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Dissociation of Cycloheximide's Effects on Activity from its Effects on Memory[1]

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GUTWEIN, B. M., D. QUARTERMAIN AND B. S. MCEWEN. *Dissociation of cycloheximide's effects on activity from its effects on memory.* PHARMAC. BIOCHEM. BEHAV. 2(6) 753–756, 1974. – Two doses of cycloheximide (CYC) were used to dissociate the effect of this drug on locomotor activity from its effect on memory. Results indicated significant short-term (30′–40′) and long-term (24 hr) increases in locomotor activity occur at both CYC doses, whereas significant short- and long-term decreases in step-out latencies in a passive-avoidance task occur primarily at the higher CYC dose. Pargyline, a monoamine oxidase inhibitor, significantly increases locomotor activity but does not decrease step-out latencies in the passive-avoidance task. It is concluded that the decreases in step-out latencies shown by CYC-treated animals in the passive avoidance task are the result of an amnesic effect of CYC rather than its effect on locomotor activity.

Cycloheximide Passive avoidance Activity Amnesia

A NUMBER of studies have demonstrated that mice treated with cycloheximide (CYC) shortly before activity sessions show significant increases in locomotor activity up to 45 min after injection [3, 7, 10]. Recently, evidence has been presented which indicates that mice treated with CYC 30 min before training in a one-trial inhibitory avoidance task show significantly shorter step-out latencies when retention is tested 5 min to 24 hr after training [5]. The present study was carried out to examine the possibility that short step-out latencies are the result of an effect of CYC in increasing locomotor activity levels rather than any amnesic effect.

EXPERIMENT 1

The aim of Experiments 1 and 2 was to correlate the occurrence of retrograde amnesia for a one-trial inhibitory avoidance response resulting from CYC administration with a CYC-induced enhancement of locomotor activity. It was expected that by varying the dose of CYC, one might dissociate the effects of CYC on amnesia from its effect on activity.

Method

Animals. The animals were male mice, 25 g in weight, of the C57BL/6J strain, maintained on a fixed 12 hr light–dark schedule, housed in individual cages, with food and water ad lib.

Apparatus. The apparatus was a two-compartment passive-avoidance box. The small compartment, made of clear Plexiglas, was 10 cm long and 7 cm wide. A circular hole, 5 cm in dia., served as an entrance to the large compartment which had black Plexiglas sides and was 15 cm long and 10 cm wide. The floor was made of stainless steel rods 3 cm in dia. and set 12 cm apart. A black Plexiglas guillotine door separated the two compartments. The entire apparatus was 20 cm high and both compartments were covered with separate hinged lids.

Design and procedure. Basic procedure was as follows. Mice were placed in the small compartment; after they entered the large compartment, the connecting door was closed and a 2 sec 0.2 mA shock was automatically delivered through the bars. Latencies to enter the large compartment were recorded both on the training trial and the retention

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TABLE 1

MEAN STEP-OUT LATENCIES FOR MICE INJECTED WITH BOTH DOSES OF CYC AND TRAINED IN THE PASSIVE-AVOIDANCE TASK

Group	N	Time of test	Mean retention latency	S.D.	Significance compared with saline control
1: CYC–120 mg/kg	10	10 min	52.7	0.32	$p \leq 0.01$
2: CYC–40 mg/kg	15	10 min	200.7	0.27	NS
3: SALINE	25	10 min	203.6	0.31	
4: CYC–120 mg/kg	10	24 hr	76.5	0.19	$p \leq 0.01$
5: CYC–40 mg/kg	10	24 hr	127.0	0.26	NS
6: SALINE	10	24 hr	147.1	0.37	

test trial. Mice were injected subcutaneously in the back of the neck 30 min before training and tested for retention either 40 min or 24 hr after injection.

For testing at 40 min, the groups were: saline (N = 25); CYC, 120 mg/kg (N = 10); CYC, 40 mg/kg (N = 15). Both doses of drug were dissolved in 0.3 cc solution of physiological saline. For testing at 24 hr, the groups were: saline (N = 10); CYC, 120 mg/kg (N = 10); CYC, 40 mg/kg (N = 10).

Results

A one-way analysis of variance with Scheffe pairwise comparisons were used to analyze the results of this experiment and Experiment 4 [12]. The results of Experiment 1 indicate that mice treated with CYC 120 mg/kg exhibited significantly shorter retention latencies when tested 10 min after training compared to saline controls (Scheffe pairwise comparisons of one-way ANOVA, df = 1/56, F 20.21, $p<0.01$).

Mice treated with 40 mg/kg of CYC were not significantly different from saline controls when retention was measured 10 min after training (Scheffe pairwise comparisons of one-way ANOVA, df = 1/56, F = 0.21). CYC-injected mice tested for retention 24 hr after training exhibited significantly shorter re-entry latencies compared to saline controls (Scheffe pairwise comparisons of one-way ANOVA, df = 1/36, F = 20.42, $p<0.01$), but mice treated with 40 mg/kg of CYC did not exhibit significantly shorter retention latencies compared to saline controls at this time (Scheffe pairwise comparisons of one-way ANOVA, df = 1/36, F = 0.003).

EXPERIMENT 2

In the following experiment, we investigated the effects of the same two doses of CYC on locomotor activity. As noted in the introduction to Experiment 1, this procedure might be expected to clarify whether the increased locomotor activity induced by CYC contributed to the memory deficit observed in CYC-treated animals in the passive-avoidance task.

Method

Locomotor activity was measured by placing a cardboard insert on the floor of the large compartment of the passive-avoidance apparatus. A line was drawn across the middle of the cardboard, dividing the apparatus into three sections when the connecting door to the start box was opened. Mice were placed in the small compartment and given 1 point each time they crossed a line. Cumulated activity counts were recorded each minute for a 10-min period.

Design and procedure. Locomotor activity was recorded for 10 min for groups of mice starting either 30 min or 24 hr after subcutaneous injection of either 120 mg/kg CYC, 40 mg/kg CYC, or saline alone. At 30 min: CYC, 120 mg/kg (N = 10); CYC, 40 mg/kg (N = 10); and saline (N = 10). At 24 hr: CYC, 120 mg/kg (N = 10); CYC, 40 mg/kg (N = 10); and saline alone (N = 10). The timing of activity testing for all groups coincided with the time of training and testing in the passive-avoidance experiment.

Results

The locomotor activity scores are presented in Fig. 1. A two-way analysis of variance with repeated measures and Scheffe pairwise comparisons were used to analyze the results of this experiment. This analysis indicates that mice injected with CYC 120 mg/kg and CYC 40 mg/kg 30 min before activity sessions had significantly higher locomotor activity levels compared to saline controls (two-way ANOVA, repeated measure, df = 9/324, F = 10.76, $p<0.01$). Scheffe pairwise comparisons indicate that both doses of CYC significantly increased locomotor activity levels compared to saline controls when activity was measured for 10 min starting 30 min following drug administration. (CYC 120 mg/kg, df = 1/36, F = 8.10, $p<0.01$, and CYC 40 mg/kg, df = 1/36, F = 43.49,

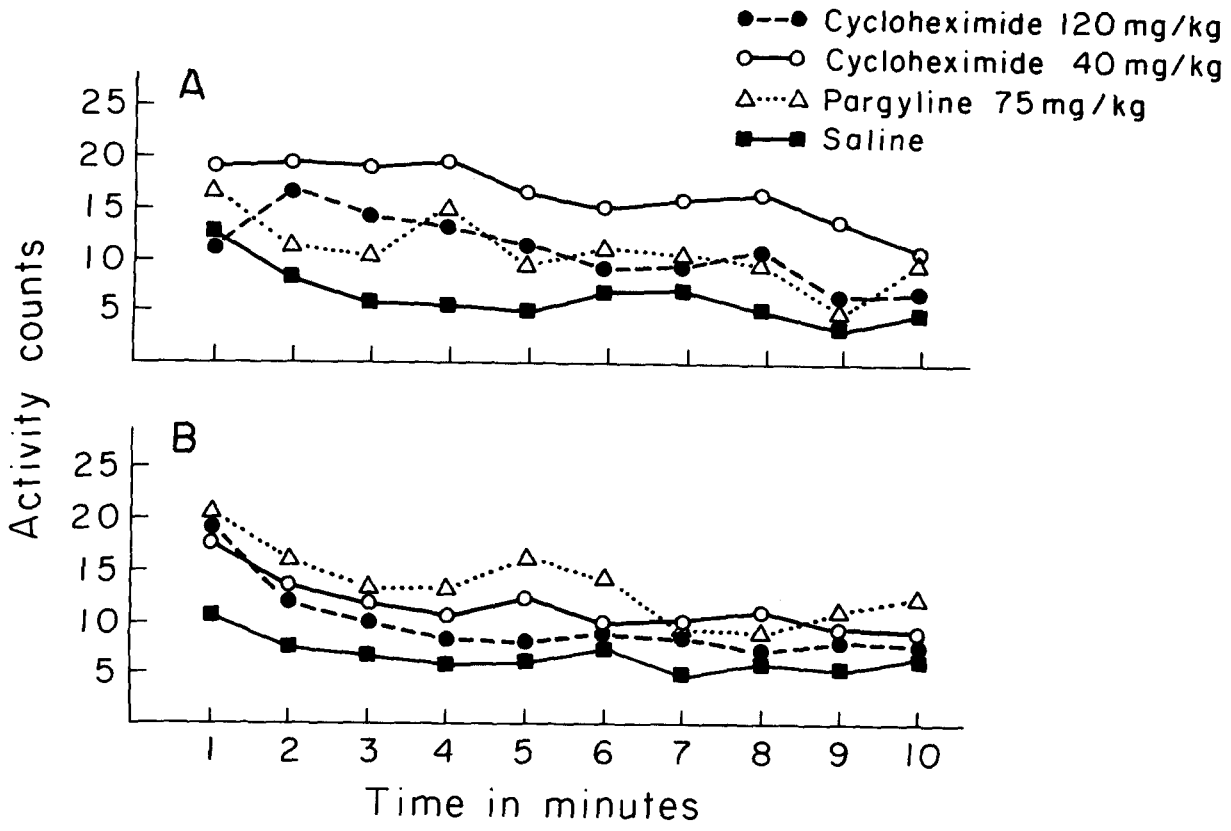

FIG. 1. Cumulated activity counts for mice treated with 120 mg/kg cycloheximide, 40 mg/kg cycloheximide, pargyline 75 mg/kg, or saline. The timing of activity testing for groups coincided with the time of training and testing in the passive-avoidance experiment. (A) tested 30 min after injection. (B) tested 24 hr after injection.

$p<0.001$). Mice treated with either of the CYC doses and given activity sessions 24 hr following drug administration also exhibited significantly increased locomotor activity when compared to saline controls (two-way ANOVA, repeated measures, $df = 9/324$, $F = 19.01$, $p<0.001$, and Scheffe pairwise comparisons: CYC 120 mg/kg, $df = 1/36$, $F = 7.66$, $p<0.01$ and CYC 40 mg/kg, $df = 1/36$, $F = 30.63$, $p<0.001$).

EXPERIMENT 3

In an attempt to further dissociate activity from amnesia, we attempted to determine whether a monoamine oxidase inhibitor, pargyline, which has been observed to increase locomotor activity [2] but which has no known amnesic properties would significantly reduce step-out latencies in the passive-avoidance task. The object of Experiment 3 was to record activity scores following pargyline injection using the test conditions previously employed with CYC in Experiment 2. In Experiment 4, we attempted to determine the effect of pargyline administration on step-out latencies in the passive-avoidance task.

Method

In this experiment, our basic procedure was identical to that previously described in Experiment 2. Group 1: Mice were injected with pargyline (75 mg/kg, i.p. in 0.2 cc saline solution) (N = 10) or with saline alone (N = 10) 1 hr before activity sessions. Group 2: Mice were similarly treated as in Group 1; pargyline (75 mg/kg in 0.2 cc saline solution, injected i.p.) (N = 10) and saline alone (N = 10). Activity sessions were given 24 hr after drug administration. Activity counts were recorded each minute for a 10-min period.

Results

Activity scores for the pargyline-treated animals are shown in Fig. 1. The results of Experiment 3 indicate that pargyline significantly increased locomotor activity 1 hr and 24 hr after injection compared to saline controls (two-way ANOVA with repeated measures; at 10 min, df 9/324, $F = 10.76$, $p<0.01$. At 24 hr, $df = 9/324$, $F = 19.01$, $p<0.001$. Scheffe pairwise comparisons of two-way ANOVA repeated measures: pargyline, 1 hr, df 1/36, $F = 8.14$, $p<0.01$; pargyline, 24 hr, $df = 1/36$, $F = 62.74$, $p<0.001$).

EXPERIMENT 4

Apparatus. The apparatus was the same as described in Experiment 1.

Design and procedure. In this experiment, our basic procedure was identical to that previously described in Experiment 1. Group 1: Mice were injected with either pargyline (75 mg/kg, i.p.) in 0.2 cc saline solution (N = 10), or saline alone (N = 10), and trained in the passive-avoidance task 50 min later. Retention was tested 10 min

after training. This dose level and injection time were selected so that greater than 95% inhibition of MAO was achieved at time of testing [4,6] and also coincided with the time of testing employed in Experiment 1. Group 2: Mice were similarly treated to those in Group 1; pargyline (75 mg/kg, i.p. in 0.2 cc solution) (N = 10) and trained 50 min later. Retention was tested 24 hr after drug administration.

Results

The results of Experiment 4 indicate that pargyline-treated mice tested for retention at 1 hr and at 24 hr following drug administration did not have step-out latencies significantly different from saline controls (Scheffe pairwise comparisons of one-way ANOVA, at 1 hr: $\bar{X}$ = 163.6 sec, df = 3/56, F = 0.16; at 24 hr: $\bar{X}$ = 99.1 sec, df = 3/56, F = 0.28).

DISCUSSION

The results of Experiments 1 and 2 indicate a partial dose dependent separation of CYC's effect on step-out latencies from its effect on locomotor activity. The results of Experiments 3 and 4 provide additional support for this hypothesis by showing that pargyline significantly increases locomotor activity, but does not significantly decrease step-out latencies in the passive-avoidance task. These results clearly indicate that enhancement of locomotor activity is not always accompanied by a decrease in step-out latencies. It is important to point out that our data differs from the results reported in previous experiments [3, 7, 10], which demonstrated that the effects of 120 mg/kg CYC on locomotor activity were short-lasting (increases up to 45 min and a return to baseline at 24 hr). The failure of these studies to detect an enhancement of locomotor activity at 24 hr might be attributable to habituation, since the same animals were tested at the 45 min and at the 24 hr activity sessions.

A recent experiment [2] has shown that mice injected with 120 mg/kg CYC 30 min before passive-avoidance training and treated with pargyline 2 hr prior to the 24 hr retest show significantly increased retention latencies compared to a CYC-SAL group but not a SAL-SAL group. The results of additional control groups led the authors to conclude that the increased step-out latencies of the CYC-pargyline group was due to the drug's attenuation of CYC-induced retrograde amnesia. They also observed a considerable increase in locomotor activity in the pargyline-treated mice when they were placed in the start compartment of the passive-avoidance box for the retention test. Segal, Squire and Barondes [7] have recently shown that iso-cycloheximide, an isomer of CYC, increases locomotor activity but does not affect retention in a multiple-trial shock avoidance task. These results provide additional support for the separation of CYC's effects on locomotor activity from its effects on memory.

A number of studies have shown that CYC can produce amnesia rapidly after training. Quartermain and McEwen [5] have shown that mice injected with CYC and trained in a one-trial passive-avoidance task under low shock (0.16 mA) show a well-developed amnesia when tested at 1 min and 5 min after training. It should be noted that with high shock (1.6 mA), there were no significant differences in the latencies between the saline and CYC-injected animals until 24 hr after training. Squire and Barondes [8] and Squire, Smith and Barondes [11] reported that a CYC-sensitive component of memory could be detected within minutes after training onset in the multiple-trial shock-avoidance task. These results, therefore, indicate that in both passive-avoidance and multiple-trial shock escape tasks, CYC-injected mice show amnesia when retention is tested within an hour following training. It thus appears that an earlier hypothesis [1,8] which postulated that short-term memory was insensitive to CYC will have to be revised.

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