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Discriminative Stimulus Effects of Nicotine and Chronic Tolerance

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SHOAIB, M., E. THORNDIKE, C. W. SCHINDLER AND S. R. GOLDBERG. *Discriminative stimulus effects of nicotine and chronic tolerance.* PHARMACOL BIOCHEM BEHAV **56**(2) 167–173, 1997.—Tolerance to discriminative stimulus (DS) effects of drugs, as observed by a shift of the dose-response curve to the right, has been observed with many addictive drugs (e.g. amphetamine, cocaine and morphine). Chronic administration of nicotine has been reported to produce tolerance to the locomotor depressant effects and aversive stimulus properties of nicotine; however, the DS effects of nicotine have not been examined for development of tolerance following chronic treatment. We report on experiments utilising a cumulative-dosing drug discrimination paradigm. Eight, male Sprague–Dawley rats were trained to discriminate nicotine (0.4 mg/kg s.c.) from saline under a fixed ratio (FR 10) schedule for food reinforcement. Multiple training sessions were given daily, and once criteria was met, cumulative doses of nicotine (0.025–1.2 mg/kg s.c.) were evaluated. Rats acquired the nicotine discrimination after 80 sessions. During this period, rats developed tolerance to the rate-depressing effects of nicotine after 20 nicotine-training sessions. Chronic treatments of nicotine in the rat's home cage for 7 days during suspended training failed to shift the dose-response curve for nicotine. Increasing the frequency to three daily injections also had no effect on nicotine discrimination. Furthermore, continuous infusions of nicotine (6.4 mg/kg/day) delivered via osmotic minipumps failed to shift the dose-response curve. No physical signs of withdrawal were apparent, particularly on lever responding, following removal of the minipump. These results suggest that under the conditions described, chronic tolerance to nicotine's DS does not develop readily. **Copyright © 1997 Elsevier Science Inc.**

Nicotine Discrimination Tolerance Chronic treatment Acute treatment

TOLERANCE is a characteristic feature associated with repeated administration of many drugs. Pharmacologically, this adaptation is observed as a parallel shift of the dose-response curve; thus, larger doses are required to produce the same magnitude of response. Tolerance to the behavioural effects of psychoactive drugs has been examined extensively (9). However, the majority of the observations have centered upon either unconditioned behaviours, for example upon locomotor activity (3,23) or on operant responding maintained by food reinforcement (10).

Nicotine can also serve as a stimulus in a number of different paradigms. In addition to its positive reinforcing (7,20) and aversive (6,8) stimulus properties, nicotine's discriminative stimulus (DS) properties are also thought to be important in tobacco addictions (21). Rats can be trained to discriminate nicotine from saline with high pharmacological specificity (18,21). Once a discrimination is established, stimulus control remains stable for long periods of time, during which the dose required to produce the stimulus does not change. However, there are some studies which report that acute or chronic administration of drugs such as cocaine, amphetamine or morphine can produce tolerance to the DS effects of that drug (25). Many of these studies have involved the chronic administration of drugs during periods of suspended training. Typically, tolerance is reflected by a right-ward shift of the dose-response curve. In many cases, chronic treatments lasting 3–7 days have been sufficient to significantly shift dose-response curves to the right (1,12,24).

Chronic tolerance is observed with repeated injections of nicotine and can persist for up to 90 days (23). Tolerance to the initial depressant effects of nicotine on locomotor activity is acquired if three injections of nicotine are given daily (23), but it may also occur with daily or twice weekly administrations (16,22). After chronic exposure for 5 days (0.2 mg/kg/day) the nicotine dose-response curve shifted upward, so that greater locomotor stimulation was produced at each test dose of nicotine (14).

A limited number of studies have examined the develop-

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ment of tolerance to the DS effects of nicotine. One series of experiments has shown that acute tolerance can develop to the nicotine discrimination following single boluses of nicotine (0.8 mg/kg) injected 90 min apart (13) or for several days (19). Attenuation of stimulus control exerted by the training dose of nicotine (0.4 mg/kg s.c.) was attributed to desensitisation occurring at the nicotinic acetylcholine receptor. However, in these two studies, full dose-response curves were not constructed and thus the magnitude of tolerance was not assessed.

In addition to development of tolerance, the repeated administration of nicotine can also produce physical dependence on the drug, which is demonstrated by appearance of signs or symptoms of behavioural disruption when administration of the drug is stopped (5). Malin et al (15) have described an abstinence syndrome in rodents. Dependence was induced by continuously infusing nicotine for 7 days via a subcutaneous osmotic minipump. Marked changes in behaviours were observed when nicotine was stopped; teeth chattering, writhing, wet shakes/tremors and ptosis (15). Apart from such directly observed signs of a nicotine abstinence syndrome, however, little attention has been paid to the possibility that more subjective effects of nicotine might also be modified during abstinence.

The present experiments were designed to examine the effects of chronic treatment regimens of nicotine during suspended training using a cumulative-dosing drug discrimination paradigm as previously described by Young et al. (26). The experiments test the hypothesis that discriminative stimuli induced by all abused substances are capable of showing tolerance. In addition, the study also investigated the consequences of continuous infusions of nicotine, delivered via osmotic minipumps in this paradigm, particularly since withdrawal from nicotine may modify the subjective properties of nicotine.

METHODS

Animals

Eight male Sprague–Dawley rats (Charles River, Wilmington, MA) were used. Rats initially weighing 250–350 g were housed individually, and their body weights were gradually reduced to 80% of free feeding by limiting daily access to food; water was available ad libitum. The rats were maintained on a 12 h light/dark cycle with lights on at 0800 h.

Apparatus

Standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) were used. Each chamber contained two levers, one on either side and equidistant from a food cup. The experiments were controlled by microcomputers connected to the chambers through appropriate interfaces using a MED-PC (Med Associates, Inc) program.

DISCRIMINATION TRAINING

Rats were trained to lever press for food using a fixed ratio that increased progressively. Once animals had reached a fixed ratio (FR) of ten responses for each food pellet, drug and vehicle training sessions began. Rats were trained to discriminate nicotine (0.4 mg/kg s.c.) from saline under a fixed-ratio (FR10) schedule of food reinforcement, using a procedure similar to that described by Young et al. (26). Training sessions consisted of a 5 min time-out (TO) interval followed by a 5-min ratio trial. For about half the rats in the group, the right lever was the drug lever; for the other half, the left lever was the drug lever. After a nicotine injection, responding on only one of the levers (the drug lever) was reinforced; responses on the other lever (saline lever) were recorded but not reinforced. Similarly, following an injection of saline, responding on the saline lever was reinforced and responses on the nicotine lever were recorded but not reinforced. The house lights were on only during the FR trial and were off during the TO intervals. At the end of each trial, the subject was removed and administered an injection and the next TO interval was initiated. Each daily training session varied in length and in the number of nicotine or saline trials; 0-6 saline trials preceded up to 2 drug trials. Sessions that began with a drug training trial consisted of two trials during which responses were reinforced on the drug-appropriate lever, with saline administered before the second trial. Sessions that began with a saline trial consisted of multiple trials with saline followed by a maximum of two drug trials, with saline administered before the final drug trial. The sequence of trials (up to a maximum of 6 per day) was varied so that an equal number of drug and saline trials were conducted each week. During each trial, the first ten presses on either lever designated the 'selected lever', a measure used to ascertain acquisition of stimulus control. Once rats met the criteria set for stimulus control, that is eight consecutive correct lever selections, they were tested under a cumulative dosing procedure.

DISCRIMINATION TESTING

Once stimulus control was established, dose-response curves were generated for nicotine. At least 18 h was allowed for nicotine to 'wash-out' before further tests with nicotine were performed. During a dose-response determination, saline was followed by cummulative doses of nicotine (0.025, 0.1, 0.4, 0.8, 1.2 mg/kg s.c.) administered before the start of each TO period. Following these tests, rats were trained for a minimum of 1 wk, and testing only proceeded if the performance of the animal met criterion. Before treatments were evaluated, successive dose-response curves were generated. In addition, tests with repeated saline injections were performed to make sure that rats were not switching from the saline lever over succesive trials.

Chronic treatments. During suspended training, in which rats remained in their home cages for the entire chronic treatment period, the effects of repeated nicotine injections (0, 0.1, 0.4, 0.8 and 1.2 mg/kg s.c.) administered daily as either a single injection or a series of three injections (3×0, 3×0.1, 3×0.4, 3×0.8 mg/kg s.c.) spaced 90 min apart were evaluated. Dose response curves were performed at least 18 h after the last dose of nicotine. Cumulative dose-response curves were conducted before and after the chronic treatment.

Continuous infusions. Using the same procedure as that described above for chronic treatments, the constant infusion of nicotine administered via subcutaneously implanted osmotic minipumps (Alzet, Model no. 2401) was examined. Six hours following completion of the 'before' dose-response curve, osmotic minipumps were implanted under brief halothane anaesthesia. Constant infusions of nicotine (3.2 and 6.4 mg/kg/day s.c.) or saline lasting for 7 days were evaluated by removing the pumps at least 18 h prior to generating the 'after' dose-response curve.

Plan of Experiments

All animals were trained until they satisfied the criterion for stimulus control. All rats were subjected to the three tests

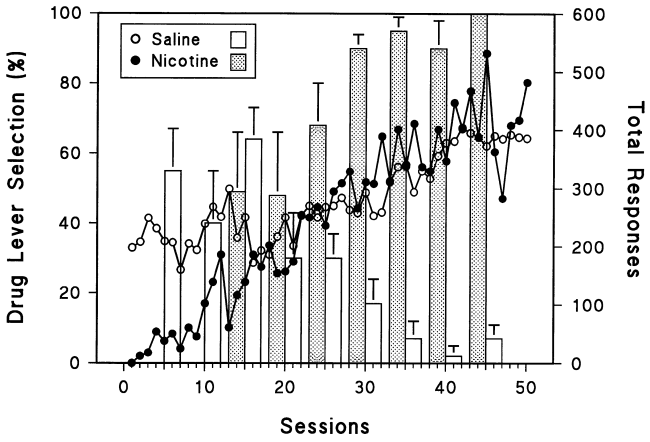

FIG. 1. Training data for acquisition of nicotine (0.4 mg/kg s.c.) as a discriminative stimulus. Each block represents the mean ± sem accuracy of correct lever selections for 5 trials (sessions) of nicotine (closed bars) or saline (open bars). Each point represents the mean ± sem number of responses following each nicotine (●) or saline (○) training session.

described. The first experiment examined the effects of chronic nicotine injections (daily single injection) during suspended training. The next experiment investigated the effects of chronic injections of nicotine with three injections given daily during suspended training. Finally, continuous infusion of nicotine was tested upon the discrimination of nicotine. While the ED_{50} was noted to gradually decrease over experiments, it was found to remain stable during the test periods, thus allowing accurate evaluation of the treatments.

Statistical Analysis

Discriminative performance was analysed as percentage of drug-appropriate responses. Dose-response curves were constructed for each animal, and analysed in groups resulting in a mean and SEM for each data point, which is plotted in the figures. For each treatment regimen, comparisons were performed between the 'before' and 'after' dose-response curves. Doses required to evoke 50% drug-appropriate responses (ED_{50} and 95% Confidence Intervals, CI) were determined by regression analysis and analysis of variance with repeated measures (Bioassay, ARC Baltimore). Significant differences between dose-response curves were determined by paired Students *t*-tests. Subjects not demonstrating good stimulus control, defined as at least 90% difference between nicotine- and saline-appropriate responding in the 'before' dose-response test, were excluded from analyses for that treatment regimen.

TABLE 1

EFFECTS OF CHRONIC TREATMENTS (SINGLE DAILY INJECTIONS) ON THE DS EFFECTS OF NICOTINE

Nicotine (mg/kg s.c.)	ED_{50} (95% CI) Before	After
Saline	0.42 (0.30–0.53)	0.35 (0.23–0.45)
0.4	0.27 (0.20–0.34)	0.28 (0.15–0.41)
0.8	0.26 (0.16–0.36)	0.30 (0.20–0.39)
1.2	0.39 (0.23–0.55)	0.23 (0.05–0.41)

TABLE 2

EFFECTS OF CHRONIC TREATMENTS (THREE DAILY INJECTIONS) ON THE DS EFFECTS OF NICOTINE

Nicotine (mg/kg s.c.)	ED_{50} (95% CI) Before	After
Saline	0.17 (0.03–0.31)	0.15 (0.08–0.21)
0.1	0.23 (0.13–0.33)	0.24 (0.14–0.33)
0.4	0.20 (0.11–0.29)	0.13 (0.07–0.19)
0.8	0.16 (0.11–0.22)	0.18 (0.11–0.25)

Drugs

(–)-Nicotine hydrogen tartrate (Sigma, St Louis, MO) was dissolved in isotonic saline. The pH of nicotine solutions was adjusted to 7 with dilute NaOH. All doses were expressed as those of the base. Subcutaneous injections were given in a volume of 1 ml/kg into the left flank.

RESULTS

Acquisition of Discrimination

Approximately 80 trials of training (40 nicotine and 40 saline) given to rats over 30 days, were required before they could discriminate nicotine from saline. The pattern of acquisition to discriminate nicotine is shown in Fig. 1. The first few injections of nicotine were markedly suppressant on food-maintained responding to a point where rats were not completing the fixed ratio. After 16 drug sessions tolerance developed to the suppressant effects of nicotine and lever selection was observed to be around 50%. It took a further 10 sessions before discrimination was evident. Further training sessions with nicotine and saline gradually improved the accuracy of lever selection. By the 40th drug and vehicle session robust stimulus control was apparent (Fig.1). Generalisation tests to cumulative doses of nicotine engendered dose-related responding on the nicotine-appropriate lever. The ED_{50} for stimulus control by nicotine was 0.30 mg/kg (CI, 0.20–0.35) and was stable over repeated determinations (ED_{50}s range from 0.42–0.26 mg/kg s.c.) over 3 wk. Tests with saline administered 6 times every 10 min, as in the cumulative dosing regimen, revealed robust stimulus control as all the rats responded primarily on the saline-appropriate lever throughout the test.

TABLE 3

EFFECTS OF CONTINUOUS INFUSIONS (OSMOTIC MINI-PUMPS) ON THE DS EFFECTS OF NICOTINE

Nicotine (mg/kg/day)	ED_{50} (95% CI) Before	After
Saline	0.18 (0.12–0.23)	0.11 (0.06–0.17)
3.2	0.16 (0.10–0.22)	0.19 (0.14–0.24)
6.4	0.12 (0.06–0.18)	0.30* (0.22–0.39)

* Denotes significant shift of dose-response curves by nicotine treatment.

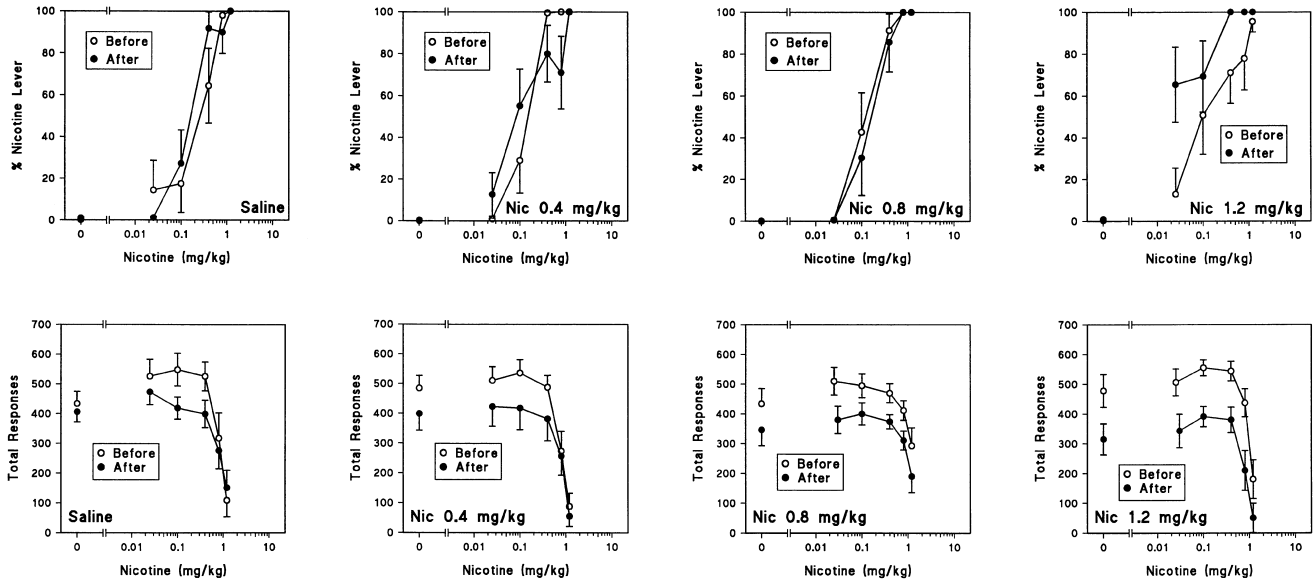

FIG. 2. Dose-response curves for the discriminative stimulus effects of nicotine in subjects treated chronically for 7 days with single daily injections of nicotine. The doses of nicotine administered chronically are indicated in each panel. The top section shows the responses to the nicotine-appropriate lever as a percentage of total trial responses. The cumulative doses of nicotine are shown on the abscissae. Open circles represent control tests conducted 'before' the start of repeated treatments. The closed circles represent tests conducted 'after' the period of chronic treatment. The bottom section shows the total number of responses made made following each cumulative dose of nicotine.

Evaluation of Chronic Treatments of Nicotine

Daily single injections of nicotine for 7 days during suspended training failed to significantly shift the dose-response curve. Fig. 2 shows the dose-response functions of nicotine 'before' and 'after' chronic nicotine treatments. Even large doses of nicotine (0.8–1.2 mg/kg s.c.) that produced marked sedation and convulsions during the chronic treatments failed to induce any changes in the ED_{50} (Table 1). Irrespective of the nicotine dose tested, response rate was observed to be higher before the suspended training than after the chronic treatments (Fig. 2). Increasing the frequency of nicotine injections to three

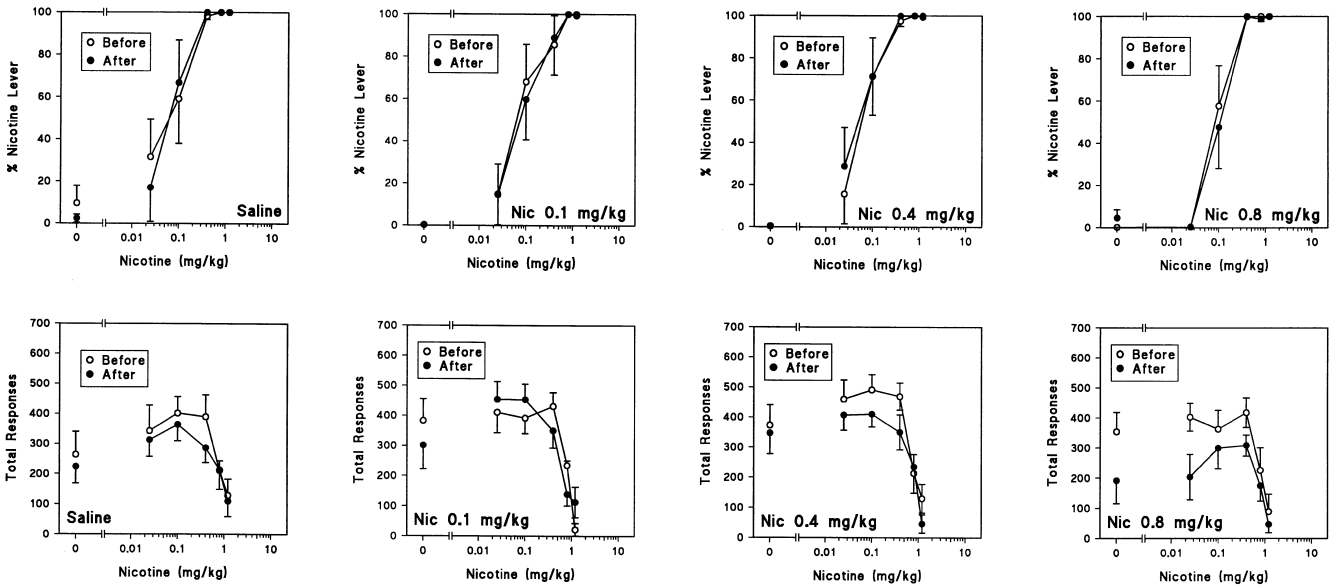

FIG. 3. Dose-response curves for the discriminative stimulus effects of nicotine in subjects treated chronically for 7 days with three daily injections of nicotine. The doses of nicotine administered chronically are indicated in each panel. The top section shows the responses to the nicotine-appropriate lever as a percentage of total trial responses. The cumulative doses of nicotine are shown on the abscissae. Open circles represent control tests conducted 'before' the start of repeated treatments. The closed circles represent tests conducted 'after' the period of chronic treatment. The bottom section shows the total number of responses made made following each cumulative dose of nicotine.

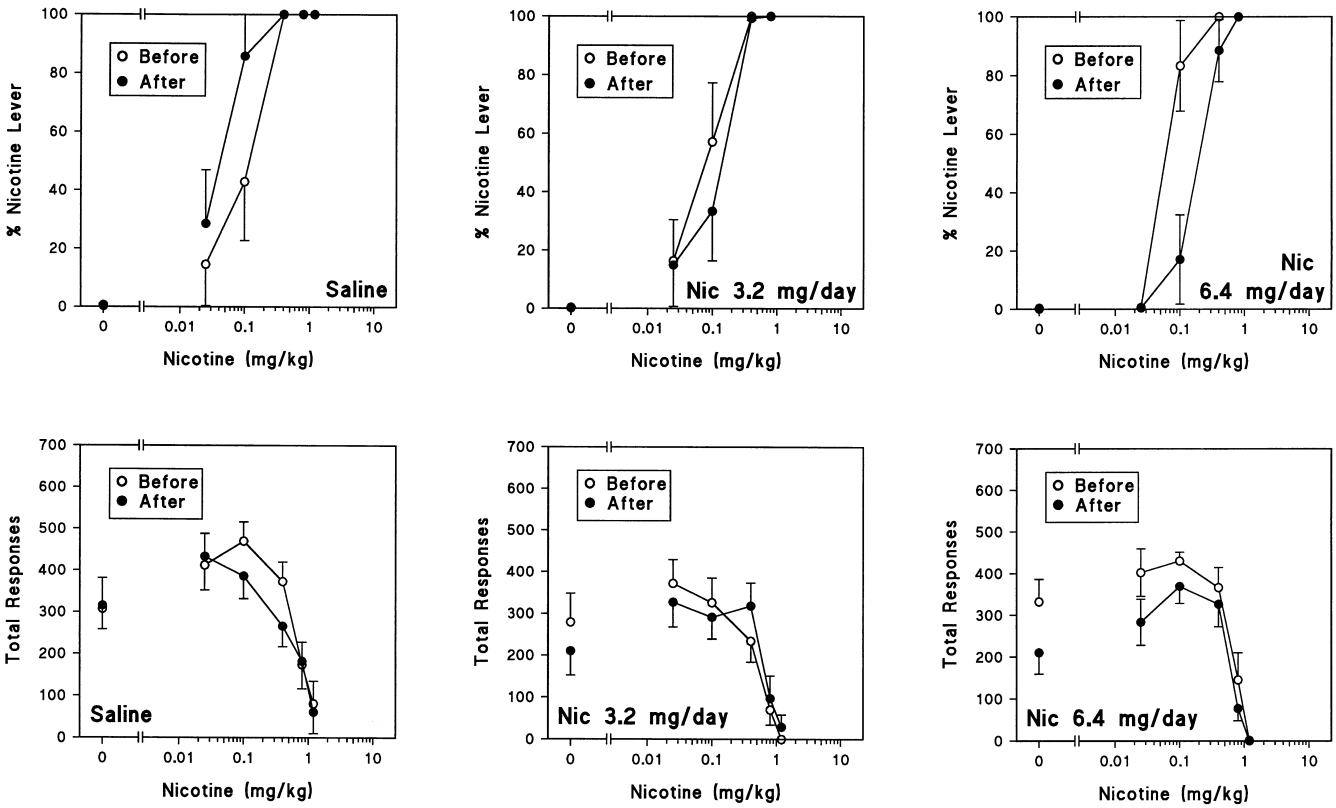

FIG. 4. Dose-response curves for the discriminative stimulus effects of nicotine in subjects treated chronically for 7 days with continuous infusions of nicotine. Infusion rates are indicated in each panel. The top section shows the responses to the nicotine-appropriate lever as a percentage of total trial responses. The cumulative doses of nicotine are shown on the abscissae. Open circles represent control tests conducted 'before' the start of repeated treatments. The closed circles represent tests conducted 'after' the period of chronic treatment. The bottom section shows the total number of responses made made following each cumulative dose of nicotine.

times daily over the 7 day period failed to significantly modify the cumulative dose-response curve. Figure 3 shows the dose-response patterns following exposure of three different doses of nicotine and saline. The ED_{50}s calculated from these data confirmed the lack of shift by these chronic treatments (Table 2). No significant effects of chronic nicotine treatment were apparent on the total number of lever responses (Fig. 3).

Evaluation of Continuous Infusions of Nicotine

Figure 4 shows the dose-response curves generated following the continuous infusion of nicotine or saline for 7 days. The infusion of saline produced a small shift of the nicotine dose-response curve to the left, but this was not significant (Table 3). Infusion of nicotine produced a dose-dependent shift to the right of nicotine discrimination. Delivery of nicotine at a rate of 6.4 mg/kg/day produced a 2.5 fold increase in the ED_{50} of nicotine discrimination [$t(5) = 4.8$, $P < 0.05$]. This effect was partly due to an unusually high % drug lever value at 0.1 mg/kg nicotine in the 'before' condition. Nevertheless, this modification occurred in the absence of any other behavioural effects. Eighteen hours after removal of the osmotic minipumps, the rats withdrawn from nicotine exhibited no signs of 'withdrawal' as described by Malin et al (15). This absence was also apparent with lever responding, which was no different from response rates measured before implantation of the minipumps. Infusion of a smaller dose of nicotine (3.2 mg/kg/day) did not induce tolerance to the discriminative stimulus effects of nicotine (Table 3). Again, no effect of continuous nicotine infusion was apparent on total lever responding (Fig. 4).

DISCUSSION

Using multiple-trial training sessions, the present experiments demonstrated that injections of nicotine can serve as discriminative stimuli. Robust stimulus control was obtained, which engendered dose-dependent generalisation to increasing doses of nicotine. The median dose that produced approximately 50% nicotine-appropriate responding was 0.30 mg/kg s.c. (CI, 0.20–0.35 mg/kg). This index of discrimination remained relatively stable throughout the testing period. The ED_{50} generated using multiple training trials per session was very similar to that found by Pratt et al (17) using conventional single trial training procedures with 0.4 mg/kg nicotine (ED_{50} approximately 0.20 mg/kg s.c.). One advantage with the multiple training trials procedure is the ability to construct full dose-response curves within hours. Such an approach has been used successfully to demonstrate chronic tolerance to the DS effects induced by a range of abused substances (1,12,24,26).

The main aim of this study was to examine the effects of chronic nicotine treatment during suspended training. Previous experiments with other drugs of abuse have shown that chronic exposure to drugs with training halted has readily produced shifts of dose-response curves (1,12,24). In most

cases, these shifts to the right have been observed following 3 to 7 days of chronic treatment. Hence, in the present experiment, a seven day chronic dosing procedure was utilised. Single daily injections of a range of pretreatment doses of nicotine failed to significantly shift the dose-response curves. Increasing the frequency of injection to three times per day, failed to have any impact on the ED_{50}'s of nicotine discrimination. The chronic treatments were behaviourally active, especially doses above 0.4 mg/kg s.c. which were markedly depressant and the 1.2 mg/kg s.c. dose induced convulsions. It is conceivable that tolerance may have developed with higher doses of nicotine, but due to the toxicity observed at the 1.2 mg/kg s.c. dose, it was difficult to test this. With morphine and cocaine, the doses found to shift the dose-repsonse curves have been approximately 5–10 times larger than the training dose (26). With nicotine, the largest dose examined was three times the training dose. This difference may have some implications, particularly for the development of dependence. It is conceivable that tolerance may have developed if rats were trained to discriminate a lower dose of nicotine. A shift of the dose response curve following a period of chronic drug exposure may be interpreted as a withdrawal-induced effect. For example with morphine, the chronic treatment may induce dependence, and in the period prior to the re-determination of the dose-response curve, the animal may undergo spontaneous withdrawal. Alleviation of these withdrawal effects are therefore manifested as tolerance to the DS effects since the nicotine is simply reinstating baseline conditions, i.e. a saline-like state. It is also conceivable that by the time the rats attained robust stimulus control, having received approximately 60 injections of nicotine, chronic tolerance may have already developed to the DS effects of nicotine in the same manner as shown on locomotor activity (23) which was found to persist for up to 90 days. This explanation may also explain the lack of effect for chronic treatments on response rates, since tolerance was observed to the rate suppressant effects of nicotine during acquisition of discrimination.

The effect of withdrawal from nicotine was examined using a regimen reported to induce dependence to nicotine (15). Continuous infusions of nicotine from osmotic minipumps produced a small two-fold shift of the dose response curve. However, the effect observed with the largest rate of infusion (6.4 mg/kg/day) was due partly due to an unusually high % drug-lever responding in the control 'before' condition. Therefore, this effect was not robust, which was also confirmed by the lack of physical signs of spontaneous withdrawal, especially during the period prior to the re-determination of the dose-response curve. Previous studies examining withdrawal from nicotine in rats have shown that abstinence can be behaviourally disruptive on lever responding (5). In the present study, no deficits in responding were apparent. Tolerance to the DS effects may be an artifact. As discussed by others (4), tolerance to DS effects should occur regardless of suspended training. A number of studies have failed to find tolerance when training was continued during the phase of chronic injection (2,4,11).

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