior

The purpose of this experiment was to determine whether the effects of continuous nicotine infusion are specific to NSA or they merely produce nonspecific suppression of operant behavior. To accomplish this, the effects of 3.2 mg/kg/day nicotine on food-maintained behavior were examined. Six rats were trained to lever press for food pellets under a FR 3 schedule in operant chambers identical to those used in the NSA experiments. For 24 h prior to the first day of training, rats were food deprived. On the first day of training rats were exposed to a conjoint variable time (VT) 60 s FR 1 schedule of food delivery for 3 h. Under this schedule, a single 45-mg food pellet (PJ Noyes, Lancaster, NH) was delivered, on average, every 60 s, and each lever press on the active lever also produced a food pellet. Responses on the inactive lever had no programmed consequence. During this period, all rats learned to procure food pellets from the food aperture and learned to lever press. At the end of this 3-h period, the schedule was changed to a simple FR 1 schedule (i.e., the VT 60 s component was terminated), which remained in effect for the remainder of the 23-h session. On the second and third day of training, the response requirement was increased to FR 2 and FR 3, respectively. During the three training sessions, rats had unlimited access to food pellets and were not given any supplemental lab chow. On Day 4, and for the remainder of the experiment, rats responded under a FR 3 schedule identical to that used in the NSA experiments except that a food pellet served as the reinforcer and sessions terminated after 23 h elapsed or 111 pellets (5 g) were earned, whichever occurred first. Limiting the number of pellets that could be earned per session prevented weight gain (and thus dose reductions) during the nicotine infusion phase. As in Experiments 3 and 4, data were collected, chambers were serviced, and rats were fed supplemental lab chow (15 g) prior to the beginning of each session. Supplemental chow was provided because making all of the rats daily food ration contingent upon responding could have resulted in rats being relatively more motivated to respond than the NSA rats and, thus, more resistant to nicotine infusion.

After responding stabilized under the FR 3 schedule (no statistically significant trend in response rates over five consecutive sessions), rats were anesthetized and osmotic pumps (model 2ML4) were implanted subcutaneously in the intrascapular area to provide a nicotine infusion rate of 3.2 mg/kg/day. Following surgery, rats were immediately returned to their operant cages and responding continued for an additional 9 days. Data from the first 2 days after pump implantation were not analyzed to allow time for recovery from surgery.

2.11. Statistical analyses

For all statistical analyses, an alpha level of $P < .05$ was used. For Experiment 1, a paired t test was used to determine if nicotine concentration was significantly higher following the first 0.03-mg/kg/inf unit dose compared to the 0.01-mg/kg/inf unit dose. A multiple regression analysis with nicotine concentration as the dependent variable and model and dose time point (1st vs. late session) as covariates was used to determine if concentrations were significantly higher under the 1-h model than the 3-h model. For Experiment 2, individual t tests were used to determine whether significant increases in venous and arterial nicotine levels occurred following a single unit dose of nicotine under each of the simulated NSA models. For Experiment 3, a repeated-measures ANOVA with Bonferroni post hoc tests was used to determine whether NSA during extinction was significantly lower than baseline. For Experiment 4, a two-factor ANOVA with NSA unit dose and osmotic-pump dose as factors was conducted on the number of infusions expressed as a percentage of baseline. This analysis was done to determine whether NSA was significantly reduced by continuous nicotine infusion, and whether the effects of continuous nicotine infusion differed as a function of NSA unit dose. Following a significant main effect of osmotic-pump dose, one-factor ANOVAs were conducted for each NSA unit dose with Tukey post hoc comparison tests. For Experiment 5, a one-factor repeated-measures ANOVA was conducted followed by Bonferroni comparison tests to determine whether daily response rates during continuous nicotine infusion were significantly lower than mean baseline response rates.

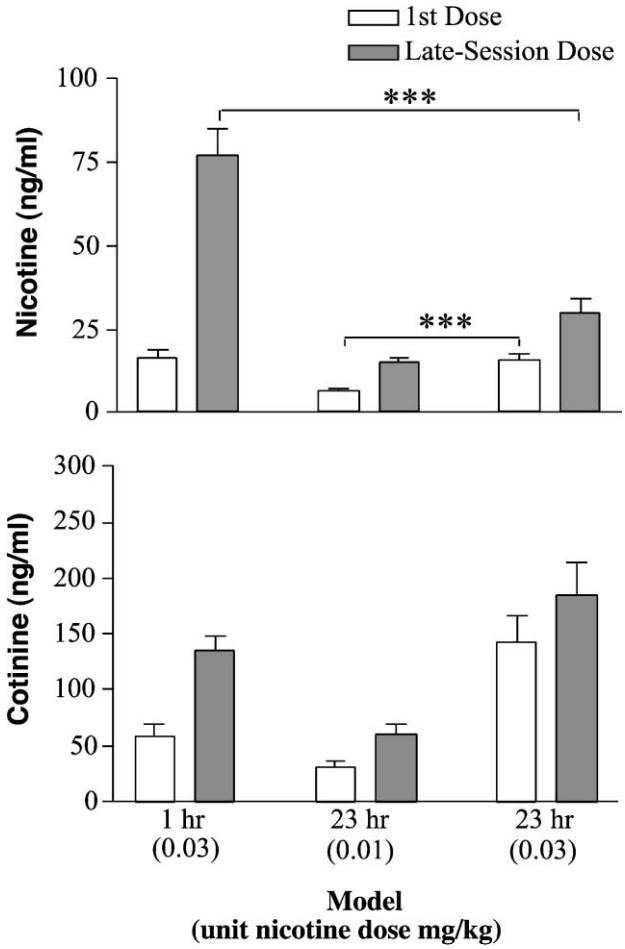

Fig. 1. Venous serum nicotine (top) and cotinine (bottom) concentrations associated with simulations of three schedules of NSA. The 1-h session consisted of 15 nicotine doses delivered at 4-min intervals. The 23-h sessions consisted of 60 nicotine doses of 0.01 mg/kg delivered at 12-min intervals over the 12-h active/dark cycle of each day (since this is the usual pattern of NSA with this model) or 30 doses of 0.03 mg/kg delivered at 25-min intervals over the 12-h active/dark cycle. Nicotine and cotinine concentrations (mean ± S.E.) were measured 3 min after the first dose of the session for each of the three models. Concentrations were also measured after the last dose of the 1-h session to estimate the peak venous serum nicotine concentration, after the 26th dose of the 23-h 0.01-mg/kg nicotine session and after the 13th dose of the 23-h 0.03-mg/kg nicotine session to estimate steady-state venous serum nicotine concentrations. Venous serum nicotine concentrations were substantially higher after the final dose of the 1-h session than after the steady-state doses during the 23-h sessions. ***$P < .001$.

3. Results

3.1. Experiment 1. Simulation of NSA—venous nicotine and cotinine concentrations

Venous serum nicotine concentrations during simulated NSA for the three different dosing regimens are shown in Fig. 1. For the 23-h/day access regimens, venous serum nicotine concentrations were higher after the first 0.03-mg/kg dose than after the first 0.01-mg/kg dose ($q = 4.91$, $P < .01$). Although the mean nicotine concentration after the late-session 0.03-mg/kg dose was more than twofold higher than that after the late-session 0.01-mg/kg dose, this difference was not statistically significant. For each dose, nicotine levels were approximately twice as high at steady state (after the 13th or the 26th dose) than after the first dose. All of the mean venous serum nicotine concentrations using the 23-h/day access model were within the range (10–40 ng/ml) reported for regular cigarette smokers (Benowitz and Jacob, 1984). For the 1-h/day access model, the venous serum nicotine concentration after the first dose was similar to that of the 23-h/day access model at the comparable 0.03-mg/kg

dose, but levels after the final dose of the 1-h session were almost three times as high as the steady-state level for the 23-h/day access model ($q = 12.51$, $P < .001$). Venous serum cotinine concentrations were also nicotine dose related.

3.2. Experiment 2. Simulation of NSA—arterial nicotine concentrations

For both NSA dose sizes, the postdose arterial nicotine concentrations exceeded the concurrent venous nicotine concentrations (Fig. 2). The postdose arterial/venous concentration ratio was modest (< 2), because it was the result of both the most recent nicotine dose and previous doses. When the boost in nicotine concentrations from the most recent dose is considered (postdose minus predose) the arterial/venous boost ratio was 3.6 for the 0.01-mg/kg NSA dose and 2.8 for the 0.03-mg/kg NSA dose. These ratios are within the range reported for the arterial/venous boost ratio for cigarette smokers (Henningfield et al., 1993; Rose et al., 1999a). The postdose venous nicotine concentrations were higher than those measured in Experiment 1 (Fig. 1) but were measured earlier (30 s vs. 3 min) and closer to the time of expected peak venous serum nicotine concentration (Hieda et al., 1999).

3.3. Experiment 3. Extinction of nicotine responding

Fig. 3 demonstrates extinction and reacquisition of nicotine responding under the 23-h/day NSA model with a unit dose of 0.01 mg/kg/inf. Substitution of saline for nicotine on Days 6 through 12 resulted in a 69.2 ± 6% (mean ± S.D.) reduction in mean active-lever responding during the extinction phase relative to baseline levels ($t = 5.47$, $P < .001$). Substitution of nicotine for saline on Day 13 resulted in reacquisition of active-lever responding, with all rats returning to or exceeding their preextinction levels within the 5-day reacquisition period.

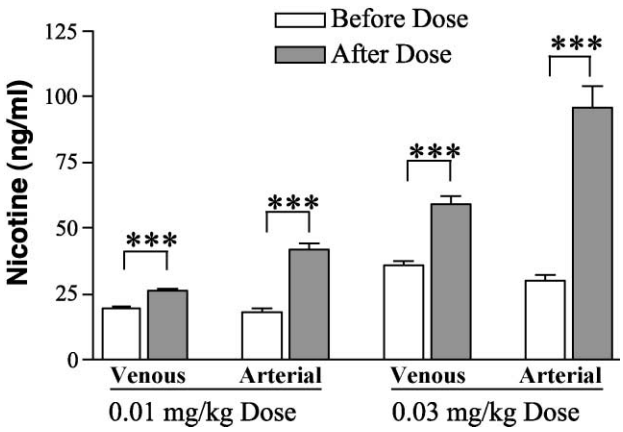

Fig. 2. Arterial and venous serum nicotine concentrations associated with simulations of the 23-h/day access model of NSA. Levels were measured before and after the 27th dose of a session (0.01 mg/kg/inf nicotine) or before and after the 14th dose of a session (0.03 mg/kg/inf nicotine). Arterial and venous serum nicotine concentrations prior to a dose were nearly equal, while after a dose the arterial concentrations were higher. Venous nicotine concentrations are higher than those shown in Fig. 1 because blood was obtained sooner after the nicotine dose (30 s vs. 3 min). *** $P < .001$.

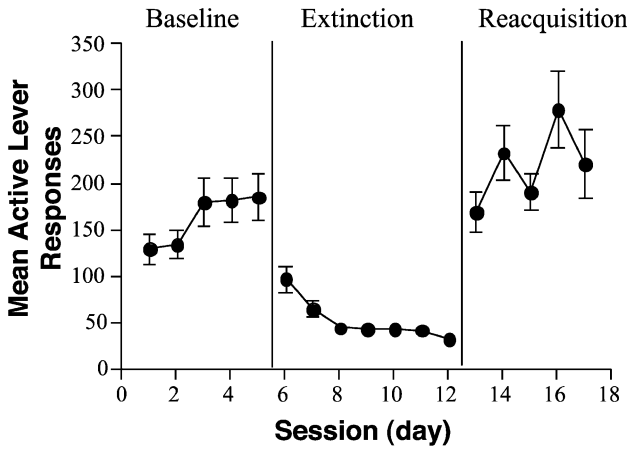

Fig. 3. Extinction of NSA. Animals received 0.01 mg/kg/inf nicotine during the baseline period of NSA on an FR 3 schedule during 23-h sessions. During extinction, the FR 3 schedule was maintained, but saline was substituted for nicotine, and during reacquisition, nicotine was again provided. Mean daily responding during extinction decreased by 69 ± 6% (mean ± S.D.) compared to the mean baseline value.

3.4. Experiment 4. Serum and brain nicotine concentrations associated with osmotic pump infusions

Serum arterial and venous nicotine concentrations at the end of the NSA experiments (when NSA had been terminated for 2 days but osmotic pump infusion continued) were dose related (Table 1). Arterial nicotine concentrations were slightly lower than the corresponding venous concentrations. The serum nicotine concentrations attributable to osmotic pump delivery in the first experiment, which used 2ML2 pumps, were lower than in the second experiment, which used 2ML4 pumps, in keeping with observations from pilot studies.

The relationship between venous serum nicotine concentrations produced by subcutaneous osmotic pump infusion and the arterial and venous serum nicotine concentrations produced by simulations of NSA are shown in Fig. 4. For both the 0.01- and 0.03-mg/kg NSA doses, osmotic pump infusion at 1 mg/kg/day produced serum nicotine concentrations greater than those associated with the first nicotine dose of a NSA session, and approximately equal to venous nicotine concentration measured 3 min after a nicotine dose at steady state. The 2.1-mg/kg/day infusion rate produced serum nicotine concentrations approximating the peak venous serum nicotine levels produced by NSA simulation (30 s after a nicotine dose). The 3.2-mg/kg/day infusion rate produced serum nicotine concentrations exceeding the peak venous serum nicotine levels produced by NSA simulation and (for the

Table 1
Serum and brain nicotine and cotinine concentrations associated with subcutaneous osmotic pump infusion of nicotine

Osmotic pump model and nicotine infusion rate	Venous nicotine (ng/ml)	Arterial nicotine (ng/ml)	Brain nicotine (ng/g)
(A) Nicotine concentrations			
2ML2			
0	<5	<5	<5
1.0 mg/kg/day	18±5	17±6	78±39
2.1 mg/kg/day	27±6	25±5	134±37
3.2 mg/kg/day	40±8	34±6	151±34
2ML4			
0 mg/kg/day	<5	<5	<5
1.0 mg/kg/day	26±8	24±8	102±41
2.1 mg/kg/day	46±14	41±11	157±20
3.2 mg/kg/day	77±15	69±13	327±49
(B) Cotinine concentrations			
2ML2			
0 mg/kg/day	6±6	6±7	<30
1.0 mg/kg/day	153±26	154±23	135±19
2.1 mg/kg/day	317±86	296±57	237±70
3.2 mg/kg/day	500±53	498±49	372±62
2ML4			
0 mg/kg/day	23±9	24±8	<30
1.0 mg/kg/day	171±41	172±36	130±28
2.1 mg/kg/day	328±36	331±55	234±28
3.2 mg/kg/day	518±152	514±167	324±110

Concentrations are from Experiment 3 and were measured 2 days after NSA has stopped (but while osmotic pump infusion continued) in order to measure nicotine and cotinine concentrations resulting from osmotic pump infusion alone. (A) Nicotine concentrations were infusion rate related. The very low nicotine concentrations in control (saline infusion) group demonstrate that there was little residual nicotine remaining 2 days after NSA was stopped. The 2ML2 pumps were used for the NSA 0.01 mg/kg/infusion groups, and the 4 ML2 pumps were used for the NSA 0.03 mg/kg/infusion groups. Nicotine concentrations associated with the 2ML4 pumps were higher than those from the 2ML2 pumps despite the same calculated infusion rates, consistent with pilot data showing higher actual infusion rates from the 2ML4 pumps. Thus, the higher infusion rates needed for the NSA 0.03-mg/kg/infusion groups were provided by using the 2ML4 pumps. Values are the means±S.D. (B) Cotinine concentrations 2 days after NSA was stopped. Very low values in the control (saline infusion) group show that little cotinine remained from the NSA that was terminated 2 days earlier. Serum cotinine concentrations at the highest (3.2 mg/day) nicotine infusion rate were higher than those associated with simulated NSA alone (Fig. 1).

0.01-mg/kg/inf NSA dose) equaling the peak arterial nicotine concentrations associated with simulated NSA.

3.5. Nicotine intake during NSA

The mean±S.D. daily nicotine intake during the baseline FR 3 period for NSA at the 0.01-mg/kg/inf unit dose was 0.66±0.22 mg/kg/day (range 0.23–1.06) and for the 0.03-mg/kg/inf unit dose was 1.18±0.43 mg/kg/day (range 0.53–1.84). These values compare favorably with the mean doses delivered during simulated NSA (see Experiments 1 and 2) of 0.6 mg/kg/day for the 0.01-mg/kg/inf unit dose and 0.9 mg/kg/day for the 0.03-mg/kg/inf unit dose.

3.6. Effects of nicotine infusion on NSA

Fig. 5 shows absolute levels of responding for each unit nicotine dose during each acquisition phase and the nicotine treatment phase. Fig. 6 shows the effects of nicotine treatment as the percent reduction in responding relative

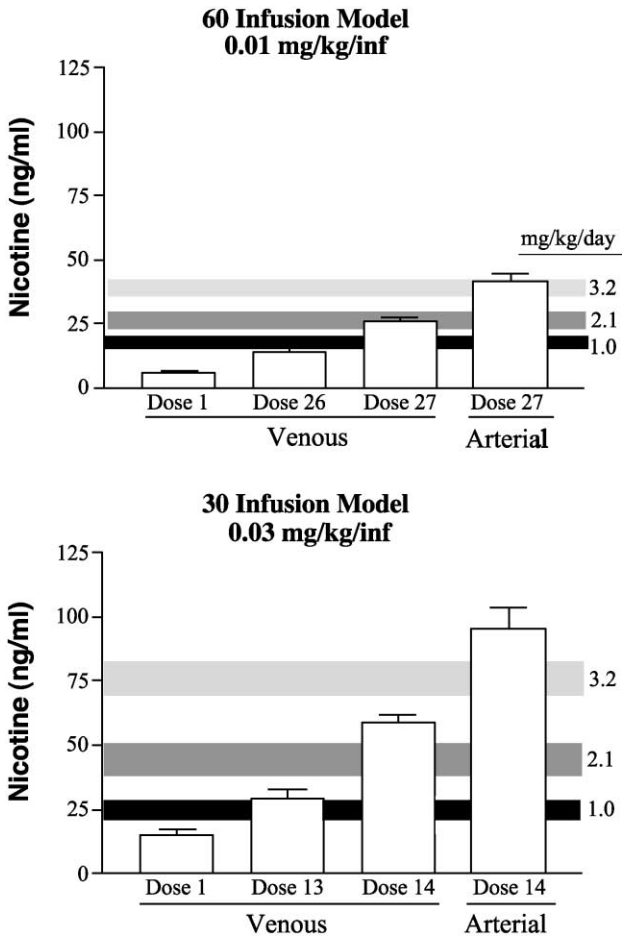

Fig. 4. Relationship between serum nicotine concentrations during simulated NSA and nicotine concentrations produced by continuous subcutaneous osmotic pump infusion. Vertical bars represent the mean±S.E. values of serum nicotine concentrations during simulations of NSA. For the 0.01-mg/kg/inf NSA unit dose (top), venous serum values were measured 3 min after the 1st and 26th nicotine doses and 30 s after the 27th nicotine dose (data also presented in Figs. 1 and 2). Venous nicotine concentrations were higher after the 27th dose than after the 26th dose because blood was sampled sooner. Arterial concentrations were measured 10 s after the 27th dose to represent the peak arterial concentration. For the 0.03-mg/kg/inf NSA unit dose (bottom), venous serum concentrations were measured 3 min after the 1st and 13th dose, 30 s after the 14th dose, and the arterial concentrations were measured 10 s after the 14th dose. The shaded horizontal bars represent the mean±S.E. serum nicotine concentrations produced by osmotic pump infusion alone at various rates. Note that similar osmotic pump infusion rates produced higher serum nicotine concentrations in the NSA 0.03-mg/kg groups than in the nicotine 0.01-mg/kg groups because a different model infusion pump was used. For both NSA regimens, the venous serum nicotine concentrations produced by continuous subcutaneous nicotine infusion spanned a range from lower than to higher than the peak venous concentrations associated with NSA.

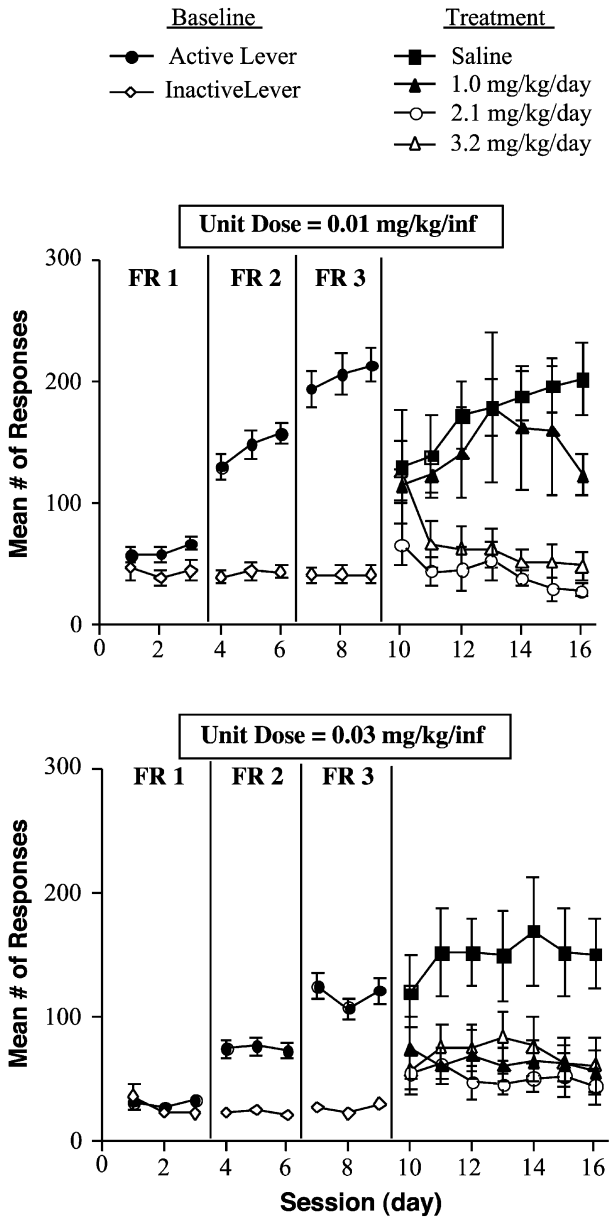

Fig. 5. Effects of nicotine infusion on NSA with a nicotine dose of 0.01 (top) or 0.03 mg/kg/inf (bottom). Data from all four groups are combined for the 3 weeks prior to osmotic pump implantation. For the first 2 days after osmotic pump implantation, while rats were recovering from this surgery, data were collected but were not used in subsequent statistical analysis. Nicotine infusion decreased active lever pressing at both NSA unit doses. Data are the means ± S.E. Percent reductions and statistical analysis are presented in Fig. 6. Note that similar osmotic pump infusion rats of 1.0, 2.1 and 3.2 mg/kg/day produced higher serum nicotine concentrations in rats responding for 0.03 mg/kg/inf because a different model of osmotic pump was used. These higher concentrations were specifically intended, and the use of different pumps was simply the means used to achieve them (see Experiment 4 in Methods).

to baseline levels under the FR 3 schedule. Two-factor ANOVA indicated a significant main effect of continuous nicotine infusion rate ($F = 29.11$, $P < .0001$) but no main effect of NSA unit dose ($F = 3.15$, $P = .084$). That is, NSA at either unit dose was significantly reduced by continuous

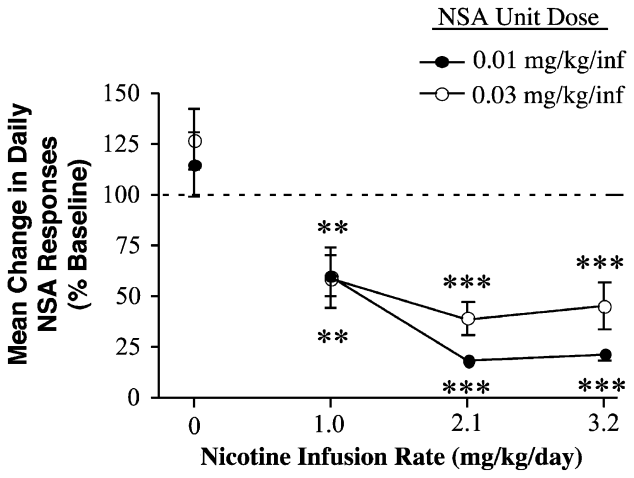

Fig. 6. Effects of continuous nicotine infusion on NSA expressed as the percent of mean daily baseline responding on the active lever (mean ± S.D.). The control groups (saline infusion) showed no change from baseline. All groups receiving continuous nicotine infusions showed significant decreases in NSA. For the NSA 0.01-mg/kg/inf group, nicotine infusion produced a modest decrease in NSA at the 1.0-mg/day infusion rate, and significantly greater reductions at 2.1 or 3.2 mg/day. For the NSA 0.03-mg/kg/infusion group, all nicotine infusion rates produced comparable decrements in NSA. The decrements at 2.1 and 3.2 mg/day were less than those observed in the NSA 0.01-mg/kg/infusion groups, but this difference was not significant. ** $P < .01$, *** $P < .001$ compared to the 0-mg/kg/day nicotine infusion group.

nicotine infusion at all treatment doses, while NSA was not significantly affected in rats treated with saline. In rats responding for 0.01 mg/kg/inf, continuous nicotine infusion

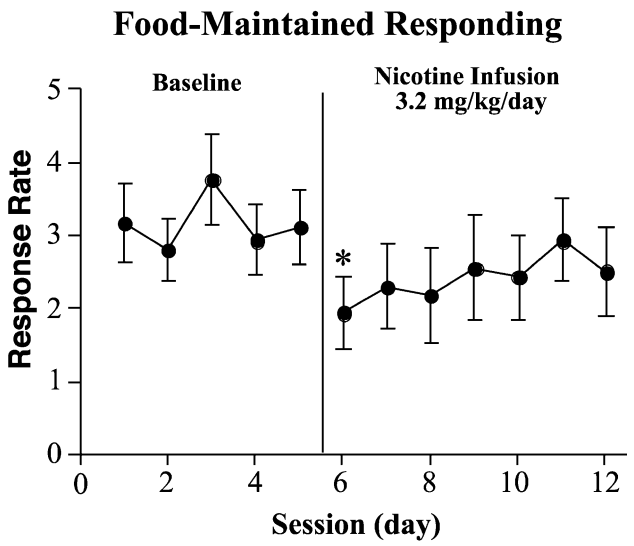

Fig. 7. Effects of nicotine infusion at 3.2 mg/day on food maintained behavior. Rats received 15 g of noncontingent food daily and 111 pellets (5 g) of response-contingent food under an FR 3 schedule. At the end of the baseline period, osmotic pumps were implanted for nicotine infusion. During nicotine infusion, all rats earned all of the available food pellets. The data shown are the mean ± S.E. response rates on the active lever. Nicotine infusion had no effect on total food intake and reduced response rate only for the first day of data collection after osmotic pump implantation. * $P < .05$.

produced dose-related decreases in the mean number of active lever presses. The effect of 2.1 and 3.2 mg/kg/day was significantly different from 1.0 mg/kg/day ($q = 4.39$, $P < .05$ and $q = 4.06$, $P < .05$, respectively), but the effect of 3.2 mg/kg/inf was not different from that of 2.1 mg/kg/inf. Such dose-dependent effects were not observed in rats responding for 0.03 mg/kg/inf (i.e., the percent reduction in NSA at the 2.1- and 3.2-mg/kg/day treatment doses was not significantly different from that at the 1-mg/kg/day dose).

3.7. Experiment 5. Effects of nicotine infusion on food-maintained behavior

Mean rates of responding during the baseline and nicotine-infusion phases are shown in Fig. 7. Continuous nicotine infusion at the highest infusion rate (3.2 mg/kg/day) did not suppress total food intake. All rats earned all 111 food pellets (5 g) under the FR 5 schedule on all days. A significant ($37 \pm 15\%$ S.E.M.; $t = 3.37$, $P < .05$) decrease in response rate was observed only on the first day of nicotine infusion.

4. Discussion

This study expands existing models of NRT by (1) using a 23-h/day NSA model to provide nicotine at daily doses comparable to cigarette smoking and in a temporal pattern of nicotine access which resembles that of cigarette smoking, (2) characterizing the venous and arterial serum nicotine concentrations associated with simulated NSA, which then allowed quantitative simulation of NRT, (3) demonstrating substantial suppression of NSA by continuous nicotine infusion and (4) demonstrating the behavioral specificity of continuous nicotine infusion by its lack of sustained effect on food-maintained behavior. Within the range of nicotine infusion rates studied, the hypothesis that higher infusion rates would result in greater suppression of NSA was not clearly supported since nicotine infusion rates producing venous serum nicotine concentrations exceeding the peak venous levels associated with simulated NSA were more effective than lower infusion rates only at the lower NSA unit dose.

Observations of 23-h/day access NSA in this study confirmed those of Valentine et al. (1997) that mean daily nicotine intake with this model (0.66 mg/kg/day with the 0.01-mg/kg NSA dose, 1.18 mg/kg/day with the 0.03-mg/kg NSA dose) is comparable to the reported range of 0.15–1.1 mg/kg/day in cigarette smokers (Benowitz and Jacob, 1984). In addition, the mean serum nicotine concentrations 3 min after the first dose of a simulated NSA session (6–15 ng/ml) or 3 min after a midsession dose representing steady-state levels (14–29 ng/ml) were similar to the 10–40-ng/ml range reported in cigarette smokers (Benowitz and Jacob, 1990; Foulds et al., 1992a; McNabb et al., 1982). Venous serum nicotine concentrations measured in rats just 30 s after a midday dose were higher than concentrations measured 3 min after the dose, as expected from a previous study of single nicotine doses administered to rats in a comparable manner (Hieda et al., 1999). We are aware of no comparable data in humans, i.e. blood obtained within less than a minute after a mid- or late-day cigarette. In contrast, simulation of the more commonly used 1-h/day access NSA model produced venous nicotine concentrations (mean 75 ng/ml) that were nearly three times as high and which exceeded venous levels typical of cigarette smokers. These venous nicotine concentrations were similar to a mean venous level of 65 ± 6 ng/ml reported in rats at the end of a 2-h session with a NSA unit dose of 0.03 mg/kg/inf (Shoaib and Stolerman, 1999). The 23-h/day access model may therefore have advantages for studies in which quantitative approximations of serum nicotine levels or dosing patterns during cigarette smoking are important. As previously reported (Shoaib and Stolerman, 1999), venous serum concentrations of the major nicotine metabolite cotinine were lower than those typical of cigarette smokers, perhaps because less nicotine is converted to cotinine in rats than in humans (Kyerematen et al., 1988; Shulgin et al., 1987) or because of species differences in rates of cotinine elimination.

Arterial nicotine concentrations in smokers within the first 10 min after a cigarette have been reported to be 2–10 times higher than concurrent venous levels (Henningfield et al., 1993; Rose et al., 1999a). In the current study, the peak arterial nicotine concentration during simulated NSA was higher than the peak venous concentration. The boost in the arterial/venous nicotine concentration ratio after a midsession nicotine dose was 2.8 for the 0.03-mg/kg dose and 3.6 for the 0.01-mg/kg dose. Thus, administering nicotine via the jugular vein provided the higher initial arterial nicotine concentrations typical of cigarette smoking. A similar arterio-venous nicotine concentration ratio was previously observed in rats receiving a single nicotine dose in this manner (Hieda et al., 1999); the current study extends these findings to rats receiving repeated nicotine doses.

The subcutaneous nicotine infusions in this study produced a range of nicotine concentrations in rats responding for both the 0.01- and 0.03-mg/kg/inf unit NSA doses that spanned the range of peak venous serum nicotine concentrations produced by simulated NSA alone at these unit NSA doses. The highest subcutaneous nicotine infusion rates equaled or exceeded the highest peak venous levels associated with NSA. As intended, the nicotine concentrations associated with subcutaneous nicotine infusion were higher for the 0.03-mg/kg/inf groups than for the 0.01-mg/kg/inf groups. In contrast to nicotine administered as intravenous bolus doses, the corresponding arterial nicotine concentrations produced by subcutaneous nicotine infusion were slightly lower than concurrent venous concentrations, likely because some of the nicotine, which was infused subcutaneously was absorbed into veins draining into the femoral venous site of blood sampling. Thus, the venous nicotine concentrations produced by subcutaneous nicotine

infusion represent the highest blood levels to which rats were exposed by this intervention.

At the 0.01-mg/kg/inf NSA unit dose, continuous nicotine infusion produced a dose-related suppression of NSA, with the 2.1- and 3.2-mg/kg/day infusion rates suppressing NSA to a greater extent than the 1-mg/kg/day rate. There was no additional effect from the 3.2- compared to the 2.1-mg/kg/day rate. However, these two infusion rates produced 81% and 78% suppression of NSA, respectively, which is similar to the decrease in responding observed when saline was substituted for nicotine and which may therefore represent the maximum selective suppression of NSA that is achievable with this protocol.

At the 0.03-mg/kg/inf NSA unit dose, comparable suppression of NSA was observed at all three infusion rates. Taken together with results from the 0.01-mg/kg/inf experiments, these data provide only equivocal support for the hypothesis that suppression of NSA is nicotine infusion rate related over the range of infusion rates studied. Rather, they suggest that it is not necessary to equal or exceed the highest nicotine concentrations in blood during NSA in rats in order for NRT to substantially reduce the rewarding effects of NSA. However, even the highest nicotine infusion rates used in this study did not produce serum nicotine concentrations exceeding the peak arterial concentrations association with simulated NSA. It is possible that such higher nicotine infusion rates might be more effective than those used in this study.

There are limited animal data to compare with these results. Pretreatment of rats with one dose of subcutaneous nicotine produced dose-related suppression of NSA during a subsequent 1-h NSA session, but nicotine blood levels were not measured (Corrigall and Coen, 1989). In humans, intravenous nicotine at a rate calculated to match each smoker's daily nicotine intake from cigarettes reduced ad lib smoking by only 25% (Benowitz and Jacob, 1990). Three nicotine patches (producing venous serum nicotine concentrations of 45–60 ng/ml) reduced ad lib smoking to a greater extent than one or two patches, but suppression was still incomplete (40%). These results from human studies contrast with those of the current study in that NRT of roughly comparable magnitude appeared to produce greater suppression of NSA in rats.

While the 23-h/day access NSA model mimics several features of cigarette smoking in humans, important differences still remain. The lowest NSA dose used, 0.01 mg/kg/inf, is equivalent to the nicotine absorbed by a smoker from about 2/3 of a cigarette. In the rat, this dose is delivered as a single bolus, while smokers divide it into 5–10 puffs over 5–10 min. The 0.01-mg/kg/inf NSA dose was used because it is the lowest NSA dose that is reliably self-administered using this model. Lower NSA doses have been reported to support NSA (Valentine et al., 1997), but the fraction of rats acquiring NSA at this dose is lower (unpublished data). Smoking also differs from NSA in rats in that smoking provides local sensory stimulation of the pharynx and respiratory tract (Rose et al., 1999b) and is associated with different behavioral and social cues (Rose and Corrigall, 1997). Thus, the model of NSA used in this study incorporates several important features of cigarette smoking but lacks others that could be important in predicting responses to interventions.

The safety of using high nicotine dosing rates has not been systematically studied in rats, but subcutaneous osmotic pump infusion rates of up to 4.3 mg/kg/day have been tolerated in rats without apparent adverse effects (Winders et al., 1988). In humans, doses of two or three nicotine patches at a time (42 or 63 mg/day) have been well tolerated (Dale et al., 1995; Fredrickson et al., 1995; Hughes et al., 1999; Jorenby et al., 1995). In the current study, the 3.2-mg/kg/day nicotine infusion rate did not suppress total food intake and produced only a transient decrease in the response rate for food. These data argue that nicotine infusion even at the highest rate used is well tolerated and that the effects of nicotine infusion on NSA are behaviorally specific.

In summary, venous and arterial serum nicotine concentrations associated with simulated NSA using a 23-h/day access model were similar to those reported in cigarette smokers. NSA in rats with access to nicotine for 23 h/day was suppressed by continuous infusion of nicotine, which is comparable to the effects of NRT in human smokers. Nicotine infusion rates producing serum nicotine concentrations equaling or exceeding the highest venous concentrations associated with NSA were more effective than lower infusion rates in suppressing NSA only at the lower NSA unit dose. High infusion rates of nicotine were well tolerated, and their effects were specific for NSA. This model of NSA, which has some quantitative and temporal features resembling cigarette smoking in humans, may prove helpful for studying the mechanisms and therapeutic use of NRT. It may also be useful for studying questions for which clinically relevant nicotine concentrations and dosing patterns are important, such as the evaluation of potential therapeutic agents, which modify nicotine pharmacokinetics.

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References

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

Benowitz NL, Jacob P. Intravenous nicotine replacement suppresses nicotine intake from cigarette smoking. J Pharmacol Exp Ther 1990;254:1000–5.

Benowitz NL, Jacob P, Savanapridi C. Determinants of nicotine intake while chewing nicotine pilacrilex gum. Clin Pharmacol Ther 1987;41:467–73.

Benowitz NL, Zevin S, Jacob P. Suppression of nicotine intake during ad libitum cigarette smoking by high-dose transdermal nicotine. J Pharmacol Exp Ther 1998;287:958–62.

Blondal T, Gudmundsson LJ, Olafsdottir I, Gustavsson G, Westin A. Nicotine nasal spray with nicotine patch for smoking cessation: randomised trial with six year follow up. Br Med J 1999;318:285–8.

Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 1989;99:473–8.

Dale LC, Hurt RD, Offord KP, Lawson GM, Croghan IT, Schroeder DR. High-dose nicotine patch therapy: percentage of replacement and smoking cessation. JAMA, J Am Med Assoc 1995;274:1353–8.

Donny EC, Caggiula AR, Knopf S, Brown C. Nicotine self-administration in rats. Psychopharmacology (Berlin) 1995;122:390–4.

Fagerstrom KO, Schneider NG, Lunell E. Effectiveness of nicotine patch and nicotine gum as individual versus combined treatments for tobacco withdrawal symptoms. Psychopharmacology 1993;111:271–7.

Fiore MC, Smith SS, Jorenby DE, Baker TB. The effectiveness of the nicotine patch for smoking cessation. A meta-analysis. JAMA, J Am Med Assoc 1994;271:1940–7.

Fiore M, Bailey WC, Cohen SJ, Faith Dorfman S, Goldstein M, Gritz ER, Heyman RB, Roberto Jaen D, Kottke TE, Lando HA. Treating tobacco use and dependence. Clinical practice guideline Public Health Service, U.S. Department of Health and Human Services, 2000.

Foulds J, Stapleton J, Feyerabend C, Vesey C, Jarvis M, Russell MA. Effect of transdermal nicotine patches on cigarette smoking: a double blind crossover study. Psychopharmacology 1992a;106:421–7.

Foulds J, Stapleton J, Feyerabend C, Vesey C, Jarvis M, Russell MAH. Effect of transdermal nicotine patches on cigarette smoking: a double-blind crossover study. Psychopharmacology 1992b;106:421–7.

Fredrickson PA, Hurt RD, Lee GM, Wingender L, Croghan IT, Lauger G. High dose transdermal nicotine therpay for heavy smokers: safety, tolerability and measurement of nicotine and cotinine levels. Psychopharmacology 1995;122:215–22.

Gourlay SG, Benowitz NL. Arteriovenous differences in plasma concentration of nicotine and catecholamines and related cardiovascular effects after smoking, nicotine nasal spray, and intravenous nicotine. Clin Pharmacol Ther 1997;62:453–63.

Henningfield JE, Stapleton JM, Benowitz NL, Grayson RF, London ED. Higher levels of nicotine in arterial than in venous blood after cigarette smoking. Drug Alcohol Depend 1993;33:23–9.

Hieda Y, Keyler DE, VanDeVoort JT, Niedbala RS, Raphael DE, Ross CA, Pentel PR. Immunization of rats reduces nicotine distribution to brain. Psychopharmacology 1999;143:150–7.

Hughes JR, Lesmes GR, Hatsukami DK, Richmond RL, Lichtenstein E, Jorenby DE, Broughton JO, Fortmann SP, Leischow SJ, McKenna JP, Rennard SI, Wadland WC, Heatley SA. Are higher doses of nicotine replacement more effective for smoking cessation? Nicotine Tob Res 1999;1:169–74.

Hurt RD, Dale LC, Fredrickson PA. Nicotine patch therapy for smoking cessation combined with physician advice and nurse follow-up: one-year outcome and percentage of nicotine replacement. JAMA, J Am Med Assoc 1994;271:595–600.

Jacob P, Wilson M, Benowitz NL. Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. J Chromatogr 1981;222:61–70.

Jorenby DE, Smith SS, Fiore MC, Hurt RD, Offord KP, Croghan IT, Hays JT, Lewis SF, Baker TB. Varying nicotine patch dose and type of smoking cessation counseling. JAMA, J Am Med Assoc 1995; 274:1347–52.

Keyler DE, Hieda Y, St.Peter J, Pentel PR. Altered disposition of repeated nicotine doses in rats immunized against nicotine. Nicotine Tob Res 1999;1:241–9.

Kyerematen GA, Taylor LH, Debethizy JD, Vesell ES. Pharmacokinetics of nicotine and 12 metabolites in the rat. Application of a new radiometric high performance liquid chromatography assay. Drug Metab Dispos 1988;16:125–9.

Lawson GM, Hurt RD, Dale LC, Offord KP, Croghan IT, Schroeder DR, Jiang NS. Application of serum nicotine and plasma cotinine concentrations to assessment of nicotine replacement in light, moderate, and heavy smokers undergoing transdermal therapy. J Clin Pharmacol 1998; 38:502–9.

Lucchesi BR, Schuster CR, Emley GS. The role of nicotine as a determinant of cigarette smoking frequency in man with observations of certain cardiovascular effects associated with the tobacco alkaloid. Clin Pharmacol Ther 1967;8:789–96.

McNabb ME, Ebert RV, McCusker K. Plasma nicotine levels produced by chewing nicotine gum. JAMA, J Am Med Assoc 1982;248:865–8.

Perkins KA, Grobe JE, Stiller RL, Fonte C, Goettler JE. Nasal spray nicotine replacement suppresses cigarette smoking desire and behavior. Clin Pharmacol Ther 1992;52:627–34.

Rose JE, Corrigall WA. Nicotine self-administration in animals and humans: similarities and differences. Psychopharmacology 1997;130: 28–40.

Rose JE, Behm FM, Westman EC, Coleman RE. Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: implications for addiction. Drug Alcohol Depend 1999a;56:99–107.

Rose JE, Westman EC, Behm FM, Johnson MP, Goldberg JS. Blockade of smoking satisfaction using the peripheral nicotinic antagonist trimethaphan. Pharmacol, Biochem Behav 1999b;62:165–72.

Shoaib M, Stolerman IP. Plasma nicotine and cotinine levels following intravenous nicotine self-administration in rats. Psychopharmacology 1999;143:318–21.

Shulgin AT, Jacob P, Benowitz NL, Lau D. Identification and quantitative analysis of cotinine-*N*-oxide in human urine. J Chromatogr 1987;423: 365–72.

Stratton KSP, Wallace R, Bondurant S, editors. Clearing the smoke. Assessing the science base for tobacco harm reduction. Washington, DC: National Academy Press, 2001 (Ed.: Medicine I o).

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

Winders SE, Grunberg NE, Benowitz NL, Alvares AP. Effects of stress on circulating nicotine and cotinine levels and in vitro nicotine metabolism in the rat. Psychopharmacology 1988;137:383–90.