Pharmacology Biochemistry & Behavior, Vol. 6, pp. 631–635.

Comparison of the Cycloheximide and Food Satiation Effects on a Discrimination Task[1]

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(Received 19 November 1976)

BECKER, J. AND E. K. MICHAELIS. *Comparison of the cycloheximide and food satiation effects on a discrimination task.* PHARMAC. BIOCHEM. BEHAV. 6(6) 631–635, 1977. – The effects of intraventricular cycloheximide (CHX) pretreatment were examined on the ability of rats to recall a light position discrimination task. The CHX pretreatment used inhibited brain protein synthesis at the time of training by 71%. Following treatment with CHX the animals demonstrated no retention of the task 18 hr later. Free feeding for 24 hr prior to treatment with cerebro-spinal-like fluid (CSF) produced similar effects as CHX. It is proposed that the amnesic effect of CHX in this behavioral paradigm may be a nonspecific action resulting from decreased motivation. Both the CHX-treated and satiated animals exhibited low responding levels during training, despite comparable behavioral activity levels for the CHX- and CSF-treated groups.

Cycloheximide Memory Discrimination training Motivation Behavioral activity
Protein synthesis inhibition

THE HYPOTHESIS that proteins are synthesized as a result of learning and that this process is in some way responsible for memory formation has been widely accepted. Investigations to determine the role of proteins have followed two different avenues. One approach has been to demonstrate and purify newly synthesized proteins specific for the learned task [2, 14, 21]. The other and most frequently employed method has involved the study of the effects of protein synthesis inhibitors on memory formation (e.g., [1, 3, 5, 7, 16]). Despite the general agreement in the literature that protein synthesis inhibitors administered prior to or immediately after training produce amnesia of the trained task, some studies have reported no effect of these agents on memory formation [8, 15, 20, 22], or that have reported the spontaneous recovery of memory [18,19].

There are several factors that can account for the failure to produce an amnesic effect with protein synthesis inhibitors, including the percent of protein synthesis inhibition achieved [1] and the amount and quality of training employed [6, 7, 8, 13]. For example, reduced amnesia has been observed after protein synthesis inhibition if too little or too much training has been employed [7], or if longer shock duration [6] or increased intensities were used [8] in shock-motivated training paradigms. These observations might indicate that some of the effects of protein synthesis inhibitors on memory represent a non-specific interaction of this type of treatment with the training procedures used.

In order to study the specificity of the effects of one such protein synthesis inhibitor, cycloheximide (CHX), we selected a food-reinforced discrimination task rather than a shock-motivated training paradigm. With this type of positively motivated learning paradigm it was expected that one could easily compare the behavioral effects of cycloheximide with the effects of a procedure that reduces the motivational state of the experimental animals, such as food satiation before training. In addition, this study was designed so that the effects of CHX on a task which required prolonged training sessions could be explored, and so that each animal functioned as its own control for the CHX and no-CHX treatment conditions.

METHOD

Chemicals

Cycloheximide was obtained from Sigma Chemical Co., St. Louis, MO. (^{3}H)-Leucine (5.0 Ci/mmole) was obtained from New England Nuclear Corporation, Boston, MA. All other chemicals were of reagent grade.

Animals

Thirty-eight male Sprague-Dawley rats were housed in

[1] This research was supported in part by Grant AA 01900 from the National Institute of Alcoholism and Alcohol Abuse, by Grant 22357 from the National Institute of General Medical Studies, and by Grant G–007507226 from the U.S. Office of Education, HEW, to the University of Kansas, Department of Human Development, Behavior Analysis Follow Through (the opinions expressed herein do not necessarily reflect the position of the U.S.O.E., and an official endorsement by U.S.O.E. should not be inferred). The authors wish to thank W. Freed for his expert help with the electromechanical wiring, R. Grubbs for assistance with the injections, and M. L. Michaelis, E. Ramp and J. Sherman for their valuable discussions.

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individual cages, kept on a 12 hr light, 12 hr dark schedule and maintained at 85% of their free feeding weight throughout the experiment. All animals had been subjects in a student behavioral psychology class prior to the experiment and had similar training histories.

Apparatus

A Gerbrands operant conditioning unit was altered to accommodate two lights, one on each side of the lever. The push rod was removed from the chamber. The control unit was attached to a stepper and a cumulative recorder. Condition changes were made manually.

Behavioral Methods

The animals were randomly divided into four groups of six animals in each group. Each group received two injections separated by one week's time. Each injection was followed in 2 hr by a training session and a postcheck session 18 hr later. The first group (Group 1) received a cerebro-spinal fluid-like injection [4] (CSF) as the first injection and CHX as the second injection. The second group (Group 2) received CHX as the first and CSF as the second injection. The third group (Group 3) received CSF in both injections and the fourth group (Group 4) was satiated prior to their first injection of CSF but food deprived prior to their second CSF injection. The injections were administered under light ether anesthesia employing a free-hand injection procedure [11] into the right ventricle. A single blind design was used for the injection of Groups 1 and 2. All injections were 10 μl in volume and consisted of either CSF or 18.75 μg CHX/μl CSF solution. The injection site was checked in a number of animals by sacrificing the animals 12 hr after their last injection and checking for the needle blood tracks. In several animals the site was localized by injection of 10 μl of methylene blue dye through the previously used track just prior to sacrifice. Only one animal had to be discarded because of improper injection site placement.

Preceding all injections each animal was established on a fixed ratio of 25 bar presses to one 45 mg Noyes food pellet (FR 25). Following that training the animals were taught to discriminate light position. One light functioned as a discriminative stimulus (S^D) for bar pressing on a FR 5. The second light signified that bar pressing would not be reinforced (S^Δ). For the first discrimination training session, rats in all four groups were trained while the left light (the light illuminated in the initial FR 25 training) functioned as S^D and the right light served as the S^Δ. Following the first postcheck session each animal was reestablished on a FR 25 in the presence of the illumination of the right light. During the second discrimination training session the right light functioned as the S^D while the left light signified the S^Δ component. The discrimination training session following either injection consisted of four periods of S^D and three periods of S^Δ; the first was always an S^D and it alternated with three S^Δ conditions. Each S^D condition was five minutes long. Each S^Δ condition continued until the rat had not responded for three consecutive clock minutes (3 min change-over-delay). The postcheck administered 18 hr after each training session was a repeat of the seven training conditions the animal had been exposed to 18 hr earlier. All discrimination training sessions were preceded by a 24 hr food deprivation period except for the first training session of Group 4. During all sessions a cumulative recorder was operated and the number of responses were recorded (from electromechanical counters) at one minute intervals by the experimenter.

A discrimination ratio was used to determine the degree to which each animal had made the discrimination between S^D and S^Δ conditions [12]. This ratio was calculated as:

$$\frac{\text{the number of responses in the } S^D \text{ conditions}}{\text{Total responses in all conditions}}$$

for an entire session. A perfect discrimination would be a ratio of one. Discrimination ratios were also calculated for each of the three exposures to the two stimuli to determine if there was improvement across the session in the animals' ability to distinguish the cues. These ratios were calculated as:

$$\frac{\text{the number of responses in the } S^D \text{ component}}{\text{responses in } S^D + \text{responses in the preceding } S^\Delta \text{ component}}$$

The difference in each animal's discrimination ratio in the postcheck session minus the discrimination ratio in the training session was used in calculating the group average discrimination ratio difference under a given treatment condition. In addition to discrimination ratios the response rate of each animal in the S^D and S^Δ conditions were computed as responses per minute.

Activity Measures

A Lehigh-Valley photocell activity cage (61 cm diameter) was employed to measure general activity in two groups of four rats each. One group received 10 μl CSF first, the other 10 μl CHX. One week later the group that received CSF the first time received CHX and the other group received CSF. Two hours after each intraventricular injection, the rat was placed in the activity cage for 30 min. In computing average activity all animals receiving CHX were considered together as were all animals receiving CSF.

Biochemical Measures

Six rats were used for the measurement of brain protein synthesis activity; three rats were treated with CSF and three were injected with CHX as previously described. Each animal was returned to its home cage and 2 hr later it was injected intraventicularly with 10 μl of (^{3}H)-leucine (10 μCi). The animal was decapitated 15 min later and the brain was sliced in the cold in a dorsoventral fashion. Slices from the forebrain, mid-brain, and hind-brain areas were used for homogenization, protein precipitation, and washing of the protein pellet as previously described [1]. The average weight of starting brain tissue material was 1.08 g (range 1.0–1.2 g). The washed protein pellet was resuspended in 1.1 ml of a 1.9 N NaOH-20% Triton X-100 solution and triplicate 20 μl aliquots of the suspension were used to measure the radioactivity by means of scintillation spectrometry employing a Triton X-100-Toluene (1 : 2 v/v) scintillation fluid (average tritium efficiency was 39%). Three 0.1 ml samples of the deproteinized supernatant were also removed and the radioactivity of free (^{3}H)-leucine was determined employing a scintillation fluid with cab-o-sil [9] (average tritium efficiency was 55%). Protein concentration in the tissue homogenate, the redissolved protein

precipitate, and the supernatant was measured by the Lowry method [10].

RESULTS

Protein Synthesis Inhibition

The dose of cycloheximide selected (187.5 μg per injection) was found to be the largest dose that the animals could tolerate without exhibiting overt manifestations of sickness. Even at this dose level three animals failed to bar press for the first five minutes of the FR 5 component and they had to be excluded from the study. At the dose level employed in this study, protein synthesis was found to have been inhibited by 71% in the brain tissue. The average (^{3}H)-leucine radioactivity incorporated into the protein fraction of the control animals was 14,016 DPM/mg protein, whereas that for the CHX-treated animals was 4,390 DPM/mg protein.

Behavioral Effects of Treatment

A comparison of the discrimination ratios between the training and postcheck sessions for Group 1 which received CSF in its first injection, revealed an increase in the discrimination ratio of the postcheck session of +0.330 (standard error, SE 0.06). This can be interpreted as indicative of some retention of the discrimination between the two sessions. One week later when these same animals received CHX, the average difference between the postcheck session minus that of the training session was −0.059 (SE 0.08) which shows that these animals did not retain the training that they had received 18 hr earlier. These results were replicated with animals in Group 2 that received CHX first. The difference in discrimination ratio between postcheck and training session was +0.059 (SE 0.05), again indicating no retention of the learned discrimination. One week later when this same group of animals was injected with CSF, the difference in discrimination ratios between postcheck and training sessions was +0.257 (SE 0.04). A statistical comparison of the differences in discrimination ratios between postcheck and training sessions under the two treatment conditions of CSF and of CHX, showed that the two conditions produced significantly different effects. On a two-tailed *t*-test, the difference found in Group 1 was significant at the 0.005 level, and in Group 2 it was significant at the 0.02 level ($p<0.05$ considered as significance limit, $df = 10$).

The animals in Group 3 served as injection-scheduling controls in order to allow for the examination of any effects that might have been produced by the procedures used with Groups 1 and 2. Following the first CSF injection of the Group 3 animals, the postcheck session produced an average increase in the discrimination ratio of +0.216 (SE 0.102) when compared to the training session. One week later the same procedures resulted in an increase in the discrimination ratio between the postcheck and training session of +0.209 (SE 0.08). Statistical analysis revealed no significant difference between the two treatment procedures for Group 3.

Unlike the animals of Group 3, the animals in Group 4 that had a 24 hr free feeding period preceding their first injection of CSF, showed an average decrease in their discrimination ratio between postcheck and training of −0.111 (SE 0.077). However, one week later these same animals, when given CSF following 24 hr of food deprivation, had an average increase in discrimination ratio between the postcheck and training sessions of +0.386 (SE 0.031). The difference between the two treatment conditions was statistically significant at the 0.001 level, $df = 10$.

In summary, then, across all four groups, those animals that received CSF treatment showed improved ability to discriminate between S^D and S^Δ conditions as manifested by an increase in the discrimination ratios between training and subsequent postcheck sessions. In contrast the animals that were allowed to free feed or were treated with CHX prior to the training session showed little or no retention of the discrimination task in postcheck sessions.

The response rates for each group of animals during the three S^Δ and the last three S^D conditions of all the sessions are shown in Fig. 1. It should be recalled that the S^D conditions were always 5 min long whereas the S^Δ conditions were of variable length because of the 3 min change-over-delay. Generally, it is apparent that the response rate during the S^D conditions in the training sessions that followed CHX treatment or the 24 hr free feeding period prior to the CSF injection, were lower than the response rates in the same condition and session after the food deprivation-CSF injection treatment. In addition, while there is a decrease in the response rate in the S^Δ conditions from training to postcheck session after CSF treatment, this trend is not apparent in the CHX or satiation conditions.

The discrimination ratios for each S^Δ/S^D presentation are also shown in Fig. 1. In all groups of animals under all conditions, one can observe an increase in the discrimination ratio between the first exposure to the discriminative stimuli and the third exposure in each training and postcheck session. It should also be noted that in the CSF conditions there is a trend toward increased discrimination ratios that is carried over from training session to postcheck session. However, in animals that underwent CHX or freefeed-CSF treatment the trend is not observed to carry over from the training to the postcheck session, even though the discrimination ratios for these animals at the end of the training session are as high as those for the CSF-treated animals.

Activity Measures

The effect of CHX on the response rate that was observed in the animals treated with this agent might have indicated more than a suppression of motivation. An actual neuromuscular debilitation as a result of treatment could have produced a similar decrease in the rate of responding. This possibility was explored by measuring the effects of CSF and CHX treatment on general locomotor activity. In terms of general exploratory behavior the animals treated with CSF interrupted the activity cage photocells an average of 487 ± 192 times in 30 min while the animals treated with CHX interrupted the beam an average of 443 ± 239 times in 30 min. There was no statistical significance in the difference between exploratory behavior of CSF or CHX treated animals.

DISCUSSION

In this study, as in many earlier ones, protein synthesis inhibition led to the blockade of memory formation of a learned task. However, unlike the majority of the studies that have examined the effect of protein synthesis inhibitors on memory formation, this study involves a food-reinforced discrimination task rather than a shock avoid-

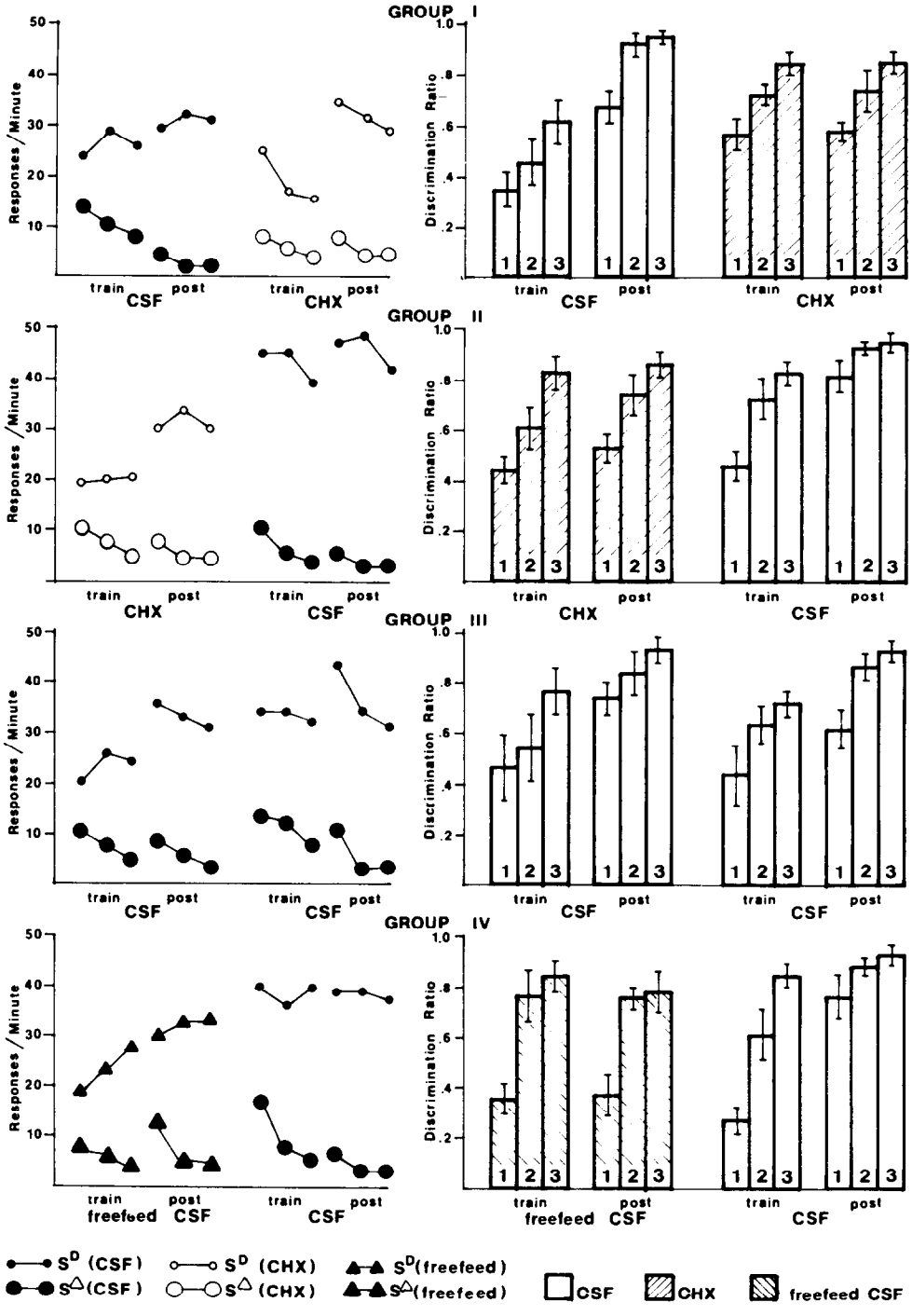

FIG. 1. Response rates and discrimination ratios for each of the three S^{Δ}/S^{D} stimulus presentations during training and postcheck sessions. All values are group means. For the discrimination ratios the vertical lines with cross bars indicate the standard error of the mean.

ance paradigm. The CHX treatment prior to training for the discrimination task blocked almost completely the retention of the trained behavior in the treated animals. This suppression of memory formation was achieved with a 71% inhibition of protein synthesis as determined by the decrease in (^{3}H)-leucine incorporation into brain proteins two hours following the CHX treatment. Thus, for this particular behavioral paradigm and these testing conditions, and for the animals employed in this study (rats), there appeared to be no need to inhibit 85–95% of the brain protein synthesis in order to observe the blockade of memory formation, as has been reported for mice [1]. As a matter of fact, the CHX dose employed was the highest dose that could be tolerated by the experimental animals

and would still allow them to go through their training session. It is possible that the behavior that was being examined is more sensitive to the effects of protein synthesis inhibition than the avoidance procedures commonly employed. Or otherwise, it may simply be a matter of species differences in the response to this type of treatment.

The CHX treatment employed in this study did not only produce a loss of retention of the trained discrimination, but it also caused a decrease in the response rates during the training session. This decrease in responding could not be directly ascribed to a generalized decrease of behavioral activity, since the CHX-treated animals maintained a normal level of exploratory activity two hr after CHX treatment. The decreases in response rates during the training session following CHX treatment were more like the decreases in responding observed in the group that had been food-satiated 24 hr before training. Thus, the CHX effect approximated more closely that of a change in the level of motivation to respond for food reinforcement. Even more interesting was the similarity between CHX treatment and food satiation on the retention of the trained discrimination, since both manipulations produced a blockade of memory formation.

The similarities between protein synthesis inhibition and decreases in the motivational state of the animals described in this study point to the need for a careful evaluation of the specificity of the effects of protein synthesis inhibitors on memory formation. It is possible that even in studies employing avoidance paradigms some of the effects of protein synthesis inhibitors are due to changes in the intensity of emotional responses in the experimental animals.

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