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Changes Occurring in Self Administration of Nicotine by Rats Over a 28-Day Period

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SMITH, L. A. AND W. J. LANG. *Changes occurring in self administration of nicotine by rats over a 28-day period.* PHARMAC. BIOCHEM. BEHAV. 13(2) 215–220, 1980.—After rats at reduced body weight had established responding by lever pressing for nicotine injections under a food delivery schedule (FT60 sec) for 1 hr daily sessions for 14 days, the rate of responding was maintained over a second 14-day period even after removal of the schedule. However, the rate was not maintained by rats lever pressing for normal saline without the schedule over the second 14-day period after self administration had been established for nicotine under the schedule. Other rats maintained at reduced body weight were allowed to lever press for nicotine over a 28-day period without the food delivery schedule. Their rate of self administration increased from initially low levels until at the end of the 28-day period the rate had reached that of rats self administering nicotine adjunctive to the food delivery schedule throughout the same period. Without the schedule, rats at reduced body weight self administering normal saline or rats at normal body weight self administering nicotine, continued to lever press only at very low rates throughout the 28-day period. It is suggested that rats maintain self administration of nicotine if the behavior can be established for a critical intake of nicotine over a critical period of time. The food delivery schedule appears only to hasten the establishment of the behavior but is not essential for self administration of nicotine by rats.

Self administration of drug Nicotine Duration of experiment Influence of schedule

IT has been shown that a large range of narcotic drugs [6, 10, 15] and various central stimulants, such as dexamphetamine, methamphetamine and cocaine [3, 5, 12] are self-injected by experimental animals. Nicotine, however, is self administered by rats in significant amounts only under certain conditions.

Lang, Latiff, McQueen and Singer [11] found that rats at reduced body weight self injected nicotine at a greater rate than they did normal saline. It was further found that rats at a reduced body weight and under the influence of an FT60 sec food delivery schedule self administered even greater amounts of nicotine. These studies have shown that nicotine is capable of maintaining responding even though the reinforcing efficacy of nicotine may be low. It was concluded that there was an interaction between pharmacological and environmental variables that led to the initiation and maintenance of responding for nicotine by rats.

These variables have been investigated further in the present experiments. In Experiment 1, the food delivery schedule was removed after self administration of nicotine had been established, in order to investigate the importance of the schedule in maintaining responding for nicotine. In Experiment 2 both the schedule and the drug were removed to investigate any interaction between pharmacological and environmental factors. In Experiment 3 the nicotine self infusion rates of rats at 80% body weight without the schedule were investigated over a longer period of days than in the earlier study [11]. This was done to determine the effect of variables in altering acquisition of self administration. In Experiment 4 the effect of body weight on the acquisition of self administration was investigated over a longer period of days. The experiments were continued over a 28-day period and the schedule used was an FT60 food delivery schedule in which a food pellet is delivered every 60 sec regardless of responding.

METHOD

Animals

Thirty one male and 6 female Lister hooded rats with no prior drug experience and weighing 240–400 g were housed individually in a room kept at 23 ± 1°C with a 12 hr light-12 hr dark cycle. Water was available at all times in the home cage.

The male rats were randomly assigned to Groups 1–5 and were reduced to 80% of their free-feeding weight over 7 days by restricting their food. The female rats were assigned to Group 6 and maintained at free-feeding weight. The female rats were assigned to this group because they were smaller and to reduce their weight would have enabled them to escape the harness described below. In addition, over the 28-day period, free-feeding male rats were found to outgrow their harness. No differences have been observed in our experiments with respect to bar pressing for nicotine injections by rats of either sex.

All animals were weighed and anesthetized with pentobarbitone sodium or a mixture of methohexitone and amylobarbitone, injected intraperitoneally. An intravenous cannula of SP 28 (Dural Plastics and Eng. Pty. Ltd.) was surgically implanted into the right jugular vein of each rat so that the tip of the cannula lay just above the right ventricle of the heart. The cannula was passed subcutaneously and left the rat through the skin between the ears. The cannula was

maintained in place by a lightweight leather harness [13] worn by each animal.

The cannulae were kept patent by flushing twice weekly with 2 ml of heparin 200 units/ml. Following a 3-day recovery period the animals were placed in a Skinner box for 1 hr/d training session, for 3 consecutive days, at approximately the same time each day. The session commenced with an initial priming dose of the drug or saline. While in the Skinner box, the harness was connected to a swivel system [17] for infusion of the drug, which allowed the animal relatively free movement. The FT60 food delivery schedule operated during the training session if it were to operate in the experimental sessions.

Apparatus

The experimental chamber was a Skinner box (R.S. Hales Equipment Pty. Ltd.) of clear Plexiglas measuring 35×32×32 cm with a lever and a pellet dispenser unit attached to adjacent walls. The lever was 8 cm from the corner and 10 cm from the floor. The dispenser was 8 cm from the corner and 5 cm from the floor.

Depression of the lever resulted in delivery of 83 μl, over a 5 sec period, of either a nicotine solution or saline by an infusion pump (Sage Instruments, Model 341). A 5 sec time delay was incorporated so that during this interval further lever presses did not result in drug infusion. The pellet dispenser was set to deliver 1 pellet every 60 sec, thus operating an FT60 food delivery schedule. The pellets used were Noyes 45 mg food pellets. Water was not available during the experimental session and the vacant hole was left uncovered.

The delivery of the drug, contingent on lever pressing, was monitoried by a National Semiconductor Microprocessor. The microprocessor also recorded both lever presses and infusions over consecutive 5 min intervals for the 60 min experimental session.

Drugs

The anesthetic used during surgery was pentobarbitone sodium (Nembutal, Abbott Laboratories Pty. Ltd.) or a mixture of amylobarbitone (Sodium Amytal, Eli Lilly and Co.) and methohexitone (Brietal, Eli Lilly and Co.). The combination of amylobarbitone (3.0 mg/ml) and methohexitone (17.6 mg/ml) was given intraperitoneally at a dose of 1.7 ml/kg.

A solution of nicotine hydrogen tartrate (B.D.H. Ltd.) in 0.9% sterile saline was made up daily prior to the experimental sessions. The volume of the injection was 83 μl. The injection dose was 0.1 mg/kg.

EXPERIMENT 1: EFFECT OF REMOVING THE SCHEDULE ON THE RATE OF SELF ADMINISTRATION OF NICOTINE

In this experiment, the rate of self infusion of nicotine after removal of the schedule after 14 days was compared to that of rats continuing to respond under the influence of the FT60 schedule throughout the 28-day period.

METHOD

Rats of Groups 1, 2 and 3 were given access to nicotine injections via lever pressing with an FT60 schedule for 1 hr/day. After 14 days rats of Group 1 continued under the same conditions while rats of Group 2 continued without the schedule. The rats were at 80% of their free-feeding weight.

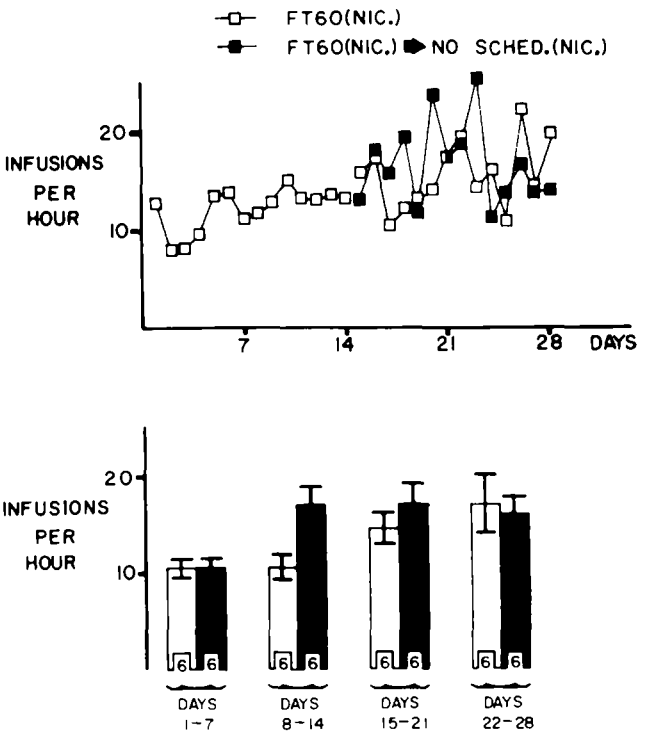

FIG. 1. The upper panel shows mean daily rates of infusion of nicotine. Over the first 14 days the mean rates of infusion of Groups 1, 2 and 3 were combined (open squares □). These rats were at 80% of free-feeding weight and under the influence of the FT60 food schedule. After Day 14, Group 1 (open squares □) continued lever pressing for nicotine under the influence of the schedule, whereas in Group 2 (filled squares ■) nicotine injections were available but the schedule was discontinued. The lower panel shows histograms of mean infusions with standard errors of nicotine over each 7-day period. The open histograms are of Group 1 and the closed histograms are of Group 2 rats.

RESULTS

Over the first 14 days rats on the FT60 schedule self administered nicotine at 11.89 infusions/hr (SEM=0.57, 18 rats). This corresponds to an intake of 0.36 mg nicotine/hr (SEM=0.02, 18 rats). Rats continuing to bar press for nicotine under the FT60 schedule over Days 15–21 had a mean infusion rate of 14.19/hr (SEM=1.51, 6 rats) and a corresponding nicotine intake of 0.43 mg/hr (SEM=0.09, 6 rats) and for Days 22–28, an infusion rate of 16.67/hr (SEM=2.98, 6 rats) and an intake of 0.50 mg nicotine/hr (SEM=0.23, 6 rats). Rats self administering nicotine without the schedule, over the second 14 days, maintained their rate of infusion with 16.91 infusions/hr (SEM=2.14, 6 rats) and a corresponding nicotine dose of 0.51 mg/hr (SEM=0.13, 6 rats) over Days 15–21 and 16.14 infusions/hr (SEM=1.72, 6 rats) and a nicotine dose of 0.48 mg/hr (SEM=0.14, 6 rats) for Days 22–28. Over Days 15–21 the rate of self administration without the schedule was not significantly different from that of animals lever pressing under the schedule ($p>0.05$, t test, 10 df). Over Days 22–28 the rate of self administration without the schedule was maintained and again did not differ

from that of rats lever pressing under the schedule ($p>0.05$, t test, 10 df). Results are shown in Fig. 1.

In summary, after rats established a rate of self-administration of nicotine on a food delivery schedule for 14 days, the rate of self-administration was maintained over a subsequent 14 day period even though the schedule was removed.

EXPERIMENT 2: EFFECT OF REMOVING BOTH SCHEDULE AND NICOTINE ON SUBSEQUENT SELF ADMINISTRATION

The rate of self administration of nicotine after removal of the food delivery schedule was compared to the rate of self administration after both the schedule and the drug had been removed.

METHOD

After 14 days rats of Group 2 continued to lever press to obtain nicotine whereas in the case of rats of Group 3 the nicotine solution was replaced by saline. The food delivery schedule was withdrawn from both groups. The rats were at 80% of their free-feeding weight throughout the 28-day period.

RESULTS

Rats in Group 3 self administering saline without the schedule had an infusion rate of 7.29/hr (SEM = 1.00, 6 rats). The rate fell further during Days 22–28 to 6.14 injections/hr (SEM = 0.74, 6 rats).

Over Days 15–21, the rate of rats in Group 2 self administering nicotine without the schedule was not significantly different from that of saline without the schedule ($p>0.05$, t test, 10 df). However, the rates of the animals in Groups 2 and 3 were significantly different over Days 22–28 ($p<0.05$, t test, 10 df). Results are shown in Fig. 2.

In summary, although rats maintained their rate of self administration of nicotine over a second 14 day period after removal of the food delivery schedule, this was not the case if normal saline replaced the nicotine injections during the second 14 day period. The rate of lever pressing for normal saline fell significantly.

EXPERIMENT 3: GRADUAL ACQUISITION OF SELF ADMINISTRATION AT 80% BODY WEIGHT WITHOUT THE SCHEDULE

In this experiment, the rate of self infusion of rats at 80% body weight under the food delivery schedule was compared with that of rats without the schedule over a 28-day period.

METHOD

Rats of Group 1 were given access to nicotine injections under the influence of the schedule for 28 days. Rats of Group 4 were also given access to nicotine injections for 28 days but the schedule did not operate. Rats of Group 5 had access to saline injections for 28 days without the schedule. All rats were maintained at 80% body weight.

RESULTS

Rats at 80% body weight self administered saline at a constant low rate. The mean infusion rate of saline over 28 days was 4.88/hr (SEM = 0.47, 6 rats). Rats self administering nicotine at 80% body weight without the schedule had an initial infusion rate of 3.98/hr (SEM = 0.61, 7 rats) with a

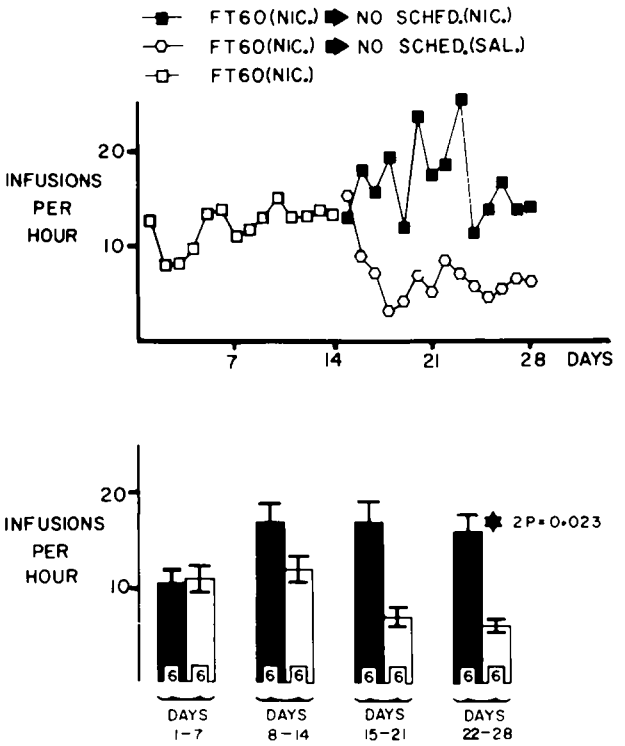

FIG. 2. The upper panel shows mean daily rates of infusion of nicotine. Over the first 14 days the mean rates of infusion of Groups 1, 2 and 3 were combined (open squares □). These rats were at 80% of free-feeding weight and under the influence of the FT60 food schedule. After 14 days Group 2 (filled squares ■) had nicotine solution available for self injection and Group 3 (open hexagons ⬡) had nicotine solution replaced by saline. Neither group was under the schedule over the second 14-day period. The lower panel shows histograms of mean infusions with standard errors of nicotine over each 7-day period. The closed histograms are of Group 2 and the open histograms are of Group 3 rats.

corresponding nicotine intake of 0.12 mg/hr (SEM = 0.01, 7 rats) for Days 1–7. This increased to 6.43 infusions/hr (SEM = 0.87, 7 rats) and 9.84 infusions/hr (SEM = 1.25, 7 rats) with nicotine intakes of 0.19 mg/hr (SEM = 0.05, 7 rats) and 0.30 mg/hr (SEM = 0.07, 7 rats) for Days 8–14 and 15–21, respectively. Over Days 22–28 the mean number of infusions was 13.16/hr (SEM = 1.76, 7 rats) and nicotine intake was 0.39/hr (SEM = 0.11, 7 rats).

Over Days 1–7 the infusion rate without the schedule was not significantly different from the rate of self injection of saline ($p>0.05$, t test, 11 df). The rates of saline administration over Days 8–14 and 15–21 are not different from those of nicotine self administration ($p>0.05$, t test, 11 df) and ($p>0.05$, t test, 11 df), respectively. Over Days 22–28, the rate of self administration of nicotine without the schedule was significantly different from that of saline ($p<0.05$, t test, 11 df).

As reported in Experiment 1, animals self administering nicotine under the influence of the schedule maintained a high intake of nicotine over 28 days. Over Days 1–7, the infusion rate of animals self administering nicotine without the schedule was significantly different from the rate of self administration with the schedule ($p<0.05$, t test, 11 df). Over Days 8–14 and 15–21 the rate of nicotine self administration

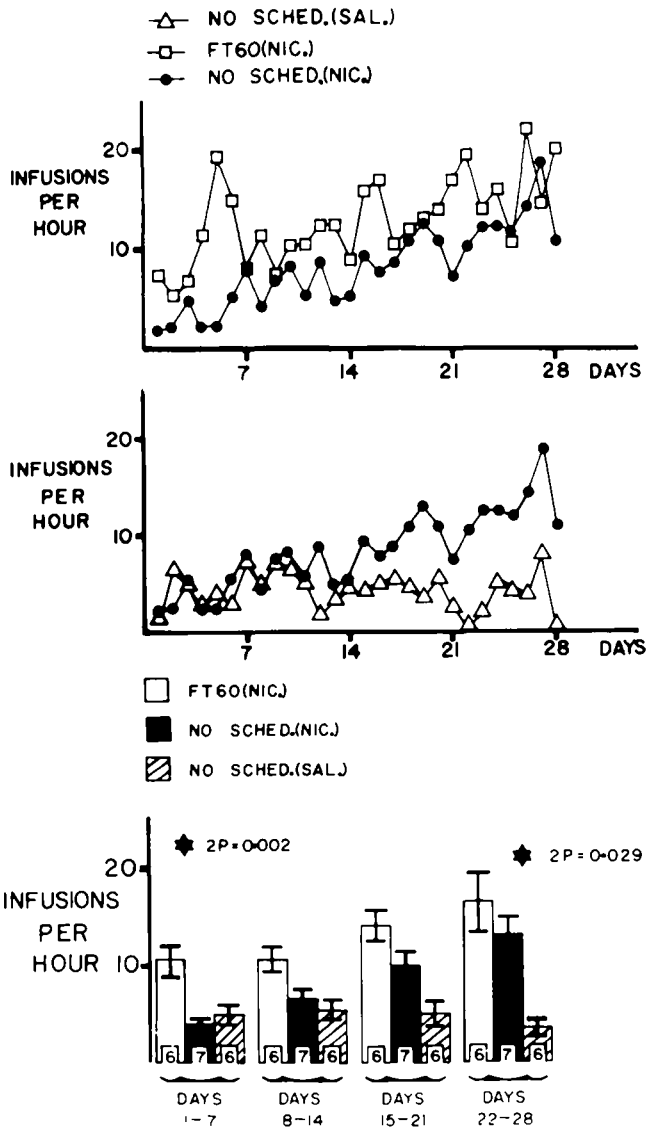

FIG. 3. The upper two panels show mean daily rates of infusion of nicotine of rats at 80% free-feeding weight. Group 1 (open squares □) self administered nicotine under the influence of the schedule. Group 4 (closed circles ●) had access to nicotine and Group 5 (open triangles △) had access to normal saline; neither Groups 4 or 5 were under the influence of the schedule. The bottom panel shows histograms of mean infusions with standard errors of nicotine over each 7-day period. The open histograms are of Group 1, the closed histograms of Group 4 and the hatched histograms of Group 5 rats.

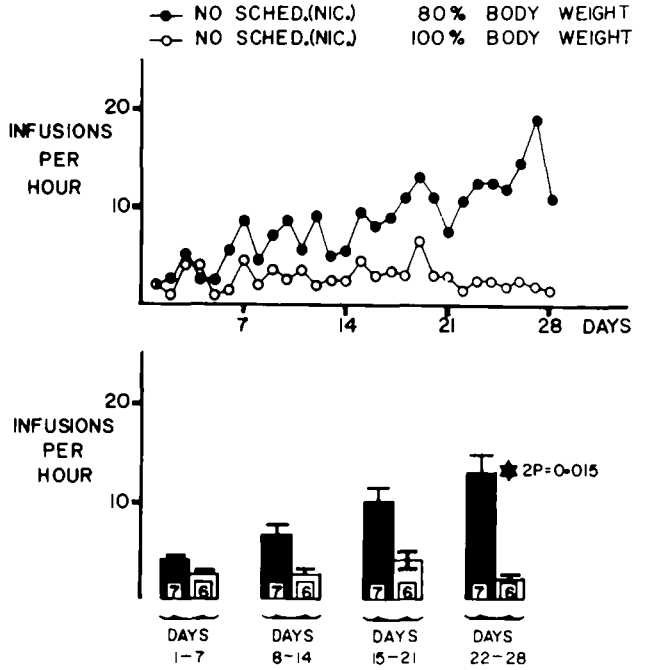

FIG. 4. The upper panel shows mean daily rates of infusion of nicotine. Rats in Group 4 (closed circles ●) were at 80% of free-feeding weight and Group 6 (open circles ○) were at 100% of free-feeding weight. Both groups had access to nicotine self injection and responded without the schedule. The lower panel shows histograms of mean infusions with standard errors of nicotine over each 7-day period. The closed histograms are of Group 4 and the open histograms are of Group 6 rats.

without the schedule was not different from that of rats under the influence of the schedule ($p>0.05$, t test, 11 df) and ($p>0.05$, t test, 11 df), respectively. Also over Days 22–28, the rates of nicotine self administration with and without the schedule were not significantly different ($p>0.05$, t test, 11 df). Results are shown in Fig. 3.

In summary, initially, rats at 80% of normal body weight without the FT60 schedule, self administered nicotine at a rate not different from that for normal saline. During 28 days, however, the infusion rate for nicotine had increased so that it was not significantly different from that of rats responding for nicotine under the influence of the FT60 schedule.

EXPERIMENT 4: EFFECT OF BODY WEIGHT ON THE ACQUISITION OF SELF ADMINISTRATION

In this experiment the rate of self administration of nicotine without the schedule of rats at 80% body weight was compared to that of rats at 100% body weight over a 28-day period.

METHOD

Rats of Group 4 had nicotine injections available without the food delivery schedule. These rats were at 80% body weight. Rats of Group 6 at 100% body weight were also given access to nicotine injections without the schedule.

RESULTS

The self infusion rate of nicotine by animals at free-feeding weight is maintained at a constant low rate over 28 days. The mean infusion rate is 2.76/hr (SEM=0.25, 6 rats). As reported in Experiment 3, the self administration of nicotine without the schedule increases over the 28-day period. Over Days 1–7 and 8–14 the infusion rate of animals at free-feeding weight was not significantly different from rats at 80% body weight ($p>0.05$, t test, 11 df) and ($p>0.05$, t test, 11 df), respectively, nor over Days 15–21 ($p>0.05$, t test, 11 df). However, over Days 22–28 the two groups were significantly different ($p<0.05$, t test, 11 df). Results are shown in Fig. 4.

In summary, the infusion rate of animals at free-feeding weight without the schedule is low and constant over 28 days

and did not reach the level achieved by rats at 80% body weight without the schedule.

DISCUSSION

It has been suggested that the self injection of nicotine by rats reduced to 80% body weight under an FT60 schedule involves an interaction of pharmacological, environmental and nutritional factors [14]. The results of the present experiments can be discussed in terms of these parameters.

Earlier findings indicated that animals at 80% body weight would self administer nicotine at higher rates than saline under an FT60 schedule. It has been found in this study that animals at 80% body weight that have self administered nicotine for 14 days under an FT60 food delivery schedule will maintain self administration for at least another 14 days after removal of the schedule. These results indicate a pharmacological role of nicotine in maintaining self administration behavior.

Rats at reduced body weight, without the schedule, initiate and increase their rate of self infusion until it is not different from the rates recorded under the influence of the schedule. The pharmacological effects of nicotine reinforce the lever pressing and increase the incidence of further lever pressing. It appears, therefore, that the schedule is not important in the achievement of self-administration behavior under 80% body weight conditions. The pharmacological effects are sufficient to achieve this behavior, given adequate experience with the drug.

Although rats at 80% body weight will increase their self infusion rates over 28 days without the schedule, rats at free-feeding weight will not. Nicotine could be thought to cause this increase in self administration by acting as an appetite suppressant. Alternatively, there is evidence that states of deprivation, such as food deprivation in this case, can cause both electrical stimulation and physiological imbalances. These in turn are capable of increasing spinal reflex excitability resulting in increased readiness to respond. This level of activity is further increased by the occurrence of a response [16]. This theory of general activation further suggests that the lateral hypothalamus is involved in the integration of the signals that result in increased excitability. Therefore, rats at reduced body weight may have a greater readiness to respond than animals at free-feeding weight. These animals would then display more exploratory behavior and would lever press at a slightly higher rate at the beginning of the experiment. Nicotine may then reinforce the lever pressing and lead to an increase in self administration. Animals at free-feeding weight do not display a great amount of exploratory behavior, and so do not receive much nicotine. It would follow from this that there is a critical amount of nicotine that must be self administered by the animal before high rates of responding are achieved. This is supported by the observation that rats will maintain self administration after removal of the schedule after self administering nicotine under the influence of the schedule.

These high rates of nicotine infusion can be assured either by FT60 food delivery schedule or by allowing a longer period of time for self administration to gradually increase. It would appear from these experiments that the initiation of responding is sensitive to the pharmacological, environmental and nutritional factors. The FT60 food delivery schedule is necessary to generate high levels of responding during the acquisition phase, but if the schedule is removed after establishment of self administration, there is no alteration in the rate of self administration. It has been found that free-feeding rats will not self administer nicotine at high rates. If, however, rats at 80% body weight are allowed to establish responding and are then brought back to free-feeding weight, they maintain responding [14].

It appears that the most important factor in generating the high level of self administration is to ensure a critical nicotine intake. If this critical intake occurs during the acquisition of responding, self administration of nicotine by rats is maintained.

In humans, nicotine is self-administered by smoking, sniffing and chewing tobacco. Evidence exists implicating nicotine in the aetiology of smoking. Nicotine occurs in higher concentrations than other alkaloids in tobacco smoke, and it has been demonstrated that nicotine is also the most potent alkaloid at several physiological sites [4]. Armitage, Hall and Morrison [1] have suggested that some people smoke to achieve a certain level of nicotine. The hypothesis has been supported by studies in which it was observed that smokers modified their behavior to achieve certain doses of nicotine [2,7]. Nicotine administration prior to smoking produces a small, but significant decrease in the number of cigarettes smoked [8]. It has also been observed that intravenous infusion of nicotine to smokers was a pleasurable sensation and alleviated the desire to smoke for some time after administration [9]. These results which provide further evidence for nicotine titration by smokers support the view that the tobacco habit in man is in part a form of nicotine dependence. The reinforcing properties of nicotine seem to be similar in the rat.

ACKNOWLEDGEMENTS

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REFERENCES

1. Armitage, A. K., G. H. Hall and C. F. Morrison. Pharmacological basis for the tobacco smoking habit. *Nature* **217**: 331–334, 1968.
2. Ashton, H. and D. W. Watson. Puffing frequency and nicotine intake in cigarette smokers. *Br. Med. J.* **3**: 679–681, 1970.
3. Balster, R. L., M. M. Kilbey and E. H. Ellinwood Jr. Methamphetamine self-administration in the cat. *Psychopharmacologia* **46**: 229–233, 1976.
4. Clark, M. S. G., M. J. Rand and S. Vanov. Comparison of pharmacological activity of nicotine and related alkaloids occurring in cigarette smoke. *Archs Int. Pharmacodyn.* **156**: 363–379, 1965.
5. Deneau, G. J., T. Yanagita and M. H. Seevers. Self-administration of psychoactive substances by the monkey. *Psychopharmacologia* **16**: 30–48, 1969.
6. Downs, D. A. and J. H. Woods. Codeine and cocaine reinforced responding in Rhesus monkeys: effects of dose on response rates under a fixed ratio schedule. *J. Pharmac. exp. Ther.* **191**: 179–188, 1974.
7. Frith, C. D. Effect of varying nicotine content in cigarettes on human smoking behaviour. *Psychopharmacologia* **19**: 188–192, 1971.

8. Jarvik, M. E., S. D. Glick and R. K. Nakamura. Inhibition of cigarette smoking by orally administered nicotine. *Clin. Pharmac. Ther.* **11**: 574–576, 1970.
9. Johnston, L. M. Tobacco smoking and nicotine. *Lancet* **2**: 742, 1942.
10. Kumar, R., H. Steinberg and I. P. Stolerman. Inducing a preference for morphine in rats without premedication. *Nature* **218**: 545–565, 1968.
11. Lang, W. J., A. A. Latiff, A. McQueen and G. Singer. Self-administration of nicotine with and without a food delivery schedule. *Pharmac. Biochem. Behav.* **7**: 65–70, 1977.
12. Pickens, R. and T. Thompson. Cocaine-reinforced behaviour in rats: effects of reinforcement magnitude and fixed-rate size. *J. Pharmac. exp. Ther.* **161**: 122–129, 1968.
13. Ross, J. W. Pharmacological and conditional factors associated with cocaine dependence. Doctoral thesis at University of Melbourne, 1977.
14. Singer, G., F. Simpson and W. J. Lang. Schedule induced self injections of nicotine with recovered body weight. *Pharmac. Biochem. Behav.* **9**: 387–389, 1978.
15. Van Ree, J. M., J. L. Slanger and D. de Wied. Intravenous self-administration of drugs in rats. *J. Pharmac. exp. Ther.* **204**: 547–557, 1978.
16. Wayner, M. J. Motor control functions of the lateral hypothalamus and adjunctive behaviour. *Physiol. Behav.* **5**: 1319–1325, 1970.
17. Weeks, J. R. The pneumatic syringe: a simple apparatus for self-administration of drugs by rats. *Pharmac. Biochem. Behav.* **7**: 559–562, 1977.