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Effects of Pretraining on Subsequent Cycloheximide Induced Amnesia

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QUINTON, E. E. *Effects of pretraining on subsequent cycloheximide induced amnesia.* PHARMAC. BIOCHEM. BEHAV. 2(5) 585–588, 1974. – In the first experiment, mice were trained on a passive avoidance (PA) task, given one extinction trial, and then were injected with cycloheximide or saline shortly before retraining on the PA task. On a subsequent test trial, the performance of the cycloheximide group was inferior to the saline group, but superior to a cycloheximide group not given the pretraining experience. In the second experiment, one group of mice was given cycloheximide before each of two training sessions while another group received cycloheximide before the first training session and saline before the second. The group given cycloheximide before each training session was amnesic for both sessions to an equal degree, while the other group was amnesic for only the first session. The final test performance of the latter group was similar to that of a saline group not given any pretraining experience. These data seem to indicate that pretraining has limited effect on subsequent cycloheximide induced amnesia, and that such amnesia is the result of impaired memory formation rather than impaired memory retrieval.

Cycloheximide Amnesia Memory Passive avoidance

SEVERAL studies have reported that experimental amnesia of a passive avoidance (PA) response will be considerably attenuated if the animal is given preexposure to the training apparatus (familiarization), or given a pretraining foot shock or electroconvulsive shock (ECS) [8, 11, 12, 13]. These results have been interpreted as indicating that amnesic agents, particularly ECS, induce amnesia by impairing memory retrieval rather than memory consolidation [14]. The familiarization effect has been explained in terms of an "elaboration" hypothesis which contends that preexposure to any of the training conditions establishes a functional memory system into which the memory of the actual training event will be incorporated [8]. This incorporation of the memory of the specific learning event into the preexisting memory system protects the former from the retrieval disrupting effects of the subsequent amnesic treatment [8, 11, 13].

Whether familiarization affects the degree of experimental amnesia is a matter of dispute, as is the general question of whether ECS induces amnesia by impairment of memory formation or impairment of memory retrieval [3, 5, 7]. However, the present study was initiated to determine whether familiarization with the apparatus can protect against amnesia induced by the antibiotic cycloheximide (cyc). Earlier studies investigating the familiarization phenomenon have utilized ECS as the amnesic agent almost exclusively. The amnesia induced by cyc or ECS has many characteristics in common [6, 16, 17], but there are sufficient differences to suggest that the two agents differ in their mechanism of action [1,2]. Nevertheless, it has been suggested that cyc, like ECS, induces amnesia by impairing information retrieval rather than storage [17]. If this is indeed the case, then familiarization should protect against cyc induced amnesia as it has been claimed to do against ECS induced amnesia.

It has been stated that preexposure to any portion of the training trial–amnesic agent sequence will be sufficient to preestablish a memory system into which the memory of the specific learning event will be incorporated and thus be protected from the amnesic agent [8]. It would seem to follow that the closer the preexposure conditions were to the actual training conditions, then the stronger would be the association between the two memory systems, and the less effective would be the amnesic treatment [13].

Experiment 1 was designed to maximize the similarity between the pretraining and training conditions, and to establish a strong preexisting memory system. The procedure was similar to that employed by Jensen and Riccio [9]. Animals were first trained on the PA task and then given one extinction trial, then they were retrained under the influence of cyc. Thus, the animals would have extensive familiarization with the task before the training-amnesic treatment, and a memory system specific to the training paradigm would have been preestablished. If cyc impairs retrieval according to the elaboration hypothesis, then there should be little or no amnesia expressed on a test trial subsequent to the cyc-retraining treatment. However, if cyc impairs memory formation, then there should be amnesia of the relearning trial just as there is amnesia of the initial learning trial [18].

It has also been claimed [8] that the elaboration hypothesis is supported by studies which have found that if an amnesic agent, such as ECS [9,15] CO^2 [15], or hypothermia [20], is administered after each of two spaced

PA training sessions, then there is amnesia of the PA response after the first training session but not the second. Experiment 2 of this study was designed to determine whether amnesia is present after the second training session when cyc is administered before both training sessions.

METHOD

Animals

A total of 138 male C57BL/6J mice (Jackson Laboratories), 12–14 weeks old, were used in this study. They were housed 6/cage, given free access to food and water, and maintained on a 12 hr light/dark cycle.

Apparatus

The behavioral apparatus has been described in detail elsewhere [19]. Basically it consisted of an illuminated platform affixed to one side of a vertical panel and a box (or chamber) affixed to the other side of the panel. An opening in the panel permitted the mouse to leave the platform and enter into the darkened chamber. The platform and floor of the chamber were metal and connected in series to a shock source (Grason-Stadler shock generator, Model 700) set to deliver 2.0 mA a.c. constant current. The animal completed the circuit when it entered the chamber by bridging the platform and chamber floor, and thus received a very brief foot shock.

Drugs

Either 125 mg/kg of cyc (approximately 0.3 ml of a 10 mg/ml solution) or 0.3 ml of 0.15 M NaCl (sal) was injected subcutaneously in the dorsal thoracic area 30 min before the appropriate training sessions. This dose of cyc has been reported to be an effective amnesic dose in the C56BL/6J, and results in an inhibition of cerebral protein synthesis of 90% or more 30 min after injection [5].

EXPERIMENT 1

Procedure

To insure that a strong PA response was established, the mice were given two training trials initially on the PA task. The mice were placed on the platform and permitted to spontaneously enter into the chamber, at which time they received a foot shock. The latency to enter the chamber was recorded as the step-through-latency (STL). The mice remained in the chamber for 10 sec and were then returned to the entrance platform for a second training trial. They were gently pushed into the chamber if they did not spontaneously enter within 10 sec. The mice again remained in the chamber for 10 sec before being removed and placed in a holding cage where they remained until all mice in the same housing group were run. Twenty-four hr later the mice were given an extinction trial with a criterion STL of 60 sec. If a mouse did not enter the chamber within 60 sec, it was gently pushed into the chamber and given an STL of 60 sec.

Each housing group was then randomly assigned to one of 2 drug treatment groups (a total of 24 mice in each group). For one treatment group, saline was injected subcutaneously 1 hr after the extinction trial. For the other, cyc was injected subcutaneously 1 hr after the extinction trial. All mice were then given one retraining trial 30 min after the injection. On that trial, if an animal did not step into the chamber within 60 sec, then it was gently pushed into the chamber. Thus, all animals received a foot shock on the retraining trial. All mice were given the final test trial 72 hr after the retraining trial. The criterion STL for that test trial was again 60 sec.

Two additional groups of mice (N = 25/group) were not given the pretraining experience, but were injected with cyc or sal 30 min before being given one training trial and tested 72 hr later.

Results

The pretraining groups which later received cyc or sal before retraining did not differ on the first training trial STLs ($\bar{X}$ STLs: sal, 9.0 sec; cyc 6.7 sec; $p>0.1$, two-tail t), extinction trial STLs (Median STLs: sal, 52.5 sec; cyc, 58.5 sec; $p>0.9$, Mann-Whitney U), or retraining trial STLs (Median STLs: sal, 13.5 sec; cyc, 25.5 sec; $p>0.23$, Mann-Whitney U). However, on the final test trial the sal injected group exhibited stronger PA than did the cyc injected group (Table 1). Only 4 sal and 5 cyc mice had to be forced into the chamber on the retraining trial. For the sal group, PA performance improved from the retraining trial to the test trial (sign test, $p<0.02$), but the cyc group did not show any improvement ($p>0.1$).

The two groups which did not receive the pretraining experience also did not differ on the initial training trial STLs ($\bar{X}$ STLs: sal, 3.68; cyc, 5.68; $p>0.1$, two-tailed t), but were significantly different on the test-trial STLs (Table 1).

Table 1 summarizes the results of this experiment. The median STLs on the final test trial for each treatment group are given along with the p values for the Mann-Whitney comparisons (two-tail) between each group.

TABLE 1

MEDIAN STLs ON THE FINAL TEST TRIAL FOR EACH GROUP AND p VALUES (TWO-TAIL) ASSOCIATED WITH MANN-WHITNEY U COMPARISONS BETWEEN GROUPS

		p		
Groups†	STL*	2	3	4
1	57.5	0.016	0.01	<0.001
2	31.0		>0.1	0.001
3	28.0			<0.001
4	7.0			–

*STL in seconds

†1 = pretrain/sal; 2 = pretrain/cyc; 3 = non-pretrain/sal; 4 = non-pretrain/cyc.

EXPERIMENT 2

Procedure

Two groups of mice were injected with cyc 30 min before being given one training trial on the PA task.

Seventy-two hours later, one group (N = 20) was again injected with cyc and the other group (N = 20) was injected with sal. Both groups of mice were given a test/retraining trial 30 min after the injection. The animals were given a final test trial 72 hr later.

Results

The two groups did not differ on the initial training trial STL ($\bar{X}$ STLs: cyc, 6.1 sec; sal, 6.2 sec; $p>0.1$, two-tail t) or the test/retrain trial (median STLs: cyc, 11.5; sal, 7.5; $p>0.1$, Mann-Whitney U, two-tail). However, the group which received sal before the test/retrain trial demonstrated greater PA on the final test trial than did the group given cyc before the test/retrain trial (median STLs: cyc, 13; sal, 31; $p = 0.02$, Mann-Whitney U, two-tail). No animal in either group reached criterion latency (60 sec) on the test/retrain trial. On the final test trial, one mouse from the cyc group and 5 mice from the sal group reached criterion latency (60 sec). Additionally, the PA performance of the sal group increased significantly from the test/retrain trial to the final test trial (sign test, $p<0.001$), whereas the performance of the cyc group did not (sign test, $p>0.1$). Additional comparisons (Mann-Whitney U, two-tail) between the cyc and sal groups from this experiment and the nonpretrain cyc and sal groups from Experiment 1 revealed that the test performance of the two sal groups were not significantly different ($p>0.1$), nor was the test performance of the two cyc groups significantly different ($p>0.1$).

DISCUSSION

When the final test performance of the pretrain and nonpretrain groups in Experiment 1 are compared, it is apparent that the pretraining experience enhanced the test performance of both groups given cyc or sal before the retraining trial. This suggests that the original training did establish a memory system for the PA response which survived the extinction training and potentiated the effectiveness of the retraining trial in reestablishing the PA response.

However, it is equally apparent that the test performance of the pretrain/sal group was superior to that of the pretrain/cyc group. This suggests that cyc given before retraining effectively induced amnesia of the retraining experience, even though the degree of amnesia was less than in nonpretrain/cyc animals. This interpretation is supported by the finding that the number of mice in the cyc group whose performance improved from the retraining trial to the test trial was not significant.

These data do not clearly support either the impaired memory retrieval [14] or impaired memory formation [4, 6, 19] interpretations of cyc induced amnesia. The superior test performance of the pretrain/cyc group compared to the nonpretrained/cyc group is consistent with the elaboration hypothesis [8] and the impaired memory retrieval interpretation of cyc induced amnesia.

However, the impaired memory formation interpretation is supported by the difference in test performance between the pretrain/cyc and pretrain/sal groups. It could be argued that cyc does not induce total amnesia and that the weak memory that does develop is enhanced by transfer from the latent memory of the strong original training sufficiently to elevate test performance above that of the nonpretrain/cyc group, but not equal to that of the pretrain/sal group.

The results of the second experiment are less ambiguous. They indicate that if cyc is injected before each of two PA training session, it induces amnesia of each training session to an equal degree. This effect of cyc is different from that reported with other amnesic agents [9, 10, 15, 20] and suggests that cyc is a more potent amnesic agent. These results are also contrary to a state dependent interpretation of the cyc impairment.

According to the impaired retrieval hypothesis of experimental amnesia, the amnesic agent does not block memory formation of the experience, but rather blocks memory retrieval. This hypothesis would seem to be supported by several studies which have reported that posttraining exposure to cues associated with the training experience permits the memory to be expressed on subsequent test trials [14]. If memory formation is not impaired by an amnesic agent and memory can be reactivated by mere exposure to training cues, then one would expect that additional training, following the original training-amnesic treatment, should enhance the response tendencies established during the original training. Performance on a subsequent test trial should therefore be stronger than it would have been had the animal not received the training-amnesic treatment.

However, this expectation was not supported in this study. The test performance of the group given cyc before the first training trial and sal before the second was no better than that of a group which received only one training trial. (non-pretrain/sal group from Experiment 1). These results strongly support the interpretation of cyc induced amnesia as being the result of impaired memory formation, rather than impaired memory retrieval.

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