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$ACTH_{4-10}$ and improved use of information in rats

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Water-deprived rats were trained to approach one of four tables in an elevated X-maze for water reward. Before the first trial of each daily session, the experimenter provided information about the location of water by placing the rat on the table that was correct for that day. A correction procedure was used. The same animals served as subjects in two experiments in which either three trials (Experiment I) or seven trials (Experiment II) were given each day. In Experiment I all subjects were tested without injections, and 1 h after injection of saline or 20-, 50-, or 75-μg doses of $ACTH_{4-10}$. In Experiment II subjects were tested 1 h after injection of 75 μg of $ACTH_{4-10}$ on 3 days, and 1 h after injection of saline on the 3 previous days and the 3 following days. In both experiments the percentage of correct responses on the first trial was greater after 75 μg of $ACTH_{4-10}$. Performance on subsequent trials was not affected. The improvement could reflect a better use of the information provided prior to the first trial or a reduced tendency to visit the table that had been correct on the previous day.

ACTH and several analogs of ACTH inactive on the adrenal glands are capable of influencing behavior. For example, $ACTH_{4-10}$ seems to delay extinction of avoidance behavior (for reviews see de Wied, 1969, 1974) and ACTH has also been reported to enhance the retention of appetitive responses (Guth, Levine, & Seward, 1971). There are also some reports of improved acquisition of tasks following ACTH administration (Beatty, Beatty, Bowman, & Gilchrist, 1970; Guth, Levine, & Seward, 1971; Pagano & Lovely, 1972), although hypophysectomized rats can acquire conditioned responses in the absence of pituitary ACTH (e.g., Lissák & Bohus, 1972; Stone & King, 1954). The ways in which ACTH and its analogs influence behavior are still most uncertain. Further research is needed to determine the range of tasks in which ACTH is effective.

In the study of the biological substrates of learning and memory, it is desirable to distinguish the incorporation of information from the execution of motor acts based on the information. In this experiment we wanted to study the effects produced by $ACTH_{4-10}$ on behavior guided by a single informative event as contrasted with a more general effect on acquisition or retention of behaviors learned over the course of repetitive trials. When an animal acquires a task after multiple training trials, it is difficult, if not impossible, to establish just when "learning" takes place or what is being learned, e.g., associations between stimuli, responses, or a combination of the two. Furthermore, an increase in the number of conditioned responses emitted by an animal during training may reflect processes that are related to performance rather than to learning itself.

Accordingly, the testing paradigm selected was one in which water-deprived animals were "informed" at the start of training each day about the location of water in an elevated maze. Thus, performance on the first trial of each day's training would reflect information obtained before a response had been made, and subsequent trials would reflect the enhancement of learning over the course of the day's training. For this purpose, we modified the "three-table problem" used by Maier (1932). In the original task, rats were shown the location of the reward, one of the three tables, and then allowed to choose among the tables when started from a different location. To make the problem somewhat more difficult for the animals, we used four tables instead of three.

EXPERIMENT I

Method

Materials. The $ACTH_{4-10}$ (OI 63, Batch WB 1199 K1) was generously provided by Dr. H. Van Reizen of Organon International BV, Oss, The Netherlands.

Subjects. Ten male Long-Evans hooded rats from Charles River Breeding Laboratories were used as subjects. At the beginning of the experiment, they were from 40 to 60 days of age. During shaping and training procedures, the rats were maintained on a 23-h water-deprivation schedule, with water provided ad lib in the home cage for 30 min after each session.

Apparatus. The apparatus used in the study was a modification of the Maier (1932) three-table maze. In this study, however, four tables were used. The tables were arranged as corners of a rectangle, with a 1.5-in.-wide elevated pathway running from each table to a central intersection. The distance from each table to the center position was 2.5 ft. The table tops and runways were 30 in. above the floor.

Each of the tables differed from the rest in its position in the room, as well as its shape, size, and top (metal, wood, paper, or vinyl). On the side facing the center of the maze, each table had a plywood wall, 8 in. high and 30.5 in. wide, containing a 3 x 3.75 in. entrance. Whenever present, a small Petri dish of water was placed just inside the entrance and to the right. The tables were illuminated by two large operating room lights.

Procedure. One testing session lasting about 20 min was given each day. During initial shaping procedures, each rat was placed on a table with a dish of water. After it drank for a few seconds,

Table 1
Mean Number of Incorrect Responses on the Three Trials Given Each Day in Experiment I

Days	Treatment	Trial 1	Trial 2	Trial 3
1-4	None	1.05	.83	1.37
5-7	None	1.27	.77	.70
8-10	Saline	1.27	.80	.70
11-16 (3 days)	A/B Saline	1.20	.73	.43
11-16 (3 days)	A/B $ACTH_{4-10}$ 20 μg	1.00	.67	.67
17-18	$ACTH_{4-10}$ 50 μg	1.44	.61	.50
19-22	$ACTH_{4-10}$ 75 μg	.72	.90	.46

Note–Treatment A/B refers to the condition in which half of the animals received saline and half received 20 μg of $ACTH_{4-10}$ initially and after 3 days of this treatment had the solutions reversed (see text for further details). The data from the two groups have been combined in the table.

it was placed close to the door on one of the runways and permitted to run back to the table and drink again. This procedure was repeated several times. On each subsequent run, the rat was started slightly farther out on the runway, until eventually each run began just outside an entrance to another table with the rat facing toward the center of the maze. Initially, water was available on all of the tables except the one at which the trial started.

When all rats were moving rapidly among all tables, the first part of the training began. Water was placed on only one of the four tables. The animal was placed on the table with the water and permitted to drink a small amount. Then the rat was removed and placed on one of the other runways, just outside the door, facing toward the center of the maze. A response was recorded whenever an animal placed all four paws onto a table. If the rat went directly from the starting table to the table with the water, the response was correct. If it went to another table, the run was incorrect. Occasions on which the animals turned around on the runway and went onto the starting table were noted, but a correct or incorrect response was only counted when the animal left the original start table and made a choice among the three other tables.

This testing procedure was repeated for 7 days. The table to have the water was determined randomly each day. Three daily trials were started from each of the three other tables in succession, the order being also determined randomly. A correction procedure was used throughout.

On Days 8 and 9, .2 ml of physiological saline was administered s.c. to each rat 10 min before training. On Day 10 the saline was administered 60 min before training. To half of the animals (Group A), .2 ml of saline was administered for 3 days (11-13) followed by 20 μg of $ACTH_{4-10}$ in .2 ml of saline for the next 3 days (14-16). The other half of the animals (Group B) received the 20-μg dose of $ACTH_{4-10}$ for the first 3 days (11-13) followed by saline on the next 3 days (14-16). The nature of the solutions given the rats was unknown to the experimenter (B. W.) at the time of administration. On Days 17 and 18, all animals were tested 60 min after administration of 50 μg of $ACTH_{4-10}$ in .2 ml of saline, s.c., and on Days 19-22 all animals were tested 60 min after 75 μg of $ACTH_{4-10}$ in .2 ml of saline.

Results

The data generated by all of the animals were examined for response or table preferences. Neither type of preference was found that was exhibited by all of the animals or by any treatment group, although some animals had specific preferences. Overall, responses to the four tables were equally distributed, as were the tendencies to turn right, left, or go straight forward, both in the normal state and after injection. Returns to the starting table, considered to be a possible indication of emotionality, were not related to any particular phase of training or to the administration of ACTH at any level.

First Trial Performance. Table 1 shows the mean number of tables visited before the correct table on the three trials given each day in Experiment I for each condition of testing. Condition A/B refers to the condition in which 20 μg of $ACTH_{4-10}$ was administered to one half of the animals and saline to the other half for 3 days. Then the solutions were switched between the groups for 3 more days. For purposes of analysis, the data from all animals administered 20 μg $ACTH_{4-10}$ both before and after saline have been combined, as have the data from animals administered saline before and after $ACTH_{4-10}$. Table 2 shows the percentages of first trials that were correct under the various conditions, and the percentages of first-choice responses to the table that had been correct on the previous day. Animals performing under the 75 μg of $ACTH_{4-10}$ were more likely to make a correct first choice than they were under the other conditions. Statistical analysis was made by ranking for each animal the number of correct first trial responses under the saline control treatment, the 20-μg dose of $ACTH_{4-10}$, 50-μg $ACTH_{4-10}$, and 75-μg $ACTH_{4-10}$ for each animal. The ranks were then evaluated by the Friedman two-way analysis of variance by ranks (Siegel, 1956). This revealed a χ_r^2 of 9.48 ($p < .01$). By the use of the sign test (Siegel, 1956) to pairs of ranks for the treatment conditions, it was found that the only ones significantly different from each other were the 50- and 75-μg conditions ($p < .01$), although there was a tendency for the 75-μg condition to produce better first trial performance relative to all other conditions.

An examination of first trial responses before the higher dose levels of $ACTH_{4-10}$ were given reveals that all of the animals were consistent in their method of responding. For example, one animal had a preference for Table D. All but three of the responses made by this subject before injections began were to this one table. Other animals exhibited preferences for tables or turns which lasted for some number of trials.

Table 2
Percentage of First Trials that were Correct and Percentage of First Trials Made to Table Correct on Previous Day at Different Times and Under Different Treatments in Experiment I

Days		Percentage of Animals Making Responses to: Correct Table	Table Correct on Day Before
6-7	End of initial testing	37	30
8-10	Saline	37	10
11-16	Saline (3 days)	30	44
11-16	ACTH 20 μg (3 days)	37	44
17-18	ACTH 50 μg	17	72
19-22	ACTH 75 μg (4 days)	51	44

Table 3
Mean Number of Incorrect Responses During Experiment II

Days	Treatment	Trials 1	2	3	4	5	6	7
1-3	Saline	1.33	.52	.52	.22	.15	.30	.15
4-6	ACTH 75 μg	1.00	.48	.41	.41	.19	.26	.33
7-9	Saline	1.78	.30	.37	.07	.19	.19	.04

Incorrect responses would then be altered in favor of a different table or turn for another few trials. However, it should be noted that even before the higher dose levels were administered, the majority of first responses were either to the correct table or to the table correct on the previous day.

From this analysis it would appear that the execution of correct responses on first trials on any particular day was impeded by a tendency to approach the table rewarded on the previous day and by individual tendencies to make specific turns or to approach specific tables.

Second and Third Trial Performance. Table 1 presents the mean number of tables selected by each animal before the correct table on the second and third trials for all treatments. A general improvement can be observed on the performance of animals over the course of training on the second and third trials (except for the second trial under 75 μg $ACTH_{4-10}$). Performance improved on the second trial, probably because by the second trial all of the animals had not only been exposed to the reward before the first trial but, in addition, had also made a response to the correct table and were rewarded for it. No effects of $ACTH_{4-10}$ could be found on performance on the second and third trials.

EXPERIMENT II

Method

For 17 days after the end of the first experiment, the animals received food and water ad lib. They were then put on a 23-h water-deprivation schedule and reshaped to make approach responses to the tables. This preliminary training lasted for 6 days, by the end of which all animals were performing well. One animal that failed to respond under 50 μg $ACTH_{4-10}$ in Experiment I continued to exhibit poor performance and was not included in further tests.

After the preliminary training, the animals were again tested in the four-table problem. Several changes were made in the training procedures. The number of trials given each day was increased from three to seven. The start tables were randomly determined with the stipulation that on the first three trials each nonreward table would be represented once. The order of the first three trials was repeated for the next three trials. The 7th and last trial was begun from the starting table used in the first trial. In Experiment II a response was recorded to a table whenever an animal extended its head and forepaws onto the table. Training was given for 9 days. On the first and last 3 days, the animals were run 1 h after injection of .2 ml of saline. On the middle 3 days the animals were run 1 h after an injection of the same volume of saline containing 75 μg of $ACTH_{4-10}$. The order of table selection and the latency of response to the correct table were recorded.

Results

The mean number of incorrect responses made after saline and after 75 μg $ACTH_{4-10}$ are given in Table 3. As would be expected on the basis of the correction procedure used in the experiment, the animals improved over the course of the seven trials given each day. The percentages of first trial responses made to the correct table and to the table correct on the day before are given in Table 4. The data indicate that more correct responses were made on the first trial after 75 μg $ACTH_{4-10}$ than after saline. This was evaluated by an analysis of the number of correct first trial responses made after $ACTH_{4-10}$ relative to the number made after the first and second saline series. A Friedman two-way analysis of variance by ranks indicated a significant effect of the ACTH treatment ($\chi_r^2 = 15.03$, $p < .01$). Since every animal after $ACTH_{4-10}$ performed at least as well as it did after saline and six animals performed better relative to the first saline series and five performed better relative to the second series, the probabilities of this effect could be established at .016 and .032, respectively, for these comparisons using a binomial expansion.

A Friedman two-way analysis of variance by ranks also showed that the ACTH treatment altered the animal's tendency to approach the table rewarded on the previous day relative to the saline treatment days before and after ($\chi_r^2 = 8.05$, $p < .05$). No animal made more responses to the previously rewarded table on the 3 days of $ACTH_{4-10}$ treatment than it did after saline treatment on the 3 days either before or after the peptide treatment. During the $ACTH_{4-10}$ treatment, eight of the nine animals made fewer responses to the table that was correct on the previous day than they did on the 3 previous days. Five animals made fewer such responses after $ACTH_{4-10}$ treatment than after saline on the 3 days following the peptide treatment. Evaluation of these differences by estimates based on the binomial expansion indicates $p < .01$ and $p < .05$ for the two comparisons, respectively. The Friedman two-way analysis of variance of the numbers of correct responses made by animals on Trials 2 through 7 failed to reveal differences between the saline and $ACTH_{4-10}$ treatments.

Table 4
Percentage of First Trials that were Correct and the Percentage of First Trials Made to the Table that Had Been Correct on the Previous Day in Experiment II

Treatment	Percentage of Animals Making Responses to: Correct Table	Table Correct on Day Before
Saline	30	67
$ACTH_{4-10}$ 75 μg	56	22
Saline	30	44

DISCUSSION

From the results of the present experiments, one observation is of special interest, that is, the improved performance on the first trial under 75 μg of $ACTH_{4-10}$. This was associated with a corresponding reduction in the number of visits to the table that had been correct on the previous day. It was this tendency that represented the major source of errors. Before the first trial of each session, the experimenter provided information about the location of the water by placing the rat on the correct table. The better use of this information as reflected in more correct responses would produce some reduction in the number of incorrect responses including those to the table that had been correct on the previous day. Therefore, the effect of 75 μg of $ACTH_{4-10}$ could have been to improve the use of the information provided prior to the first trials or to reduce the tendency of animals to perseverate responses acquired on the previous day.

No dose of $ACTH_{4-10}$ used in the present experiment produced a general enhancement of learning or performance as measured on trials after the first. Improvements in performance were noted over the course of training but no special effect of the peptide was observed.

It might be suggested that the improved performance of the animals treated with the high dose of the ACTH peptide could be a consequence of an enhanced motivation for the water reward. This is unlikely for two reasons. First, there were no differences in the latencies of response on the first trials or on later trials following $ACTH_{4-10}$ administration in Experiment II. Second, there was no facilitation by the peptide of the performance on Trials 2 through 7 in Experiment II. An increase in motivation to obtain water would probably both reduce response latency and facilitate performance on all trials given each day.

In general, the 20- and 50-μg doses of $ACTH_{4-10}$ seemed to be without effect. However, there was some suggestion of a possible interaction between the level of training and the peptide, since the subgroup that received the peptide first in Experiment I demonstrated improvement which continued throughout the later saline trials. However, the subgroup that received the saline for 3 days before the peptide did not demonstrate this improvement in performance.

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