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Nicotine self-administration in rats

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1 Female Wistar rats were allowed to self-administer nicotine solutions through indwelling jugular vein cannulae for 23 h per day for periods from three to five weeks.

2 Two response levers were available to the rats; responding on one lever, designated the active lever, produced an immediate infusion of nicotine solution or saline. A second lever for which responding had no programmed consequences was introduced as a control for the locomotor stimulant action of low doses of nicotine.

3 Baseline lever response rates were determined over a period of one week, in which active lever responding produced an infusion of saline. Rats were then allowed access to varying doses of nicotine or saline for a further two or three weeks. Response rates on the active lever increased significantly in rats with access to nicotine at a dose of 30 $\mu g\,kg^{-1}$ per response. However, control lever response rates were also significantly elevated.

4 The role of nicotine-induced locomotor stimulation in the self-administration behaviour was further evaluated in a dose-reduction experiment, in which the dose of nicotine available to rats responding for 30 $\mu g\,kg^{-1}$ per response was reduced to 3 $\mu g\,kg^{-1}$ per response. This resulted in a significant differential increase in active lever responding relative to control lever responding.

5 The results suggest that nicotine is positively reinforcing in rats which had not previously been deprived of food or water or received prior drug treatment, but also indicate that nicotine induced locomotor stimulation may contribute to the observed increases in lever response rates when rats self-administer nicotine.

Introduction

The self-administration of nicotine in drug naive rats with free access to food and water has not been extensively studied. Clark (1969) found that rats given a choice of water or nicotine solutions drank progressively more of the nicotine solution, but in this brief abstract, the magnitude of the preference, the quantities of nicotine consumed, and the possible role of taste in influencing the preference are not discussed. Hanson *et al.*, (1979), in a study in which rats self-administered nicotine through indwelling venous cannulae, also found that rats would self-administer nicotine solutions at a greater rate than saline, and that the preference would continue to develop over more prolonged exposures.

These studies seemed to indicate that nicotine is positively reinforcing in rats not subjected to prior food or water deprivation or prior treatment with nicotine. However, even after a month or more of access to nicotine the response rates were low compared with the rates at which rats will self-administer morphine, amphetamine or cocaine (Pickens *et al.*, 1978; Collins *et al.*, 1984). Since nicotine is known to increase locomotor activity when administered to rats at low doses (Pradhan, 1970; Bättig *et al.*, 1976; Clarke & Kumar, 1983), the possibility that the apparent self-administration of nicotine resulted from an initial accidental lever response leading to more frequent responses as a consequence of nicotine-induced increased activity could not be excluded. The results of Hanson *et al.*, (1979) and Collins *et al.*, (1984) suggest that the rat may be a relatively cheap and convenient experimental model for the pharmacological analysis of nicotine reinforcement. It is therefore important that the potential role of drug-induced locomotor stimulation in nicotine self-administration be explored. In the present experiments, a design that provides a measure of

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non-specific increases in motor behaviour was employed: a second lever, depression of which had no programmed consequences, provided a measure of the rate of responding not directly coupled to nicotine reinforcement. The results we report here suggest that nicotine self-administration in rats at normal body weight depends upon the reinforcing properties of the drug and is not an artifact of drug induced increases in accidental depression of the lever. Preliminary accounts of part of this study have been presented to the International Study Group Investigating Drugs as Reinforcers, 1981, and the Society for Neuroscience, 1981.

Methods

Forty-nine female Wistar rats (Simonsen Laboratories, Gilroy, California), weighing 250 to 300 g at the time of surgery, were implanted with intravenous cannulae (Weeks, 1972) in the right jugular vein under sodium pentobarbitone (50 mg kg^{-1}) anaesthesia. These rats had no prior exposure to either nicotine or the operant chambers. Food and water were available *ad libitum*.

Rats were tested in six Plexiglas boxes (20 × 23 cm, 19 cm high), each housed in an illuminated, sound attenuated chamber. Small exhaust fans provided ventilation and masking of ambient noise. On opposite ends of the test chamber were retractable levers (5 × 1.5 cm) extending 1.7 cm into the test chamber, 4 cm above the grid floor.

The self-administration technique employed has been described by Weeks (1977). Responding on the active lever resulted in an infusion by a 100 μl Hamilton microsyringe of either 0.9% W/V NaCl solution (saline) or nicotine solution, 100 μl kg^{-1}. Responding on the control lever was recorded, but had no programmed consequence. There was a 5 s time-out associated with activation of the syringe, during which lever presses were not recorded and did not result in a second infusion. All tubing connecting the drug solution reservoir to the rat's jugular cannula was replaced when post-drug saline solution replaced nicotine. The internal volume of the jugular cannulae was less than 25 μl.

Procedure

The results of Hanson *et al.*, (1979) indicated that self-administration of nicotine was likely to develop slowly. Therefore, we employed a paradigm in which the rate of lever pressing with drug or saline solutions was measured over sessions of seven days. In the initial session the base-line rates of responding for saline were recorded for each animal. The saline solution was then replaced by nicotine for two sessions. At the end of this period some animals continued to have access to nicotine for a further week, while others were returned to saline. Eventually, all animals were returned to saline. One group of seven rats were allowed access to saline without drug throughout five weeks of testing.

One week after surgery, a rat was placed in a light-weight harness attached to a fluid swivel that allowed freedom of movement, and was placed in the testing chamber. Cannula patency was assessed 30 min before placing the rat in the chamber on the first day, and again at the end of the post-drug saline sessions by infusing 25 μl of methohexital sodium (5 mg kg^{-1}) and observing the latency to loss of consciousness. Rats responding within 5 s were judged to have patent cannulae; only the data from those rats are included here.

Rats were tested for 23 h a day. Data were collected twice daily in conjunction with the lighting change of the test chambers and experimental room (white light on 08 h 00 min–20 h 00 min, dim red lights on in test chambers 20 h 00 min–08 h 00 min). Previous studies had shown that animals self-administer nicotine primarily during their active periods (Yanagita, 1977, Hanson *et al.*, 1979), in this case at night. Data collected at 08 h 00 min recorded the number of lever presses (on each lever) for the 12 h of night; those collected at 20 h 00 min recorded the number of lever presses for 11 h of the day. Recording of body weight, animal care and systems check took place from 19 h 00 min–20 h 00 min daily. All saline and drug solutions were replaced weekly, at which time infusion volumes were adjusted for changes in body weight. Nicotine solutions were prepared from nicotine tartrate, and amounts administered are expressed as nicotine free base. All solutions were kept under positive nitrogen pressure. Results were analyzed by repeated measures analysis of variance, followed by post-hoc Scheffe tests, with an α level of 0.05.

Results

Self-administration of saline and nicotine solutions

Virtually all responding with saline in the pre-drug baseline session occurred at night. The mean number of responses on the active lever (resulting in a saline injection) was 11.4 per night and 1.8 per day ($t[48] = 6.49$, $P < 0.001$). There was no significant difference between response rates on the active lever (12.3 responses per 23 h) and the control lever (14.2 responses per 23 h) ($t[48] = 1.86$, NS), during this period of exposure to saline.

A total of 16 animals were tested under identical conditions with access to nicotine, 30 μg kg^{-1} per

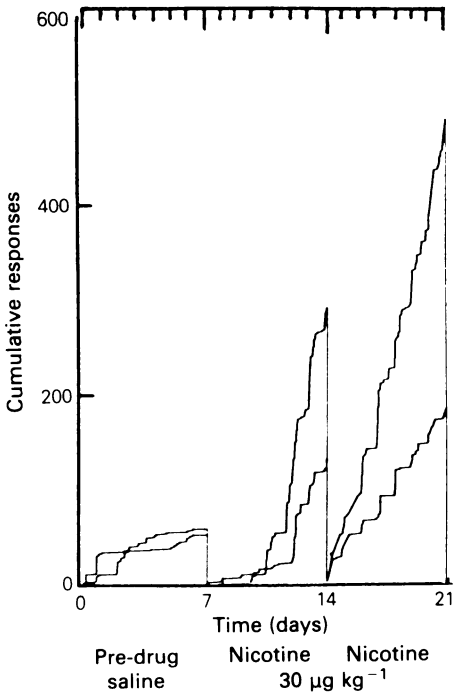

Figure 1 A cumulative response record for rat 086. Each upward deflection of the pen represents one lever press. The pens were reset after each seven day period of consecutive testing. The active lever in each condition is the one with the highest cumulative response rate. There was no difference in the rates on the two levers during the pre-drug saline session.

response on the active lever, for two weeks after the initial session with saline. The responses of a rat showing a substantial increase in its rate of responding with nicotine are illustrated in Figure 1. About five days after the solution injected following responding on the active lever was changed from saline to nicotine, responding on this lever began to increase above response rates on the control lever, and above pre-drug saline response rates. By the second week of nicotine exposure active lever response rates for this rat increased to more than four times the pre-drug saline response rate. A less marked increase in the rat of responding on the control lever was also observed. The scallops in the records, most evident from day 10 onwards, result from substantially more frequent responding at night than during the day. For this reason, the data presented subsequently refer only to night lever response rates.

Mean rates of nicotine or saline self-administration by rats allowed access to 3, 10, or 30 $\mu g\,kg^{-1}$ per response nicotine, or saline, are shown in Figure 2. There was a significant difference in response rates among sessions ($F = 11.8$, d.f. = 2,66, $P < 0.001$) and a significant treatment session lever interaction ($F = 2.38$, d.f. = 6,66, $P < 0.05$). Active lever response rates during access to the high dose of nicotine were significantly higher than pre-drug saline administration rates ($P < 0.001$). The mean rate of control lever responding also increased above pre-drug saline levels during the second week of exposure to the high dose of nicotine ($P < 0.001$). Inspection of the data from individual animals showed that five

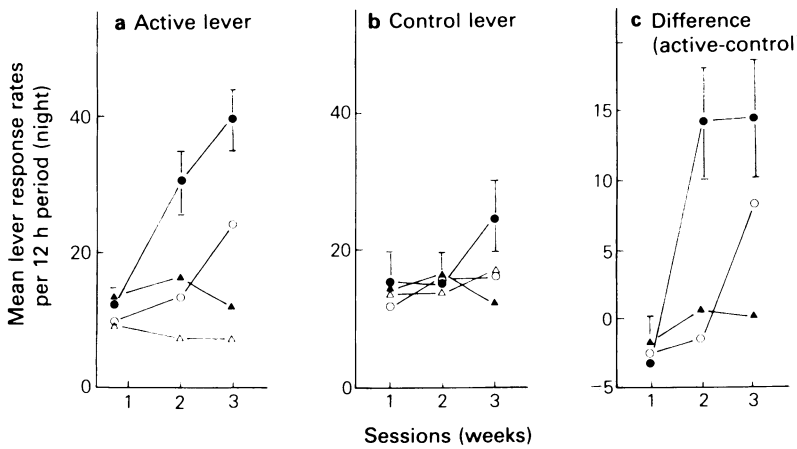

Figure 2 Effect of nicotine dose on the rate of nicotine self-administration. Rats were tested over three sessions of seven days, with access via the active lever to saline solution during week 1 (pre-drug saline), and nicotine solutions, 30 $\mu g\,kg^{-1}$ per response ($n = 16$, ●), 10 $\mu g\,kg^{-1}$ per response ($n = 7$, 0), 3 $\mu g\,kg^{-1}$ per response ($n = 7$, ▲), or saline ($n = 7$, △), for weeks 2 and 3. (a) Shows the mean response rates, averaged over the last six nights of each session, on the active lever (delivering nicotine or saline solution). (b) Shows the response rates on the control lever (with no programmed consequences), and (c) shows the mean within animal differences between active and control levers. s.e. mean values are shown by vertical lines for the group receiving the highest nicotine dose but are omitted from the other points for clarity.

of the sixteen rats given access to the high dose of nicotine showed a significant increase in responding ($P<0.05$) on the control lever. Rats allowed access to nicotine at $10\,\mu g\,kg^{-1}$ per response showed an increase in responding on the active lever during their second week of nicotine exposure ($P<0.05$). Three of the seven rats allowed access to this dose showed a significant increase in responding ($P<0.05$) on the active lever; only one of these animals also increased responding on the control lever. Responding was not increased on either lever during access to $3\,\mu g\,kg^{-1}$ per response nicotine or to saline. These results confirm that rats will self-administer nicotine at rates significantly greater than for saline. However, some animals receiving the highest dose also showed a significant increase in responding on the control lever.

Effect of reducing the dose of nicotine

A possible approach to evaluation of the role of nicotine-induced locomotor stimulation in the self-administration of nicotine is to determine the effects on response rates of a reduction in the dose of nicotine administered per response after establishment of nicotine self-administration. If rats respond on the active lever as a result of the reinforcing consequences of nicotine injection, then a reduction in the nicotine dose should lead to an increase in responding on the active lever if the rat compensates for the reduced amount of nicotine injected for each response, while responding on the control lever should either fall or show no change. The locomotor activity hypothesis, on the other hand, predicts that when the dose of nicotine is lowered, responding on both levers should decline since the nicotine-induced stimulation of motor activity will be reduced.

The results of such an experiment are illustrated in Figure 3. There were significant differences in response rates between sessions ($F=7.09$, d.f. = 5, 74, $P<0.001$), between levers ($F=8.16$, d.f. = 1,16, $P<0.05$), and there was a significant session/treatment/lever interaction ($F=3.24$, d.f. = 9,74, $P<0.01$). Rats given access to saline only responded at a low rate throughout the experiment, and response rates during the first week (saline) were not significantly different between the treatment groups. Rats allowed access to nicotine $30\,\mu g\,kg^{-1}$ per response for weeks two, three and four responded on the active lever more frequently than during access to saline in week one ($P<0.001$) Response rates of these rats on both active and control levers remained relatively constant throughout the three weeks of exposure to nicotine. The third group of rats, allowed access to nicotine $30\,\mu g\,kg^{-1}$ per response for weeks two and three, and then to a lower nicotine dose ($3\,\mu g\,kg^{-1}$ per response) during week four, showed significantly elevated response rates on

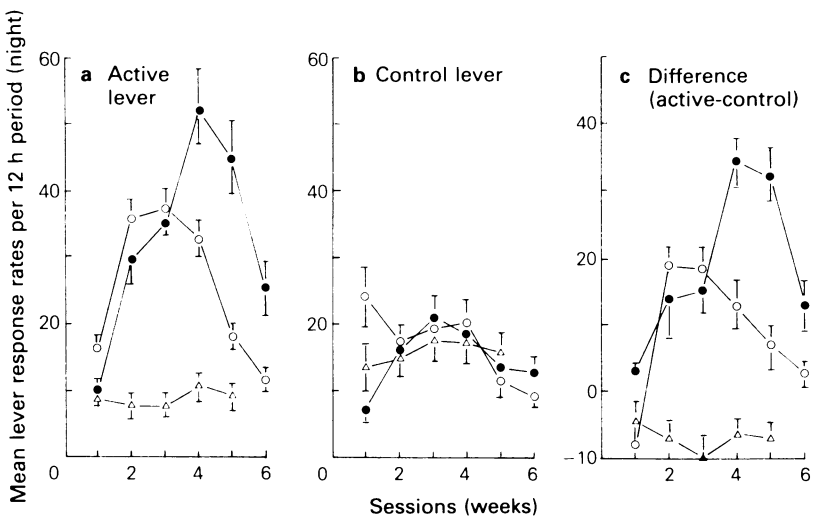

Figure 3 Effect of a reduction in the dose of nictoine per response on the rate of responding for nicotine. Three groups of rats were tested over six weeks. Group A ($n=7$, △) received saline injection in response to responding on the active lever throughout the experiment. Group B ($n=6$, ○) received saline during week 1, nicotine $30\,\mu g\,kg^{-1}$ per response during weeks 2, 3 and 4, and saline during weeks 5 and 6. Group C ($n=6$, ●) received the same treatment as group B until week 4, when the nicotine dose was reduced from 30 to $3\,\mu g\,kg^{-1}$ per response. In weeks 5 and 6 group C received saline, as the other groups. The three panels report lever response rates, as described in the legend to Figure 2.

the active lever in each of these weeks ($P<0.001$). A significant elevation in control lever responding was also observed in the second week of access to nicotine 30 $\mu g\,kg^{-1}$ per response ($P<0.05$). Thus the difference between active and control lever response rates was not significant in the second week of nicotine availability in this group of rats. However, when the dose of nicotine delivered by each active lever response was reduced from 30 to 3 $\mu g\,kg^{-1}$ in week four, the mean response rate on the active lever increased while responding on the control lever declined slightly (Figure 3, group C), and a significant difference between active and control lever response rates was now observed ($P<0.05$). The overall active lever increase in responding was two fold for a tenfold reduction dose. Thus, a dose of nicotine that was not reinforcing in rats with no previous exposure to nicotine was avidly self-administered in rats already self-administering the drug at a higher dose. This demonstrates that rats will partially compensate their rate of responding to adjust for a reduction in dose.

Extinction of nicotine self-adminstration

At the end of the third week of exposure to nicotine, the drug solutions were replaced by saline (extinction). The return to pre-drug rates of responding was gradual, requiring more than a full week (Figure 3). The resistance to extinction was particularly marked in the group of rats subjected to a dose reduction prior to withdrawal of the nicotine, in which the rate of responding on the active lever remained significantly elevated above the pre-drug saline rate during the first week of exposure to post-drug saline (week five; $P<0.001$). There was no indication in either group which had received nicotine of an 'extinction burst' of responding during the first 12 h after saline substitution for nicotine, as has been observed for other reinforcing agents (Pickens *et al.*, 1978). There were also no obvious signs of physical dependence, such as the diarrhoea, rhinorrhoea and the 'wet dog shakes' of opiate withdrawal. No detailed tests for physical dependence were carried out; however, the rats did not display irritability or aggressiveness when handled during the early evening. The only recorded symptom was an increase in body weight from a mean of 269 g in the last nicotine session to 283 g in the post-drug period. The body weight gain of the rats withdrawn from nicotine had been suppressed during the nicotine self-administration (267 g in pre-drug saline session to 269 g in the last nicotine session) compared with that of the saline control group (269 g in week 1 to 279 g in week 3, to 285 g in week 5). Thus the weight gain after nicotine withdrawal was no more than a recovery to the weight of untreated animals.

Discussion

The role of nicotine in cigarette smoking behaviour in man is still unclear (Russell, 1979). A number of studies have sought to demonstrate that nicotine has reinforcing properties in experimental animals (see review by Dougherty *et al.*, 1981). However, rates of nicotine self-administration are generally low, and questions regarding the mechanisms of nicotine self-administration remain. Most studies of nicotine reinforcement in rats have coupled availability of the drug with a food delivery schedule in rats at reduced body weight (Lang *et al.*, 1977; Singer *et al.*, 1978; Latiff *et al.*, 1980; Smith & Lang, 1980). Sanger (1978) demonstrated that a schedule of induced drinking could be used as a method of producing self-administration of nicotine by rats. An important role of the schedule itself in facilitating the acquisition of nicotine self-administration has been noted by Latiff *et al.* (1980), using rats, and Spealman & Goldberg (1982) using squirrel monkeys. The present study has confirmed the observation of Hanson *et al.* (1979), that rats at normal body weight with free access to food and water will self-administer nicotine at a greater rate than saline.

All drug intake in these experiments required that the rat depress the appropriate lever in the experimental chamber. No priming or passive infusions were given. Drug self-administration was, therfore, not initiated in an attempt to suppress drug withdrawal symptoms, and no withdrawal symptoms were observed when saline was substituted for nicotine after two or three weeks exposure to the drug. However, total nicotine intake was low; during the second and third weeks of access to 30 $\mu g\,kg^{-1}$ response of nicotine, the animals self-administered a total of about 1 $mg\,kg^{-1}$ of the drug during each 12 h nighttime period. Stable elevated response rates were observed during this period. The rats were allowed access to food and water *ad libitum* throughout the experiment, and nicotine administration was not associated with food reinforcement. Nor was any attempt made to increase responding by increasing the number of lever presses required for each injection of nicotine. Almost all nicotine self administration occurred during the night (active period). This may explain the inability of some groups to show nicotine self-administration in rats at normal body weight allowed access to nicotine only for a limited period during daytime.

Doses of nicotine as low as 50 $\mu g\,kg^{-1}$ have been shown to produce significant increases in locomotor activity in rats (Pradhan, 1970; Bättig *et al.*, 1976). Conventional measures of activity do not provide a good index of the frequency of inadvertent depression of a lever in the experimental chamber. We have estimated the increase in activity of the rats that

might contribute to nicotine self-administration by including a second lever in the chamber. Responding on this lever was recorded, but did not result in drug administration, and should not, therefore, be reinforcing. Provided that self-administration of the drug produces a sufficient drug effect to allow discrimination of the levers, responding on the control lever should record the rate of responding unrelated to direct nicotine reinforcement.

When allowed access to nicotine, $30\,\mu g\,kg^{-1}$ per response, some rats showed an increased rate of control lever responding. This could be a consequence of a nicotine-induced increase in motor activity resulting from an initial accidental depression of the active lever, or of a reinforcing effect of nicotine coupled with an inability to discriminate between the levers, or of a combination of these factors. Procedures which might facilitate discrimination between the levers, such as the provision of a cue light over the active lever, or increasing the distance between the levers, did not increase the difference between response rates on active and control levers (unpublished observations). We have assessed the role of nicotine-induced increased motor activity by reducing the dose of nicotine delivered by each response in rats actively self-administering nicotine. If responding resulted only from a drug-induced increase in motor activity, the rate of responding on both active and control levers should decrease. In fact, the difference in response rates between active and control levers was increased by the dose reduction. The simplest explanation of these results is that the rats were able to discriminate between the levers, and increased responding on the active lever in an attempt to maintain nicotine intake at a level providing positive reinforcement. The results indicate the nicotine-induced reinforcement is a significant factor in the observed self-administration behaviour. This conclusion is also supported by the resistance to extinction of the nicotine self-administration behaviour.

However, nicotine-induced motor stimulation might have some facilitatory effect. Five of 16 rats self-administering nicotine at $30\,\mu g\,kg^{-1}$ per response showed a significant increase in control lever responding. Since they can apparently discriminate between the levers, drug-induced motor stimulation may be responsible for this effect. The increases in control lever responding became significant in the second week of exposure to nicotine at $30\,\mu g\,kg^{-1}$ per response, when active lever response rates were substantially elevated. It is possible that nicotine reinforcement occurs at a lower plasma level of drug than required for stimulation of otor activity. Nevertheless, once sufficient drug is self-administered, nicotine induced motor stimulation may contribute substantially to lever response rates.

In summary, we have shown that rats will self-administer nicotine without prior treatment with the drug, and without food deprivation. Our results suggest that while a nicotine-induced increase in motor activity may contribute to the self-administration, the major factor in initiating self-administration is the positive reinforcement provided by the drug. There was no evidence of physical dependence on nicotine, and no indication that suppression of aversive features of nicotine withdrawal contributed to the maintenance of self-administration. These results suggest that the rat may be a useful experimental animal for the pharmacological analysis of nicotine reward.

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