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Effect of ACTH 4-10 on Copulatory Behavior and on the Response in a Test for Socio-Sexual Motivation in the Female Rat

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MEYERSON, B. J. AND B. BOHUS. *Effect of ACTH 4–10 on copulatory behavior and on the response in a test for socio-sexual motivation in the female rat.* PHARMAC. BIOCHEM. BEHAV. 5(5) 539–545, 1976. – Female copulatory behavior and the urge of a female rat to seek contact with a sexually active male was studied after treatment with the peptide ACTH 4–10. No effects of this peptide were seen on the copulatory response of ovariectomized females treated with estradiol benzoate (EB) alone or EB and progesterone combined. An increasing barrier technique was used to measure how much of an aversive stimulus (crossing an electrified grid) a female was willing to endure to gain contact with a sexually active male. It has been shown in previous investigations (15) with ovariectomized rats that after a certain time of adaptation and training in this apparatus the response attained remains at a certain basal level from which it is increased by treatment with EB. Animals treated with ACTH 4–10 during the phase of adaptation and training attained a significantly higher basal response level than controls. This effect was only observed when the peptide treatment covered the period of adaptation and was not seen when the treatment was started after this critical period. The EB-induced response was not influenced by ACTH 4–10 given during the period of adaptation or given in connection with the EB treatment. It is concluded that ACTH 4–10 influenced the acquired response level but had no effect on steroid induced responses, which are probably controlled by more innate mechanisms.

Female copulatory behavior Socio-sexual motivation ACTH 4–10 ACTH 1–39 Acquired response level

CONDITIONING experiments have provided evidence that the heptapeptide ACTH 4–10 influences brain functions involved in the acquisition and extinction of avoidance and approach conditional behavior (for review see de Wied *et al.* [4]). The peptide, which has a sequency common to ACTH and to the melanocyte stimulating hormone (MSH), corrects imparied ability of hypophysectomized rats to acquire a conditional avoidance response (CAR) and inhibits the extinction of CAR in intact rats [1]. ACTH and related peptides such as MSH and ACTH 4–10 facilitate acquisition and delay extinction of appetitive responses in the rat [7, 8, 10, 11]. The behavioral effects are more likely to be due to a direct action of the peptide than to be achieved by adrenal activation, as the peptide has been shown to lack a stimulatory effect on the adrenal cortical secretion.

Recently Bohus *et al.* [2] studied the effect of ACTH 4–10 on copulatory behavior and on sexually motivated approach responses of male rats. The peptide increased intromission and ejaculation latencies and this effect was related to the dose of testosterone proprionate. The urge to seek contact with a female was tested in a runway situation. ACTH 4–10 delayed extinction of the runway response, but had no effect on the drive to seek contact with a female.

Elements of the copulatory behavior of the female rat are dependent on ovarian hormones. The lordotic response to the male mounting disappears after ovariectomy but can be restored by estrogen followed after a certain time by progesterone [3, 13, 19]. The effect of hormones on the female sexual motivation, i.e. the eagerness to seek contact with a sexually active male, has recently been investigated [5, 12, 15, 17]. The three methods used differed from each other with respect to the approach offered to the animal to reach the male (overcome an aversive barrier, approach in an open-field, runway-choice situation). Cyclic changes in the response, with the highest response rate during estrus was found in females with a 5 day cycle. In the ovariectomized female estrogen induced an increase in the urge to seek contact with the male, this effect being dose-dependent. The present study uses one of these techniques, namely the increasing barrier method, in which the animal has to pass an electric grid to gain contact with the male. The grid current is increased stepwise every second time the female crosses. Thus in consecutive trials the animal has to endure progressively aversive stimuli to reach the goal cage. The test procedure may be divided into 3 phases: (1) the animal becomes adapted to the test situation without any aversive stimulus being applied, (2) the animal becomes

trained in the aversive test situation in daily tests to a fairly constant basal level of response and (3) the estrogen treatment is then given, which induces an increase in response lasting for a few days, hereupon the basal level is regained.

In the present investigation the influence of ACTH 4–10 on the socio-sexual motivation, when given at the different phases of the experimental procedure of the increasing barrier method, was examined, and also the effect of the peptide on the estrogen + progesterone activated copulatory behavior in female rats.

MATERIAL AND METHODS

Animals

About 240 ovariectomized albino Sprague-Dawley rats weighing 300–350 g were used. They were kept under a reversed day-night schedule (12 hr light – 12 hr darkness) at 22–24°C and provided with commercial food pellets and tap water ad lib. The rats were ovariectomized on arrival at the laboratory and were used in the experiments not earlier than 3 weeks after surgery.

Procedure

Details of the environmental conditions and procedures have been described elsewhere [15]. The tests were performed 3–9 hr after the beginning of the dark period, under dimmed lighting conditions.

Lordotic behavior. The female was brought to an observation cage (40 × 40 × 30) containing a vigorous male rat. The occurrence of tail deflection and elevation of the back (lordotic response) of the female when mounted by the male was assessed. Females which displayed lordosis at at least two consecutive mounts out of six were scored positive. The results are expressed as a percentage based on the number of animals which showed a positive response in each test.

Sexual motivation. The apparatus and training procedure employed have been described in detail previously [15]. To obtain contact with a male, the female rat had to cross an electrified grid. The length of time for which the rat remained in the starting cage before crossing to the goal cage was measured (hesitation time), as well as the number of crossings made. In the present study the hesitation times before crossing the grid for the first and second time were added together. After every second crossing the intensity of the electric current was increased stepwise (0–0.01–0.05–0.07–0.12 mA and thereafter by a factor of 1.3). A hesitation time exceeding 5 min terminated the test. The time allowed in the goal cage was 15 sec. The contact with the male was restricted, i. e. contact was allowed but the animals were separated by a wire mesh. The apparatus is depicted in Fig. 1.

Injected materials. Estradiol benzoate and progesterone (Organon through Erco, Stockholm, Sweden) were dissolved in olive oil, isopregenone (6-dehydro-retro-progesterone, Ferrosan, Malmö, Sweden) in propylene glycol and ACTH 4–10 (Organon through Professor D. de Wied, Utrecht, The Netherlands) in saline. ACTH 1–39 corticotropine carboxymethyl cellulose, Ferring, Malmö, Sweden) was diluted in saline. The injection volume for estrogen and ACTH 1–39 was 1 ml/kg b.w. and for ACTH 4–10 0.5 ml, isopregnenone 0.1 ml and progesterone 0.2 ml per animal. All injections were given subcutaneously (SC) except isopregnenone, which was given intravenously (IV).

Statistical tests. The following statistical tests were used: Friedman two-way analysis of variance (χ^2), Mann Whitney U test (z), Wilcoxon matched-pairs signed-ranks test (T), and Student's *t*-test (*t*). The test used in a particular case will appear from the statistical symbols given in brackets in

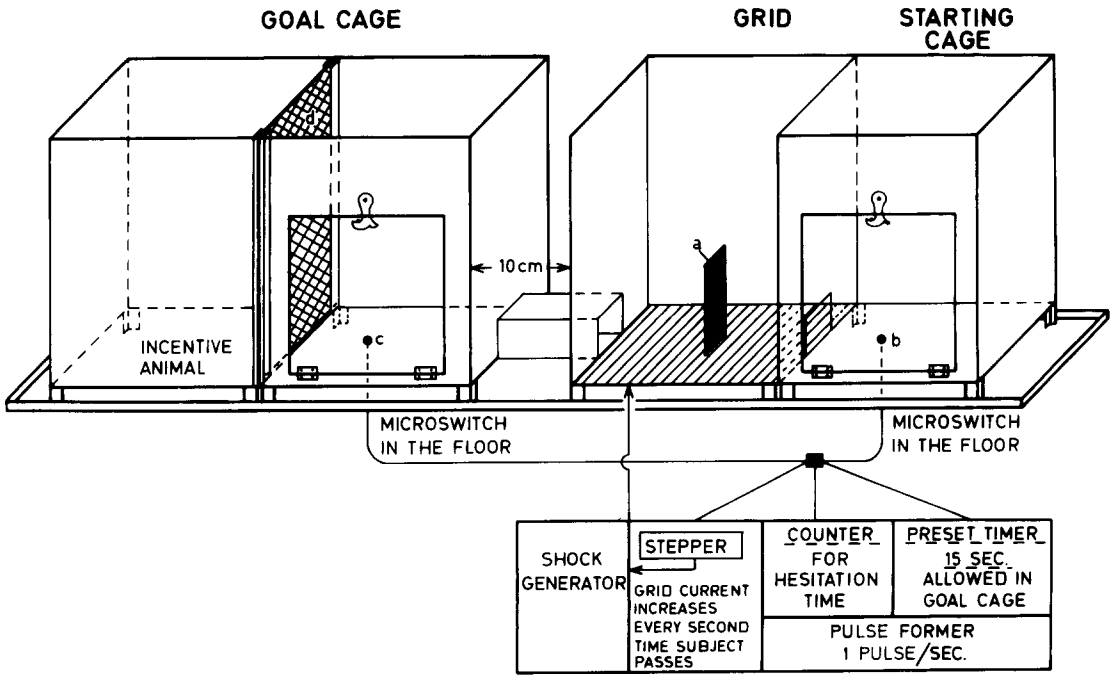

FIG. 1. The apparatus used to measure the urge of ovariectomized rats to seek contact with a male. For testing procedure see Method and [15].

TABLE 1

LORDOTIC RESPONSE IN OVARIECTOMIZED RATS

Treatment	Percentage Lordosis Min after last injection Pretest	30	90	180	240	N
A. ACTH 4-10 at 52h	4	4	4	0		24
B. Prog. at 48h and saline at 52h	75	79	79	79		24
C. ACTH 1-39 at 48h	7*			85		14
D. ACTH 4-10 at 52 1/2h and isopr. at 53h	6	6	33	83		18
E. Saline at 52 1/2h and isopr. at 53h	6	6	22	83		18
F. ACTH 4-10 at −2, 1/2, 2, 4h and prog. at 48h					67†	12
G. Saline at −2, 1/2, 2, 4h and prog. at 48h					83†	12

Estradiol benzoate, 5 μg/kg SC was given at 0h; ACTH 4-10 20 μg/rat SC; ACTH 1-39 20 IU/kg SC Progesterone (prog.) 0.4 mg/rat SC; Isopregnenone (isopr.) 0.5 mg/rat IV.
The pretest was conducted 30–60 min before the last injection.
*The animals were tested with estradiol benzoate alone 2 weeks prior to the present test.
†Cumulated response in 3 tests made 4, 6 and 8 h after the progesterone injection.

Results below. For the non-parametric tests see Siegel [18].

RESULTS

Lordotic Behavior

ACTH 4–10 was given 52 hr after administration of estradiol benzoate (EB) to investigate whether the peptide could replace the progesterone treatment, which is necessary to obtain a response. As is evident from Table 1, B and C, both progesterone and ACTH 1–39 given 48 hr after the EB treatment induced lordotic behavior in about 80% of the animals. In contrast, ACTH 4–10 was not effective in inducing a lordotic response. Tests were made to investigate whether ACTH 4–10 influenced the time latency between an IV injection of a progestin and the appearance of the lordotic response. It has been shown earlier that the anesthetic property of progesterone influences the time it takes for this hormone to produce its lordosis activating effect [14]. Isopregnenone was chosen in the present test situation, as this progestin lacks anesthetic properties. It was given IV into one of the lateral tail veins and tests for lordotic behavior were conducted 30, 90 and 180 min after the injection. ACTH 4–10 given 30 min before the progestin injection did not influence the latency of the response or the total response (Table 1, D and E).

The effect of ACTH 4–10 administered in connection with the estrogen treatment was investigated. The peptide was given 2 hr before and 30 min, 2 hr and 4 hr after the estrogen injection. Progesterone was given at 48 hr and the behavior was tested 4, 6, and 8 hr later. The cumulated response is shown in Table 1 F. No significant difference was found between ACTH 4–10 treated females and controls.

Sexual Motivation

ACTH 4–10 During Adaptation to the Test Apparatus – Experiment 1

The animals (n = 42) were treated as follows: Day 1: The female was placed in the starting cage and left free to explore the apparatus for 5 min. No grid current was on. The goal cage held a sexually active male. This procedure was repeated on Day 2. On Day 3 the female rat was brought back to the starting cage after having crossed the grid and entered the goal cage. Fifteen seconds were allowed in the goal cage and after 6 crossings the session was ended that day. All animals performed 6 crossings. On Day 4 the grid current was switched on and the procedure followed the description given in Methods. The experimental animals received 20 μgACTH 4–10 1 hr prior to the test, while controls were treated with saline. One session was conducted each day on the days indicated in Fig. 2. Peptide treatment was continued until Day 10. There was an overall effect among the test sessions on Days 4–10 in the ACTH 4–10 group (χ^2 = 19, *df* 8, $p<0.02$) but not in the saline group (χ^2 = 6.2, *df* 8, $p>0.5$). In the first test session the responses were lower than in the later ones. It was a consistent finding that ACTH 4–10 treated animals crossed the grid significantly more often than controls. This was evident from the first day recordings were made (Day 4, z = 2.4, p = 0.016; Days 4–6, z = 3.9, $p<0.001$). The hesitation times for the first two crossings (see Methods) were combined. The difference increased from Day 7 and lasted till Day 30 (Day 11, z = 2.5, p = 0.012; Day 30, z = 3.4, $p<0.001$). The hesitation time was not significantly different in the ACTH 4–10 group (Days 4–10) from that in the controls (see Table 2).

ACTH 4–10 after the Adaptation to the Apparatus – Experiment 2

The animals (n = 21) were tested here in the same way as in Experiment 1 except that saline was given instead of ACTH 4–10 to the experimental animals from Days 1–4. On Days 5–10 ACTH 4–10 (20 μg/animal) was injected 1 hr before the day's test. No test session was conducted on Day 6 but ACHT 4–10 was given (Fig. 3 and Table 3). There was no difference in the number of crossings between experimental and control animals on Day 4 (z = 0.18, $p>0.4$) or 5 (z = 0.52, $p>0.3$) nor in the average response

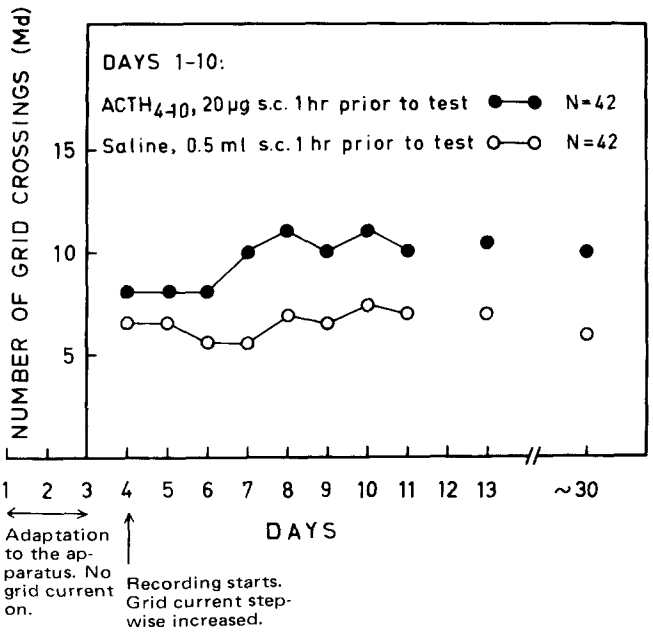

FIG. 2. Effects of treatment with ACTH 4–10 given on Days 1–10 on the number of grid crossings amde by ovariectomized rats to gain contact with a sexually active male (Experiment 1).

of Days 5 through 11 ($z = 0.28$, $p>0.5$). The differences between the results of test sessions within the same group for Days 5 through 11 were not statistically significant (ACTH 4–10 group: $\chi^2 = 5.48$, df 5, $p>0.3$; saline controls $\chi^2 = 3.6$, df 5, $p>0.5$). The hesitation times were about the same in the two groups on Day 4 ($t = 0.4$ NS) and similar to those in Experiment 1 for that day. The average hesitation times for the test sessions on Days 5, 6, 8, 9, 10 and 11 in the ACTH group were slightly higher than those in the saline treated groups, and also higher than in Experiment 1. However, this difference did not reach statistical significance.

Estradiol Benzoate to Previously ACTH 4–10 Treated Animals – Experiment 3

About 30 days after the beginning of Experiment 1 estradiol benzoate 10 µg/kg was given to animals (n = 24) which had been treated with ACTH 4–10, as well as to subjects used as controls, in Experiment 1 (Fig. 4). The estrogen treatment significantly increased the number of crossings in both groups (ACTH 4–10 group $\chi^2 = 15.3$, $df = 4$, $p<0.01$; saline controls $\chi^2 = 41.2$, $df = 4$, $p<0.001$). The response for Day 2 and 3 conbined did not differ between the two groups ($z = 1.0$, $p>0.1$). The relative increase from Day 0 to Days 2 and 3 in the former control group (Experiment 1) seemed to be higher than in the former ACTH 4–10 treated group. However, on Day 0 there was a significant difference between the two groups ($z = 3.2$, $p<0.001$). As the two groups started at a different level before the hormone treatment, a direct comparison of the extent to which estrogen increased the number of grid crossings in these groups is difficult. The hesitation times were significantly higher in the saline controls on Day 0 of EB treatment than in the ACTH 4–10 group (Table 4). On the third day of the EB treatment the hesitation times had significantly decreased compared to Day 0 (ACTH 4–10 group: $T = 31$, $p<0.01$; saline controls $T = 6$, $p<0.01$). No difference was seen at this time between the two groups.

ACTH 4–10 and Estradiol Benzoate Treatment – Experiment 4

Animals which had participated in Experiments 1 and 3 were also used in Experiment 4. This experiment was conducted about 120 days after the beginning of Experiment 1. Experimental animals and controls from Experiment 1 were divided into two groups. One group was treated with ACTH 4–10 plus estradiol benzoate and the other group received saline instead of ACTH 4–10. In all categories estradiol benzoate induced a significant (Friedman analysis of variance $p<0.001$) increase in the number of crossings and reduced hesitation times (Fig. 5 A and B and Table 5). The grid crossings performed by animals which had received ACTH 4–10 in Experiment 1 are depicted in Fig. 5A. It is clear that ACTH 4–10 given 1 hr before the test on Days 0, 1, 2, 3 and 4 did not influence the estrogen induced increase in crossings (Day 0 $z = 0.1$, NS, Days 2 and 3 $z = 0.9$ NS). Figure 5 B shows the analogous experiment carried out on animals not treated earlier with ACTH 4–10. No differences between rats treated with ACTH 4–10 and those treated with estrogen alone were seen (Day 0: $z = 0.8$ NS; Days 2 and 3: $z = 0.3$ NS). It was also seen from the scores on Day 0 that the difference between the ACTH 4–10 treated animals of Experiment 3 (Fig. 4) and the saline treated animals of Experiment 4 (Fig. 5) at 120 days had decreased as compared with Day 30 after the early ACTH 4–10 treatment (Day 0, Experiment 3 compared to Experiment 4: $z = 2.6$, $p<0.01$; Days 2 and 3: $z = 1.9$, $p = 0.012$). An analogous comparison between the controls of Experiment 3 and the rats treated with estradiol benzoate alone in Experiment 4 showed no significant difference in crossings

TABLE 2

HESITATION TIMES IN S (MEAN ± SEM)

	Day								
	4	5	6	7	8	9	10	4-10	
ACTH 4-10	65 ± 10	80 ± 15	69 ± 10	49 ± 8	45 ± 8	53 ± 11	68 ± 12	61 ± 7	t = 0.9 NS
Saline	64 ± 14	102 ± 14	79 ± 11	58 ± 9	66 ± 13	76 ± 13	78 ± 13	71 ± 7	

The experiment was repeated four times, with a new batch of animals each time.

TABLE 3

HESITATION TIMES IN S (MEAN ± SEM)

	Day								
	4	5	6	8	9	10	11	5-11	
ACTH 4-10	63 ± 15	118 ± 26	128 ± 23	101 ± 25	125 ± 26	60 ± 12	79 ± 20	98 ± 11	t=1.5 NS
Saline	71 ± 15	82 ± 19	61 ± 13	56 ± 13	88 ± 23	112 ± 26	40 ± 7	74 ± 11	

The experiment was repeated twice with a new batch of animals each time.

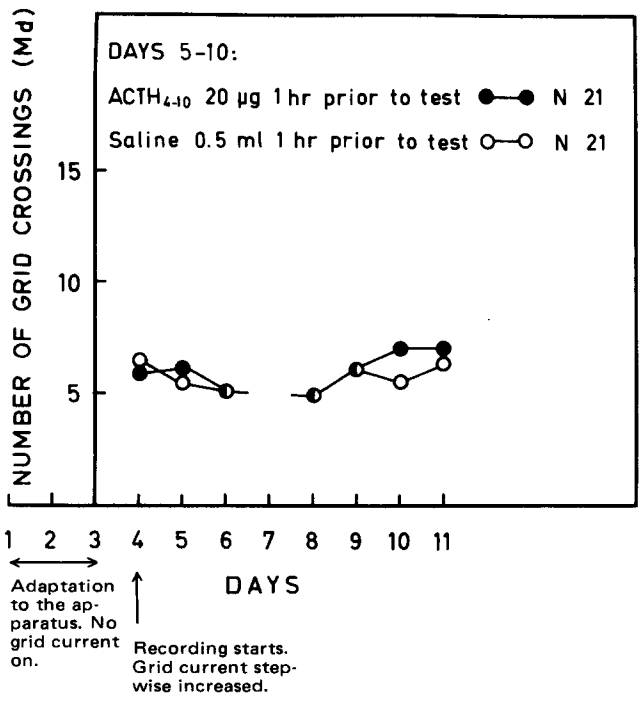

FIG. 3. Effects of treatment with ACTH 4–10 given on Days 5–10 on the number of grid crossings made by ovariectomized rats to gain contact with a sexually active male (Experiment 2).

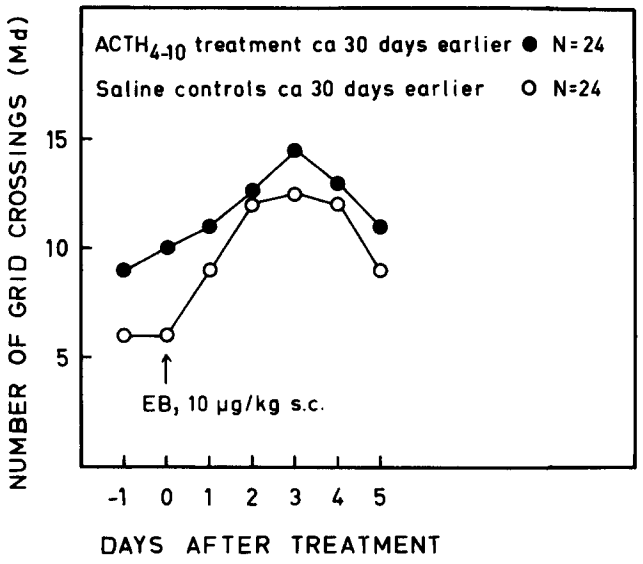

FIG. 4. Effects of treatment with estradiol benzoate on the number of grid crossings made by ovariectomized rats to gain contact with a sexually active male (Experiment 3).

on Day 0 (z = 0.1, NS). However, on Days 2 and 3 the number of crossings was somewhat higher in Experiment 3 than in Experiment 4 (z = 2.5, $p = 0.01$).

A comparison of the estradiol benzoate plus saline treated animals in Groups A and B revealed no statistical difference in the number of crossings on Day 0 or Days 2 and 3 (z = 1.2 and 1.7, NS). The ACTH 4–10 treated animals differed significantly, however, in crossings on Day 0 (A compared to B, z = 2.3, $p<0.02$) and Days 2 and 3 (z = 2.8, $p = 0.005$). The animals treated earlier with ACTH 4–10 had a higher score.

DISCUSSION

Lordotic Behavior

ACTH 4–10 proved to be no substitute for progesterone treatment in eliciting lordotic behavior. Nor did it influence the latency from a progestin injection to the appearance of the lordotic response. The peptide given in connection with the estrogen treatment did not influence the estrogen + progesterone induced response. Feder and Ruf [6] reported that ACTH treatment facilitated lordotic behavior in estrogen-primed ovariectomized rats by releasing progesterone from the adrenals. That ACTH 4–10 could not replace progesterone, whereas ACTH 1–39 was effective in inducing a lordotic response in estrogen-primed females, indicates that ACTH 4–10 did not provoke a release of steroids from the adrenals.

TABLE 4

HESITATION TIMES IN S (MEAN ± SEM)

	Day		
	0	3	5
ACTH 4-10 group	49 ± 11	17 ± 3	32 ± 8
Saline controls	106 ± 22	17 ± 4	22 ± 7
Significant difference	t = 2.38 $p<0.05$	NS	t = 0.89 NS

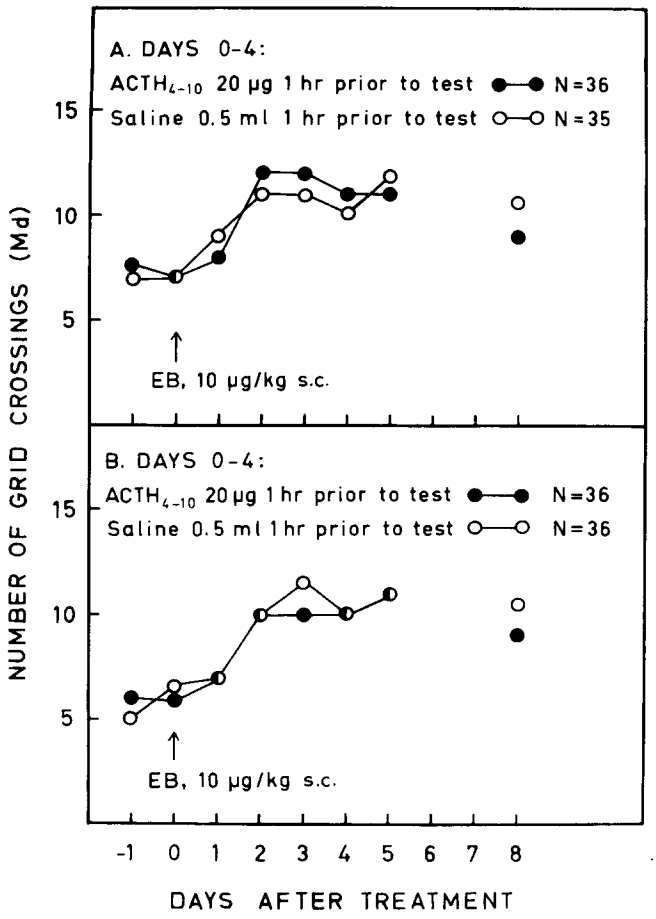

FIG. 5. Effects of treatment with estradiol benzoate (EB) plus ACTH 4–10 on the number of grid crossing made by ovariectomized rats to gain contact with a sexually active male (Experiment 4). The animals had been treated with (A) ACTH 4–10 and (B) saline in Experiment 1.

Grid Crossings

There was a significant increase in the number of crossings made over the grid by the ovariectomized rats following treatment with ACTH 4–10. This effect was only obtained when the ACTH 4–10 treatment covered the period when the animals were adapting to the apparatus. When administration of ACTH 4–10 was started one day after the animal was first subjected to the grid current it had no effect. The effect of ACTH 4–10 given on Days 1–10 still persisted 30 days after Day 1, but had decreased at 120 days.

How is this effect of ACTH 4–10 to be interpreted? Before reasoning let us consider the animal's test situation. The incentive for the animal's activity in the apparatus probably consists of several elements such as general locomotion, exploration and socio-sexual approach. On the basis of earlier data [15] we assume that the increase in the response after estrogen treatment mainly has a sexual significance. In the first three sessions (Days 1–3) the animal is having to habituate to a novel environment. Even if no electric current is passed through the grid in these sessions, some insecurity and emotional involvement have

TABLE 5

HESITATION TIMES IN S (MEAN ± SEM)

		Day 0	3	8
A.	EB + ACTH 4-10	40 ± 9	18 ± 6	31 ± 8
	EB + saline	52 ± 15	21 ±6	24 ± 4
B.	EB + ACTH 4-10	46 ± 14	16 ± 6	20 ± 3
	EB + saline	38 ± 10	16 ± 3	22 ± 5

For all treatments the differences between Days 0 and 3 were significant ($p<0.05$). Comparisons between the effects of EB + ACTH 4-10 and EB + saline revealed no significant differences.

to be overcome by the animal to explore and move around in the apparatus. After this initial period on Day 4 it is faced with the new situation of having to endure a stepwise increasing aversive barrier in order to reach the goal box. Let us then consider the two main results obtained: (1) treatment with ACTH 4–10 did not influence the response when given on Days 5–10 (Experiment 2) and (2) the increased response level seen when ACTH 4–10 was given on Days 1–10 was still obvious on Day 30, i.e. 20 days after the last injection of the peptide. The long lasting effect of ACTH 4–10 excludes the possibility that the activity level or sensory functions were influenced directly. It is more likely that when the treatment was given from Day 1 the response level attained after the first days in the apparatus was influenced by the experience acquired during the initial phase of the experiment (Days 1–4). It is conceivable that the peptide might affect the capacity of the animal to adjust (habituate) to the environment in such a way that by the experience acquired during the first sessions the animal takes a higher response level at the subsequent part of the experiment. Since behavioral adjustment to a solitary situation like the open-field is not affected by ACTH fragments [20] it seems that the improved adjustment to the present behavioral situation possibly caused by the peptide involved some kind of social interaction. The nature of this interaction is probably not of sexual significance in the absence of estrogen priming.

Estradiol benzoate, as has been shown earlier, increases the number of grid crossings and decreases the hesitation times. ACTH 4–10 given on Days 1–10 did not result in an increase of the estradiol benzoate induced response 30 days later. The maximal response level permitted by the technique is 24 crossings, which means that some increase was still possible. A direct comparison of the experimental animals and controls is rendered difficult, however, by the fact that the response was already significantly different on the day of hormone treatment. If anything, the relative increase from Day 0 to Day 3 was slightly lower in the ACTH 4–10 group.

The hormone induced response is probably based mainly on innate mechanisms or mechanisms organized early postnatally. Female rats, treated with testosterone at the age of 5 days do not show any estrogen induced response when tested with this technique [16]. It is obvious from Experiment 4 that ACTH 4–10, when given together with estradiol benzoate at a time when the effect of the peptide given on Days 1–10 had almost disappeared, did not influence the estrogen induced increase in grid crossings.

From this observation, together with the result of Experiment 1, it would seem that ACTH 4–10 influenced an acquired response level but to have no effect on steroid induced responses (lordosis or grid crossing), which are probably governed by more innate mechanisms.

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