Pharmacology Biochemistry & Behavior, Vol. 14, pp. 683–688, 1981. Printed in the U.S.A.

Caffeine Discrimination in the Rat[1]

HAROLD E. MODROW, FRANK A. HOLLOWAY

Department of Psychiatry and Behavioral Sciences

AND

JOHN M. CARNEY

Department of Pharmacology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

Received 6 October 1980

MODROW, H. E., F. A. HOLLOWAY AND J. M. CARNEY. *Caffeine discrimination in the rat.* PHARMAC. BIOCHEM. BEHAV. 14(5) 683–688, 1981.—Rats were trained to discriminate 32 mg/kg caffeine from saline in a two-lever appetitive task. Across a range of caffeine test doses (1–32 mg/kg) rats showed a dose related generalization to the training cue. At intermediate caffeine dose levels, caffeine appeared to produce a more potent cue on tests following saline-training days than after drug-training days. Several psychomotor stimulants (d-amphetamine, methylphenidate, nicotine and TRH) failed to generalize to the caffeine cue. In contrast, theophylline did generalize to caffeine at a dose roughly twice that of the caffeine training dose.

Drug discrimination Operant behavior Caffeine Theophylline d-Amphetamine Methylphenidate Nicotine TRH

CAFFEINE is a psychoactive compound found in a variety of sources including coffee, tea, cocoa and cola beverages [7]. For example, one cup of brewed coffee may contain as much as 100–150 mg caffeine, one cup of tea 9–91 mg and cola drinks 25–45 mg/10 oz [7]. In a sample of 110,000 persons, 19.5% of females and 21.4% of males reported drinking 7 or more cups of coffee per day [14] producing an estimated daily dosage greater than 1000 mg of caffeine.

In spite of its widespread consumption, research examining the behavioral effects of caffeine has been limited. Changes in both the rate and pattern of operant behavior have been reported after caffeine injections. Both Ando [2] and Webb and Levine [25] examined the effects of caffeine upon differential reinforcement for low rates of responding (DRL). Both studies reported a decrease in reinforcements due to an increase in the number of responses with relatively short interresponse times (IRT). Davies *et al.* [6] compared the effects of caffeine, amphetamine and nicotine on fixed interval (FI), fixed ratio (FR) and Sidman avoidance schedules in squirrel monkeys. Though amphetamine increased the response rate for all three schedules, nicotine increased responding only during the first half of the interval on the FI schedule. Caffeine increased responding on both the FR and FI schedules. Wayner, *et al.* [24] examined the effects of caffeine on both operant and adjunctive behaviors and found a biphasic dose response curve for both lever pressing and schedule-induced licking after injections of caffeine. Similar results were obtained by McKim [16] who reported that low doses would increase response rates while higher doses lowered response rates under both FI and FR schedules in mice. A similar inverted U-shaped dose-effect curve has been found for caffeine's effect on rat locomotor activity [1, 10, 23]. These studies indicate that caffeine shares some but not all of the behavioral effects produced by psychomotor stimulants.

The drug discrimination paradigm is unique among behavioral measures in that it permits the subject to indicate whether or not a substance is discriminably similar to the drug-training condition. The discriminative properties of a wide variety of psychomotor stimulants, including amphetamine [9], nicotine [21], cocaine and methylphenidate [8], have been described. Animals trained to discriminate one psychomotor stimulant demonstrated generalization when tested with other psychomotor stimulants [8]. However, not all psychomotor stimulants will show cross-generalization. Although thyrotropin-releasing hormone (TRH) has been found to induce hyperactivity, anorexia and disruption of operant responding [4], TRH discriminating rats do not show generalization of amphetamine to TRH [12]. The discriminative properties of caffeine have received much less attention. Krimmer [15] found that rats, trained to discriminate a depressant, pentobarbital, from saline, would respond to the saline lever when tested with caffeine. However, 20–50 mg/kg caffeine appeared to generalize to the drug cue in animals trained to discriminate the antidepressant, buprion, from saline [13].

[1]Reprint requests should be addressed to H. E. Modrow. This study was supported in part by a Research Council Grant from the Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center (H. E. Modrow, P.I.), by USPHS Grant 1 RO1 DA 02666 (F. A. Holloway, P.I.) and by USPHS Grant 5 T32 DA 07105 (R. S. Krug, P.I.).

The purpose of the first study was to determine; (1) whether rats could be trained to discriminate caffeine at a dose which produces reliable changes in operant and locomotor activity, and (2) the dose effect curve for generalization of lower caffeine doses to the caffeine training condition. The second experiment was designed to characterize the psychopharmacological properties of the caffeine cue in reference to such properties of other psychomotor stimulants (d-amphetamine, methylphenidate, nicotine, and TRH). Theophylline, a dimethyl-xanthine which is an active metabolite of caffeine, also was tested.

EXPERIMENT 1

METHOD

Subjects

Twelve naive adult male Sprague-Dawley rats weighing between 340 and 460 grams were used in this study. Prior to training, all animals were reduced to approximately 80% of their free-feeding weight and maintained at that point for the duration of the study. All animals were housed in individual rack cages with ad lib water and were maintained on a 12:12 light-dark cycle with light onset at 8:00 a.m. CST.

Apparatus

Four identical LeHigh-Valley (Model 132-02) two-lever operant chambers, measuring 25.0×30.5×32.0 cm, were used. Each chamber consisted of transparent Plexiglas composing two walls and the top with two end walls composed of stainless steel. The floor consisted of stainless steel bars separated by 1.9 cm. One end wall contained two levers mounted 4.2 cm from the floor and 14.3 cm from each other. Midway between the two levers was a food cup into which the 45 mg Noyes food pellet reinforcements were delivered by a Lafayette pellet dispenser. Each chamber was enclosed in a LeHigh-Valley sound attenuating chamber. All programming and recording was done by means of solid state equipment.

Procedure

As soon as the animals were reduced to 80% of their free-feeding weight, they were trained to lever press for food reinforcement. One-half of the animals were shaped to press the left lever; the other half were shaped to press the right lever. Upon completion of the initial shaping procedure, Phase 1 of discrimination training began with pre-session intraperitoneal (IP) injections of normal saline. All injections were administered 20 minutes prior to the training session. In Phase 1 of training, the schedule of reinforcement for the previous shaped lever response was gradually raised until the animal was consistently responding on an FR30 schedule. Session length was 20 minutes. The FR30 schedule was chosen in order to obtain a better indication of discrimination. On the FR10 schedule utilized by some researchers (e.g., [8]), one or two spurious responses could exert more statistical influence than is the case with the FR30 schedule (e.g., 9% versus 3%). All animals attained stable performance (10–15% variation) on the FR30 schedule within 8–10 days. Subsequently in Phase 2 each animal received an injection of 32 mg/kg caffeine (free-base) 20 minutes prior to the training session. Pilot work indicated peak caffeine blood levels within 20 minutes of IP injections. In these caffeine sessions each animal was shaped to press the opposite lever. This second phase of training consisted of two day blocks of caffeine-appropriate lever training interspersed with two day blocks of saline-appropriate lever training. During this double-alternation sequence, the schedule of reinforcement for saline sessions was FR30. The reinforcement schedule during caffeine sessions was gradually raised to FR30. Animals required 6–10 caffeine sessions to attain stable rates under the FR30 schedule.

Phase 3 of training also utilized a double-alternation sequence, essentially the same as Phase 2, i.e., two sessions (one/day) with prior saline injections followed by two sessions (one/day) with prior caffeine injections, etc. Training continued until the animal reached criterion (no more than 5 incorrect responses prior to the first reinforcement on 8 out of 9 consecutive days). The double-alternation procedure (used for example by Rosecrans [21]) likely provides better control over such factors as position learning [11] than is the case for the single-alternation procedure.

Other caffeine doses were tested for generalization to the training dose after the training criterion was reached. Tests were administered every third day. This test schedule was superimposed on the caffeine/saline training sequence used in Phase 3. Animals were randomly given tests of saline, 1.0, 3.2, 5.6, 10.0, 20.0 and 32.0 mg/kg caffeine. Each animal received each test dose twice, once after a caffeine training day and once after a saline training day. On test days, twenty minutes following administration of the drug, the animal was placed in the apparatus and allowed to press both levers until it had pressed 30 times on one lever. At this point it was removed from the apparatus and the test session was concluded.

RESULTS AND DISCUSSION

Once shaped to the caffeine lever (Phase 2), eleven of the twelve rats met the caffeine discrimination (Phase 3) criterion in a mean of 28.6±0.8 daily training sessions. One rat failed to reach criterion within 50 training sessions and was dropped from the study.

The dose-effect curves for generalization to the caffeine cue are presented in Fig. 1. A repeated measures analysis of variance demonstrated a significant difference among the various caffeine doses, $F(6,10)=9.119$, $p<0.001$. Analysis of the percentage of drug-appropriate lever responding revealed a significant component of the dose effect curve was linear, $F(1,60)=104.73$, $p<0.001$. A Tukey post-hoc analysis demonstrated that the animals made a significantly ($p<0.05$) greater percentage of drug-appropriate lever responses at 5.6, 10.0, 20 and 32 mg/kg caffeine than with saline or 1.0 mg/kg caffeine. However, only 20 mg/kg was not significantly different from 32 mg/kg. That is, only test doses of 20 and 32 mg/kg completely generalized to the caffeine training dose. Thus while doses below 20 mg/kg caffeine were discriminably different from saline, the threshold for complete generalization (i.e., not significantly different from the training test dose of 32 mg/kg) to the caffeine training cue, appeared to lie between 10 and 20 mg/kg caffeine.

As may be seen in Fig. 1, there was also an effect due to the sequence of testing. Rats appeared more sensitive to caffeine test doses after saline-training days than on days following caffeine. This effect was significant, $F(1,60)=4.246$, $p<0.05$, and most apparent at intermediate caffeine test doses. Post-hoc analyses revealed significant differences between tests after drug training days and after

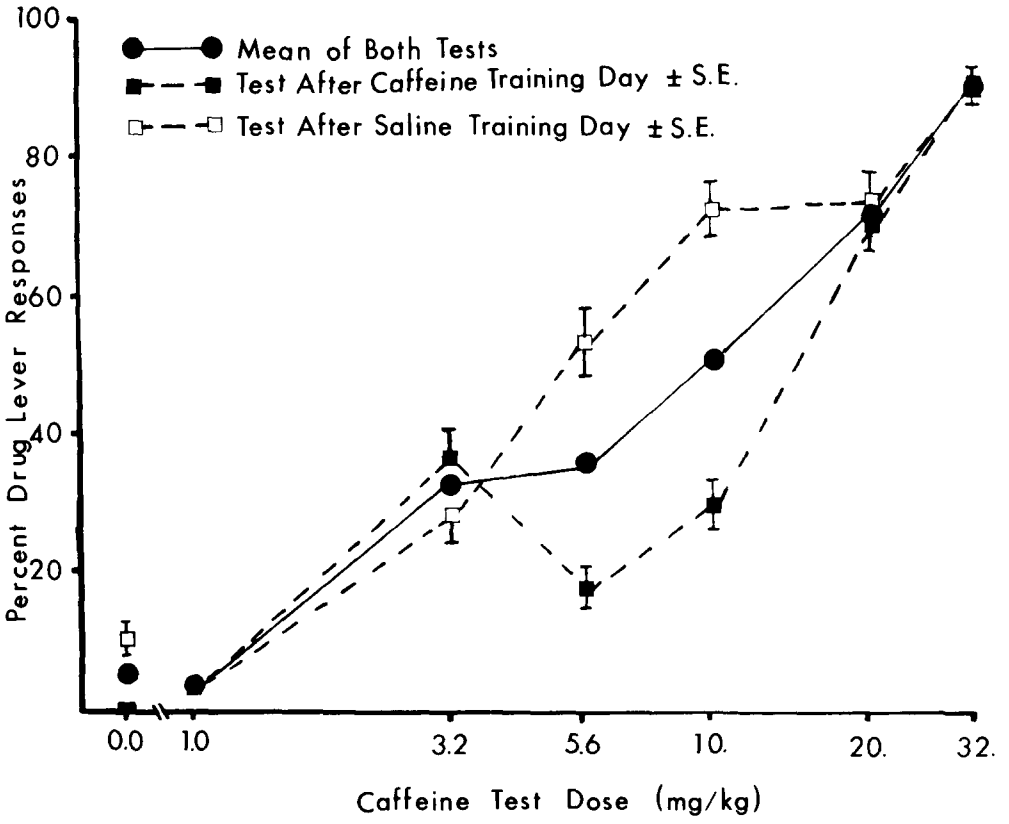

FIG. 1. Generalization of caffeine test doses to the training dose of 32 mg/kg caffeine.

saline training days when the test dose was 5.6 mg/kg caffeine, $F(1,60)=8.34$, $p<0.01$, or 10 mg/kg caffeine, $F(1,60)=12.47$, $p<0.001$. Previous research has not reported the kind of differences in drug discrimination between tests after drug and saline training days reported in the present study. Neither the biochemical nor the behavioral basis of this phenomenon is known but the presence of seemingly unequal ED_{50}s between tests after drug and saline training days suggests that rats may be sensitive to contrasts between injections on successive days.

It is clear that rats are able to learn to discriminate the cue produced by IP injections of caffeine. The intensity of this cue is directly related to the dose of injection, and furthermore, the doses are within the dose range of caffeine found to produce stimulatory effects on both locomotor activity and operant behavior. Therefore, the discriminative properties of caffeine could be related to nonspecific stimulatory properties, an issue examined in Experiment 2.

EXPERIMENT 2

METHOD

Subjects

The subjects used in this study were the eleven rats from the previous study which had achieved the criterion for caffeine discrimination. Their care remained the same as in the previous study.

Apparatus

The apparatus used in this study was the same as that used in the previous study.

Procedure

Daily drug discrimination sessions were conducted as was described in Experiment 1. Two training sessions in the caffeine drug state alternated with two training sessions in the saline drug state. Tests were again administered every third day.

Six animals were tested first with d-amphetamine (sulfate) while the remaining five were tested first with theophylline. Amphetamine tests included IP injections of 0.3, 1.0, 1.5 and 2.0 mg/kg. Theophylline tests included IP injections of 10, 20, 32, 44 and 56 mg/kg. Higher doses of d-amphetamine and theophylline were not tested due to disruption of behavior (i.e., no responding). As soon as testing was concluded for one drug, the animal began testing on the other drug. For a given drug, testing on the various doses was randomized. Following completion of testing on the first two drugs, all animals were tested on methylphenidate hydrochloride, nicotine (base) and TRH. The test doses included 1, 3, 5 and 7 mg/kg methylphenidate IP, 10 mg/kg TRH IP and 0.1, 0.2 and 0.4 mg/kg nicotine SC. Because of the well-known stimulatory effects of nicotine upon the smooth muscle receptors, and autonomic ganglia, it was necessary to adminis-

TABLE 1
GENERALIZATION OF THE CAFFEINE CUE TO OTHER DRUGS

Drug	Dose	Percent drug lever responses ± S.E.	Significance level saline*/caffeine†
d-Amphetamine			
	0.3 mg/kg IP	17.89 ± 3.62	n.s./0.001
	1.0	20.13 ± 2.95	n.s./0.001
	1.5	17.16 ± 3.99	n.s./0.001
	2.0	26.66 ± 2.90	0.05/0.001
Methylphenidate			
	1.0 mg/kg IP	2.27 ± 0.68	n.s./0.001
	3.0	9.09 ± 2.72	n.s./0.001
	5.0	13.04 ± 2.44	n.s./0.001
	7.0	9.09 ± 2.74	n.s./0.001
Nicotine			
	0.0 mg/kg SC	0.29 ± 0.09	n.s./0.001
	0.1	18.18 ± 3.68	n.s./0.001
	0.2	9.66 ± 2.73	n.s./0.001
	0.4	17.73 ± 2.90	n.s./0.001
TRH			
	10 mg/kg IP	0.83 ± 0.25	n.s./0.001
Theophylline			
	10 mg/kg IP	17.18 ± 3.34	n.s./0.001
	20	25.93 ± 3.92	n.s./0.001
	32	27.74 ± 3.82	n.s./0.001
	44	50.34 ± 4.44	0.05/0.05
	56	73.89 ± 2.79	0.001/n.s.

*Statistical comparison to saline tests.
†Statistical comparison to 32 mg/kg caffeine tests.

ter the nicotine subcutaneously. In order to control for this route of administration, a subcutaneous dose of saline also was given. Once again, for each drug the various doses were tested randomly. All drug doses were calculated as the base.

All test sessions were identical to those described in the first study. Twenty minutes following injection of the test drug the animal was placed in the operant chamber and allowed to press either lever until 30 responses were accumulated on one lever. The animal was then removed from the apparatus and the test was terminated. The 20 minute injection-test interval was used to insure a constant condition relative to the training condition. This time of testing resulted in drug-induced alteration of behavior by all drugs.

RESULTS AND DISCUSSION

If caffeine's discriminative properties were due to nonspecific stimulatory actions, then we might expect other psychomotor stimulants to generalize to the caffeine cue. Table 1 demonstrates the lack of generalization of d-amphetamine, methylphenidate, nicotine and TRH to the caffeine cue. In contrast, theophylline did generalize to the caffeine cue. Analyses of variance and post-hoc comparisons were performed on all drugs. With the exception of theophylline, all drugs produced comparable results. In the cases of d-amphetamine, methylphenidate and nicotine, no significant differences (within drug comparisons) were found among the various doses for each drug. Post-hoc comparisons revealed that no dose of nicotine, methylphenidate or TRH was different from the saline test dose. In general amphetamine also was not different from the saline test. Although 2.0 mg/kg amphetamine was found to be different from saline, the percentage of caffeine-appropriate lever responding was less than 30. Further, there was no trend for increased caffeine-appropriate lever responding as the amphetamine dose increased. As previously noted, 2.0 mg/kg amphetamine was not found to be different from any of the other amphetamine doses. In addition, all test doses of amphetamine, methylphenidate, nicotine and TRH produced significantly less caffeine-appropriate lever responding than the 32 mg/kg caffeine test dose. In conclusion, several traditional psychomotor stimulants showed no generalization to the caffeine cue, thus suggesting that the basis of the discriminative cue is due to some pharmacologically specific action of caffeine.

It is logical that theophylline, an active metabolite of caffeine, would generalize to the caffeine cue. Indeed the only drug which produced caffeine-appropriate lever responding was theophylline. An analysis of variance revealed significant differences between the different doses of theophylline, $F(4,40)=4.046$, $p<0.01$. A Tukey post-hoc analysis demonstrated significantly greater caffeine-appropriate lever responding at 56 mg/kg than at 10, 20 and 32 mg/kg theophylline. In addition 56 mg/kg theophylline was not significantly different from 32 mg/kg caffeine. Both 44 and 56 mg/kg theophylline demonstrated significantly greater caffeine-appropriate lever responding than saline injections. The remaining three doses did not significantly differ from saline.

GENERAL DISCUSSION

The results of these studies demonstrate that rats can discriminate caffeine from saline. The cue does not seem to be based on a nonspecific stimulant effect, a conclusion supported by the lack of generalizability of other psychomotor stimulants to the caffeine cue. Rather the caffeine cue appears restricted to the methylxanthines, as indicated by the generalization of theophylline to the caffeine cue. A few studies have indirectly examined the discriminability of caffeine by testing its generalizability to other drugs. Morrison and Stephenson [14] found that rats would respond to the saline-appropriate lever when tested with caffeine after training with nicotine. Caffeine also does not generalize to either a pentobarbital [15] or an alcohol [22] training cue. In the only previous study to directly examine the discriminability of caffeine, Overton and Batta [19] using the shock-escape procedure, were able to obtain caffeine discrimination at a dose of 125 mg/kg but not at 50 mg/kg caffeine. As Overton [18] has pointed out, establishment of drug discrimination in the shock-escape procedure may require higher dosages than in an appetitive operant task.

One possible basis for caffeine's cue properties could be its peripheral effects, e.g., some irritative effect or other direct action on tissue. In the present study the pH of the caffeine and saline solutions were identical (approximately 7.3), thus the irritation explanation appears unlikely. Caffeine does produce a wide variety of other peripheral effects which could provide a basis for its discriminative properties. These effects include increased coronary output, diuresis, and smooth muscle relaxation. However, theophylline is more potent than caffeine in producing these effects [20]. Therefore, if the cue were peripheral in nature, we might expect to see theophylline being equal to or more potent than caffeine in producing the caffeine cue, an effect not seen in this study.

One well known effect of the methylxanthines on the central nervous system is their ability (*in vitro*) to inhibit phosphodiesterase [7]. Again theophylline is a more potent inhibitor of phosphodiesterase than caffeine. In the present study, caffeine was found to be more potent in producing a discriminable cue. Thus it appears unlikely that the specific cue properties of caffeine are due to inhibition of phosphodiesterase.

An alternative central mechanism for the production of the caffeine cue involves interaction with various neural receptor mechanisms. One group [3] has reported that both caffeine and theophylline are able to block the *in vitro* binding of [^{3}H]-diazepam at the putative benzodiazepine receptor. In addition, caffeine is nearly twice as potent as theophylline in inhibiting [^{3}H]-diazepam binding. This order of potency is the same as that found in the present study. The data suggest that the caffeine cue may be related to its blocking activity at the putative benzodiazepine receptor. Another mechanism for caffeine's action has recently been suggested by Bruns, *et al.* [5]. In their study, both caffeine and theophylline were reported to inhibit the binding of [^{3}H]-cyclohexyladenosine to the brain adenosine receptor. Whether either one or both of these two receptor-based mechanisms are involved in caffeine's cue effects requires direct evaluation.

REFERENCES

1. Anders, N. and D. M. Jackson. Locomotor activity stimulation in rats produced by dopamine in the nucleus accumbens: Potentiation by caffeine. *J. Pharm. Pharmac.* **27:** 666–670, 1975.
2. Ando, K. Profile of drug effects on temporally spaced responding in rats. *Pharmac. Biochem. Behav.* **3:** 833–841, 1973.
3. Asano, T. and S. Spector. Identification of inosine and hypoxanthine as endogenous ligands for the brain benzodiazepine-binding sites. *Proc. natn Acad. Sci. U.S.A.* **76:** 977–981, 1979.
4. Barlow, T. S., B. R. Cooper, G. R. Breese, A. J. Prange and M. A. Lipton. Effects of thyrotropin-releasing hormone on behavior: evidence for an anorexic like action. *Soc. Neurosci. Abstr.* **1:** 334, 1975.
5. Bruns, R. F., J. W. Daly and S. H. Snyder. Adenosine receptors in brain membranes: Binding of ^{3}H-N^6-cyclohexyladenosine and ^{3}H-1,3-diethyl-8-phenylxanthine. *Proc. natn Acad. Sci. U.S.A.* **77:** 5547–5555, 1980.
6. Davis, T. R. A., C. J. Kensler and P. E. Dews. Comparison of behavioral effects of nicotine, d-amphetamine, caffeine and dimethylheptyl tetrahydrocannabinol in squirrel monkeys. *Psychopharmacology* **32:** 51–65, 1973.
7. Gilbert, R. M. Caffeine as a drug of abuse. In: *Recent Advances in Alcohol and Drug Problems*, Vol. 3, edited by R. J. Gibbons, Y. Israel, H. Kalant, R. E. Poplan, W. Schmidt and R. G. Smart. New York: Plenum Press, 1978, pp. 49–176.
8. Ho, B. T. and M. L. McKenna. Discriminative stimulus properties of central stimulants. In: *Drug Discrimination and State Dependent Learning*, edited by B. T. Ho, D. W. Richards and D. L. Chute. New York: Academic Press, 1978, pp. 67–77.
9. Ho, B. T. and P. Silverman. Stimulants as discriminative stimuli. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by J. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier/North Holland, 1978, pp. 53–68.
10. Hughes, R. N. and A. M. Greig. Effects of caffeine, methamphetamine and methylphenidate on reaction to novelty and activity in rats. *Neuropharmacology* **15:** 673–676, 1976.
11. Hunter, W. S. The temporal maze and kinaesthetic sensory processes in the white rat. *Psychobiology* **2:** 1–17, 1920.
12. Jones, C. N., L. D. Grant and A. J. Prange. Stimulus properties of thyrotropin-releasing hormone. *Psychopharmacology* **59:** 217–224, 1978.
13. Jones, C. N., J. L. Howard and S. T. McBennett. Stimulus properties of antidepressants in the rat. *Psychopharmacology* **67:** 111–118, 1980.
14. Klatsky, A. L., G. D. Freidman and A. B. Siegelaub. Coffee drinking prior to acute myocardial infarction—Results from the Kaiser-Permanente epidemiologic study of myocardial infarction. *J. Am. Med. Ass.* **226:** 540–543, 1973.
15. Krimmer, E. C. Drugs as discriminative stimuli. *Diss. Abstr. Internat.* **35:** 4572-B, 1974.
16. McKim, W. A. The effect of caffeine, theophylline and amphetamine on operant responding of the mouse. *Psychopharmacology* **68:** 135–138, 1980.
17. Morrison, C. and J. Stephenson. Nicotine injection as the conditioned stimulus in discrimination learning. *Psychopharmacologia* **15:** 351–360, 1969.
18. Overton, D. A. Optimal training compartment design, schedule of reinforcement and shaping procedures to establish 2-bar operant drug discrimination, Paper presented at First International Symposium on Drugs as Discriminative Stimuli, Beerse, Belgium, 1978.
19. Overton, D. A. and S. Batta. Relationship between abuse liability of drugs and their degree of discriminability in the rat. In: *Predicting Dependence Liability of Stimulant and Depressant Drugs*, edited by T. Thompson and K. Unna. Baltimore: University Park Press, 1977, pp. 125–135.

20. Ritchie, J. M. Central nervous system stimulants (continued): II. The xanthines. In: *The Pharmacological Basis of Therapeutics*, edited by L. S. Goodman and A. Gilman. New York: McMillan, 1975, pp. 367–378.
21. Rosecrans, J. A., M. T. Kallman and R. Glenon. The nicotine cue: an overview. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by F. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier/North Holland, 1978, pp. 69–81.
22. Schecter, M. D. Simulus properties of ethanol and depressant drugs. In: *Drug Discrimination and State Dependent Learning*, edited by B. T. Ho, D. Richards and D. L. Chute. New York: Academic Press, 1978, pp. 103–117.
23. Thithapandha, A., H. M. Boling and J. R. Gillette. Effects of caffeine and theophylline on activity of rats in relation to brain xanthine concentrates. *Proc. Soc. exp. Biol. Med.* **139:** 528–536, 1972.
24. Wayner, M. J., F. B. Jolicoeur, D. B. Rondeau and F. C. Barone. Effects of acute and chronic administration of caffeine on schedule dependent and schedule induced behavior. *Pharmac. Biochem. Behav.* **5:** 343–348, 1976.
25. Webb, D. and T. E. Levine. Effects of caffeine on DRL performance in the mouse. *Pharmac. Biochem. Behav.* **9:** 7–10, 1978.