Mirex and Behavior in the Long-Evans Rat

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Mirex (didecachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene) has been widely distributed in an attempt to control the imported fire ant (*Solenopsis invicta*) in the Southeastern United States. It is an extremely stable organochlorine compound and relatively low amounts may have toxic effects on a variety of non-target species; e.g., pink shrimp (LOWE et al. 1971), blue crabs (LOWE et al. 1971), mice (WARE and GOOD 1967).

In addition, some evidence exists for subtle behavioral changes in laboratory animals following long-term ingestion of nonlethal quantities. Thus PEELER (1975) has found a number of behavioral changes following mirex administration in rats including an increase in locomotor activity. The change was most pronounced in animals which ingested a presumed physiological dose over a 60-day period.

In a variety of studies, increased activity (and reactivity) has been associated with overresponding on a partial reinforcement schedule and a subsequent deficit in the ability to inhibit responding in the face of altered reward contingencies (AARON and THORNE 1975, THORNE et al. 1976a, THORNE et al. 1976b). BURT (1975) has stated that any alteration in behavior subsequent to exposure to a neurotoxin could be considered deleterious.

The first experiment was designed to assess the effect of long-term mirex ingestion upon the ability of adult rats to modify behavior under changing reward contingencies. Following exposure to mirex, all animals were tested for activity changes in an open-field apparatus, and were then trained on a partial reinforcement schedule (VI 30-sec) prior to testing on a differential reinforcement of low response rate paradigm (DRL 20-sec).

EXPERIMENT 1

METHODS

Subjects: The subjects were 21 adult male Long-Evans rats obtained from the breeding colony maintained by the Psychology Department at Mississippi State University.

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The animals were at least 6 months old at the beginning of the study with an average weight of approximately 543g.

All animals were singly housed in cages measuring 17.78 x 25.40 x 17.78 cm. The cages contained a feeding container consisting of a piece of 2 x 4 on which was nailed an inverted jar lid. Initially the feeding container was not attached to the cage but frequent spillage necessitated attachment. Ad lib water conditions were maintained throughout the study.

Apparatus: Activity was measured in an open-field box measuring 76.2 x 76.2 x 25.4 cm. The box was painted flat black, and the floor was divided by white lines into 25 equal squares. Hardware cloth covered the top. Operant conditioning was conducted in a standard Lehigh Valley operant chamber.

Procedure: The rats were randomly assigned to one of three different treatment groups: Group Control Chow,CC; Group Low Mirex, LM; Group High Mirex, HM. On the first day each animal was weighed and placed into a single cage containing 25g (an approximate daily feeding) of the appropriate type of chow. For Group CC rats this consisted of finely ground Purina rat chow. Group LM and Group HM animals received finely ground Purina rat chow containing either 1.78 ppm or 17.8 ppm mirex (technical grade) obtained from Allied Chemical Co. The concentration of mirex used was determined in relation to the LD 50 for rats (GAINES and KIMBROUGH 1970). Thus, a dosage of 1.78 ppm would result in ingestion of 0.3 LD 50 over a 1-yr period while a dosage of 17.8 ppm would result in ingestion of 3 LD 50 in 1 year.

For the first 10 days all subjects received 25g of the appropriate chow. Beginning on Day 11, the amount of chow was reduced in order to lower the animals' weight to approximately 85% of first day weight. Once an animal reached the desired weight, it received the amount of chow necessary to sustain this weight each day.

From Day 8 through Day 12, each rat was placed into the open-field apparatus after weighing. The number of squares crossed and the number of rearings (standing on the hindlegs) were recorded during a 1-min period. The apparatus was cleaned after each animal's testing, and the order of testing was varied so that the behavior of one animal would not consistently affect that of another. The number of subjects within each group for which data were collected in the open field was as follows: Group CC, 6; Group LM, 7; Group HM, 8.

Beginning on Day 11, several 45mg Noyes pellets were placed in each animal's food container in order to facilitate finding and eating during shaping in the

operant chamber. On Day 15 each animal was placed in the operant chamber and was trained to press the bar for 45mg Noyes pellets. During shaping, the length of the sessions ranged from 20-35 min.

After the animals had acquired the response, at least one session of adjustment to partial reinforcement was given. This consisted of training on a variable ratio schedule requiring a gradually increasing number of responses per reinforcement and was considered complete when the rat was making at least 5 responses per reinforcement.

On the day after completion of the adjustment, all animals were placed on a VI 30-sec schedule and trained until their response rate was considered stable. The criterion of stabilization was 3 consecutive days with less than 15% variability in the total number of responses. Responses during the first four days on VI 30 were not counted so that the minimum number of days any animal stayed on VI 30 was seven. All sessions beginning with the introduction of VI training were 20 min in length.

A DRL 20-sec training schedule was initiated on the day after the criterion of stabilization was achieved. On the DRL 20-sec schedule any barpress occurring with less than a 20-sec delay from the previous press reset a timer and delayed reinforcement for at least 20 sec. A barpress following a previous press by 20 sec or more resulted in reinforcement and reset the timer initiating another cycle. DRL training continued for 7 days.

Because of untrainability, some of the subjects tested in the open-field apparatus did not complete the study. Thus, the final number of subjects in each group trained on the VI 30-sec and DRL 20-sec schedules was as follows: Group CC, 5; Group LM, 6; Group HM, 7.

Tissue Analysis: Following the completion of data collection, one rat from Group CC, three from Group LM, and two from Group HM were sacrificed and a portion of the brain was removed and analyzed for mirex content using a gas chromatograph (Barber-Colman Pesticide Analyzer) with a Ni^{63} detector according to the procedure of THOMPSON (1974).

RESULTS

Open-field Test: A Kruskal-Wallis one-way analysis of variance performed on the total number of squares crossed by each animal revealed no significant differences. The group means were: Group CC, 92.7; Group LM, 101.6; Group HM, 79.9. A comparison of rearing scores revealed no evidence for group differences and the means were: Group CC, 43.7; Group LM, 40; Group HM, 43.4.

VI 30-sec Performance: A Kruskal-Wallis one-way analysis of variance performed on the average stabilized response

rates indicated that there were no significant differences among the three groups. The average rates were: Group CC, 208.9; Group LM, 103.5; Group HM, 121.9. The rather high average response rate in Group CC was attributable to the performance of one subject who responded at a rate of 417.7 responses per 20-min session.

DRL 20-sec Performance: A Kruskal-Wallis one-way analysis of variance revealed no significant differences when the subjects were compared on the total number of rewards received during the 7 days of testing. The group means were: Group CC, 94.8; Group LM, 120.5; Group HM, 123.4.

The correlation between performance on the VI 30-sec task and number of rewards obtained during DRL 20-sec testing was significant ($r_S = -0.589$, $p < 0.01$, 1-tailed test). Thus, animals responding at a relatively high rate on the VI 30-sec schedule received relatively few rewards on DRL 20 and vice versa.

Tissue Analysis: No residues of mirex were found in the brain sample from the Group CC rat. The three samples from Group LM animals were found to contain 0.20, 0.14, and 0.14 µg of mirex per gram of brain tissue (dry weight). Samples from Group HM subjects were found to contain 1.32 and 1.10 µg mirex per gram of brain (dry weight) or slightly less than 10 times the amount found in Group LM samples.

DISCUSSION

The results of the present study indicated that mirex ingestion in the dosages used over a several week period ($\bar{X} = 39.7$ days, range from 34-49) has no detectable effect upon open-field activity and operant behavior as assessed by VI 30-sec and DRL 20-sec schedules. Thus, the present experiment failed to replicate the finding by PEELER (1975) that mirex ingestion in adults increases locomotor activity in the open field.

One possible explanation for the discrepancy stems from the fact that the route of administration of the mirex was different in the two studies. PEELER (1975) administered mirex by intubation while our subjects ingested it with their food. It is possible that the intubation led to greater absorption and incorporation and correspondingly greater behavioral changes.

One methodological problem in the present experiment was that the animals did not receive a constant dosage of the mirex. This was necessitated by the requirements of a deprivation schedule to motivate the rats in the operant chamber. In order to circumvent this problem, in Experiment 2 all animals were tested on a task motivated by escape from footshock.

EXPERIMENT 2

In Experiment 1 we failed to detect any differences in performance between mirex-treated animals and control subjects. In order to further explore the possibility of neurotoxic effects of mirex ingestion, the performance of mirex-treated rats and controls on another moderately difficult task, discrimination-reversal, was examined.

In one example of the discrimination-reversal task, the animal learns to discriminate between two patterns or positions, i.e., he learns to approach one of the patterns while avoiding the other. When he reaches some arbitrary criterion on this task, the reward contingencies are changed, and he must now learn to approach the previously negative pattern or position while avoiding the previously positive stimulus. This alternation of reward contingencies continues through several reversals until improvement is shown or until some set number of reversals has been completed. The discrimination-reversal task has been used to compare the intelligence of diverse species of animals (WARREN 1965).

The discrimination-reversal task was selected because it is difficult enough to be discriminating and because conditions of food deprivation are not required if escape from footshock is the motive for learning. In this study only one concentration of mirex was administered, the high dose from Experiment 1 or 17.8 ppm.

METHODS

Subjects: The subjects were 9 adult male Long-Evans rats. The animals were at least 6 months old at the start of testing and their average weight was approximately 556g.

Housing and feeding containers were identical to that described in Experiment 1. All subjects received 25g of the appropriate chow daily and water ad lib.

Apparatus: Open-field activity was assessed in the apparatus described in Experiment 1. The apparatus used to measure discrimination-reversal performance was a Thompson-Bryant two-choice box from which the choice chamber had been removed, leaving only a startbox and a goalbox. The startbox had a grid floor through which footshock could be administered. A Plexiglas door separated the start area from a 7.6-cm grid in front of two openings into the goalbox. The doors to the goalbox were blocked by 10.2 x 16.5 cm stimulus cards. Each stimulus card consisted of heavy posterboard on which was pasted either a piece of

white or a piece of black construction paper. In order to prevent the animals from using olfactory cues to solve the discrimination, the cards were inserted into clear plastic sleeves which were washed with water after the testing of each animal. Painted gray cards were used in preliminary training.

Procedure: The rats were randomly assigned to one of the two treatment groups: Group CC, 4 rats; Group HM, 5 rats. The procedure for the first 14 days was the same as in Experiment 1 except that the animals were not placed on a deprivation schedule and were not exposed to Noyes pellets.

On the 15th day, training began on the discrimination tasks. Preliminary training consisted of five trials in which an animal was forced to leave the startbox and push aside a gray card to enter the goalbox. On the day following preliminary training, practice on the first discrimination task (white, positive; black, negative) began. On each trial, a subject was placed into the startbox for 5 sec during which the Plexiglas door separating the start area from the discriminanda was lowered. After elevation of the door, each subject was given approximately 15 sec in which to make a choice. If the rat chose the black card, a mild footshock was administered, whereas choice of the white card permitted entrance to the goalbox. The position of the cards, but not the plastic sleeves, was varied according to a modified Gellerman sequence, and a correction procedure was employed. Animals were given 10 trial/day, with an intertrial interval of about 1 min, until a criterion of 9 correct responses in one day was attained. The number of errors an animal made prior to the day it reached criterion constituted the measure of learning ability.

On the day after an animal reached criterion on the first problem, training began on the first reversal and consisted of training the rat to go to the black door and avoid the white. The alternation of tasks (white, positive; black, positive; etc.) continued until either the animal was unable to reach criterion on a task or at least 4 reversals had been completed.

After the completion of data collection, two Group HM subjects were sacrificed and a portion of the brain removed for analysis of mirex residues. The samples were assayed by the same technique used in Experiment 1.

RESULTS

Open-field Test: There were no significant differences in either the number of squares traversed or in the number of times rearing occurred. The group means were: Group CC, $\bar{X}$(activity) = 94, $\bar{X}$(rearing) = 37.75; Group

HM, $\bar{X}$(activity = 95.6, $\bar{X}$(rearing) = 42.6.

Discrimination-reversal Task: Mann-Whitney comparisons of errors made on each reversal did not indicate a trend toward a significant difference on any task. The development of performance over the first 6 tasks (5 reversals) is shown in Figure 1.

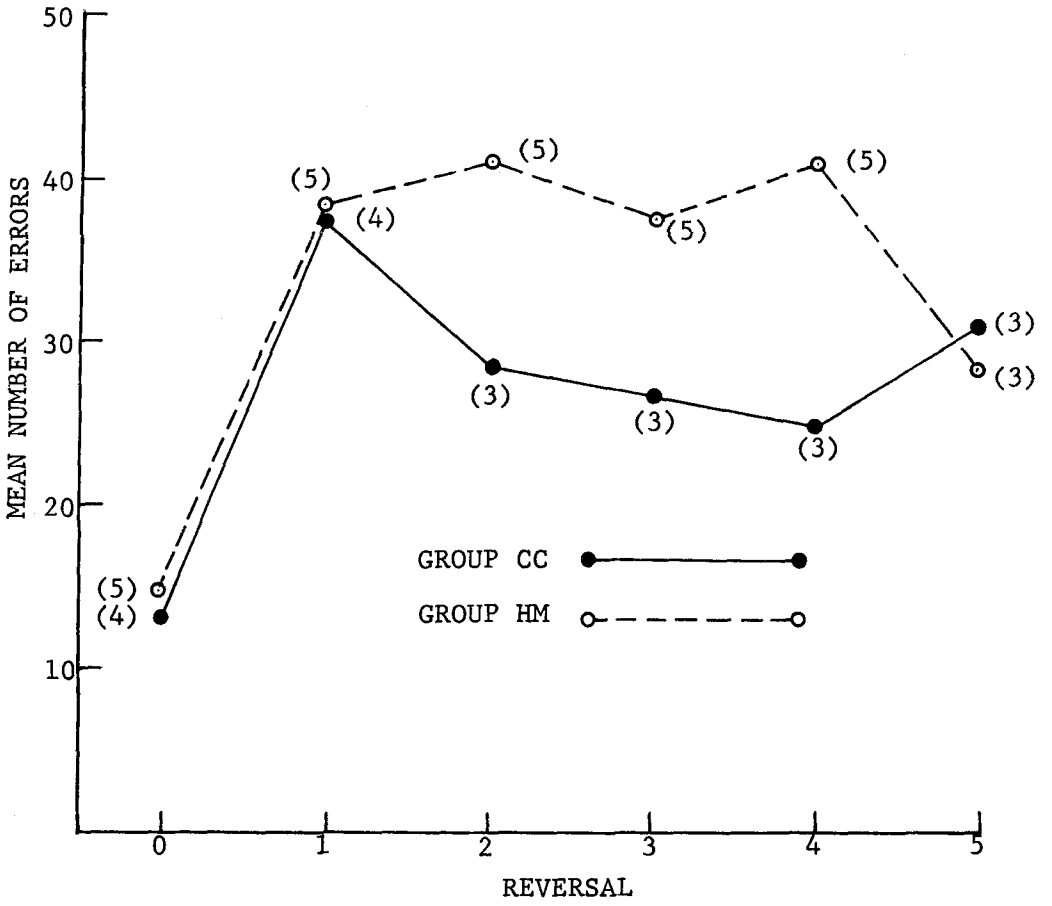

Figure 1. Mean number of errors per reversal. The numbers in parentheses indicate the number of subjects whose data contributed to each point.

Inspection of the graph indicates that performance deteriorated on the first reversal, and the average number of errors remained relatively high for the last 4 reversals. No statistical evidence for group improvement was seen.

Tissue Analysis: Samples from animals in Group HM were found to contain 1.14 and 1.86µg mirex per gram of brain (dry weight).

DISCUSSION

Again mirex ingestion in a concentration of 17.8 ppm had no effect upon behavior. In this experiment the rats received a constant amount of baited chow over a period of time that was more than twice as long as in Experiment 1 ($\bar{X}$ = 83.2 days, range 61-113 days). Thus, we conclude that long-term ingestion of mirex in the concentrations assessed has no detectable effect on the behaviors observed (open-field activity, VI 30-sec, DRL 20-sec, discrimination-reversal motivated by escape from footshock).

It is possible, of course, that modification of any of the possibly relevant parameters, e.g., concentration of mirex, length of feeding, behavior assessed, age of animals at the time of administration, might result in detection of behavioral changes. However, to the extent that the concentrations we administered and the behaviors we measured are ecologically relevant, mirex seems to have little neurotoxic effect upon the rat.

SUMMARY

In two experiments, adult male Long-Evans rats were fed chow containing mirex (1.78 ppm and 17.8 ppm in Experiment 1, 17.8 ppm in Experiment 2) over a several week period and were tested on a variety of behavioral tasks. No differences in behavior were seen between control and experimental animals tested in an open-field apparatus, on VI 30-sec and DRL 20-sec operant paradigms, or on a discrimination-reversal task motivated by escape from footshock.

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