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Discriminative Stimulus Effects of Cyclazocine in the Rat[1]

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ABSTRACT

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Discriminative effects of cyclazocine were investigated in two groups of rats trained to discriminate between saline and either 0.3 or 1.0 mg/kg of cyclazocine with a two-choice discrete-trial avoidance paradigm. Behavior was considered to be under stimulus control when a rat could reliably complete at least 18 trials of a 20-trial session on the correct choice lever. Cyclazocine engendered dose-related cyclazocine-appropriate responding in both groups of rats with the position of the dose-response curves along the abscissa reflecting the differences in training dose. The time course of the discriminative effects of cyclazocine was relatively short; drug-appropriate responding began to decline from maximal levels within 1 hr of drug administration. Although the stimulus effects of the training doses of cyclazocine could not be completely blocked by naltrexone, naltrexone (0.3 mg/kg) shifted the cyclazocine dose-response curves to the right so that a comparable degree of stimulus control was seen only with 3 times the training dose of cyclazocine. The opioids ketocyclazocine and SKF 10-047 produced stimulus effects comparable to those produced by both training doses of cyclazocine as measured by the number of trials completed on the drug-appropriate lever; ethylketocyclazocine, pentazocine and levallorphan produced stimulus effects comparable to only those of the low training dose; morphine and nalorphine produced stimulus effects which were not comparable to those of either training dose. The nonopioids ketamine and phencyclidine both mimicked the discriminative effects of cyclazocine, whereas mescaline, *d*-amphetamine and lysergic acid diethylamide D-tartrate did not. These results suggest that the discriminative effects of cyclazocine are comprised of at least two major components: 1) a narcotic component which is antagonized by naltrexone but does not appear to be mediated through the same populations of opioid receptors as morphine, and 2) a non-narcotic component which is not antagonized by naltrexone but mimicked by ketamine and phencyclidine.

The discriminative stimulus effects of morphine have now been studied extensively in several species of animals (Hill *et al.*, 1971; Shannon and Holtzman, 1976a, 1977; Schaefer and Holtzman, 1977; Järbe and Rollenhagen, 1978; Järbe, 1978). The results of these studies have been consistent with the hypothesis that the stimulus properties of the narcotic analgesics may be analagous to the subjective effects that the drugs produce in man (Colpaert, 1977, 1978; Lal *et al.*, 1977). Animals trained to discriminate morphine from saline will generalize completely only to those opioid analgesics which have been shown to engender subjective effects in man similar to those of morphine (Schaefer and Holtzman, 1977; Shannon and Holtzman, 1976a, 1977). Since the subjective effects of a narcotic appear to be a primary determinant of its abuse potential (Jasinski, 1973, 1977), drug discrimination procedures may provide an animal model for studying components of action of the narcotic analgesics which are relevant to their abuse potential.

Analgesics with narcotic antagonist activity also produce distinct subjective effects in man. However, narcotic antagonist analgesics are a relatively heterogeneous group of drugs with respect to the profile of pharmacologic activity, and some members of the group can produce subjective effects that are markedly disparate from the feelings of well-being often associated with the administration of morphine (Hill *et al.*, 1963; Martin, 1967; Haertzen, 1974). One such drug that has been well characterized in man is cyclazocine. The subjective effects produced by cyclazocine vary qualitatively with the dose administered. At low doses, cyclazocine tends to resemble morphine, whereas, at intermediate doses, the subjective effects of cyclazocine are dysphoric and characterized by drunkenness and sedation. At high doses, cyclazocine produces psychotomimetic effects which appear to be qualitatively different from those of lysergic acid diethylamide D-tartrate (LSD) (Martin *et al.*, 1965; Haertzen, 1970, 1974). Presumably, as a consequence of the unpleasantness of the subjective effects of cyclazocine, the drug is considered to have little potential for abuse (Martin *et al.*, 1965).

Cyclazocine has recently been shown to serve as a discriminative stimulus in animals. The discriminative stimulus effects of cyclazocine appear to be distinct from those of morphine consonant with the reported differences in the profile of subjective effects of the two drugs. Monkeys trained to discriminate cyclazocine from saline generalize completely to several opioids with mixed agonist and narcotic antagonist properties such as levallorphan, oxilorphan, and ketocyclazocine (Schaefer and

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1978). However, rats (Shannon and Holtzman, 1977) ...keys (S. G. Holtzman and G. J. Schaefer, unpublished ...ations) trained to discriminate between morphine and ...ne only partially generalize to these drugs. Furthermore, ...oth monkeys (Schaefer and Holtzman, 1978) and rats (Rose-crans *et al.*, 1978; Hirschhorn, 1977) trained to discriminate between cyclazocine and saline generalize only partially to morphine; conversely, animals trained to discriminate between morphine and saline generalize only partially to cyclazocine (Shannon and Holtzman, 1976a). Thus, the results of extensive cross-generalization tests suggest significant differences in the discriminative stimulus properties of cyclazocine and morphine as well as some commonalities.

Another important difference between morphine and cyclazocine is the ease with which their effects can be blocked by pure narcotic antagonists such as naloxone or naltrexone. In both the rat and monkey, the discriminative stimulus effects of morphine are readily and completely antagonized by low doses of naloxone or naltrexone (*e.g.*, 0.1 mg/kg; Schaefer and Holtzman, 1977; Shannon and Holtzman, 1976b). In contrast, doses of naloxone an order of magnitude greater are required to completely antagonize the stimulus effects of cyclazocine in the monkey (Schaefer and Holtzman, 1978) and even higher doses of naloxone do not appear to completely antagonize the stimulus ffects of cyclazocine in the rat (Rosecrans *et al.*, 1978). Based on the results of single-dose studies in man, the dose of naloxone required to block the subjective effects produced by cyclazocine is at least an order of magnitude higher than the dose required to antagonize the effects of morphine (Jaskinski *et al.*, 1968; Evans *et al.*, 1974), consonant with the results obtained in drug discrimination studies. These overall differences between morphine and cyclazocine are consistent with the hypothesis that the effects of these two drugs are mediated by different populations of "opioid" receptors (Martin *et al.*, 1976).

The discriminative stimulus properties of cyclazocine have not been studied as thoroughly as those of morphine, particularly in the rat. It is important to characterize more completely the stimulus properties of cyclazocine so that comparisons can be made with morphine which may provide additional insight into differences between the two drugs which could ultimately be related to differences in abuse potential. Therefore, the purpose of this investigation was to characterize the discriminative stimulus effects of cyclazocine in the rat by using the two-choice, discrete-trial avoidance paradigm previously used to evaluate the discriminative stimulus effects of morphine (Shannon and Holtzman, 1976a, 1977). Two different training doses of cyclazocine were used in order to determine if the stimulus properties of cyclazocine, like its subjective effects, vary with the dose administered. Furthermore, systematic antagonism experiments with naltrexone were conducted in order to determine whether the stimulus properties of cyclazocine are mediated through opioid receptors. In order to assess the possible existence of a nonopioid psychotomimetic component in the stimulus effects of cyclazocine, in addition to an opioid component, both groups of rats were tested for stimulus generalization to morphine, to drugs with mixed agonist and narcotic antagonist properties and to nonopioid psychoactive drugs, particularly those with psychotomimetic activity.

Methods

Subjects. The subjects were 22 experimentally naive CFE rats (Carworth, Division of Charles River Breeding Laboratories, Wilmington, MA) that weighed between 160 and 400 g at the start of discrimination training. Between experiments, the rats were housed either alone or in pairs in cages which were kept in a colony room with a 12-hr light-dark cycle. Food and water were continuously available in the home cage.

Apparatus. A detailed description of the apparatus used can be found in Shannon and Holtzman (1976a). Briefly, the rats were tested in a one-lever rat chamber (model 1110-L, Grason-Stadler Co., Inc., Bolton, MA) which had been modified by adding two levers (designated the "choice response levers") on the wall opposite the original lever (designated the "observing response lever"). The grid floor of the chamber was wired to receive a scrambled electric shock delivered by a constant current shock generator (model 700, Grason-Stadler Co.). The entire chamber was housed in a light-proof, sound-attenuating and ventilated chest. All scheduled contingencies were programmed and data were recorded by automatic relay equipment.

Discrimination training. The rats were trained in a discrete-trial avoidance procedure as described previously (Shannon and Holtzman, 1976a) with minor modifications. For each trial, the rat had to respond first on the observing response lever and then on one of the two choice response levers in order to avoid or escape from electric shocks delivered to the floor of the test chamber. During each training session, only a response on the appropriate choice response lever for the current drug state terminated the trial; responses on the other choice response lever (*i.e.*, the "inappropriate" lever) had no programmed consequences. A trial was defined as correct if the rat emitted the response sequence of observing lever-appropriate choice lever and as incorrect if the rat emitted the response sequence of observing lever-inappropriate choice lever-appropriate choice lever. Only the first choice response after the observing response was recorded for each trial. Responses on either of the choice levers before the first observing response and multiple observing responses had no programmed consequences.

The illumination of the house-light and the onset of white noise signaled the beginning of a trial. The white noise was terminated by the first observing response of each trial but the house light remained on until the trial was terminated by an appropriate choice response. Beginning 5.0 sec after the onset of a trial, a scrambled 1 mA electric shock (1.0-sec duration) was presented at 2.0-sec intervals for the duration of the trial. A 50-sec intertrial interval in which the chamber was dimly illuminated with a red light separated each trial from the next. A session ended after 21 trials or 30 min, whichever came first. The first trial of each session was considered to be a "warm-up" trial, and data from this trial were not included in subsequent data analyses. The cumulative latency for a rat to complete each trial after the illumination of the house light was recorded for each session (*i.e.*, response latency).

The rats were initially trained to make only the observing response in order to terminate each trial. The rats were then injected with either the training drug or saline and shaped to press the appropriate choice response lever after making the observing response. In the following session, the rats were shaped to the other choice response lever under the other drug condition. The choice lever assignments appropriate for each drug condition remained constant throughout the study for each of the rats and were counter-balanced across the group. The training sessions continued 5 days a week under a double-alternation schedule for the drug state (*i.e.*, saline, saline, drug, drug, saline, saline. . . .) until the rats achieved the criterion of four consecutive sessions in which responding was at least 90% correct for each session (*i.e.*, 18 of 20 trials were correct excluding the first trial). In the next session, the rats were administered saline or the training drug and tested with both choice levers activated so that pressing either choice lever after pressing the observing lever would terminate a trial (*i.e.*, a "test session"). The following session was also conducted as a test session but for the other drug state. The behavior of the rats was considered to be under the stimulus control of cyclazocine and saline if at least 18 of 20 trials in both of these test sessions were completed on the choice lever appropriate for the particular drug state.

Two groups of rats were trained to discriminate either 0.3 or 1.0 mg/kg of cyclazocine, respectively, from saline. All drugs were given s.c. 30

before the beginning of a session except in the time course study which the animals were injected at various intervals before each session. The animals remained in their home cages in the interval between drug administration and the start of the session. Eleven rats were trained with 1.0 mg/kg of cyclazocine and met the criteria for stimulus control of behavior in an average of 37 sessions which included several sessions for shaping the animals. After completing stimulus generalization tests, five of these rats were retrained with 0.3 mg/kg of cyclazocine. Nine additional rats were trained initially with 0.3 mg/kg of cyclazocine and met the criteria for stimulus control of behavior in an average of 38 sessions. Two of the 22 rats became ill during the training period and were deleted from the study.

Drug substitution testing. Once an animal had met the criteria for the acquisition of the discrimination, drug substitution testing was begun. In order to maintain a stable performance of the discrimination, training sessions in which only the appropriate choice response lever was activated were conducted on Mondays, Wednesdays and Thursdays. The training dose of cyclazocine and saline continued to be given on a double-alternation schedule. Test sessions with both choice levers activated were conducted on Tuesdays and Fridays with only those animals which had completed the previous four training sessions at a criterion level of 90% correct. As in the training sessions, test sessions ended after the completion of 20 trials or after 30 min had elapsed. However, test sessions were terminated early if it became evident that an animal was unable to respond as sometimes happened after the administration of the highest dose of some of the drugs. Data from sessions in which 20 trials were not completed were excluded from the data analysis and are not reported in this paper.

Data analysis. The data are expressed as the average number of trials completed on the cyclazocine-appropriate choice lever for each drug dose by using four rats per dose except where otherwise indicated. The remaining trials of the 20-trial session were completed on the saline-appropriate lever. A dose of a test drug was considered to substitute for the training dose of cyclazocine (*viz.*, to produce a comparable degree of stimulus control of behavior as measured by the number of trials on the drug-appropriate lever) or an animal was considered to have generalized to a test drug if the test drug resulted in an average of at least 90% of the trials of a test session being completed on the cyclazocine-appropriate lever. The cumulative response latencies for completing 20 trials are expressed as the mean ± S.E.M. and appropriate statistical comparisons were made using a Student's *t* test for paired observations.

Drugs. The drugs used were cyclazocine base, ketocyclazocine base, ethylketocyclazocine base and pentazocine base (generously supplied by Sterling-Winthrop Research Institute, Rensselaer, NY), morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO), mescaline hydrochloride (Regis Chemical Co., Morton Grove, IL), levallorphan tartrate (donated by Roche Laboratories, Division of Hoffmann-La Roche, Inc., Nutley, NJ), phencyclidine hydrochloride, LSD, naloxone hydrochloride and naltrexone hydrochloride (National Institute on Drug Abuse), nalorphine hydrochloride (donated by Merck and Co., Inc., Chemical Division, Rahway, NJ), *d*-amphetamine sulfate and SKF 10-047 base (N-allylnormetazocine; (a gift from Smith Kline and French Laboratories, Philadelphia PA) and ketamine hydrochloride (a gift from Parke, Davis and Co., Ann Arbor, MI). The vehicle for cyclazocine, ketocyclazocine, ethylketocyclazocine, pentazocine and SKF 10-047 was 8.5% lactic acid, saline and 1.0 N sodium hydroxide solution in a 2:1:2 ratio. The vehicle for all other drugs was 0.9% saline. All drugs doses are expressed in terms of the free base. All drugs were injected s.c. in a volume of 1.0 ml/kg b. wt., except for nalorphine (175 mg/kg), mescaline (56 and 100 mg/kg) and levallorphan (30 mg/kg), which were administered in a volume of 2.0 ml/kg b. wt. to help prevent the development of lesions at the site of injections.

Results

Dose- and time-effect curves for cyclazocine. Figure 1 (left panel) shows dose-response curves for cyclazocine in the two groups of rats: one trained to discriminate between saline and 0.3 mg/kg of cyclazocine (0.3-C-group) and one trained to discriminate between saline and 1.0 mg/kg of cyclazocine (1.0-C-group). In both groups, cyclazocine engendered dose-related drug-appropriate responding which was maximal at the training dose of each group and above. The ratio of the low training dose to the high training dose (*i.e.*, 0.3:1.0) is similar to the ratio of the estimated ED_{50} values (*i.e.*, mean dose at which 10 trials were completed on the cyclazocine-appropriate lever) for the two groups of rats (*i.e.*, .13/.35). Therefore, the difference in the positions of the two curves along the abscissa is proportional to the difference in the two training doses, with the curve for the 0.3-C-group positioned to the left of the curve for the 1.0-C-group. During these tests, the rats in both groups showed a trend toward lower cumulative response latencies after receiving the training dose of cyclazocine as compared to saline, but the differences were not significant (0.3-C-group, 245 ± 30 sec with saline and 208 ± 9 sec with cyclazocine; 1.0-C-group, 290 ± 19 sec with saline and 225 ± 19 sec with cyclazocine). The 3.0 mg/kg dose of cyclazocine caused ataxia in six of 11 animals in the 1.0-C-group which prevented them from completing the session; the cumulative response latency of the five rats that completed the session at this dose averaged 540 ± 81 sec. Ataxia was not observed at any of the lower doses of cyclazocine. Time course studies were conducted with the training dose of cyclaz-

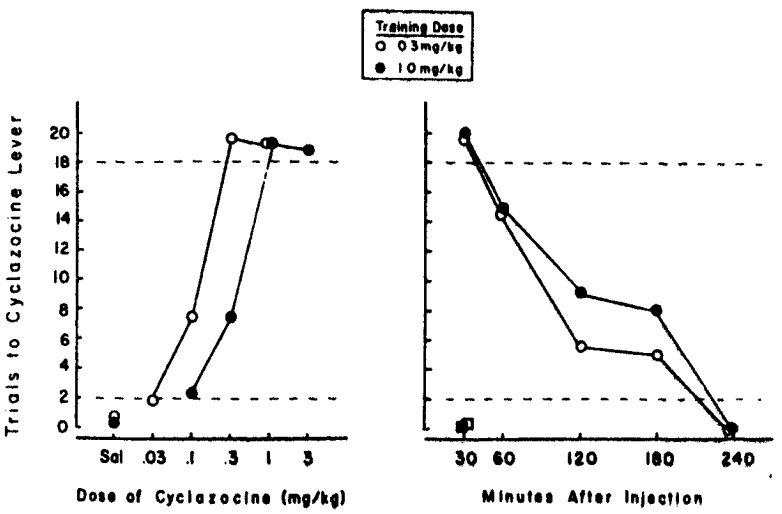


Fig. 1. Cyclazocine dose-response curves for rats trained to discriminate saline from either 0.3 or 1.0 mg/kg of cyclazocine (left panel) and time course for the stimulus control of behavior by each of the training doses of cyclazocine (right panel). Each point is the mean number of trials completed on the cyclazocine-appropriate lever in a 20-trial session; the remaining trials of the session were completed on the saline-appropriate lever. The isolated points above saline (Sal) in each panel indicate the amount of cyclazocine-appropriate responding observed after an injection of saline. Means are based upon one observation in each of four (time course curves), nine (cyclazocine curve in the 0.3-C-group) or eleven (cyclazocine curve in the 1.0-C-group) rats. Only five rats in the 1.0-C-group were able to complete the session at 3.0 mg/kg of cyclazocine. The upper and lower horizontal dashed lines indicate the minimum levels of discriminative responding at which the performance of the animals was maintained with the cyclazocine training dose and saline, respectively.

ocine in each group of rats (fig. 1, right panel). Stimulus control of behavior by cyclazocine followed a similar time course in both groups. At 30 min, the standard pretreatment time, both groups of rats were responding almost exclusively on the cyclazocine-appropriate lever. However, the mean number of trials completed on the cyclazocine-appropriate lever decreased in both groups to 15 trials at 60 min and continued to decrease at 120 and 180 min. Both groups reached saline-like levels of responding at 240 min.

Antagonism by naltrexone. In order to establish the involvement of opioid receptors in the mediation of the discriminative stimulus effects of cyclazocine, experiments were performed in which cyclazocine and naltrexone were administered in combination. When graded doses of naltrexone were administered concomitantly with the training dose of cyclazocine, the discriminative effects of cyclazocine in both groups were only partially antagonized (fig. 2). The maximal decrease in mean drug-appropriate responding observed was to 6.4 trials completed on the drug lever in the 0.3-C-group and to 8.8 trials in the 1.0-C-group with 30 and 10 mg/kg of naltrexone, respectively. The administration of naltrexone alone engendered no drug-appropriate responding in the 1.0-C-group and only slight drug-appropriate responding in the 0.3-C-group.

The antagonism of the stimulus effects of cyclazocine was studied further by determining if the dose-response curve for cyclazocine could be shifted to the right with naltrexone. First, dose-response curves with cyclazocine alone were determined in each of the two groups of rats and then the curves were redetermined with the simultaneous administration of 0.3 mg/kg of naltrexone (fig. 3). Naltrexone (0.3 mg/kg) was equieffective in shifting the cyclazocine dose-response curves of the two training dose groups to the right so that the minimum dose of cyclazocine which completely substituted for the training dose of cyclazocine in the presence and in the absence of naltrexone was increased from 0.3 to 1.0 mg/kg in the 0.3-C-group and from 1.0 to 3.0 mg/kg in the 1.0-C-group.

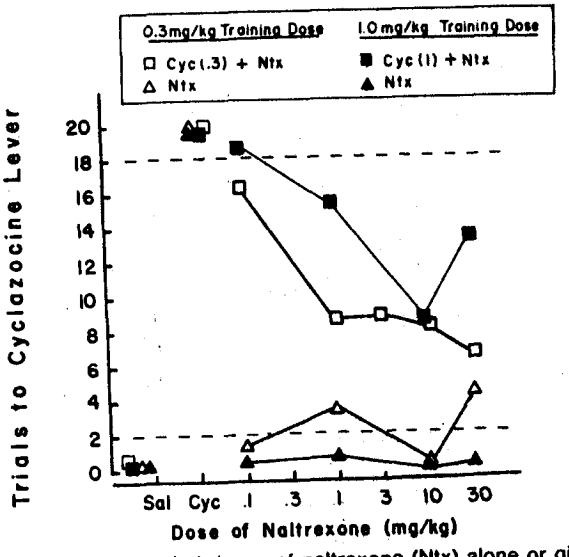


Fig. 2. Effects of graded doses of naltrexone (Ntx) alone or given in combination with the training dose of cyclazocine in rats trained to discriminate either 0.3 or 1.0 mg/kg of cyclazocine from saline. The points above saline (Sal) or cyclazocine (Cyc) indicate the amount of cyclazocine-appropriate responding after administration of saline or the training dose of cyclazocine, respectively. Means are based upon one observation in each of four (1.0-C-group) or seven to nine (0.3-C-group) rats. Other details are the same as in figure 1.

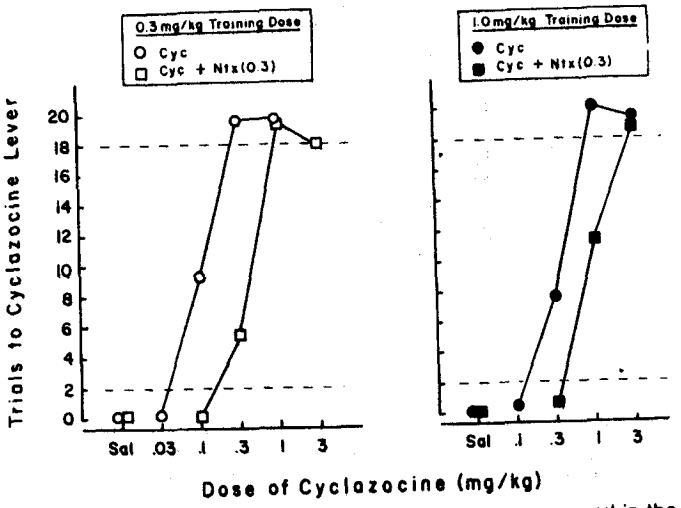


Fig. 3. Cyclazocine dose-response curves in the presence and in the absence of 0.3 mg/kg of naltrexone (Ntx) in rats trained to discriminate saline from either 0.3 (left panel) or 1.0 (right panel) mg/kg of cyclazocine (Cyc). Each point represents a mean based upon one observation in each of four rats; the same four rats in each group were used to determine the curves in the presence or absence of naltrexone. Other details are the same as in figure 1.

Stimulus generalization to opioids. Morphine and six compounds with mixed agonist and narcotic antagonist properties were tested to determine whether or not they could mimic the discriminative effects of cyclazocine. Three patterns of generalization were produced by the various opioids: 1) rats in both training groups generalized completely to the test drug; 2) rats in the 0.3-C-group generalized completely to the test drug but rats in the 1.0-C-group did not; and 3) rats from neither group generalized completely to the test drug. Those opioids which substituted completely for both training doses of cyclazocine are shown in figure 4.

Ketocyclazocine and SKF 10-047 both engendered dose-related drug-appropriate responding which was a function of the training dose of each group as reflected by the relative positions of the two sets of curves along the abscissa. The 1.0 mg/kg dose of ketocyclazocine caused a more than 2-fold increase in the cumulative response latency of the 0.3-C-group (244 ± 16 sec with saline and 542 ± 83 sec with ketocyclazocine; $P < .05$), and a trend toward an increase in the cumulative response latency of the 1.0-C-group (330 ± 40 sec with saline and 474 ± 113 sec with ketocyclazocine) was also apparent. SKF-10-047 did not affect the cumulative response latencies for either group.

Ethylketocyclazocine, pentazocine and levallorphan substituted completely for the low training dose of cyclazocine but not for the high one (fig. 5). The amount of drug-appropriate responding which was observed in the 1.0-C-group varied for each of the three drugs. Ethylketocyclazocine produced no drug-appropriate responding at the 0.3 mg/kg dose in the 1.0-C-group of rats and none of the four rats in this group that were tested with the 1.0 mg/kg dose were able to complete the session. In contrast, the rats in the 0.3-C-group completed an average of 15.7 and 20 trials on the cyclazocine-appropriate lever with 0.3 and 1.0 mg/kg of ethylketocyclazocine, respectively, although only two of the four animals could complete the session at the higher dose. Pentazocine produced a dose-related increase in drug-appropriate responding in both groups of rats. However, the number of trials completed on the drug-appropriate lever reached a maximum of only 13 to 14 trials in

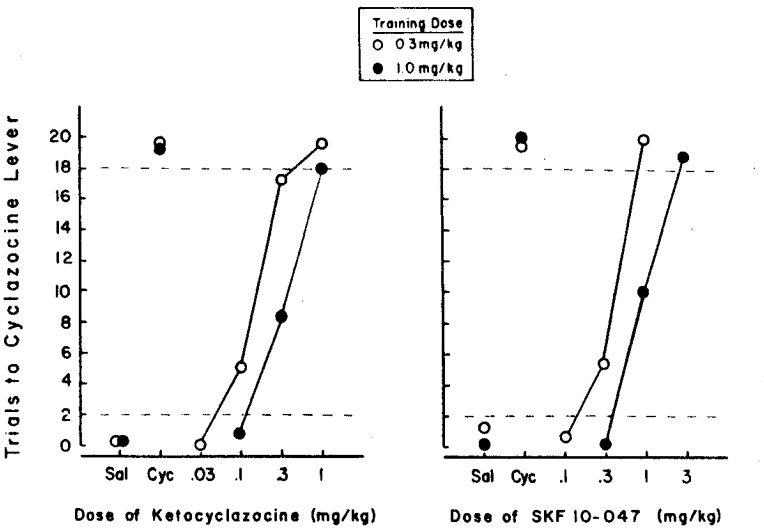


Fig. 4. Dose-response curves of opioids which mimic the discriminative stimulus effects of both training doses of cyclazocine in rats trained to discriminate saline (Sal) from 0.3 or 1.0 mg/kg of cyclazocine (Cyc). Each point represents a mean based upon one observation in each of four rats. Other details are the same as in figures 1 and 2.

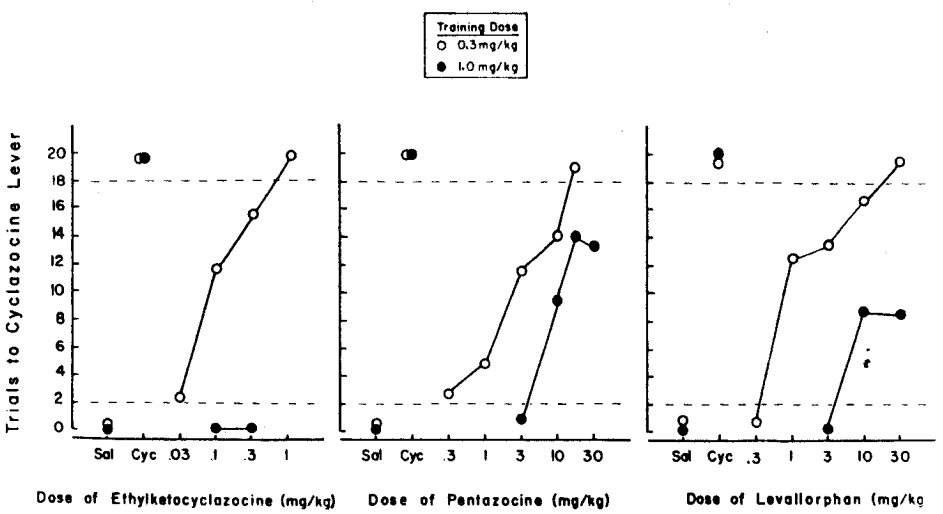


Fig. 5. Dose-response curves of opioids which substitute completely for the low training dose of cyclazocine (Cyc) but not for the high training dose in rats trained to discriminate 0.3 or 1.0 mg/kg of cyclazocine from saline (Sal). Each point represents a mean based upon one observation in each of four rats except in the following cases: due to nondrug related deaths, 0.3 and 1.0 mg/kg of pentazocine were only tested in three rats in the 0.3-C-group and 3.0 mg/kg of levallorphan was only tested in two rats in the 1.0-C-group. At 1.0 mg/kg of ethylketocyclazocine, only two of the four rats tested in the 0.3-C-group could finish the session. Only three of four rats could finish the session after 17.5 (both groups) and 30 (1.0-C-group) mg/kg of pentazocine. Other details are the same as in figures 1 and 2.

.0-C-group at the two highest doses of pentazocine. Only three of the four animals tested in each group were able to complete the session with 17.5 mg/kg of pentazocine and only three of the four animals tested in the 1.0-C-group were able to complete the session with 30 mg/kg of pentazocine. Levallorphan engendered drug-appropriate responding in the 0.3-C-group over a wide dose range and substituted for cyclazocine at 30 mg/kg. In contrast, a plateau of 8.7 and 8.2 trials completed on the drug-appropriate lever was observed with the 10 and 30 mg/kg doses of levallorphan, respectively, in the 1.0-C-group. Lesions were noted at the site of injection of 30 mg/kg of levallorphan; higher doses could not be tested safely.

Those drugs which did not substitute for either training dose of cyclazocine are shown in figure 6. The 1.0-C-group completed virtually no trials on the drug lever when tested with morphine. However, when morphine was tested in the 0.3-C-group, an average of 9.5 and 9.3 trials were completed on the cyclazocine-appropriate lever after 3.0 and 10 mg/kg, respectively. Only three of the four rats in the 0.3-C-group and two of the four rats in the 1.0-C-group were able to complete the sessions after receiving 10 mg/kg of morphine. Similarly, nalorphine did not produce any cyclazocine-appropriate responding in the 1.0-C-group at doses of up to 175 mg/kg but did produce a dose-related increase in the number of trials completed on the cyclazocine-appropriate lever in the 0.3-C-group that reached a maximum of 9.7 trials at 30 mg/kg.

Stimulus generalization to nonopioid psychoactive drugs. In order to determine if there is a non-narcotic component of the discriminative effects of cyclazocine, five nonopioid drugs with prominent psychoactive properties were tested for stimulus generalization in both groups of rats. Ketamine and phencyclidine both mimicked the stimulus effects of cyclazocine (fig. 7). Ketamine produced dose-related cyclazocine-appropriate responding in the 0.3-C-group; stimulus control of behavior comparable to that of the training dose was achieved at 10 mg/kg. The 1.0-C-group also generalized completely to 10 mg/kg of ketamine, but unlike the group trained with 0.3 mg/kg of cyclazocine, the 1.0-C-group emitted almost no cyclazocine-appropriate responses at 3.0 mg/kg of ketamine. Similarly, the 1.0-C-group generalized completely to 3.0 mg/kg of phencyclidine although very little cyclazocine-appropriate responding was observed with lower doses of phencyclidine. In contrast, in the 0.3-C-group, phencyclidine engendered dose-related cyclazocine-appropriate responding over its entire dose range. A maximum mean of 17.25 trials were completed on the cyclazocine-appropriate lever at 3.0 mg/kg of phencyclidine, but all four of the rats in the 0.3-C-group completed 18 or more trials on the cyclazocine-appropriate lever at 1.0 or 3.0 mg/kg of phencycli-

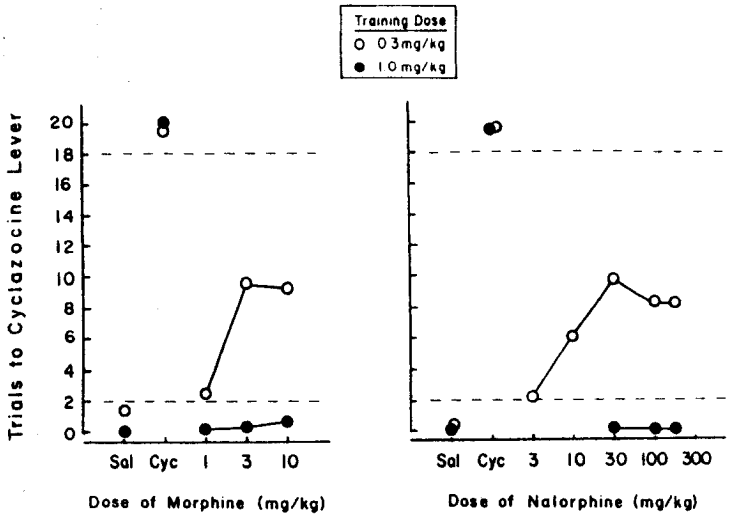
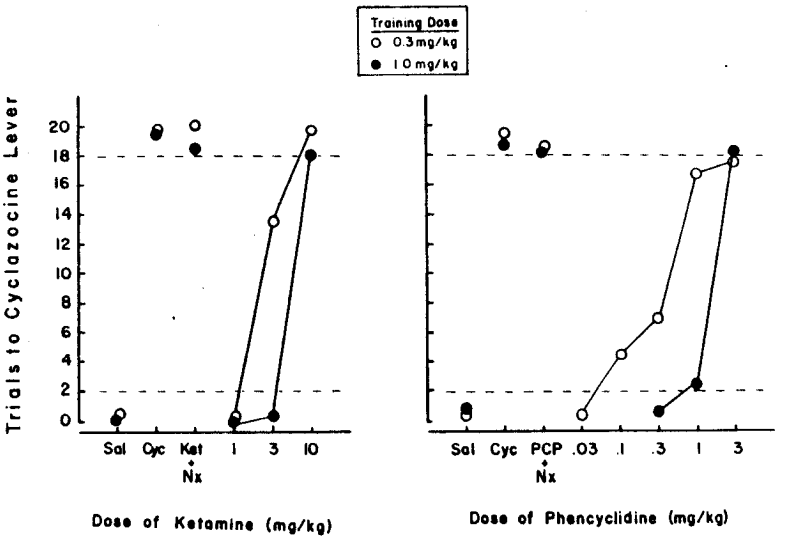


Fig. 6. Dose-response curves of opioids which did not mimic the discriminative stimulus effects of either training dose of cyclazocine (Cyc) in rats trained to discriminate 0.3 or 1.0 mg/kg of cyclazocine from saline (Sal). Each point represents a mean based upon one observation in each of four rats, except for 10 mg/kg of morphine at which only three (0.3-C-group) or two (1.0-C-group) rats of the four tested were able to complete the session. Other details are the same as in figures 1 and 2.

Fig. 7. Dose-response curves of nonopioids which were ...e to mimic the discriminative stimulus effects of cyclazocine in rats trained to discriminate saline (Sal) from either 0.3 or 1.0 mg/kg of cyclazocine (Cyc). Points above ketamine (Ket) + naloxone (Nx) and phencyclidine hydrochloride (PCP) + Nx indicate the amount of cyclazocine-appropriate responding when 10 mg/kg of naloxone was given concomitantly with 10 mg/kg of ketamine or 3.0 mg/kg of phencyclidine, respectively. Each point represents a mean based upon one observation in each of four rats. Other details are the same as in figures 1 and 2.



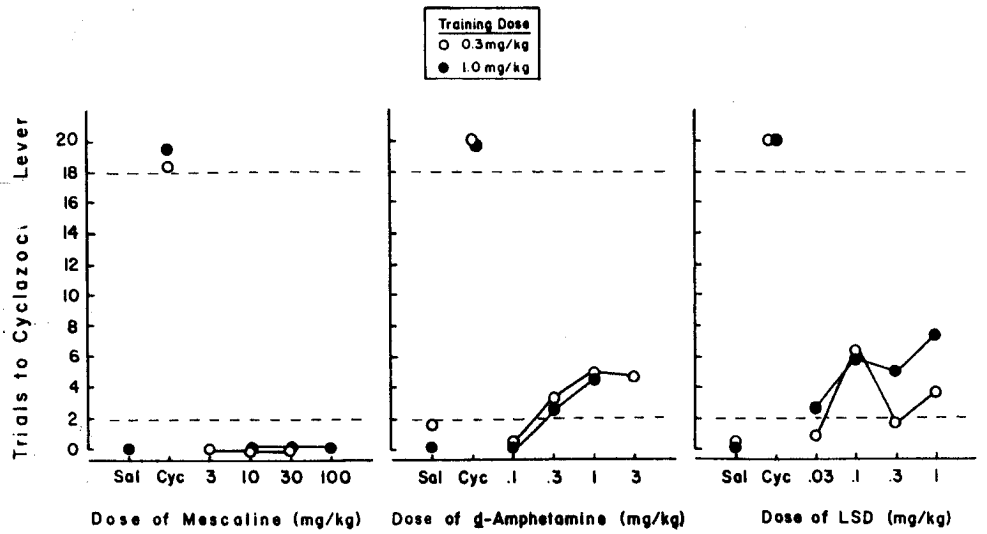


Fig. 8. Dose-response curves of nonopioids which did not mimic the discriminative effects of cyclazocine (Cyc) in rats trained to discriminate saline (Sal) from either 0.3 or 1.0 mg/kg of cyclazocine. Each point represents a mean based upon one observation in each of three (mescaline) or four (*d*-amphetamine and LSD) rats. The 1.0 mg/kg dose of LSD was only tested in three animals in the 1.0-C-group due to a nondrug related death of one of the subjects. Other details are the same as in figures 1 and 2.

dine. Ketamine (10 mg/kg) and phencyclidine (3.0 mg/kg) were tested in combination with 10 mg/kg of naloxone in both groups of cyclazocine-trained rats. The stimulus effects of both ketamine and phencyclidine were completely unaffected by this dose of naloxone.

Three other non-opioid psychoactive drugs failed to substitute for cyclazocine (fig. 8). Mescaline did not produce any drug-appropriate responding in either group of rats at doses up to 30 and 100 mg/kg. Lesions at the site of injection were often noted after a mescaline injection; consequently, only three

animals in each group were tested with each dose of mescaline. *d*-Amphetamine (0.3–3.0 mg/kg) produced only low levels of cyclazocine-appropriate responding which did not exceed an average of 5.5 trials in either group. With 3.0 mg/kg of *d*-amphetamine, only one of the four rats tested in the 1.0-C-group could complete the session; this rat completed all trials on the saline-appropriate lever (not shown). In contrast, all four of the rats in the 0.3-C-group were able to complete the session. The effects of LSD on cyclazocine-appropriate responding was not clearly dose-related in the 0.3-C-group. The maximum mean number of trials completed on the cyclazocine-appropriate lever was 6.5 at 0.1 mg/kg of LSD. The 1.0-C-group emitted approximately the same number of cyclazocine-appropriate responses as the 0.3-C-group with 0.1 mg/kg of LSD. The maximum number of trials completed on the cyclazocine-appropriate lever averaged 7.3 at 1.0 mg/kg of LSD, the highest dose tested. Only th rats in the 1.0-C-group were tested with this dose of LSD; on nimal died of nondrug related causes before it could be tested.

Discussion

This investigation has shown that both 0.3 and 1.0 mg/kg of cyclazocine can serve as a discriminative stimulus in rats. There was no difference between the average number of training sessions to achieve criterion of the two training dose groups, although other studies have found the number of training sessions to achieve criterion generally decreases as the training dose increases (Järbe and Rollenhagen, 1978; Hill *et al.*, 1971). Quantitative differences between the stimulus effects of cyclazocine in the two groups were observed when the ED_{50} values of the cyclazocine dose-response curves for the two groups were compared. The ratio of the ED_{50} values for the two groups was proportional to the ratio of the two training doses with the curve for the 0.3-C-group being positioned along the abscissa to the left of the curve for the 1.0-C-group indicating that the latter group required a higher dose of cyclazocine before the shold dose for substitution was attained. The stimulus eralization curves for the test drugs which had cyclazocine-like stimulus effects also displayed similar positioning along the abscissa for the two groups of rats. These data are consistent with other discrimination studies in which more than one training dose was used (Overton, 1974; Schaefer and Holtzman, 1978; Järbe and Rollenhagen, 1978; Shannon and Holtzman, 1979).

The time course of stimulus control of behavior by cyclazocine was similar for the two training doses of cyclazocine in their respective groups. These time courses in the rat are similar to those reported for the squirrel monkey trained to discriminate between 0.1 mg/kg of cyclazocine and drug vehicle (Schaefer and Holtzman, 1978). This is in contrast to the time course of stimulus control of behavior by morphine which is considerably longer in the monkey (Schaefer and Holtzman, 1977) than it is in the rat (Shannon and Holtzman, 1976a).

Studies on the interactions of cyclazocine and pure narcotic antagonists are a way of indirectly determining if the discriminative stimulus effects of cyclazocine are being mediated through opioid receptors. The finding that 0.3 mg/kg of naltrexone was equieffective in shifting the cyclazocine dose-response curve for both groups of rats to the right when administered concomitantly with graded doses of cyclazocine is consistent with the concept of two drugs competing for the same receptor site(s). Therefore, at least one component of the discriminative effects of both training doses of cyclazocine appears to be mediated through opioid receptors (*i.e.*, the narcotic component).

However, several lines of evidence indicate that there exist significant differences between the cellular substrates that mediate the stimulus effects of cyclazocine and those that mediate the stimulus effects of morphine. Firstly, the relative potency of naltrexone in modifying the position of the cyclazocine dose-response curve is substantially lower than its potency in shifting the position of the morphine dose-response curve. Whereas 0.3 mg/kg of naltrexone increased the minimum dose of cyclazocine which substituted for 0.3 to 1.0 mg/kg and for 1.0 to 3.0 mg/kg in the 0.3-C-group and 1.0-C-group, respectively, only one-third of this dose of naloxone (Shannon and Holtzman, 1976a) or naltrexone (unpublished observations) increased the minimum dose of morphine which substituted for 3 to 56 mg/kg. Similar relationships have also been observed in squirrel monkeys trained with cyclazocine or morphine (unpublished observations).

Secondly, our results in combination with those of previous studies show that the discriminative stimulus effects of cyclazocine are not entirely morphine-like. In our study, neither group of rats trained with cyclazocine generalized completely to morphine. Furthermore, the amount of cyclazocine-appropriate responding engendered by morphine was dependent upon the size of the training dose. Animals trained on the lower dose of cyclazocine showed intermediate levels of cyclazocine-appropriate responding with morphine, whereas animals in the 1.0-C-group did not. These results are in accord with those from other cross-generalization tests in which rats and squirrel monkeys trained to discriminate cyclazocine from its vehicle generalized only partially to morphine (Rosecrans *et al.*, 1978; Hirschhorn, 1977; Schaefer and Holtzman, 1978). Rats trained to discriminate saline from 1.75 or 3.0 mg/kg of morphine generalized partially to cyclazocine, whereas virtually no generalization to cyclazocine occurred in rats trained to discriminate between saline and 5.6 mg/kg of morphine (Shannon and Holtzman, 1976a, 1979). Thus, the partial cross-generalization between morphine and cyclazocine is symmetrical and dependent upon the size of the training dose. These data appear to parallel clinical reports of morphine-like subjective effects with low but not high doses of cyclazocine (Martin *et al.*, 1965).

The differences in the stimulus effects of cyclazocine and morphine may be due to these drugs acting at different populations of opioid receptors. Martin *et al.* (1976) and Gilbert and Martin (1976) have proposed that there are three opioid receptors, μ, κ and σ, which could subserve more than one narcotic effect. This model proposes that cyclazocine exerts its effects by acting at the κ and σ receptors. In contrast, morphine only has weak activity at the κ receptor and exerts its primary effects through the μ receptor. Opioids with both agonist and narcotic antagonist activity could have different affinities as well as different intrinsic activities for each of these three receptors which would account for the heterogenous nature of this class of compounds. This concept also affords an explanation of why not all of the opioids with both agonist and narcotic antagonist properties that were tested in this study mimicked the discriminative effects of cyclazocine.

Nalorphine did not substitute for either training dose of cyclazocine. Similarly, nalorphine does not substitute for cyclazocine in the squirrel monkey (Schaefer and Holtzman,

1978). Martin *et al.* (1976) and Gilbert and Martin (1976) have classified nalorphine as a partial cyclazocine agonist. This classification is consistent with the finding that intermediate levels of cyclazocine-appropriate responding were engendered by nalorphine only in the low training dose group which presumably has a lower threshold than does the group trained with the higher dose of cyclazocine.

Ketocyclazocine and SKF 10-047 are the only two opioids which substituted completely for both training doses of cyclazocine and they did so in a manner which reflected the differences in the training doses of the two groups. Both of these drugs are believed to have both high activity and high affinity at one or more of the same receptor populations acted upon by cyclazocine (Martin *et al.*, 1976). Squirrel monkeys trained to discriminate 0.1 mg/kg of cyclazocine from saline will also generalize completely to these two compounds (Schaefer and Holtzman, 1978; unpublished observations). Ethylketocyclazocine, pentazocine and levallorphan all substituted completely for 0.3 mg/kg of cyclazocine but not for 1.0 mg/kg of cyclazocine. The failure of these drugs to substitute for the high training dose of cyclazocine could be a consequence of our inability to test sufficiently high doses of these drugs, although each drug was tested up to the highest possible dose at which the animals could complete the session. The differences in abilities of the drugs to completely mimic one training dose and not another could be due to differences in drug-receptor interactions. Pentazocine has low affinity for the receptors which mediate the effects of cyclazocine (Martin *et al.*, 1976); levallorphan has not yet been characterized in this three receptor model. On the other hand, this receptor model does not afford a convenient explanation for the results obtained with ethylketocyclazocine since it has been proposed that this drug acts through the same receptors as ketocyclazocine and that both drugs have high affinity and high intrinsic activity, at least in the dog (Martin *et al.*, 1976).

In addition to the narcotic component of the discriminative stimulus effects of cyclazocine, there is compelling evidence that a non-narcotic component also exists. This conclusion is based upon the results of the naltrexone antagonism experiments and upon the results of stimulus generalization tests with nonopioid drugs. Naltrexone, in doses as high as 30 mg/kg, did not completely block stimulus control of behavior by either of the cyclazocine training doses, although the amount of cyclazocine-appropriate responding was reduced to approximately 40% of control. Naltrexone alone does not engender enough cyclazocine-appropriate responding to account for the inability to completely antagonize cyclazocine. Our results are in agreement with those of other discrimination studies in rats in which extremely high doses of naloxone were required to decrease cyclazocine-appropriate responding 20 to 30% of control (Hirschhorn, 1977; Rosecrans, *et al.*, 1978). The attenuation of cyclazocine-appropriate responding with narcotic antagonists is consistent with the idea that the discriminative effects of cyclazocine have a narcotic component, but the failure to completely antagonize cyclazocine suggests that the stimulus effects of cyclazocine also have a non-narcotic component. The concept that cyclazocine can produce non-narcotic effects is supported by data from other studies in which the effects of cyclazocine on locomotor activity and catecholamine levels in the rat were not blocked by naloxone (Holtzman and Jewett, 1973).

This non-narcotic component of action may be associated with the psychotomimetic effects which cyclazocine can produce (Haertzen, 1970, 1974; Jasinski, 1977). Both ketamine and phencyclidine are non-narcotic psychoactive drugs that are structurally similar to each other and are capable of producing psychotomimetic effects (Domino *et al.*, 1965). These drugs appear to produce similar stimulus effects; ketamine will substitute for phencyclidine in rats trained to discriminate phencyclidine from saline (Overton, 1975; Järbe *et al.*, 1975). Both of these drugs were found to produce stimulus effects similar to those produced by cyclazocine. Inasmuch as 10 mg/kg of naloxone did not attenuate the effects of either ketamine or phencyclidine, it would appear that these drugs mimicked the discriminative effects of cyclazocine by a mechanism that did not involve opioid receptors. This non-narcotic component of action appears to be relatively specific. Other psychoactive drugs such as mescaline, *d*-amphetamine and LSD did not mimic the stimulus effects of cyclazocine in either group of rats. These data are consistent with clinical studies demonstrating that the subjective effects of these three drugs are not like those of phencyclidine (Domino, 1964) and with animal studies in which cyclazocine was found to not substitute for LSD in rats trained to discriminate LSD from saline (Hirschhorn and Rosecrans, 1976). Therefore, the non-narcotic component of the stimulus effects of cyclazocine appears to be a specific effect and may resemble the psychotomimetic effects which have been described for phencyclidine, ketamine and cyclazocine in man.

All of the opioids tested showed the same pattern of responding with the drugs being more potent or more effective in mimicking the stimulus effects of cyclazocine in the low training dose group than in the high training dose group. This same pattern of responding was also observed with the nonopioids which completely mimicked the stimulus effects of cyclazocine (*i.e.*, ketamine and phencyclidine). In contrast, this pattern was not observed with the nonopioids which did not substitute for cyclazocine (*i.e.*, mescaline, *d*-amphetamine and LSD). This finding is consistent with the view that the partial generalization to the opioids tested reflects stimulus effects that these drugs have in common with cyclazocine since the extent of generalization covaried with the training dose of cyclazocine.

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