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ACTH, but not Corticosterone Impairs Habituation and Reduces Exploration

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FILE, S. E. *ACTH, but not corticosterone impairs habituation and reduces exploration.* PHARMAC. BIOCHEM. BEHAV. 9(2) 161–166, 1978.—The habituation of an orienting response to auditory stimuli was impaired in rats by the administration of $ACTH_{1-24}$. This impairment is unlikely to be due to the action of corticosterone because of the time course of the effect, because injected corticosterone had no effect on habituation and because the peptide fragment $ACTH_{4-10}$, which does not release corticosterone, also impaired habituation. Both $ACTH_{1-24}$ and $ACTH_{4-10}$ reduced the level of exploration measured in a holeboard, but corticosterone had no significant effect. However ACTH did not impair habituation and exploration, thus providing further evidence that there are different mechanisms underlying habituation of orienting and habituation of exploration.

Distraction Habituation Exploration ACTH Corticosterone

THERE IS some evidence that adrenocorticotrophic hormone (ACTH) can affect the rate of habituation. Administration of $ACTH_{1-24}$ and $ACTH_{1-10}$ resulted in slower habituation of the human EEG response to auditory and visual stimuli [6] and low circulating levels of ACTH, produced by hypothalamic implants of cortisol, resulted in more rapid within-session habituation of the startle response of the rat [23]. In view of these findings it is puzzling that adrenalectomy, which results in high circulating levels of ACTH [4], was without effect on habituation of startle responses [5] or on habituation of flexor withdrawal reflexes [26].

There is no evidence that the glucocorticoids themselves have any effects on habituation. The impairment in EEG habituation produced by $ACTH_{1-24}$ is unlikely to be due to release of glucocorticoids since $ACTH_{1-10}$, which does not stimulate steroid release [34], also impaired habituation. Pearson and Vickars [26] found no effect of cortisol on habituation of the rat flexor withdrawal reflex. However, it must be remembered that cortisol is not the main glucocorticoid in the rat and it is possible that corticosterone would have a different action.

The present experiments were designed to investigate the effects of ACTH and of corticosterone on two types of habituation in the rat, using doses of these hormones that would fall within the physiological range. Thus, a dose of $ACTH_{1-24}$ (5 μg/100 g) was chosen that would mimic the plasma corticosterone levels produced during moderate stress [19]. The effects of a shorter peptide fragment, $ACTH_{4-10}$, were also studied. Two forms of this fragment were used, the normal one with L-phenylalanine in position 7 (Org 0163), the other $ACTH_{4-10}$:7 Dphe (Org 0164) in which L-Phenylalanine was substituted by D-phenylalanine. These two forms were included because the substitution of D-phenylalanine in $ACTH_{1-10}$ and in $ACTH_{4-10}$ has been found to facilitate extinction, in contrast to the delay of extinction caused by $ACTH_{1-10}$ [3]. The effects of corticosterone were assessed in two ways: 1) by testing rats 30 min after injection of ACTH, at which time there would be a considerable release of corticosterone but the levels of ACTH would be low [4,20]; 2) by injecting corticosterone in a dose of 3 mg/kg. This dose of corticosterone was administered to a group of rats from the same suppliers, which had been housed and handled in a manner identical to that in the present experiment. The corticosterone injections were given at the same time of day and at the same time of year as the present experiments were conducted, and resulted, 20 min after injection, in a mean plasma corticosterone level of 26.7 μg/100 ml [13], which is equivalent to that produced in moderate stress [20].

The effects of ACTH and corticosterone were assessed in two different habituation paradigms. In Experiment 1 habituation of the orienting response was measured. Rats were engaged in a baseline activity of licking and orienting was measured by the interruption in licking that occurred when tones were presented [9,11]. In Experiment 2 exploration and its habituation were studied in a 4-hole holeboard [14,15] using the number of head-dips and the mean time spent head-dipping as measures of exploration.

EXPERIMENT 1: HABITUATION OF THE ORIENTING RESPONSE

METHOD

Animals

Ninety-eight male hooded rats *(Rattus norvegicus)* from Olac Ltd (Bicester), 300–350 g, were housed in groups of six in a room maintained at 25°C in an 11 hr light:13 hr dark cycle. The rats received an initial 48 hr period of water deprivation and thereafter received water during and immediately following the test, in sufficient quantity to maintain a steady body weight. Food was available ad lib.

Apparatus

The test box, 19×19×26.5 cm, was enclosed in an acous-

tically insulated chamber. A slit in the end wall gave access to a water spout and a drinkometer recorded the rat's licking. Tones were delivered via a loudspeaker positioned in the lid of the test box at the water spout end. The tones used were 7 kHz, 85 dB (re 0.0002 dynes. cm^{-2}); 7 kHz, 75 dB; 12 kHz, 85 dB; and 7 kHz, 85 dB pulsed at 1 sec on and 1 sec off. All the tones lasted for 9 sec.

Drugs

$ACTH_{1-24}$ (Synacthen, CIBA) was dissolved in 0.9% saline to a concentration of 25 μg/ml. A dose of 5 μg/100 g was chosen on the basis of previous behavioural work [9]. A control group received equal volume injections of 0.9% saline.

$ACTH_{4-10}$ (Organon) was dissolved in deionised water to a concentration of 20 μg/ml. This gave an acid solution and so a control group was included that received injections of water to which dilute HCl had been added until it matched in pH the drug solution. The dose of $ACTH_{4-10}$ was chosen on the basis of pilot experiments.

Corticosterone (Sigma Chemical Co.) was suspended in 5% ethanol to a concentration of 2 mg/ml. The dose of 3 mg/kg was chosen on the basis of the rise in plasma corticosterone following this dose [13]. A control group receiving 5% ethanol was included.

Procedure

Because it was impossible to test all 98 rats at the same time, the experiment was run in two phases. Fifty rats were randomly allocated to Groups 1–4 and tested in the first phase; 48 rats were randomly allocated to Groups 5–8 and tested in the second phase. All the injections were given intraperitoneally and the interval between injection and time of test is shown in Table 1.

TABLE 1

THE NUMBER OF RATS TESTED IN EACH EXPERIMENTAL GROUP AND THE TIME BETWEEN INJECTION AND THE START OF TEST

Group	N	Time (Min)
1. Saline control	5	3
	5	30
2. $ACTH_{1-24}$ (5 μg/100 g)	10	3
	10	30
3. 5% ethanol control	10	20
4. Corticosterone (3 mg/kg)	10	20
5. Acidified water control	12	3
6. $ACTH_{1-24}$ (5 μg/100 g)	12	3
7. $ACTH_{4-10}$ (D) (4 μg/100 g)	12	3
8. $ACTH_{4-10}$ (L) (4 μg/100 g)	12	3

On the day following the water deprivation no injections were given and each rat was allowed 15 min in the apparatus, with free access to the water spout.

The next day each rat received its appropriate injection and was placed in the test box after the appropriate time interval. One min after the rat was placed in the box, recording of its licking was started. When the rat had made 100 licks it entered a 9 sec control period during which the number of licks made (A) was recorded. Following this period the next 20th lick switched on the tone for 9 sec and the number of licks made during the tone (B) was counted. Control and tone periods alternated until habituation criterion was reached. This was taken as 3 successive tone presentations producing a distraction ratio of $^{A-B}/_{A} \leq 0.10$. The session terminated when criterion was reached or after 15 min. All but seven rats reached criterion on this first day. These seven rats (three injected with $ACTH_{1-24}$, two with $ACTH_{4-10}$ (D), two with $ACTH_{4-10}$ (L)) received a second day's training and all but two reached criterion on this day. The remaining two rats reached criterion the following day. Rats tested on the 2nd and 3rd days received the same injections on these days as they had on the first day.

The day after reaching habituation criterion each rat was given the same injection as before and first presented with the 7 kHz 85 dB tone, to check that it was still habituated to this tone. Each rat then received a series of tone presentations in which a novel tone was presented (to test for the specificity of habituation) and then the original tone was presented again (to measure dishabituation). The original tone was then presented until it again produced no distraction and then the next novel tone was given. In this way three novel tones were presented to each rat and dishabituation was measured on three occasions. One of the novel tones represented a change in frequency from the original tone, another an intensity change and the third was a change from a continuous to a pulsed tone. The order of presentation of the three novel tones was randomly determined for each rat.

RESULTS

As there was no significant difference in the scores for the control rats tested 3 and 30 min after saline injection the scores from these two subgroups were combined.

There were no significant differences either in the lick rate or in the initial distraction ratio between any of the drug-injected groups and their respective controls (see Table 2). However, there were differences in the rate of habituation. The rats tested 3 min after injection with $ACTH_{1-24}$ habituated significantly more slowly than did the control animals ($p<0.02$, Mann-Whitney U two-tailed test). This change in habituation is unlikely to be due to the release of corticosterone since rats tested 30 min after ACTH injection and those injected with 3 mg/kg corticosterone showed the same rate of habituation as did the controls.

The rats injected with $ACTH_{1-24}$ and tested in the second phase of the experiment also showed significantly slower habituation compared with the control rats ($p<0.002$, Mann-Whitney U two-tailed test), as also did the rats injected with the $ACTH_{4-10}$ fragments ($p<0.002$ for $ACTH_{4-10}$ (D) and $p<0.02$ for $ACTH_{4-10}$ (L)).

When the parameters of the habituated tone were changed 80–90% of the rats in every group showed a distraction response to at least one of the changes. The mean distraction ratios to the changes ranged from 0.57 for the saline controls to 0.38 in the $ACTH_{1-24}$ group tested 3 min after

TABLE 2

MEAN DISTRACTION RATIO TO FIRST TONE PRESENTATION AND MEAN TRIALS TO HABITUATE TO TONE STIMULI FOR GROUPS OF RATS INJECTED WITH $ACTH_{1-24}$, $ACTH_{4-10}$ AND CORTICOSTERONE

	Distraction Ratio $\left(\frac{A-B}{A}\right)$	Trials to Habituate
Saline	.60	4.6 ± 1.1
$ACTH_{1-24}$ (3')	.58	11.6 ± 2.7†
$ACTH_{1-24}$ (30')	.67	7.0 ± 1.8
Control (5% ethanol)	.69	5.1 ± 1.0
Corticosterone	.67	5.0 ± 1.0
Acidified water	.71	4.5 ± .67
$ACTH_{1-24}$	.68	10.5 ± 2.7*
$ACTH_{4-10}$ (D)	.49	10.8 ± 2.9*
$ACTH_{4-10}$ (L)	.56	8.5 ± 1.5†

* $p<0.002$ Mann-Whitney U two-tailed
† $p<0.02$ Mann-Whitney U two-tailed

injection, but this difference was not significant.

When the habituated tone was presented again following a stimulus change, 66–80% of the rats in each group showed dishabituation, and the mean distraction ratios ranged from 0.56 to 0.43, but none of the differences between the groups was signficant.

DISCUSSION

The ability of ACTH to retard habituation to auditory stimuli seems to be due to its direct action on the central nervous system and to be independent of its steroid-releasing function. The same results were obtained with the short peptide fragments of $ACTH_{4-10}$ as with $ACTH_{1-24}$, suggesting that the effect on habituation is likely to be due to the action of a fragment of ACTH. The data that are inconsistent with the results of this experiment are those that show no change in habituation in adrenalectomised rats, which would have high circulating levels of $ACTH_{1-39}$. Thus, either the full peptide chain is itself inactive, or cleavage may occur leaving peptide fragments, other than 1–24 or 4–10, that are inactive in an habituation test.

No differences were found between the actions of $ACTH_{4-10}$ with D- as opposed to L-phenylalanine in the 7th amino acid position. These two fragments were reported to have opposing effects on extinction performance [35], but there have been no other reports of different behavioural actions.

The delayed habituation shown by the rats injected with ACTH may be seen as a persistence in responding to tones. The tones were stimuli that were irrelevant in the sense that they did not predict reinforcement and thus the effect on habituation might be analogous to the delayed extinction produced by ACTH and related peptides [16, 17, 24, 27], i.e. a persistence in responding when the conditioned stimulus no longer predicts reinforcement. However, perseveration of responding may not necessarily be the correct interpretation of the ACTH effects. Bohus [2] has shown that not all measures of extinction are delayed by ACTH; and α-MSH (a related peptide that also retards extinction) produced more rapid reversal in discrimination learning, where the stimuli that predict reinforcement were changed. It has been suggested [33] that the behavioural effects of ACTH may be due to increased motivation or attention. It is most unlikely that increased motivation could account for the habituation results. The tone stimuli did not signal reinforcement and the amount of water available was independent of the rat's response to the tones. By continuing to distract to the tones the rats injected with ACTH would have experienced a slight delay in consumption of water: presumably, an increased motivation would, if anything, have made the rats less likely to distract to the tones. ACTH improves retrieval in several learning tasks [17, 28, 29] and this has been interpreted as showing enhanced attention. One possible interpretation of the data reported in this experiment is that the rats were showing enhanced attention to irrelevant stimuli; since ACTH did not alter the initial distraction response this would seem a less satisfactory description of the results than one in terms of delayed habituation.

EXPERIMENT 2: HABITUATION OF EXPLORATION

METHOD

Animals

One hundred and twenty-nine male hooded rats, 250–300 g, were used in this experiment. They were housed in groups of six with food and water available ad lib. They were maintained under an 11 hr light-13 hr dark cycle, with the lights on at 0700 hr.

Apparatus

Exploration was measured by the time spent head-dipping in a holeboard, because this provides a reliable measure of directed exploration relatively uncontaminated by motor activity [14,15]. The importance of distinguishing between motor activity and exploration has been stressed by many authors [1, 22, 30, 32], and exploration measured in the open-field and Y-maze is heavily confounded with motor activity. The holeboard was 55×55 cm with walls 45 cm high. In the floor were four equally spaced holes, 3.8 cm dia. The output from infrared photocells placed just below the floor went to counters and provided automated measures of the number of head-dips and the mean time spent head-dipping. Infrared cells placed in the walls of the box provided a measure of motor activity, a count being scored each time a beam was interrupted. For part of the experiment objects held in glass funnels were placed under the holes. These objects were chosen to smell and feel different from each other. They were a piece of rubber, a china pipe, burnt matches and tissue paper. The illuminance on the floor of the box was 25 scotopic lux.

Drugs

These were dissolved as described in Experiment 1.

Statistical Analysis

The data were analysed by split-plot analyses of variance [25]; in each case the drug treatment (at two levels—drug and appropriate control group) was the between-subjects factor, and days, at three levels, and trial periods, at four levels, were the factors with repeated measures. (Each 10 min test was divided into four 2.5 min trial periods, to give a measure of within-session habituation.)

Procedure

This experiment was also conducted in two phases. In the first phase 48 rats were randomly allocated to the following drug groups: corticosterone 3 mg/kg (N=9); controls receiving 5% ethanol (N=9); $ACTH_{1-24}$ 5 μg/100 g with rats tested 3 min after injection (N=10); $ACTH_{1-24}$ 5 μg/100 g with rats tested 30 min after injection (N=10); saline controls tested 3 min (N=5) and 30 min (N=5) after injection.

In this part of the experiment the rats were tested with objects placed under the holes. Each rat was placed singly in the holeboard for a 10 min trial at the same time of day for 3 successive days. Rats were given their appropriate injection before each day's test. Rats were tested only between 0800 and 1100 hr because of diurnal variations in the level of head-dipping [10] and in endogenous corticosterone levels [18]. The test order was randomized between the various drug groups and the floor and walls of the apparatus were wiped and dried after each rat had been tested.

In the second phase of the experiment 81 rats were randomly allocated to the following drug groups: corticosterone 3 mg/kg (N=9); 5% ethanol (N=9); $ACTH_{1-24}$ 5 μg/100 g after 3 min (N=9); $ACTH_{1-24}$, 5 μg/100 g after 30 min (N=9); saline controls (N=9); $ACTH_{4-10}$ (D) 4 μg/100 g (N=12); $ACTH_{4-10}$ (L) 4 μg/100 g (N=12); acidified saline controls (N=12). These rats were tested on one day only in the holeboard, without any objects under the holes.

RESULTS

The number of head-dips made and the mean time spent head-dipping provide measures of directed exploration. When the rats were tested in the presence of objects neither corticosterone nor $ACTH_{1-24}$ significantly changed the level of exploration, compared with that shown by the controls. The data for the mean time spent head-dipping are shown in Fig. 1. The rats injected with $ACTH_{1-24}$ and tested 3 min later showed reduced head-dipping, but this failed to reach significance, $F(1,18)=3.6$, $p>0.05$.

Figure 1 also shows the between-day habituation of head-dipping, which was unaffected by any of the drug treatments. Both the ethanol and corticosterone treated rats showed significant between-day habituation, $F(2,32)=7.46$, $p<0.01$, and so did the saline controls and the rats injected with $ACTH_{1-24}$ and tested 30 min later, $F(2,36)=12.08$, $p<0.001$, and those tested 3 min after $ACTH_{1-24}$, $F(2,36)=17.41$, $p<0.001$.

The scores for each 10 min test were collected separately for the four 2.5 min trial periods. Changes over these trial periods provide a measure of within-session habituation. The corticosterone-injected rats and their ethanol controls showed significant within-session habituation, measured by the time spent head-dipping, $F(3,144)=7.33$, $p<0.001$, as did those treated with $ACTH_{1-24}$ and tested 30 min later, $F(3,162)=8.60$, $p<0.001$, and after 3 min, $F(3,162)=8.66$, $p<0.001$. In no case was there any drug-induced impairment of within-session habituation.

The second phase of this experiment investigated further the possibility that ACTH and/or corticosterone might alter the level of exploration. Several drugs have been found significantly to increase exploration in the hole-board when rats were tested in the absence of objects, but failed to have a significant effect in the presence of objects [7,8]. In this experiment injected corticosterone still had no effect on the level of exploration (see Table 3), but $ACTH_{1-24}$, tested after both 30 and 3 min did significantly reduce it, $F(1,16)=8.64$, $p<0.01$ and $F(1,16)=4.45$, $p<0.05$, respectively.

TABLE 3

THE EFFECTS OF $ACTH_{1-24}$, $ACTH_{4-10}$ AND CORTICOSTERONE ON EXPLORATION IN THE HOLE-BOARD. EXPLORATION WAS MEASURED BY THE TIME SPENT HEAD-DIPPING (SECS) AND THE RATS WERE TESTED IN THE ABSENCE OF ABJECTS. THE TABLE SHOWS THE MEAN TIME SPENT HEAD-DIPPING DURING A 10-MIN TRIAL

Drug	Mean Time (sec)
Saline control	66.1
$ACTH_{1-24}$ (5 μg/100 g 3 min)	48.7
$ACTH_{1-24}$ (5 μg/100 g 30 min)	42.7
5% ethanol	68.8
Corticosterone (3 mg/kg)	71.5
Acidified saline control	69.1
$ACTH_{4-10}$ (L)	55.7
$ACTH_{4-10}$ (D)	50.1

There was no drug-induced impairment of within-session habituation, $F(3,42)=8.75$, $p<0.001$ for corticosterone, $F(3,54)=6.59$, $p<0.001$ for $ACTH_{1-24}$ after 30 min, $F(3,54)=9.18$, $p<0.001$ for ACTH after 3 min. The short peptide fragments, $ACTH_{4-10}$, also reduced the time spent head-dipping, the reduction caused by $ACTH_{4-10}$ (L) failed to reach significance, $F(1,22)=2.3$, $p>0.05$, but that caused by $ACTH_{4-10}$ (D) was significant, $F(1,22)=4.3$, $p<0.05$.

Although both $ACTH_{1-24}$ and $ACTH_{4-10}$ reduced the mean time spent head-dipping in no case was the mean number of head-dips made significantly reduced.

In neither phase of the experiment (objects present or objects absent) were there any drug-induced changes in the level of motor activity, or in its habituation. Nor did any of the drugs alter the number of foecal boli dropped.

DISCUSSION

The reduction in exploration, as a result of injected ACTH fragments, was seen only when the rats were tested in the holeboard in the absence of objects, and not when they were tested with objects placed under the holes. Whilst it is not known whether the critical difference is the complexity of the test environment, or the response levels produced in the two conditions, it has previously been found that chlordiazepoxide, ethanol and parachlorophenylalanine increase exploration only in the objects absent test condition [7,8].

In contrast to habituation of orienting, there was no evidence that ACTH impaired habituation of exploration, either within a test session or between days. This provides support for the suggestion that habituation in different behavioural situations may be governed by different neural mechanisms.

The pattern of results described in this paper is consistent with, but does not prove, the hypothesis that ACTH is anxiogenic. Two anxiolytic drugs, chlordiazepoxide and ethanol in low doses, produce an exactly opposite pattern of results to ACTH: habituation to tones is accelerated and the initial level of exploration in the holeboard (without objects) is increased. We have presented elsewhere [12] rather more

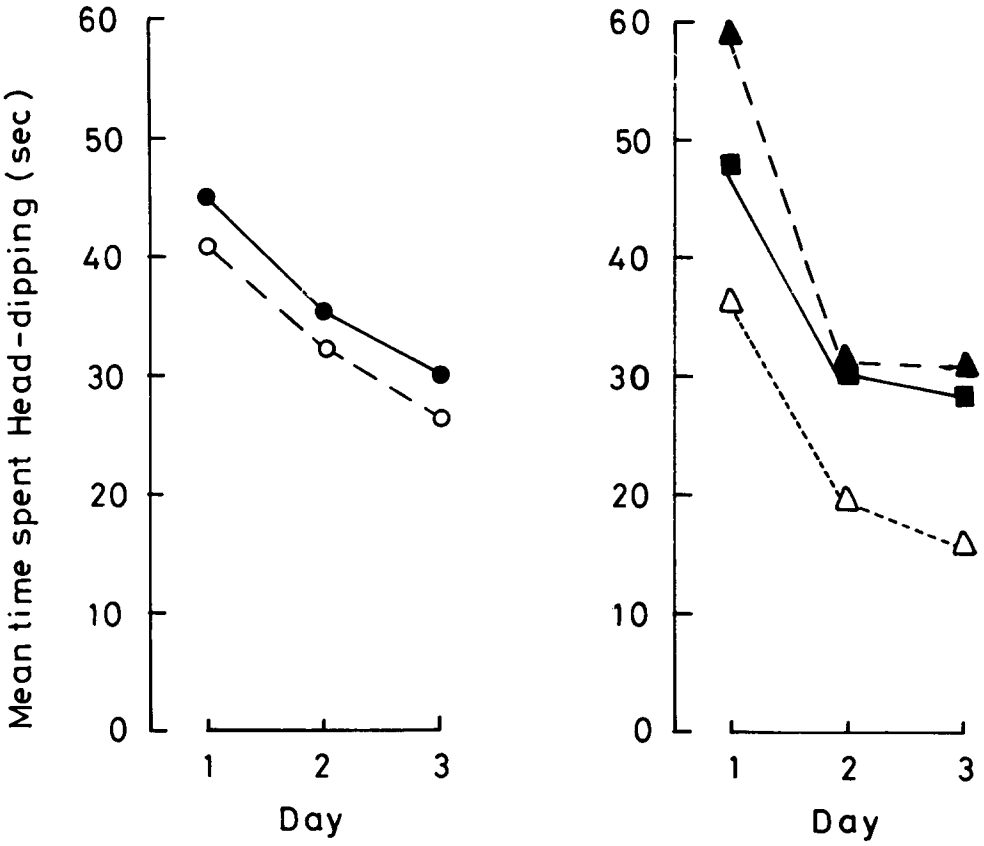

FIG. 1. Mean time spent head-dipping, in sec, during a 10-min trial in the holeboard, with objects present, on 3 successive test days. The left-hand graph shows the data for rats injected with corticosterone (3 mg/kg, 20 min) ○ - - - ○, and for the control rats injected with 5% ethanol ●——●. The right-hand graph shows the data for rats injected with $ACTH_{1-24}$ (5 μg/100 g, 30 min) ▲- - -▲, and $ACTH_{1-24}$ (5 μg/100 g, 3 min) △- - -△, and for saline controls ■——■.

direct evidence that one of the effects of ACTH is to increase the level of anxiety. It is important to note that the proposal is that the behavioural effects of ACTH are due to its direct action on the CNS and are independent of its steroid-releasing properties. This is supported by the fact that $ACTH_{4-10}$ (which is without steroid-releasing activity) has similar effects to $ACTH_{1-24}$; and also by the absence of effect of corticosterone in the present experiments and the failure to find evidence for an anxiogenic action of corticosterone [13].

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