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A Description of the Nicotine Stimulus and Tests of Its Generalization to Amphetamine

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Abstract. The discriminative stimulus properties of nicotine were investigated under a variety of conditions in three separate experiments. In each of these experiments the subject's performance was assessed using a two-lever operant procedure with liquid food reinforcement. In the first study rats were trained to discriminate between various doses of nicotine (100, 200, or 400 μg/kg) and saline under a VI-15 s schedule of reinforcement. The second experiment investigated discrimination between 400 μg/kg of nicotine and saline under different schedules of reinforcement (VI-15 s, FR-10, or DRL-10 s). Generalization of the nicotine stimulus (400 μg/kg) to the stimulus effects of several doses of *d*-amphetamine (60, 120, 240, 480, and 720 μg/kg) was investigated in the third study. Dose-generalization and time-duration studies of the stimulus effects of nicotine indicate that the sensitivity of the rats to the nicotine cue was directly related to the training dose under the VI-15 s schedule. Although response rates differed across the schedules of reinforcement, the rats' sensitivity to the stimulus effects of nicotine was not affected. Lack of complete generalization of the nicotine stimulus to *d*-amphetamine supports our previous findings that these drugs were qualitatively different in relation to their discriminative control of behavior. This research has, in addition, suggested approaches necessary to the proper evaluation of the stimulus properties of test compounds.

Key words: Discriminative stimulus – Nicotine – Amphetamine

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In certain experimental paradigms, nicotine and *d*-amphetamine appear to elicit similar behavioral effects (Morrison, 1967). Orsingher and Fulginiti (1971) showed that both drugs facilitated conditioned avoidance behavior which was, in turn, antagonized by the reversible inhibitor of catecholamine synthesis, alpha-methyl-para-tyrosine (AMPT). This antagonism by AMPT of conditioned avoidance behavior suggests that nicotine, like *d*-amphetamine, may be producing behavioral effects through interactions with neurons containing norepinephrine or dopamine. Using a discriminative stimulus procedure, Schechter and Rosecrans (1972, 1973) further investigated these possible interactions but reported no generalization of the stimulus properties of either drug to the other one. Furthermore, it has been observed that catecholamine depletion reduces the sensitivity of rats to the stimulus properties of these drugs (Rosecrans et al., 1976).

While these findings suggest that nicotine and *d*-amphetamine have neurochemical and behavioral similarities, additional evidence indicates that these drugs have different effects on motor activity (Morrison and Armitage, 1967). The present series of experiments was designed to investigate further the stimulus properties of nicotine as well as its similarity to *d*-amphetamine. Using a discriminative stimulus (DS) paradigm, the degree of generalization of the nicotine stimulus effects to those of *d*-amphetamine were investigated across several training doses of nicotine and under different schedules of reinforcement. The major objectives of this investigation were: (1) to examine the strength of the nicotine stimulus across a variety of conditions; (2) to determine under what conditions generalization of the stimulus effects of nicotine to those of amphetamine might occur; and (3) to obtain information from the nicotine-amphetamine generalization tests that would be useful in

the evaluation of compounds that elicit behavioral effects similar to nicotine.

MATERIALS AND METHODS

Subjects. Male Sprague-Dawley rats (175–200 g) with no previous drug or experimental experience were purchased from Flow Research Animals, Dublin, Virginia. These rats were individually housed in a temperature-controlled environment under a 12-h light/dark cycle. Water was freely available in the home cages and adjusted amounts of commercial rat chow were offered after each experimental session to maintain the animals at 70–80% of their expected free-feeding weight.

Apparatus. The experimental space was a standard operant test chamber (Lehigh Valley Electronics, Model 1417). One wall of the chamber contained two levers with a dipper for delivery of liquid reinforcement centered between them. A force of approximately 15 g was necessary to depress the levers. Sweetened condensed milk, diluted 2:1 with tap water and delivered by the dipper (0.1 ml), was the reinforcement. The experimental chamber was located in a larger sound-insulated and light-proof isolation cubicle (Lehigh Valley Electronics, Model 132-02) equipped with an exhaust fan. Solid-state and electro-mechanical programming equipment were used to control and record the data generated during the test sessions.

Procedure. Discrimination training was similar to that previously reported by Hirschhorn and Rosecrans (1974). At approximately 10 weeks of age, the food-deprived rats were shaped to press first one, then the other lever in the box for liquid food reinforcement. Drug discrimination training began with four preliminary training sessions of 15-min duration in which every correct lever press was reinforced. Subsequent sessions were initiated by a 2.5-min period during which no responses were rewarded, and a partial reinforcement schedule (VI-15 s, FR-10, or DRL-10 s) was imposed for the remaining 12.5 min. Every session was preceded by 10 min with s.c. injections of either nicotine (free base of the bitartrate; BDH Chem. Ltd.; Poole, England) or saline. During the four preliminary training sessions, nicotine and saline injections were alternated daily. For the duration of the experiments, however, 2 days of one treatment were followed by 2 days of the other treatment. By means of this double alternation schedule of drug administration, each treatment was preceded equally often by a session with the same and opposite treatment. One lever was reinforced after the injection of nicotine and the other lever was reinforced following saline treatment. For half of the subjects in each experiment, the right lever was rewarded after nicotine and the left lever was rewarded after the injection of saline. These conditions were reversed for the remaining animals in the studies. The injections of nicotine or saline as well as the subsequent training and testing sessions were administered one a day, 5 days a week. Inferences of learning the discrimination were made from response data collected during the initial nonreinforced portion (2.5 min) of each session. The data in these experiments are expressed as percent nicotine-correct lever choices (number of responses on nicotine-correct lever/total number of responses). Percent discrimination is the mean difference in responding on the nicotine-correct lever between the drug and nondrug states. Statistical evaluations of the data were accomplished using analysis of variance (ANOVA) techniques, with individual comparisons made by *t*-tests or Duncan's New Multiple Range test. Response comparisons in dose-generalization and drug-generalization tests were assessed by ED_{50} and ED_{75} calculations based on mean percentage of nicotine-correct responding at the training dose (Litchfield and Wilcoxin, 1949).

Experiment I. In the first experiment three groups of eight rats each were trained to discriminate between the stimulus effects of 100, 200, or 400 µg/kg of nicotine and saline under a VI-15 s schedule of reinforcement. Thus, one group was consistently trained under 100 µg/kg, one under 200 µg/kg, and the other under 400 µg/kg of nicotine. After the rats had learned these discriminations (approximately 20 sessions under each drug state), dose-generalization and time-duration parameters of the nicotine stimulus were investigated. During these additional investigations, the rats continued to be trained to respond to the original dose of nicotine, except that a test session was substituted for the training session on every fourth day. During the test session, the animals were run for 2.5–5.0 min with no reinforcement for lever pressing. This procedure was used to avoid contamination of the test data with reinforced responding and also to insure that the original discrimination was not being biased by reinforcing responding to the test dose. The dose-generalization studies were conducted during these test periods by assessing the nicotine-correct responding of the rats following the administration of 1/16, 1/8, 1/4, and 1/2 of the training dose. Thus, the rats trained at 100 µg/kg were tested at 6.25, 12.5, 25, and 50 µg/kg; those trained at 200 µg/kg were tested at 12.5, 25, 50, and 100 µg/kg; and those trained at 400 µg/kg were tested at 25, 50, 100, and 200 µg/kg of nicotine. Each of these doses was administered once to every rat in a random order. At the conclusion of these tests, generalization to doses higher than the training dose were investigated. Thus, generalization to 2, 4, and 8 times the training dose (or up to the point of behavioral disruption) were studied.

The next study investigated the time-duration parameters of the nicotine stimulus. These tests were also conducted during the fourth day test session and investigated the effects of increasing the time interval between the injection of nicotine and the beginning of a test session. In addition to the standard 10-min delay, the effects of 20, 40, 80, and 160 min injection-test intervals were studied. Again, each of these test intervals was investigated in every rat in a random order.

Experiment II. In the second experiment another three groups of six rats each were trained to discriminate the stimulus effects of nicotine (400 µg/kg) and saline. In this study, however, each of the groups of rats was trained under a schedule of continuous reinforcement. After the subjects had shown evidence of having learned the discrimination ($\geq 80\%$ nicotine-correct responding following the injection of nicotine), each group was switched to a different schedule of partial reinforcement. Thus, training continued with one group under a FR-10, one under a VI-15 s, and one under a DRL-10 s schedule of reinforcement. Again, these studies investigated parameters of dose-generalization (25, 50, 100, and 200 µg/kg) and time-duration (10, 20, 40, 80, and 160 min), as in the first experiment.

Experiment III. The third experiment investigated the generalization of the stimulus effects of nicotine to various doses (50, 120, 240, 480, and 720 µg/kg; i.p.) of the free base of *d*-amphetamine sulfate (City Chem. Corp., New York). The rats used in this experiment were the subjects from the previous two studies and included subjects that were trained to discriminate the stimulus effects of three doses of nicotine (100, 200, and 400 µg/kg) from saline under a VI-15 s schedule as well as those rats discriminating the stimulus effects of 400 µg/kg of nicotine from saline under three different schedules of reinforcement (FR-10, VI-15 s, and DRL-10 s). As in the preceding experiments, the drug-generalization tests were conducted during the fourth day test sessions and every animal was tested for generalization to each dose of amphetamine once in a random order. Thus, this experiment investigated dose-generalizations of the stimulus effects of nicotine to amphetamine across a variety of doses for each drug. In addition, the design permitted

the study of the generalization of the stimulus effects of 400 μg/kg of nicotine to different doses of amphetamine under a variety of schedules of reinforcement.

RESULTS

Experiment I. Mean nicotine-correct responding following the injection of 100, 200, and 400 μg/kg of nicotine or saline is presented in Figure 1. As can be observed, the rats learned to discriminate the stimulus effects of the various doses of nicotine and saline by the fifth trial-block (10 exposures to training under each drug state). The rate of acquisition and the degree of discrimination depended on the training dose of nicotine. A repeated measures ANOVA of mean trial block data showed that the rats trained at 400 μg/kg of nicotine learned faster and were better able to discriminate the drug state than rats trained at 200 μg/kg of the drug [$F(1,14) = 20.20$, $P < 0.01$]. The rats trained at 200 μg/kg of nicotine also discriminated significantly better than those trained at the lowest dose [$F(1,14) = 14.26$, $P < 0.01$]. Although these differences tended to decrease with more training, they were still evident up to the twelth trial-block (Table 1). Evaluation of the drug discrimination under each dose of nicotine indicates that the percent discrimination (% D, Table 1) is also dose-related. Comparison of total responding for all groups during the 15-min training sessions in trial-blocks one vs. trial-block 12 suggests a dramatic increase in mean responding across the training days [1015 vs. 2071; $t(23) = 5.1$, $P < 0.01$].

An estimate of the sensitivity of these rats to the nicotine stimulus was obtained from the dose-generalization tests (Fig. 2), conducted after the rats had shown evidence of having learned the discriminations (after the 11th trial-block, Fig. 1). Differences in the sensitivity of the three groups to nicotine are indicated by the ED_{75} values of these generalization tests (Table 1). Thus, the ED_{75} of the group trained at

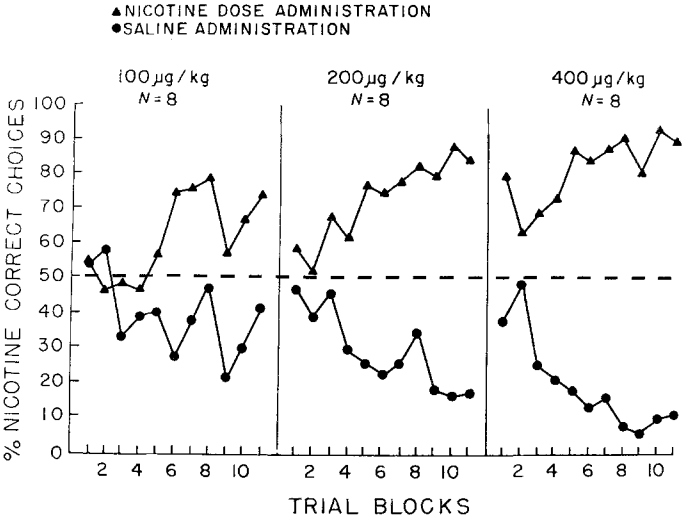

Fig. 1
Mean nicotine-correct responding following injection of 100, 200, or 400 μg/kg of nicotine and saline. Each trial-block represents data collected across two drug and two saline sessions in double alternation sequence. Data were collected under VI-15 s schedule of reinforcement during 2.5 min nonreinforced periods prior to each training session

Table 1. Strength of the discriminative stimulus produced by different doses of nicotine

Nicotine training dose (μg/kg) (*N*)	% Total responses[a] on nicotine-correct lever (± SEM)			Response rates on correct lever (± SEM)		Dose generalization gradients[c] (95% confidence limits)	
	After nicotine	After saline	%D[b]	Nicotine lever	Saline lever	ED_{75} (μg/kg)	ED_{50} (μg/kg)
100 (8)	75 ± 2	25 ± 2	49 ± 3	11.6 ± 2.3	11.7 ± 2.6	93 (34–189)	26 (9–71)
200 (8)	80 ± 1	29 ± 5	57 ± 3	9.1 ± 1.4	8.3 ± 1.9	174 (112–288)	80 (39–161)
400 (8)	89 ± 1	16 ± 2	73 ± 2	10.2 ± 0.6	12.8 ± 1.9	306 (195–505)	87 (40–185)

[a] These data were obtained during nonreinforced 2.5-min periods that preceded training blocks 8–12. Each value is mean of trial blocks (± SEM)

[b] Percentage of responses on nicotine-correct lever when given nicotine minus percentage of responses on nicotine-correct lever when given saline

[c] Transfer doses were 1/2, 1/4, 1/8, and 1/16 of the training dose at each dose level. ED_{50} and ED_{75} values were determined using the procedures of Litchfield and Wilcoxin (1949)

100 µg/kg was lower than those trained at either 200 or 400 µg/kg of the drug. The rats trained at 200 µg/kg also showed a lower ED_{75} than those trained at 400 µg/kg of nicotine. Evaluation of the data in terms of the ED_{50} dose again suggested that the 100 µg/kg group was more sensitive to the nicotine stimulus (ED_{50} = 26 µg/kg), while there was no difference between the ED_{50} doses of the rats trained at 200 (ED_{50} = 80 µg/kg) or 400 µg/kg (ED_{50} = 87 µg/kg) of nicotine. Furthermore, a repeated measures ANOVA across 12.5, 25.0, and 50.0 µg/kg showed that the group trained at 100 µg/kg generalized more than the group trained at 200 µg/kg of the drug [$F(1,14) = 9.51$, $P < 0.01$]. No such difference between the 200 µg/kg and 400 µg/kg groups were observed across 25.0, 50,0, and 100.0 µg/kg of nicotine. Evaluations of doses above the training dose indicate that the stimulus effects of the 100 µg/kg dose generalized to 200 µg/kg, which in turn generalized to 400 µg/kg of nicotine. Doses of 800 µg/kg of nicotine severely depressed response rates of the rats trained at 100 µg/kg, while the depression of response rates of the two groups trained under the higher doses was not evident until they received 1600 µg/kg of the drug.

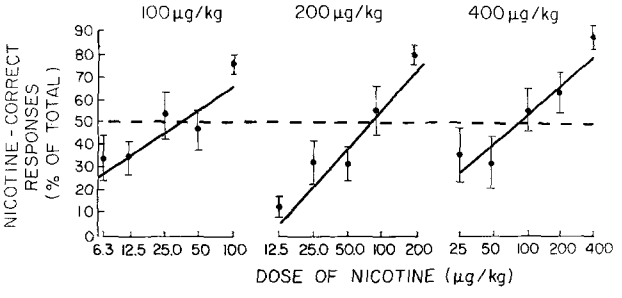

Fig. 2. Mean (± SEM) dose-generalization gradients of rats trained to discriminate 100, 200, or 400 µg/kg of nicotine from saline. Data were collected under a VI-15 s schedule of reinforcement during the 2.5–5.0 min test sessions conducted every fourth day after rats had learned discrimination. For each point, $N = 8$

The results of the time-duration study, conducted after the completion of the dose-generalization experiment, suggest that the discriminative stimulus was present for at least 40 min in the low-dose group but lasted for at least 80 min in the two groups trained under the higher doses of nicotine (Fig. 3).

Experiment II. Three different groups of rats were trained to discriminate nicotine (400 µg/kg) from saline under a schedule of continuous reinforcement. After the rats had learned the discrimination, the effects of different rates of responding on discrimination were investigated by continuing training under three different schedules of partial reinforcement (FR-10, VI-15 s, and DRL-10 s). The mean total number of responses during the 5-min test sessions were observed to vary according to the schedule of reinforcement (FR = 89.8, VI = 35,4, and DRL = 13.8; based on 3 trial-blocks). Although these response rates were significantly different [$F(2,15) = 16.66$, $P < 0.01$], no difference in nicotine-correct responding was observed across the groups (FR = 83.9%, VI = 84.7%, and DRL = 81.4%). Re-

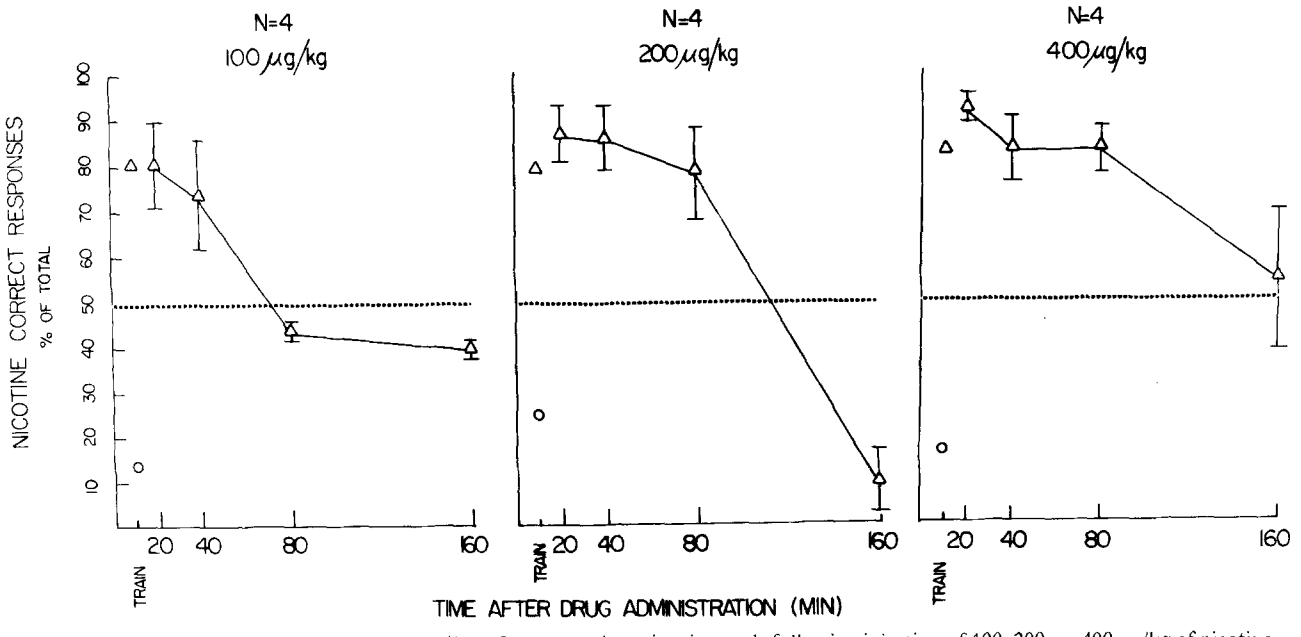

Fig. 3. Mean (± SEM) nicotine-correct responding of rats at various time intervals following injection of 100, 200, or 400 µg/kg of nicotine. Data were collected during 2.5–5.0 min nonreinforced test sessions after 130 training sessions under each drug state

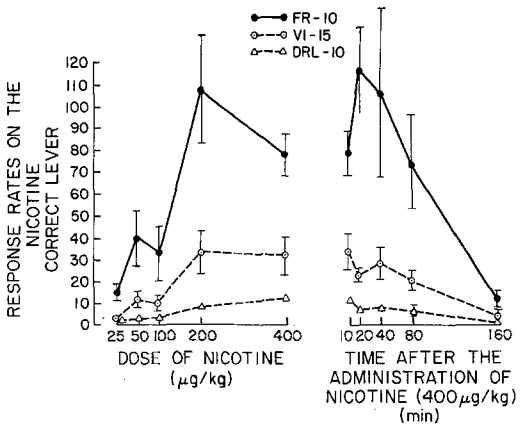

Fig. 4. Mean (± SEM) total responses on the nicotine-correct lever following the injection of nicotine (400 μg/kg). These dose-generalization and time-duration tests were conducted on three groups ($N = 6$) of rats responding under various schedules of reinforcement (FR-10, VI-15 s, and DRL-10 s)

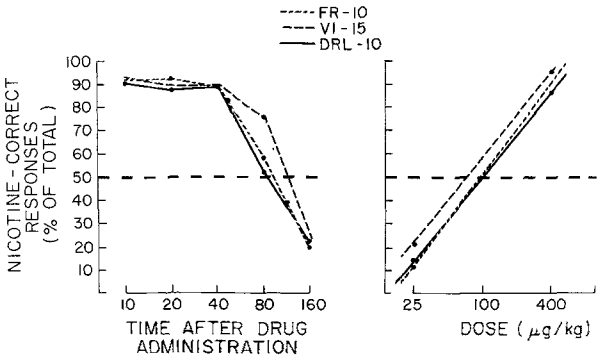

Fig. 5. Mean nicotine-correct responding during the dose-generalization and time-duration tests of Experiment II. Discrimination of stimulus effects of 400 μg/kg of nicotine from saline did not differ across various schedules of reinforcement. For each point, $N = 6$

sponse rates were also different across schedules in both the dose-generalization [$F(2,15) = 14.31$, $P < 0.01$] and time-duration [$F(2,15) = 24.49$, $P < 0.01$) studies (Fig. 4). Furthermore, the rates increased with higher doses of nicotine [$F(5,75) = 10.54$, $P < 0.01$) and decreased with longer post-injection intervals [$F(4,60) = 6.71$, $P < 0.01$]. As Figure 4 indicates, the significant group-dose interaction [$F(10,75) = 3.56$, $P < 0.01$] as well as the group-interval interaction [$F(8,60) = 6.71$, $P < 0.01$] are due to the dramatic effects of these manipulations on responding under the FR-10 schedule. Although the response rates were greatly influenced by the schedule of reinforcement, the dose of nicotine, and the postinjection interval, there were no differences in nicotine-correct responding across these measures (Fig. 5). Thus, the rats made a similar percentage of their responses on the nicotine-correct lever, regardless of their particular schedule of reinforcement, across the dose and injection interval treatments.

One unexpected observation in this experiment was the increase in response rates (on the nicotine-correct lever) as the dose of nicotine was decreased from 400 μg/kg to 200 μg/kg on the FR-10 schedule (Fig. 4), suggesting that the 400 μg/kg dose was suppressing response rates on this schedule. The response rates also increased from 10–20 min in the time-duration test (Fig. 4) for the FR-10 group, again indicating an initial suppression of responding by the 400 μg/kg dose of nicotine.

Experiment III. Groups of rats that previously discriminated the stimulus effects of nicotine and saline under various doses of nicotine and different schedules of reinforcement received different doses (60, 120, 240, 480, and 720 μg/kg) of amphetamine. The mean

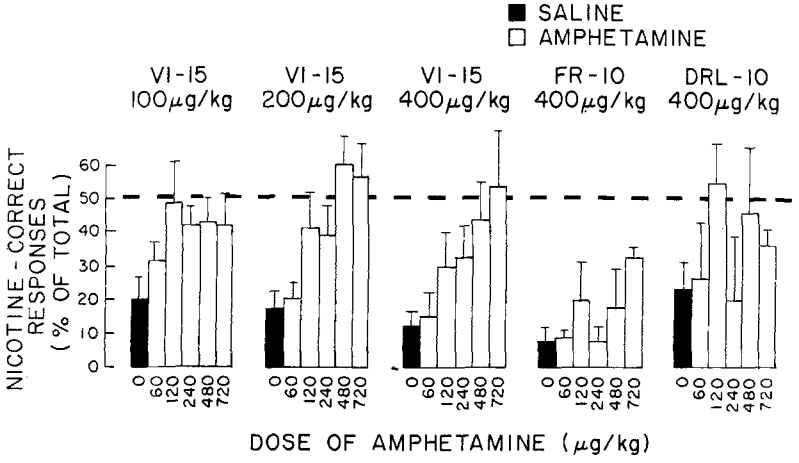

Fig. 6. Mean (± SEM) generalization, by rats trained to discriminate nicotine from saline under a variety of doses (100, 200, and 400 μg/kg) and schedules of reinforcement (FR-10, VI-15 s, and DRL-10 s), to different doses (60, 120, 240, 480, and 720 μg/kg) of amphetamine. For each VI-15 s group, $N = 6$ to 8 (depending on dose of amphetamine), while $N = 6$ for FR-10 and DRL-10 s groups

Table 2
Mean response rates following the injection of saline, nicotine (400 μg/kg), or various doses of amphetamine under a FR-10 or VI-15 schedule of reinforcement

FR-10						
Treatment	*N*	Dose of amphetamine (μg/kg)				
		60	120	240	480	720
Saline	6	70	46	49	25	8
Nicotine	6	89	78	72	39	28
Amphetamine	6	112	133	136	111	24
VI-15						
Treatment	*N*	Dose of amphetamine (μg/kg)				
		60	120	240	480	720
Saline	6	22	27	22	32	32
Nicotine	6	30	28	27	26	28
Amphetamine	6	23	25	33	18	31

[a] Values are mean response rates for both levers combined, i.e., total response rates under each drug state. The saline and nicotine data were collected on the 4 trials preceding the injection of amphetamine

percentage of nicotine-correct responding to these doses of amphetamine for those rats that maintained responding is presented in Figure 6. Although some of the rats failed to respond to all doses of amphetamine, statistical evaluations are based on the total *N* per group (VI = 8/dose, FR = 6, and DRL = 6) with nonresponse values entered as 0% nicotine-correct responses. There were no differences in generalization to amphetamine across the three nicotine-training doses (100, 200, and 400 μg/kg) under the VI-15 s schedule of reinforcement. The overall ANOVA did reveal significant differences between the response to nicotine and the various doses of amphetamine [$F(5,105) = 15.61$, $P < 0.01$] and a Duncan's Multiple Range test showed that the response to nicotine was different from each dose of amphetamine ($P < 0.05$). However, the rats responded more on the nicotine-correct lever following the injection of amphetamine than saline [$F(5,105) = 3.65$, $P < 0.01$) with post hoc tests indicating that every dose except 60 μg/kg of amphetamine was different from saline ($P < 0.05$). Therefore, the rats under the VI-15 s schedule of reinforcement were responding to amphetamine as unlike both nicotine and saline.

As indicated in Figure 6, performance on both the DRL-10 s and FR-10 schedules of reinforcement was characterized by high variability. Under neither of these schedules was the overall nicotine-correct responding to amphetamine significantly different from saline values. Therefore, the rats trained on the FR-10 schedule appear to be less sensitive to the stimulus effects of amphetamine. Thus, a surprising difference appears in the degree of nicotine-correct responding by amphetamine-treated animals between the VI-15 s and FR-10 schedules of reinforcement. This difference is further exemplified when the response rates under the various doses of amphetamine are examined. Table 2 presents the mean response rates for the animals tested under the FR-10 and the VI-15 s schedules of reinforcement across the various doses of amphetamine and the accompanying doses of nicotine and saline. The subjects under the FR-10 schedule responded at higher rates across almost all conditions than those under the VI-15 s schedule. Furthermore, amphetamine potentiated the response rates under the FR schedule but did not affect rates under the VI schedule of reinforcement. Thus, apart from the stimulus effects of the drug, amphetamine showed a differential increase in rate of responding depending upon the schedule of reinforcement.

DISCUSSION

The results of the experiments reported here indicate that the nicotine stimulus can be perceived by a rat and may be used to exert control over behavior much as any external stimulus (Fig. 1). This stimulus effect is generally dose-related both in terms of magnitude (Table 1) and duration of the response (Fig. 3). Furthermore, the stimulus effect shows a gradient of generalization to different doses of nicotine, suggesting varying degrees of dose-dependent tolerance (Fig. 2). Although the degree of discrimination between nicotine and saline was dose-dependent (Table 1), there was no difference in response rates across the three doses. This similarity in rates of responding suggests that these animals were tolerant to the behaviorally disruptive effects of nicotine and were discriminating the drug state rather than an effect of the drug on behavior. The twofold increase in response rates under the nicotine state during training (trial-block 1 vs. 12; Fig. 1) supports the development of tolerance to the disruptive effects of the drug. The development of

tolerance to behaviorally disruptive effects of drugs is a particular advantage of DS procedures. Furthermore, it appears that learning to discriminate between drug states is contingent on the subject becoming behaviorally tolerant to the specific drug (Rosecrans and Chance, 1977).

Observation of Figure 2 suggests that the dose-generalization gradient depends on the training dose and not the absolute dose of nicotine. Thus, the groups trained under 400 and 200 μg/kg showed less generalization to lower doses ($\leq$ 50 μg/kg) of nicotine than the group trained at 100 μg/kg of the drug. These differences in sensitivity suggest that the efficacy of the stimulus is specifically related to the degree of the stimulus present when the discrimination is learned and may be due to differential tolerance to nicotine across the training doses. Differences in tolerance were also suggested by the ED_{75} values for the three training doses (Table 1) as well as by the results of dose-generalization tests conducted with doses of nicotine higher than the training doses. Thus, although the group trained at 100 μg/kg generalized well to 200 μg/kg, and those trained at 200 μg/kg of nicotine generalized well to 400 μg/kg of the drug, the onset of behavioral disruption was dose-dependent. Doses of 800 μg/kg of nicotine severely depressed response rates of the rats trained at 100 μg/kg, while this disruption was not observed in the other two groups until they received 1600 μg/kg of the drug. This gradient of tolerance has also been noted in other studies in which intraventricular (ivt.) injections of nicotine produced dose-related convulsions contingent upon the training dose (Rosecrans, 1976; unpublished observations). Thus, the ivt. injection of 16 μg of nicotine elicited convulsions in rats trained at 100 μg/kg of the drug, while application of at least 32 or 64 μg was required to produce convulsions in rats trained respectively at 200 or 400 μg/kg of nicotine. Although not negating qualitative differences in stimulus effects, the observation that rats trained at lower doses generalize well to higher doses of nicotine suggests that the initial difference between groups (100, 200, and 400 μg/kg) may have been due to quantitative differences in the nicotine stimulus. Thus, the rats trained at the lower doses may have taken longer to learn the discrimination because of the diminished intensity of the nicotine stimulus. The above data further indicate that higher training doses of nicotine produce rats more tolerant and less sensitive to its stimulus effects.

Analysis of the discrimination over time indicates that although the initial learning and sensitivity were dose-dependent (Table 1), there were no differences in the stimulus effects of nicotine by 130 days of training (Fig. 3). Analysis of the stimulus effects in terms of time-duration at 130 days showed that although the nicotine stimulus effects did not differ across the training doses, the duration of the stimulus was dose-dependent (Fig. 3). These differences in duration of the stimulus effect may reflect differences in brain concentration of nicotine, since Hirschhorn and Rosecrans (1974) observed different brain levels of nicotine following the injection of 200 and 400 μg/kg of the drug.

The results of the discriminative stimulus studies under different schedules of reinforcement suggest that the nicotine stimulus is an appropriate cue both in schedules that elicit low levels of responding (DRL) and higher response rates (FR). These results show that there were no differences between the sensitivity of the rats responding under these schedules in terms of dose-response and time-duration parameters (Fig. 5).

Tests of the generalization of the nicotine stimulus to various doses of amphetamine indicate that the rats did not perceive these two drugs as the same. Complete generalization of the nicotine stimulus to amphetamine was never observed (Fig. 6). Across the different schedules of reinforcement, the VI-15 s trained rats appeared to be the most sensitive in terms of generalization. Even under this schedule, however, generalization to the stimulus effects of nicotine was never much greater than 60%. That nicotine-correct responding following amphetamine on the VI-15 s schedule was significantly different from that following nicotine or saline suggests that the rats perceived the amphetamine state as different from both the nicotine or saline states.

The differences in the degree of generalization of the stimulus effects of nicotine to various doses of amphetamine between the FR-10 and VI-15 s schedules of reinforcement may reflect the arousal level of the subjects. Previous research with nicotine (Rosecrans, 1971) has indicated that animals with low arousal (activity) levels were more responsive to the behavioral effects of nicotine than were a highly active group. The rats under the FR-10 schedule exhibited much higher response rates than those subjects responding under the VI-15 s schedule (Table 2). Furthermore, the injection of amphetamine further increased these response rates. Thus, the rats may have been too highly aroused, following the injection of amphetamine, in the FR-10 schedule to respond appropriately. These data suggest that the VI-15 s schedule may be both more sensitive to the detection of 'different-from-saline' responses and more resistant to extraneous drug effects than the FR-10 schedule of reinforcement. While the VI-15 s schedule may have been more sensitive, complete generalization between nicotine and amphetamine was not observed, sug-

gesting that the nicotine stimulus is also very specific under this schedule. Thus, rats trained on the VI-15 s schedule did not appear to be responding nonspecifically to the amphetamine effect.

Another objective of this investigation was to obtain information that would be useful in the evaluation of compounds possessing behavioral effects similar to nicotine. This information could be useful when studying the stimulus properties of psychoactive drugs as well. The results of the amphetamine generalization study (Fig. 6) suggest that to evaluate properly the stimulus properties of an unknown compound, it is advisable to use more than one training dose and at least two different schedules of reinforcement. These drug-generalization tests have also suggested a means of interpreting generalization data. Thus, unknown compounds may present three levels of generalization to training drug states: (1) complete generalization (80–100% drug-correct responding); (2) partial generalization (40–60% drug-correct responding); or (3) no generalization (0–20% drug-correct responding). Complete generalization indicates that the animal perceives the drug stimuli as similar. Such generalizations have been demonstrated for most psychoactive drugs within the same pharmacological class. Partial generalization of stimulus effects suggests that the test drug is perceived as unlike both the drug and nondrug states and indicates that it does have a CNS effect. Since the drug does have CNS effects and is perceived as different from saline, it should induce stimulus control over behavior and could be studied alone. Thus, in these studies amphetamine produced a maximal partial generalization at the higher doses (480–720 μg/kg), which also exert the strongest discriminative control over behavior (Rosecrans et al., 1976). Very little generalization suggests that such a compound has no stimulus effects or no CNS activity. However, such a drug should also be investigated as a possible antagonist of the training drug, especially if the chemical structures of both compounds are similar. We have observed that narcotics, such as morphine, will not generalize to nalorphine or cyclazocine but are readily antagonized by them (Rosecrans, 1976; unpublished observations). While these effects were predicted by knowledge of the pharmacology of narcotic antagonists, both effects may be examined using DS procedures.

Thus, it is suggested that a DS technique may be valuable in the evaluation of the behavioral effects of unknown compounds. This procedure may also be useful in the area of drug dependence to study drugs that are not readily self-administered by experimental animals. Overton (1971) has provided convincing data indicating good positive correlations between drug abuse potential and potency as DS. Therefore, drug abuse potential of specific drugs may be predicted and detected using a DS paradigm.

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REFERENCES

Hirschhorn, I. D., Rosecrans, J. A.: Studies of the time course and the effect of cholinergic and adrenergic receptor blockers on the stimulus effect of nicotine. Psychopharmacologia (Berl.) **40**, 109–120 (1974)

Litchfield, J. T., Wilcoxin, F.: A simplified method of evaluating dose-response experiments. J. Pharmacol. Exp. Ther. **96**, 99–113 (1949)

Morrison, C. F.: Effects of nicotine on operant behavior of rats. Int. J. Neuropharmacol. **6**, 229–240 (1967)

Morrison, C. F., Armitage, A. K.: Effects of nicotine upon the free operant behavior of rats and spontaneous motor activity of mice. N. Y. Acad. Sci. **142**, 268–276 (1967)

Orsingher, O. A., Fulginitti, S.: Effects of alpha-methyl tyrosine and adrenergic blocking agents on the facilitating action of amphetamine and nicotine on learning in rats. Psychopharmacologia (Berl.) **19**, 231–240 (1971)

Overton, D. A.: Discriminative control of behavior by drug states. In: Stimulus properties of drugs, T. Thompson and R. Pickens, eds., pp. 87–110. New York: Appleton-Century-Crofts 1971

Rosecrans, J. A.: Effects of nicotine on behavioral arousal and brain 5-hydroxytryptamine function in female rats selected for differences in activity. Eur. J. Pharmacol. **14**, 29–37 (1971)

Rosecrans, J. A., Chance, W. T.: Cholinergic and non-cholinergic aspects of the discriminative stimulus properties of nicotine. In: Discriminative stimulus properties of drugs, H. Lal, ed., pp. 155–185. New York: Plenum 1977

Rosecrans, J. A., Chance, W. T., Schechter, M. D.: The discriminative stimulus properties of nicotine, *d*-amphetamine and morphine in dopamine depleted rats. Psychopharmacol. Commun. **2**, 349–356 (1976)

Schechter, M. D., Rosecrans, J. A.: Nicotine as a discriminative cue in rats: inability of related drugs to produce a nicotine-like cueing effect. Psychopharmacologia (Berl.) **27**, 379–387 (1972)

Schechter, M. D., Rosecrans, J. A.: *d*-Amphetamine as a discriminative cue: drugs with similar stimulus properties. Eur. J. Pharmacol. **21**, 212–216 (1973)

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