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RAPID COMMUNICATION

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Self-administration in rats allowed unlimited access to nicotine

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Abstract The purpose of the present study was to develop an animal model of nicotine self-administration that more closely approximates the conditions of human nicotine use than do existing models. In most nicotine self-administration models, rats acquire self-administration during brief daily sessions in which rapid injections of a relatively high dose of the drug, 0.03 mg/kg, serve as the reinforcer. The present study examined nicotine self-administration in rats that acquired the behavior while having virtually unlimited access to injections of a relatively low dose of the drug; the rats did not have any prior operant training or shaping. Under these conditions, rats readily acquire nicotine self-administration at doses at least as low as 0.00375 mg/kg per injection, and they self-administer throughout the active portion of their light cycle. The daily nicotine intake of rats, which ranged from 0.18 to 1.38 mg/kg per day, appears to be comparable to that of human smokers.

Key words Nicotine · Self-administration · Intravenous · Dose-response · Nicotine intake · Unlimited access · Rats

Introduction

The development of animal models of nicotine self-administration has progressed more slowly than for other abused drugs (Rose and Corrigall 1997). This is in part due to the fact that animals do not self-administer nicotine under the same conditions in which they readily self-administer drugs such as cocaine and heroin (e.g., Ator and Griffiths 1983; Bozarth and Pudiak 1996; reviewed by Henningfield et al. 1983; Slifer and Balster 1985). This has led some to conclude that the reinforcing properties of nicotine are relatively low (Griffiths et al. 1979) or that special conditions are required for animals to self-administer the drug (Corrigall 1991; Dougherty et al. 1981). Thus, nicotine self-administration was sometimes studied using special procedures to induce self-administration of the drug (Lang et al. 1977; Slifer 1983; Slifer and Balster 1985).

The work of others, however, has shown that animals readily acquire nicotine self-administration if the drug is delivered rapidly as a high concentration bolus containing 0.03 mg/kg nicotine (Cox et al. 1984; Corrigall and Coen 1989; Donny et al. 1995; Shoaib et al. 1997). However, this dose is approximately equal to the human intake from two cigarettes (Benowitz and Jacobs 1984). When cocaine-experienced cigarette-smokers blindly self-administered similar doses of IV nicotine, they identified the drug as cocaine (Henningfield and Goldberg 1983). Thus, nicotine's effects on the CNS at relatively high doses may differ considerably from its effects at lower doses. The present study was conducted to examine nicotine self-administration in naive rats allowed to acquire the behavior while having unlimited access to injections of doses ranging from 0.00375 to 0.03 mg/kg per injection.

Materials and methods

Twenty-eight male Holtzman rats (250–350 g; HSD, Madison, Wisc., USA) were anesthetized (3.75 mg/kg droperidol +0.075 mg/kg fentanyl) and implanted with a catheter (PE 90 and silastic) in the right jugular vein and a polysulfonyl "button" (Instech, Plymouth Meeting, Pa., USA) just posterior to the shoulder blades. Following surgery, the rats were placed into operant chambers (Coulbourn, Allentown, Pa., USA) for the duration of the experiment. Each chamber was equipped with two levers on one wall (5 cm above the floor) and a water bottle on the opposite wall. A small cue light was located 1 cm over each lever.

The chambers were enclosed in sound- and light-attenuating boxes equipped with ventilation fans and timer-controlled lights (12 h light/12 h dark). The catheters exited the animals via the buttons and connected to fluid-through swivels (Instech) by way of

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flexible spring coils. During recovery, food and water were freely available; daily injections of gentamycin (4 µg/kg, IV) and hourly injections of saline (50 µl containing 200 units/ml heparin, IV) were given.

The rats were randomly assigned to one of four groups (n=7/group). At the beginning of the dark cycle on day 3 of recovery from surgery, the small cue light (45 millicandles) over each lever was turned on, thus signaling that IV nicotine (0.00375, 0.0075, 0.015 or 0.03 mg/kg per injection (expressed as the free base of nicotine sulfate; Sigma) was available contingent upon a press of one of the two levers (the rats did not receive any prior shaping or training with food reinforcement.) A 7-s time-out, during which the cue light over the active lever was off, followed each injection. Pressing the alternate lever had no consequence. Nicotine was delivered in a volume of 50 µl/350 g body weight at a rate of 50 µl/0.81 s by high-speed pumps (PHM-100-15, Med Associates, Georgia, Vt., USA) using 6-ml disposable syringes. To maintain the high degree of catheter patency needed to establish and maintain reliable nicotine self-administration (see Discussion), the drug solutions contained 400 units/ml heparin. Computers and interfaces (Coulbourn) located in an adjoining room were used to record the time of occurrence of all lever presses and injections, and to deliver the injections. Nicotine was always available except for a 1- to 2-h period at the end of the light portion of the light cycle, when the drug solutions were changed and the animals were cared for; the cue lights over the levers were not on during this period. Every week the rats were weighed and provided with a freshly sanitized operant chamber. Water was always available, but daily food rations were limited to 20–25 g to prevent the rat's weights from rapidly increasing between each weighing, because that would have resulted in their nicotine dose/injection considerably decreasing over the week. However, any rat not gaining 5% body weight since its prior weighing had its daily food ration increased.

Each rat was allowed to self-administer nicotine until it met a criterion of 3 consecutive days, during which the number of injections it self-administered per day had a coefficient of variation of 15% or less, although a minimum of 14 days of self-administration was required. A rat in the 0.0075 mg/kg group had to be dropped from the study due to a broken catheter, and a rat in the 0.00375 mg/kg group was dropped because it failed to demonstrate any evidence of self-administration (one to three responses per day for 2 weeks); it was possible to withdraw blood from its catheter throughout.

Log transforms of the number of injections, the total drug intake (i.e., the number of injections×the nicotine dose), the total number of lever presses and the nicotine dose per injection were analyzed using analyses of variance (ANOVAs; SAS) and linear regression (Systat 6.0, SPSS). Repeated measures ANOVAs were performed on the mean number of injections self-administered per day over the first 14 days of acquisition with dose and day serving as factors. The mean number of injections and total drug intake per day during the 3 days in which each rat met the criterion for stable responding (above) were analyzed separately using both ANOVAs and linear regression, with dose serving as the independent variable. The total number of lever presses occurring during these 3 days were also analyzed using repeated measures ANOVAs with dose and lever as the factors. The number of lever presses occurring during one of these days was also analyzed using repeated measures ANOVAs, with dose, lever and time period serving as the factors. Post-hoc tests were conducted using Duncan's multiple range test. Alpha was set at 0.05 for all tests.

Results

Nicotine self-administration was rapidly acquired at all of the doses tested, with the self-administration rates of all of the groups significantly increasing over the first few days of acquisition (see Fig. 1). Thereafter, the rates of the three lowest dose groups continued to increase,

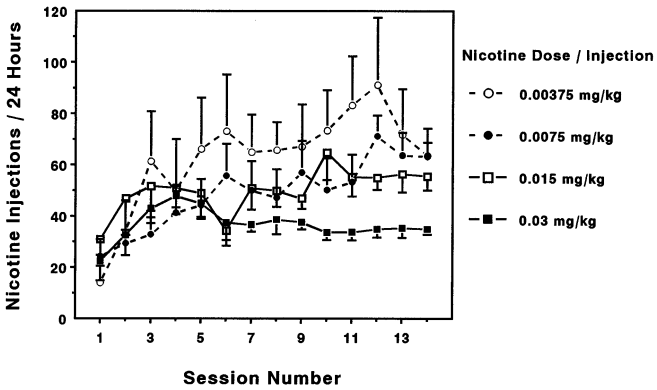

Fig. 1 The mean number of nicotine injections self-administered per day at each dose during the first 14 days of self-administration

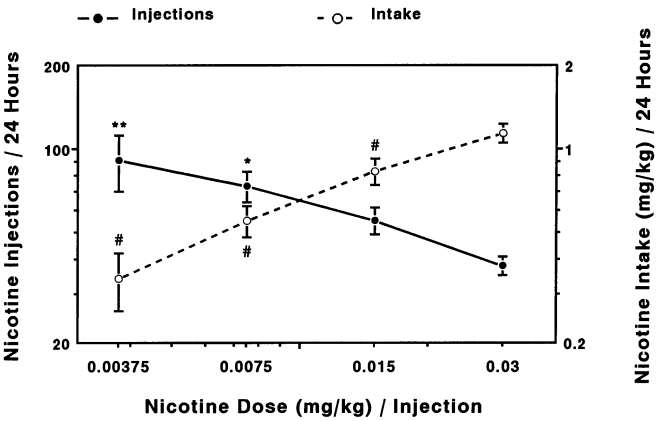

Fig. 2 The effect of nicotine dose per injection on self-administration rates and total nicotine intake when stable responding was achieved. $^{**}P<0.05$ versus 0.015 and 0.03 mg/kg. $^{*}P<0.05$ versus 0.03 mg/kg. #$P<0.05$ compared to other groups designated #

while that of the highest dose group began to decline slowly. ANOVAs indicated that, overall, the rate of self-administration on day 3 was significantly higher than that on day 1 [$F(13, 286)=8.90$, $P<0.00001$, for the main effect of day]. However, ANOVAs failed to indicate any significant differences in the self-administration rates of the different groups (Ps>0.12 for the effect of dose and the dose×day interaction).

All of the rats met the criterion for stable responding (see Materials and methods) within 14–26 days of self-administration; the mean days to criterion for the two lowest dose groups (0.00375 mg/kg=17.2±1.8; 0.0075 mg/kg=16.3±0.9) were only slightly higher than those for the two highest dose groups (0.015 mg/kg =14.1±0.1; 0.03 mg/kg=14.8±0.8). Analyses conducted on the means from the three sessions in which the rats met the criterion showed that their self-administration rates were dose-dependent; the number of injections self-administered per day was inversely related to the dose (see Fig. 2). Linear regression analyses indicated that the correlation between the nicotine dose and the number of injections per day was –0.67 [$F(1, 24)=19.6$, $P<0.0002$].

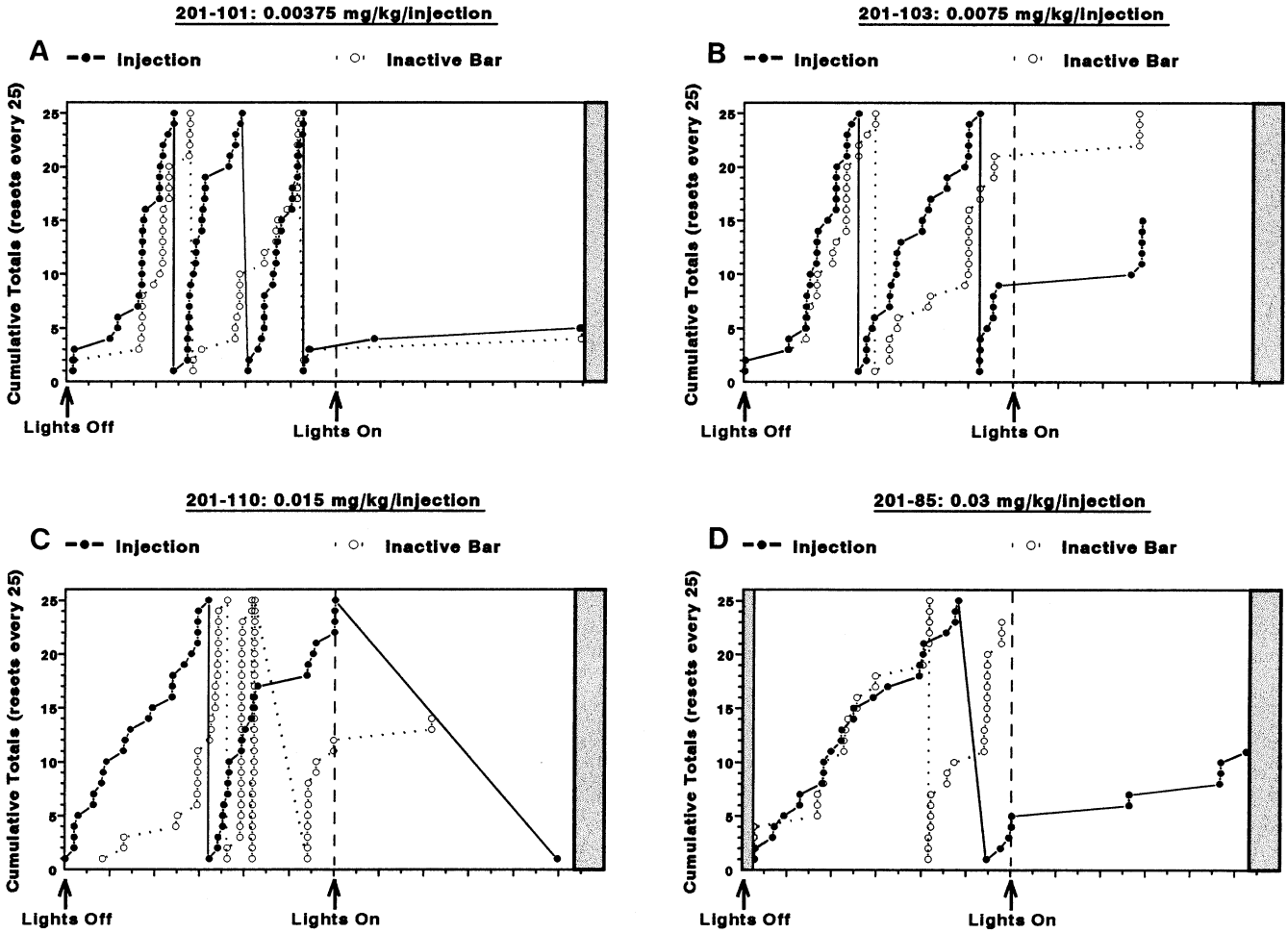

Fig. 3A–D Pattern of nicotine self-administration over 24-h period. **A**–**D** Graphic representations of cumulative records for one rat from each group on the day it met the criterion for stable responding. *Closed circles*, injections. *Open circles*, inactive lever presses. *Shaded region* indicates session was inactive

In addition, ANOVAs revealed that the self-administration rates of the 0.00375 and 0.0075 mg/kg groups were significantly higher than the 0.03 mg/kg group, and the rate of the 0.00375 mg/kg group was also higher than the 0.015 mg/kg group [$F(3, 22)=6.35$, $P<0.003$, for the effect of dose].

Nicotine consumption during the sessions in which the rats met criterion was also found to be dose-dependent. The total daily intake of the drug was positively related to the dose per injection and linear regression analysis showed that the correlation between nicotine dose and total intake was 0.82 [$F(1, 24)=52.41$, $P<0.00001$]. ANOVA indicated that the differences in nicotine consumption among the 0.00375, 0.0075 and 0.015 mg/kg groups were significant [$F(3, 22)=16.63$, $P<0.0001$].

The temporal pattern of self-administration for rats in each group can be seen in Fig. 3. The rat representing each group is the one with the mean rate of self-administration that was closest to the mean rate for its group, and its cumulative record shows its pattern of self-administration (closed circles) on the day it met the criterion for stable responding. Also shown in each record is the rat's pattern of responding on the inactive lever (open circles). As can be seen, most of the responding for nicotine occurred during the active (dark) portion of the rat's light/dark cycle. In addition, it can be seen that the slopes of the cumulative increases in self-administered injections are relatively stable and dose-dependent.

Analyses were also conducted on the mean number of active and inactive lever presses that occurred during the sessions in which each rat met the criterion for stable responding. ANOVAs revealed that, overall, the mean rate of responding on the active lever (112.91±12.39) was significantly higher than that on the inactive lever [84.90±15.54; $F(1, 22)=15.57$, $P<0.0007$, for the effect of lever]; the total number of responses occurring on the two levers was dose-dependent [$F(3, 22)=3.76$, $P<0.026$, for the effect of dose]. However, ANOVA failed to indicate any dose-dependent differences in the number of active, versus inactive, presses ($P>0.32$, for the dose×lever interaction). As can be seen in Fig. 4a, the rats in the 0.00375 and 0.03 mg/kg groups (doses expressed as 3.75 and 30.0 μg/kg in Fig. 4a) exhibited considerably higher rates of responding on the active compared to the inactive lever, whereas the rats in the 0.0075 and

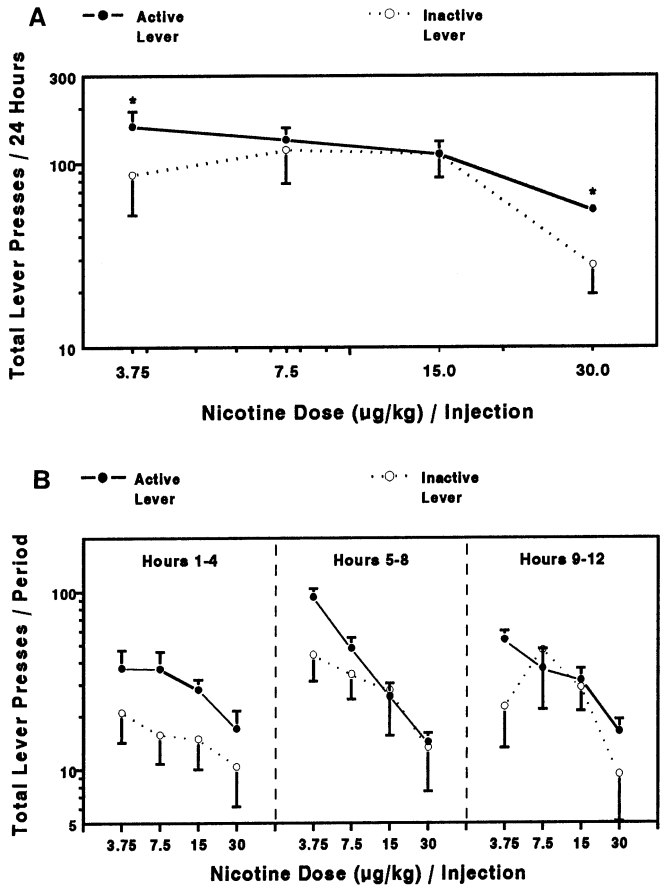

Fig. 4 **A** Mean number of total lever presses on the active and inactive levers for each group during the sessions in which each rat met the criterion for stable responding. **P*<0.05 versus inactive lever. **B** Mean lever presses occurring during different periods of the dark portion of the light cycle from one of the above sessions

0.015 mg/kg groups (expressed as 7.5 and 15.0 µg/kg in Fig. 4a) failed to exhibit evidence of selective lever pressing in their overall rates. Supplemental ANOVAs, conducted separately for each dose, confirmed that the number of responses on the two levers significantly differed in the 0.00375 [$F(1, 5)=10.84$, $P<0.022$] and 0.03 mg/kg groups [$F(1, 6)=6.68$, $P<0.042$], but not in the 0.0075 and 0.015 mg/kg groups (Ps>0.17 and 0.48, respectively).

Responding on the inactive lever was often observed to increase considerably in some animals several hours into the daily sessions (e.g., see Fig. 3c). Thus, the data from the active portion of one of the criterion sessions were divided into three equal periods. As can be seen in Fig. 4b, all of the groups appeared to exhibit a preference for the active lever during the first 4 h of this session. However, the two highest dose groups failed to exhibit any lever preference during the second 4-h period, as did the 0.0075 and 0.015 mg/kg groups during the third period. In each case, the disappearance of selective responding from one period to the next appeared to result primarily from an increase in the number of responses on the inactive lever over the two periods.

Discussion

The present study shows that experimentally naive rats rapidly learn to self-administer IV nicotine at doses at least as low as 0.00375 mg/kg per injection. Furthermore, the results also show that the rate of nicotine self-administration by rats having acquired this behavior at doses ranging from 0.00375 to 0.03 mg/kg is inversely related to the dose per injection. This is typical of the dose-response functions obtained with other self-administered drugs.

The dependence of self-administration rates and total drug intake on the dose per injection has been demonstrated with various self-administered drugs. Such studies have shown that, above the threshold dose for producing positive reinforcement, the rate of self-administration is inversely related to the dose per injection, whereas drug intake is positively related to the dose (Weeks and Collins 1964; Pickens and Harris 1968; Pickens and Thompson 1968; see Wise 1987, for discussion). Furthermore, these functions have been shown to be linear on a log-log scale (Weeks and Collins 1979), as was observed in the present study. The inverse relationship between self-administration rate and drug dose has been interpreted as indicating that animals are attempting to regulate their drug intake by reducing their self-administration rates at higher doses, or increasing them at lower doses (Yokel and Pickens 1974; Wise 1987). The dose-dependent differences in self-administration rates and nicotine intake observed herein are consistent with the hypothesis that the different groups of rats were regulating their nicotine intake.

At least one other study has been conducted in which rats were allowed to acquire nicotine self-administration while having continuous access to IV doses ranging from 0.003 to 0.03 mg/kg per injection (Cox et al. 1984). In that study, female Wistar rats with access to 0.03 mg/kg nicotine self-administered about the same number of injections per day as the rats having access to the same dose in the present study. However, in that study, rats having access to 0.01 mg/kg nicotine acquired self-administration considerably more slowly than those having access to 0.03 mg/kg, and, after 2 weeks, exhibited response rates that were still 50% lower than those at the higher dose. Moreover, rats having access to 0.003 mg/kg nicotine failed to show any increase in responding over the 2 weeks of self-administration. Interestingly, though, rats self-administering 0.03 mg/kg per injection exhibited a significant increase in their response rates when their dose was reduced to 0.003 mg/kg per injection. The reason that different dose-response functions were obtained in that study compared to the present study is not clear, but could be related to the use of different rat strains and sexes.

Another factor that may have contributed to the differences between the findings in the present study and those of previous studies (e.g., Cox et al. 1984; Corrigall and Coen 1986; Donny et al. 1995; Shoaib et al. 1997), might involve the rate at which IV nicotine is “actually”

delivered. While developing the nicotine self-administration procedure for use in this laboratory, we realized that rats that did not reliably self-administer the drug were often not receiving the full volume of their injections as quickly as was programmed. We were only able to determine this by inserting a small air bubble into the line connecting the syringe to the swivel and observing the speed and distance of its movement when an injection was administered. Our observations indicated that the efficacy of nicotine as a reinforcer declined rapidly as the "actual" injection time increased beyond 2–3 s, particularly at lower doses. In such cases, necropsies often showed that, compared to rats with reliable self-administration rates, rats exhibiting retarded injection speeds had developed fibrosis of the jugular vein that constricted the silastic catheter. Thus, for IV nicotine to be positively reinforcing, it needs to be delivered rapidly as a high concentration bolus.

Cigarette smoking has been well studied in humans, and the present findings in rats are consistent with those in humans. Studies have shown that smokers consume about 35 mg nicotine/day, with individual values varying widely over approximately 10–80 mg/day (Benowitz and Jacobs 1984, 1990). Such studies typically do not report nicotine intake in mg/kg, but based on our calculations using an average body weight of 70 kg, it would appear that smokers consume an average of 0.5 mg/kg nicotine per day, and that these values might range from about 0.14–1.14 mg/kg per day. The rats in the present study consumed 0.18–1.38 mg/kg nicotine per day. Thus, rats allowed unlimited access to IV nicotine consume daily amounts of nicotine that are comparable to human smokers. In contrast, rats allowed only limited access to 0.03 mg/kg per injection consume an average of approximately 0.5–0.6 mg/kg of the drug in a 1-h session (Corrigall 1989; Donny et al. 1995).

The model utilized in the present study shows that nicotine self-administration in rats exhibits some of the same dose-response characteristics seen with other self-administered drugs. Moreover, nicotine self-administration in rats exhibits important similarities to nicotine self-administration in humans. The model should prove to be useful for studying the mechanisms that underlie the development and maintenance of nicotine use.

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