Title
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Journal
Pharmacology Biochemistry and Behavior, 27(1)

ISSN
0091-3057

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Publication Date
1987-05-01

DOI
10.1016/0091-3057(87)90487-4

Pharmacology Biochemistry & Behavior, Vol. 27, pp. 133–142. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Neonatal Exposure to a High Level of $ACTH_{4-10}$ Impairs Adult Learning Performance

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Received 4 August 1986

McGIVERN, R. F., G. ROSE, C. BERKA, A. N. CLANCY, C. A. SANDMAN AND B. E. BECKWITH. *Neonatal exposure to a high level of $ACTH_{4-10}$ impairs adult learning performance.* PHARMACOL BIOCHEM BEHAV **27**(1) 133–142, 1987.—Male offspring of Sprague-Dawley dams were injected with 25 μg of $ACTH_{4-10}$ or the vehicle on days 2 through 7 postpartum. Peptide treated animals exhibited a marked motor response to the peptide injection. Adrenal weights of these animals were consistently heavier than littermate controls in both the developing and adult animals. $ACTH_{4-10}$ treated neonates exhibited significantly poorer learning performance in the shuttle box and were slower to acquire the reversal learning problem of a visual discrimination task under light shock levels. In addition, these animals also exhibited an exaggerated startle response and a stronger thigmotaxis response in the open field than controls. These results indicate that exposing the developing nervous system to relatively high levels of $ACTH_{4-10}$ can produce marked long-term effects on behavior.

Neonatal exposure $ACTH_{4-10}$ Learning performance Long-term effects

A large amount of evidence in recent years has demonstrated that adrenocorticotropin (ACTH) and related fragments can directly effect the nervous system [5,13]. In several studies, the peptide fragment $ACTH_{4-10}$ has been shown to produce effects on learning which parallel those observed with ACTH. Both substances can restore the impaired learning performance of hypophysectomized animals [7] as well as delay extinction in normal animals [12,23]. Retention of a passive avoidance response can also be improved by treatment with ACTH or the $ACTH_{4-10}$ fragment [14,17].

Two developmental studies which have examined the influence of $ACTH_{4-10}$ or a closely related analog, Org 2766, on learning, have reported long-term effects from postnatal peptide treatment which are similar to effects observed after acute peptide injection in the adult animal [11,24]. Champney *et al.* [11] injected 10 μg of Org 2766 intracerebroventricularly (ICV) into one day old rat pups. When tested as adults, the peptide treated animals, compared to controls, exhibited enhanced reversal learning of a visual discrimination problem similar to naive adult animals injected with $ACTH_{4-10}$ [3]. Nyakis *et al.* [24] injected rat pups daily from days 2–8 postnatally with either 2 nm or 10 nm $ACTH_{4-10}$. Neonatally treated animals, treated as adults, exhibited delayed extinction of a conditioned avoidance response (CAR) and better retention of a passive avoidance response. The results from developmental studies with ACTH fragments have been interpreted to suggest that postnatal treatment with these fragments may influence early organization of CNS substrates associated with learning [4,5].

The consistent data demonstrating positive effects of ACTH fragments on attentional and/or motivational processes has lead to a number of clinical studies in human populations with suspected deficiencies in these processes. Most clinical studies in human populations have used $ACTH_{4-10}$ or Org 2766. Treatment with these fragments has been examined in a variety of populations including the following: elderly persons with senile dementia [9,15], retarded adults, normal adult volunteers [28], and hyperactive, learning disabled children ([10,27]; cf. [26]). Results from human studies have been mixed [5,25], but two recent reviews have noted that the strongest effects of the peptide treatment in humans appears to be on improvement of mood [6,25]. Moreover, both reviews point out that positive effects of peptide treatment appears to be most pronounced when subchronic dose regimens are employed.

In the animal literature, the effects of both $ACTH_{4-10}$ and ACTH have frequently been reported to be biphasic or exhibit a U-shaped function depending upon the dose employed. Lichtensteiger and Monnet [20] reported a U-shaped dose response function for $ACTH_{4-10}$ in affecting activity of nigral dopamine neurons. Schotman and Allaart [31] found that low doses of $ACTH_{4-10}$ stimulated protein synthesis whereas high doses inhibited synthesis. Behaviorally, $ACTH_{4-10}$ treatment enhances intracranial self-stimulation in the rat at low doses, but inhibits it at high doses [19]. Similarly, low doses of ACTH enhance retention of a passive avoidance response, but impair retention at high doses [17, 18, 30].

In light of these reported biphasic effects of $ACTH_{4-10}$ in animals, as well as the experimental use of this peptide as a treatment for children with attentional deficits [10,27], we examined the long-term effects of exposure to relatively high levels of $ACTH_{4-10}$ during the neonatal period. We hypothesized that a high dose of $ACTH_{4-10}$, administered from days 2–7 postnatally, would impair adult learning performance. Additionally, we examined a spectrum of physiological and behavioral parameters not directly related to learning, but which might be influenced by neonatal $ACTH_{4-10}$ treatment based upon reported effects after acute injection in the adult animal. Included among these effects are influences on sexual behavior [8], pain sensitivity [42], pituitary-adrenal responsiveness [43], and the ability of $ACTH_{4-10}$ to weakly bind to opiate receptors [16].

METHOD

Animals and Injection Procedure

Male offspring from Sprague-Dawley dams (Harlan, Indianapolis, IN or Simonsen, Gilroy, CA) were used throughout these experiments. Dams were either impregnated in the laboratory vivarium or ordered from the supplier to arrive on the 14th day of gestation. The animals were maintained on ad lib Purina lab chow and tap water. On day 2 following parturition in the laboratory vivarium, litters larger than 8 were trimmed to 8 animals, primarily at the expense of female offspring. Litters smaller than 5 animals were not used, but female pups were raised with the smaller litters when necessary to meet the minimal size criterion. Male offspring were randomly divided within each litter to receive SC injections of $ACTH_{4-10}$ (25 μg in 50 μl) or an equal volume of the vehicle (0.9% saline adjusted to pH 5.9 with 0.1 M acetic acid). One injection per day was administered on days 2 to 7 following parturition. Animals were marked with colored dyes on the rear flanks and hind limbs to distinguish treatment group. Each animal was removed from the nest for injection or marking and then immediately returned. Animals were marked every other day from day 8 to 21, when they were subsequently weaned. At weaning, animals were group housed by treatment (3–5/cage). Animals were maintained on ad lib Purina lab chow and tap water for the remainder of the experiment. Vivarium lighting conditions were 12 hours on/off beginning at 0600. All testing was done between 1100 and 1800 hours except testing of sexual activity which was conducted between 1900 and 2100 hours. Except where noted, all animals were experimentally naive.

Activity and Eye Opening

Forty-two animals (23 ACTH treated and 19 controls from Harlan dams) were checked daily to establish the postnatal day when both eyes were open. Seventeen $ACTH_{4-10}$ treated animals and 16 controls were also measured for activity every third day from day 9 to day 30 postnatally. Activity measures were determined using four radio-frequency activity counters (Quartec) for 10 minutes/animal/test. Each animal was removed from the litter at the time of testing, following which it was placed on a heating pad until all littermates were tested. Subsequently, the litter was returned to the mother or to the home cage (after day 21). Animals used to observe developmental activity patterns were later used to study Thompson Box performance or two-way shuttle box performance.

Acoustic Startle

Ten experimental and 10 control animals from Harlan dams were tested at 90 days of age for an acoustic startle response in an 18 × 10 × 12 cm startle chamber designed to prevent lateral movement. Each animal was exposed to 30, 100 dB, 50 msec rapid rise time, 2500 Hz tones. Tones were automatically presented, via solid state programming equipment, in a fixed pseudo-random sequence with interatrial intervals varying between 30 and 70 sec. Each animal's jump response was recorded on a polygraph (Grass, Model 7) through a DC preamplifier and driver amplifier. The response was reflected in mm of pen deflection generated by displacement of a speaker coil attached to the base of the startle chamber. Subsequent scoring was done by an individual unaware of the treatment condition of each animal.

Open Field

Open field measurements were observed in nine treated and seven control animals from Harlan dams at 110 days of age according to procedures of Veith *et al.* [39]. Briefly, each animal was placed in the center of an open field and the following observations recorded for the subsequent 5 minutes: (1) time for the animal to leave center and touch wall of apparatus with body or vibrissae; (2) number of grids crossed; (3) number of rears; (4) number of episodes of grooming activity; (5) number of boli excreted. At the end of the five minutes, the animal was immediately replaced in the center of the open field and the same observations were repeated for an additional five minutes. Observations were made by a trained experimenter blind to animal's treatment condition. The apparatus was cleaned with an alcohol/vinegar solution after each animal was tested. These animals were subsequently tested 10 days later for an analgesic response to morphine using the tail flick test.

Thermal Pain Sensitivity and Morphine

At 120 days of age, nine experimental and seven control animals were tested for basal thermal pain sensitivity using the tail flick test. Procedures of this test are outlined elsewhere [22]. Briefly, each animal was given six trials at a constant heat intensity, and the latency to withdraw the tail was recorded for the last five trials. The first trial was treated as an habitation trial, and the latency was not recorded. Following the last trial, each animal was injected with an analgesic dose of morphine (7.5 mg/kg IP) and tested again 15 minutes later with three additional trials at the same heat intensity, to measure an analgesic response. A 10 sec ceiling was imposed on the tail exposure to the heat source to prevent tissue damage. Trials were spaced approximately four minutes apart.

Four additional naive experimental and four control animals from Harlan dams were tested at 120 days of age on the hotplate test. Temperature of the hotplate (Columbus Instruments, Columbus, OH) was maintained at 50°C (±0.5°C). Trials were spaced three minutes apart. Each animal was given one habituation trial, followed by three test trials to establish baseline pain sensitivity. Latency to paw, lick, or hind limb withdrawal was recorded. Subsequently, each animal was injected with morphine (7.5 mg/kg IP) and retested 20 minutes later. A 60 sec ceiling was imposed for exposure to the heat source.

Activity Avoidance Acquisition

Acquisition of a conditioned active avoidance response (CAR) was studied in a shuttle box (Lafayette Instruments, Lafayette, IN). Six experimental and six control animals were studied at approximately 90 days of age. The apparatus was arbitrarily divided into two compartments by an imaginary center line. The task demanded that the animal shuttle to the opposite side of the box from which it was situated within 10 sec after onset of a tone which served as the conditioned stimulus (CS). Unconditioned stimulus (UCS) was a 0.5 mA continuous scrambled foot shock which was initiated 10 sec after tone onset if the animal did not make an avoidance response. Both CS and UCS terminated when the animal crossed the center line into the safe compartment. The interatrial interval was 60 sec. Onset of tone and shock were initiated by mechanical programming equipment housed in a separate room. Termination of CS and UCS was initiated by an experimenter, blind to the animal's treatment condition, monitoring the animal's behavior via a video monitor in an adjacent room. Each animal was given 100 trials in a single test session. In addition to the number of CARs, the number of spontaneous interatrial crossings was also recorded. The apparatus was cleaned with an alcohol/vinegar solution after each animal was tested.

Radial Arm Maze

Nine ACTH treated and 7 controls from Simonsen dams were tested in the radial arm maze beginning at 90 days of age. The eight arm radial maze used in this study was as described by Olton, Walker and Gage [25] except that round holes 2.5 cm in diameter and 1.8 cm deep were drilled from the end of each arm to serve as food wells. The entire structure was painted a flat gray. The maze was located in a small room of dimensions 2×2×2.5 m, the walls and ceiling of which were constructed of an open-mesh (approximately 1 cm grid) wire screening. Entry into this room was through a floor-to-ceiling sliding panel, 1 meter wide; this panel was kept closed while the rats performed in the maze. The apparatus was elevated approximately 75 cm off the floor. In the middle of each wall, at a height of 1 meter, the following sensory stimuli were placed (clockwise starting with the wall containing the sliding panel): a 22 by 30 cm white card with a bold black design on it, a plain white 22 by 20 cm card, a noisy fan, and a bright fluorescent light 45 cm in length. Room lighting was provided by fluorescent ceiling fixtures.

Rats were food deprived for two days. On the third day, each animal was placed on the center platform of the maze, throughout which several 190 mg food pellets had been scattered, and was given 10 minutes to explore the maze and consume the pellets. The rats were then removed from the apparatus and given additional food to maintain their body weight at about 85% of the ad lib value. Throughout the experiment, supplemental feedings were always given just after sessions in the maze.

After three days' exposure to the maze, testing was begun. The animals were given one trial each day, according to the procedure described by Olton *et al.* [25]. A rat's arm choices were recorded until all arms had been visited once. The order of the animal's testing was varied from day to day. Preceding each trial, the floor maze was cleaned with an alcohol/vinegar solution. The animals were tested for 10 days. After this, for an additional five days, the rats were given daily trials in the maze as before, except that the ends of the arms were not baited with food. Again, each choice was recorded until all arms were visited once. Latency to visit the first eight arms was always recorded.

Errors were calculated based upon the number of repetitious arm visits made by each animal within the first 8 choices. During the first few days of testing, some animals would occasionally visit an arm but not consume the reinforcement. If the animal subsequently returned to that arm and ingested the reinforcement, the second visit was not considered a choice and an additional choice was added to the initial eight choices. An error was scored, however, if the animal returned to an arm which it had previously visited and consumed the reinforcement or if the animal returned to a previously visited arm which still contained the food pellet and the animal did not consume the reinforcement on the second visit.

Following the completion of testing, the animals were overdosed with sodium pentobarbital (Nembutal) and intracardially perfused with 0.9% saline followed by 10% formalin. The brains were refrigerated at 4°C overnight, then sectioned in the coronal plane at a thickness of 35 microns on a freezing microtome. Every sixth section was mounted on a gelatin-coated slide and stained with cresyl violet. An adjacent section was stained for AChE according to the procedure described by Lynch and Kilackey [21], and then lightly counter-stained with cresyl violet to aid structural identification.

An additional group of six ACTH treated and six control animals from Harlan dams were tested in a similar apparatus beginning at 100 days of age, but under the low illumination conditions to facilitate entry into the open arms. A 15 watt bulb was suspended 48 inches above the maze which was housed in a 6×9 foot sound-attenuated room with walls painted a yellow pastel. Procedures were the same as outlined above with the following exceptions: (1 maze was painted a flat, dull orange; (2) latency to visit the first eight arms was not recorded; (3) stimuli on the four sides of the maze were the following: a metal strip, a white card with a black design, a blank wall, and the door; (4) animals were given 10 days of exposure to the maze before formal testing. Formal testing was done for the subsequent 11 days. Thirty days later, the animals were tested for an additional 16 days. In this experiment, consummatory behavior was examined by computing a ratio of the number of available pellets in the first eight choices (corrected for errors made) to the number consumed. This measure is, hereafter, referred to as the consummatory efficiency ratio.

Original Learning and Reversal of a Visual Discrimination Problem

Four treated and four control animals from Harlan dams were tested in a visual discrimination paradigm which utilized a fixed level of shock for negative reinforcement.

The test apparatus was a black Plexiglas Thompson-Bryant box [35] which consisted of a start box, a choice compartment, and a goal box. The start box was separated from the choice compartment by a guillotine door. The goal box was separated into two sections by a partition which extended 7 cm into the choice compartment. The floor of the start box and the choice compartment consisted of a bronze grid through which shock could be administered. The apparatus was cleaned after each animal was tested with an alcohol/vinegar solution.

The first day, each animal was permitted to explore the apparatus for 15 minutes. On the second day, the animal was trained to avoid shock by running into the goal box until five consecutive correct responses were made. On day 3 the rats were trained by a shaping procedure to dislodge identical diagonally striped black and white doors to enter the goal box. Criterion was five consecutive responses elicited with no negative reinforcement.

The discrimination task required that the rat avoid electric shock by running to a solid white door, dislodging it and entering the goal box. The solid black door remained locked during this phase of the experiment. Door positions were varied according to a Gellerman series. The animals were given 25 trials per day in a mass trial procedure. Acquisition of the discrimination test was defined as nine out of 10 correct responses. An error was scored if the animal's vibrissae or body made contact with the negative door. A brief foot shock of 2 mA was administered for incorrect responses and response latencies greater than 15 sec. Following acquisition of the original learning problem, the animals were tested, beginning on the following day, for acquisition of the reversal of the black/white problem.

An additional six experimental and six control animals were tested according to the same procedures with the exception that minimal shock level was determined during training procedures for each animal to elicit a task related response. These shock levels varied for individual animals between 0.3 mA and 1.5 mA. Once the shock level was determined for an animal, it was used throughout the remainder of the testing sessions.

Sexual Behavior

Seven ACTH treated and six control animals from Simonsen dams were tested for sexual behavior in the presence of an estrous female. Testing was begun at approximately 90 days of age. Stimulus females were chronically implanted with 4–5 mm Silastic capsules containing estrogen benzoate two to three weeks before testing. Estrous was induced on the day of testing by injection (SC) of 100 μg of progesterone in a vegetable oil vehicle administered six hours before the testing session. Sexual activity was measured beginning one hour into the dark cycle between 1900 and 2100 hours.

Each male was placed in a 15 inch diameter, clear, plastic chamber illuminated by red light. After a 5 min adaptation period, a stimulus female was introduced and in her presence, the following responses were recorded: number of mounts, number of intromissions, time to ejaculation, and post ejaculatory interval (the interval between ejaculation and the next intromission).

Brain, Adrenal, and Body Weights

Whole wet brain and wet adrenal weights were measured immediately after sacrifice at several age levels. Four ACTH treated and four control animals, from three separate litters and matched for body weight, were measured at 12 days of age. Seven ACTH treated and seven control experimentally naive animals were measured at 90 days of age. The six ACTH and six control animals used in the second Radial Arm experiment were sacrificed 30 days after testing at approximately 190 days of age and also measured for brain, adrenal and body weights. During the 30 days between testing and sacrifice, the animals were left undisturbed in their home cages in the laboratory vivarium.

Body weights were recorded for animals used to study developmental activity patterns. Measurements were taken on days 2, 9, 18, 24 and 30 after parturition. Body weights on adult animals were measured for the seven naive and seven control animals described above at 90 days of age. Body weights of animals used for analgesia tests were also recorded at 120 days of age (N=10/condition). This latter group of animals was derived from four separate litters.

RESULTS

Activity, Eye Openings, Brain, Adrenal and Body Weights

Pups injected with $ACTH_{4-10}$ exhibited a markedly different response to the injection than the vehicle-injected controls. Approximately 20 sec after the injection, ACTH treated neonates showed a dramatic increase in gross motor behavior which lasted 90–120 sec. The forelimbs engaged in a vigorous swimming motion, while the hindquarters showed a tendency to rotate. This pattern was often accompanied by stereotypic head movements. These behaviors were most robust on postnatal days 2–4 and tended to diminish between days 5 and 7. Control animals injected with the vehicle exhibited none of these responses.

Developmental activity pattern was analyzed by a 2 (group) × 9 (days) ANOVA with repeated measures over the last factor. No significant group differences were detected. Date of eye opening, analyzed using a Chi square test, was also not significantly different for the two groups. The average day of eye opening for both groups was postnatal day 14, with a similar range between day 12 and day 15.

Body and brain weights were analyzed by Student's *t*-tests. No differences were detected between the groups at any age. However, adrenal weights were heavier in $ACTH_{4-10}$ treated animals. A significant difference of treatment was seen on day 12, $t(6)=3.84$, $p<0.01$ (2 tail), as well as day 190 in adult animals tested in the Radial Arm maze, $t(10)=2.85$, $p<0.01$. Experimentally naive adult animals sacrificed at day 90 showed a similar, but nonsignificant trend ($p<0.15$). The total weight of both adrenals was used for analyses. Data are presented in Table 1.

Acoustic Startle

Startle data were analyzed using a 2 (group) × 30 (trials) ANOVA with repeated measures over the last factor. A significant main effect for trials, $F(29,552)=2.70$, $p<0.001$, was revealed as well as significant group × trials interaction, $F(29,552)=2.92$, $p<0.001$. A further analysis of orthogonal trend components revealed a quadratic effect for a group × trials interaction, $F(1,18)=9.55$, $p<0.01$. These effects can be observed in Fig. 1, which shows a clearly enhanced startle response for ACTH treated animals.

Open Field

Of the five open field measures, only the time to wall

TABLE 1

ORGAN WEIGHTS OF $ACTH_{4-10}$ TREATED ANIMALS AND LITTERMATE CONTROLS AT THREE DIFFERENT AGES

Age (N/Group)		Body		Brain		Adrenal	
		Control	$ACTH_{4-10}$	Control	$ACTH_{4-10}$	Control	$ACTH_{4-10}$
190 (6)	Mean	505 g	483 g	2.06 g	2.06 g	48.53 mg*	57.31 mg
	SEM	7.84	15.81	0.025	0.03	2.12	2.23
90 (7)	Mean	439 g	436.7 g	2.04 g	2.03 g	39.55 mg	42.19 mg
	SEM	13.56	23.35	0.03	0.03	1.58	2.11
12 (4)	Mean	27.5 g	27.0 g			5.3 mg†	8.2 mg
	SEM	1.02	0.98			0.21	0.72

*$p<0.01$.
†$p<0.001$.

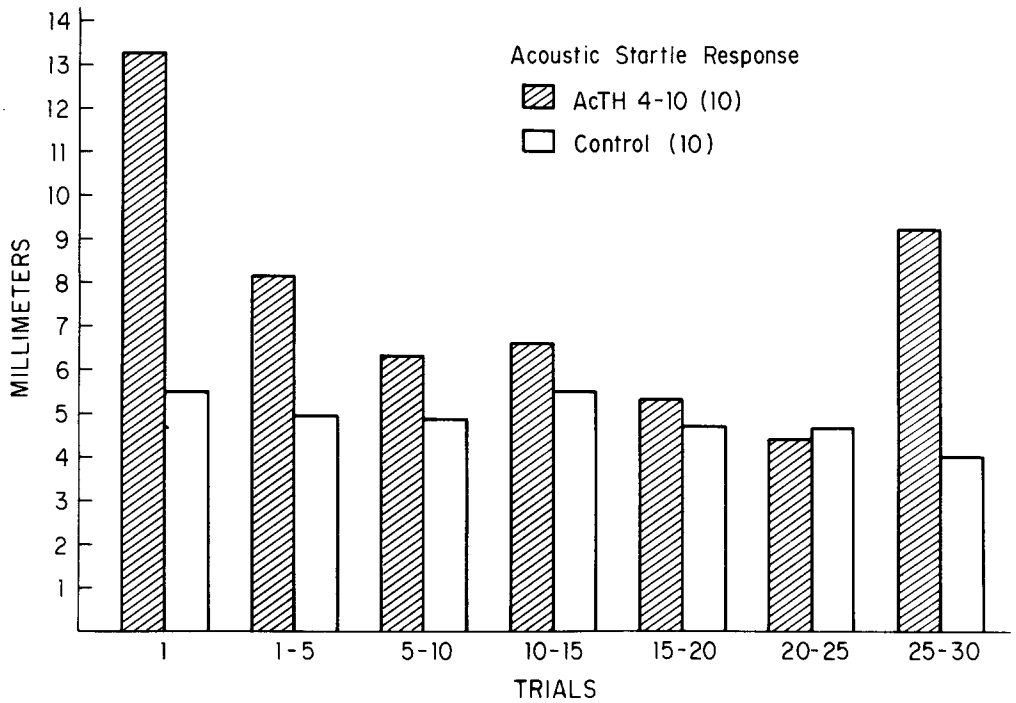

FIG. 1. Amplitude of the acoustic startle response in mm of pen deflection to a 100 dB, 50 msec, 2500 Hz tone. Thirty trials were given. N is indicated in parentheses.

measure (TTW) differentiated the two groups. ACTH treated animals moved from the center of the open field to the wall during the initial testing block significantly faster than the controls ($p<0.03$; Wilcoxon t). This measure also approached significance in the second testing block ($p<0.07$; Wilcoxon t), as shown in Fig. 2.

Thermal Pain Sensitivity and Morphine

Basal sensitivity to thermal pain, as well as analgesic responsiveness to morphine, was not significantly different for the two groups. No differences were detected using Student's t-tests for either the tail flick test or the hotplate test.

Active Avoidance Acquisition

Data were analyzed by Student's t-tests comparing the total number of avoidance responses made for each animal over the 100 trials. Animals treated with ACTH performed significantly worse than did controls, $t(10)=2.22$, $p<0.05$. ACTH treated animals also made a significantly greater number of spontaneous interatrial crossings than controls, $t(10)=2.78$, $p<0.01$. Interestingly, all ACTH treated animals developed a sterotypic pattern of responding over trials. The response pattern consisted of developing a preference for one side of the shuttle box, remaining on that side until the shock came on and then crossing to the other side. Upon

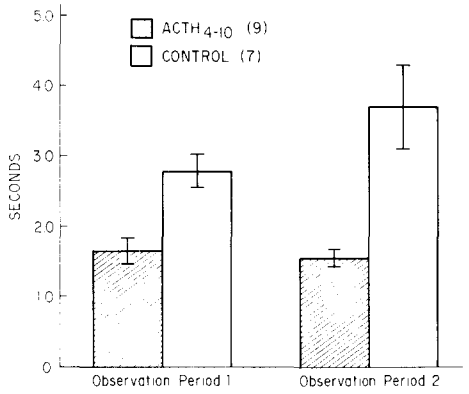

FIG. 2. Time for an animal to move from the center of an open field to the wall. Two five minute open field tests were given consecutively. N is indicated in parentheses.

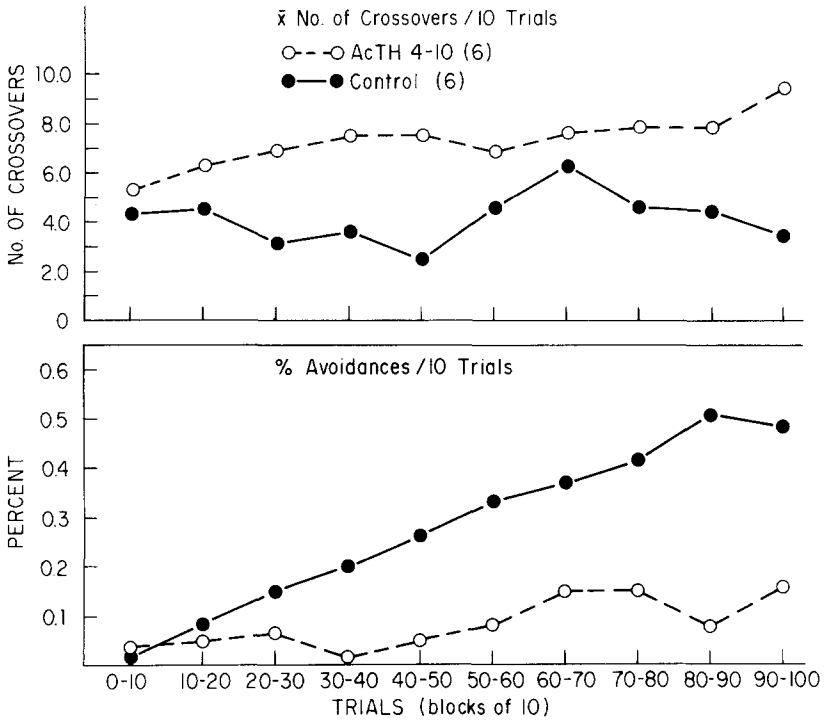

FIG. 3. Conditioned avoidance responses. Mean number of avoidances and intertrial crossovers per block of ten trials. One hundred trials were given in a single test session. ITI was 60 sec. N is indicated in parentheses.

shock termination, the animal immediately returned to the original side. This pattern was clearly evident in five of the animals after the first 60 trials and by trial 80 for the sixth animal. The pattern was not observed in any of the controls. Data are presented in Fig. 3.

Radial Arm Maze I

Experiment I did not reveal any performance decrement in ACTH animals compared to controls. Data were analyzed with a Wilcoxon *t*-test on total number of errors committed over the 10 test days. Data for days 5–10 of testing are shown in Fig. 4. However, two of the ACTH animals developed clear algorithms (repeated right turns) by the third training day and maintained them for the duration of the testing period. This pattern was not observed in any control animals. If these two animals were deleted from the analyses, the ACTH treated animals performed significantly poorer than the controls ($p<0.01$). For this reason, the experiment was repeated with a second group of animals in Experiment II (see below).

In Experiment I, data on latency to complete the first eight choices were analyzed by a 2 (group) × 10 (latency)

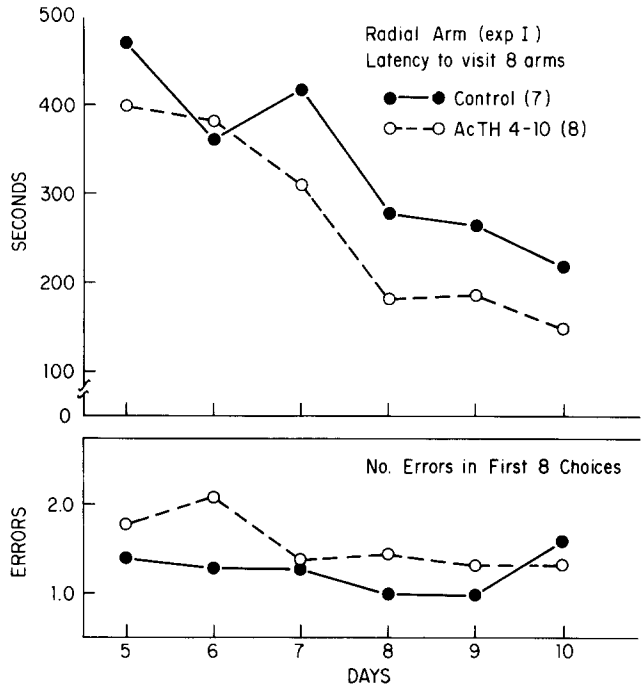

FIG. 4. Mean number of errors and latency to visit eight arms over days in the first radial arm experiment. N is indicated in parentheses.

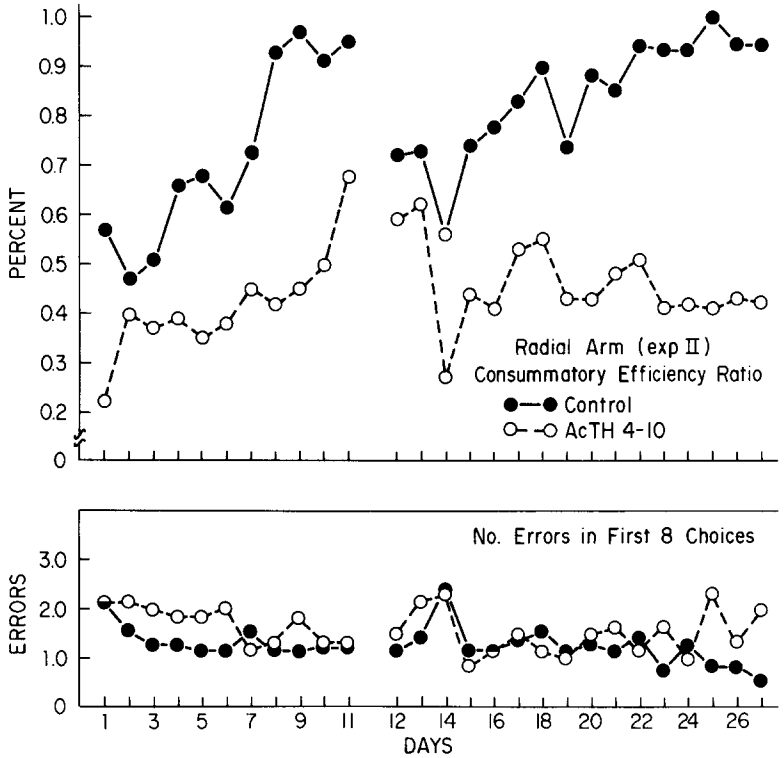

FIG. 5. Mean number of errors and consummatory efficiency ratio over days in the second radial arm experiment. Consummatory efficiency ratio was computed by dividing the number of reinforcements consumed by the number available within the first eight choices (N=8 minus the number of errors made).

ANOVA with repeated measures over the latency factor. ACTH animals tended to complete the first eight choices more quickly than controls, but this effect only approached significance, $F(1,9)=3.01$, $p<0.11$. There was also a tendency for ACTH animals to consume fewer of the food reinforcements. This effect was much more pronounced when the two animals which employed algorithms were deleted, and thus consummatory behavior was formally included in the second Radial Arm Experiment.

A qualitative histological examination of several brain areas (amygdala, septum, hippocampus, and entorhinal cortex) of these animals revealed no gross differences in organization or cell number between the control and $ACTH_{4-10}$ treated animals. The patterning of ACHE, examined particularly in the hippocampal formation, was also observed to be unchanged.

Radial Arm Maze II

As shown in Fig. 5, results from this second experiment paralleled the first with respect to the number of errors. A Wilcoxon *t*-test revealed no significant differences between groups for days 1–11 or days 12–27. However, the consummatory efficiency ratio of the ACTH treated group was consistently lower than the controls (Fig. 5). Data were analyzed using a mixed factorial ANOVA with repeated measures. Analysis of the first test period, days 1–11, revealed a significant main effect for days, $F(10,110)=5.93$, $p<0.001$, and a marginally significant effect for treatment, $F(1,11)=3.69$, $p<0.08$. Analysis of the second test period revealed main effects for days, $F(15,165)=2.43$, $p<0.01$, and for treatment, $F(1,11)=5.33$, $p<0.05$ (Fig. 5).

Sexual Behavior

Sexual behavior of ACTH treated animals was not discernably different from controls. No differences were observed in number of mounts, intromission, time to ejaculation, or post-ejaculatory interval.

Visual Discrimination Task

In the first experiment, which employed a high shock level, no differences were detected in the number of trials to criterion for the original learning problem (Control mean=30.5±5.8 SEM, ACTH mean=42.5±13.9 SEM). However, ACTH treated animals showed a marked impairment in their ability to acquire the reversal of their original black/white discrimination problem. The mean number of trials for the controls was 50.2 trials (±3.47 SEM) compared to 101 trials (±12.57 SEM) for the ACTH treated group ($t(6)=3.89$, $p<0.01$).

In Experiment II, where the minimal shock level necessary for task performance was employed, no differences were observed between the groups for either the original learning or the reversal problem. Mean number of trials to criterion for controls was 25.33 (±1.8 SEM) on the original learning problem compared to 25.0 (±1.5 SEM) for the ACTH treated group. The mean number of trials for the reversal problem was 50 (±2.8 SEM) for controls and 51.5 (±3.2 SEM) for the ACTH treated animals.

DISCUSSION

The results of the present experiment demonstrate several long-term effects after postnatal treatment with $ACTH_{4-10}$. Animals treated neonatally with a high dose of the peptide exhibited significantly poorer learning performance as adults in two of the three learning paradigms employed compared to littermate controls. Peptide treated animals were also found to exhibit an enhanced startle response as adults as well as a heightened aversion to open space, both in the open field and the radial arm maze. Additionally, adrenal weights of these animals were heavier than controls in both the developing and adult animal.

The influence of this neonatal treatment on learning capacity is difficult to assess. Peptide treated animals exhibited a marked impairment in the acquisition of the reversal learning problem of the visual discrimination task when a fixed shock level was used. However, adjusting the shock level for each animal to the minimal amount necessary to elicit a task related response normalized original learning performance of $ACTH_{4-10}$ animals, but did not influence performance of littermate controls. Reversal learning was also found to be the same as controls when shock levels were minimized. On the other hand, shuttle box performance was markedly impaired even though a relatively low level of shock for that task was used [1]. In an appetitive situation, performance in the radial arm maze was unaffected.

The results we obtained in the visual discrimination paradigm do not parallel results obtained when α-MSH, which contains the $ACTH_{4-10}$ amino acid sequence, or Org 2766 was administered neonatally [4,11]. Beckwith *et al.* [4] administered 10 μg of α-MSH on days 2–7 postnatally, whereas Champney *et al.* [11] administered a single ICV injection of 10 μg of Org 2766 on day 1 postnatally. Neither treatment affected original learning, but both studies reported enhanced reversal learning. Beckwith *et al.* [4] also reported enhanced original learning.

It may be that $ACTH_{4-10}$ is uniquely affecting the neonate in a manner not common to these other ACTH fragments. Alternatively, the relatively high dose level employed in the present study may be critical, since the effects of ACTH and related fragments can be biphasic, depending upon the dose administered. However, the apparent interaction of learning performance with shock stimulus intensity complicates an interpretation of this nature. It is not clear whether the poor learning performance we observed, when compared with the positive results cited above, suggests a biphasic effect of dose on developing CNS structures related to learning or a more global high dose effect on the CNS.

It appears that the peptide treated animals were more reactive than controls to the shock stimuli used in the visual discrimination and CAR paradigms, which could account for their poorer performance. The possibility of an exaggerated response to stress in these animals is partially supported by the enhanced startle response of these animals as well as the enlarged adrenal size. $ACTH_{4-10}$ is basically devoid of steriodogenic activity, but ICV injection of the peptide has been shown to induce pituitary-adrenal activation in the adult animal [43]. This raises the possibility that early exposure to $ACTH_{4-10}$ may have a long lasting influence on limbic structures involved in ACTH secretion. $ACTH_{4-10}$ has been demonstrated to have a variety of effects on limbic structures in adult animals [36, 37, 41]. However, its effect on the neonatal limbic system, especially the HPA axis, after peripheral injection remains to be demonstrated.

Org 2766 has been found to accumulate in the septal region after ICV injection [40]. This led us to consider the possibility that a similar accumulation of $ACTH_{4-10}$ in the

neonate, stemming from the daily injection regimen, might be detrimental to developing septal neurons or their efferent connections to other limbic areas. However, light microscopic inspection revealed no alterations in the cytoarchitecture of any limbic areas examined. Moreover, the patterning of AChE, which reflects the patterning of septal innervation in its target areas, was not disturbed. Additionally, major insult to the hippocampal structure does not appear to be implicated since damage to this structure is quite sensitive to disruption of radial arm maze choice performance [2,25].

Although $ACTH_{4-10}$ has been reported to affect copulatory and sexually motivated behavior in the adult rat [8], we did not observe any long-term effects of neonatal treatment on sexual functioning. Similarly, no long-term effects were observed on pain sensitivity or analgesic responsiveness to morphine.

Increased adrenal weights were the only difference observed between groups among the developmental parameters we examined. Similar to the report of van der Helm-Hylkama and deWeid [38], who treated female neonates with $ACTH_{4-10}$, we found no effect on data of eye opening in our male pups. Developmental activity patterns also were not altered, nor did we observe any differences in brain or body weights.

The activational effect of the peptide on the pup's motor behavior post injection has not previously been reported. We have observed a similar, but attenuated response in 2–5 day old pups injected with 10 μg of $ACTH_{4-10}$ or α-MSH (unpublished observations). The effect of the peptide on motor behavior appears to wane over time, often being absent in the 6 or 7 day old animal. A peripheral site of action is suggested by the work of Strand and co-workers [32, 33, 34], who have demonstrated that peripherally administered $ACTH_{4-10}$ enhances muscle action potentials in both immature (9–15 day old) and adult animals.

In summary, these data reveal poor learning performance, in an adversively motivated task, but normal learning in an appetitive task in addition to increased-adrenal weights and an exaggerated startle response in adult animals treated with a high dose of $ACTH_{4-10}$. These results suggest that $ACTH_{4-10}$ can act as a behavioral teratogen when administered subchronically in high doses to the developing nervous system.

ACKNOWLEDGEMENTS

$ACTH_{4-10}$ was kindly supplied by Abba J. Kastin. We are grateful to Susan Beall and Mark Cloud for their excellant technical assistance. This work was supported in part by grants from NIAAA (AA06478-03; AA06466-03) awarded to R.F.M.

REFERENCES

1. Beatty, W. W. and P. A. Beatty. Hormonal determinants of sex differences in avoidance behavior and reactivity to electric shock in the rat. *Comp Physiol Psychol* **73:** 446–455, 1970.
2. Becker, J. T., J. A. Walker and D. S. Olton. Neuroanatomical bases of spatial memory. *Brain Res* **200:** 307–320, 1980.
3. Beckwith, B. E., C. A. Sandman and A. J. Kastin. Influences of three shortchain peptides (α-MSH, MSH/$ACTH_{4-10}$, MIF-1) on dimensional attention. *Pharmacol Biochem Behav* **5:** Suppl 1, 11–16, 1976.
4. Beckwith, B. E., C. A. Sandman, D. Hothersall and A. J. Kastin. Influence of neonatal injections of α-MSH on learning, memory and attention in rats. *Physiol Behav* **18:** 63–71, 1977.
5. Beckwith, B. E. and C. A. Sandman. Central nervous system and peripheral effects of ACTH, MSH and related neuropeptides. *Peptides* **3:** 411–420, 1982.
6. Berger, P. A. and J. R. Tinklenberg. Neuropeptides and senile dementia. In: *Strategies for the Development of an Effective Treatment for Senile Dementia,* edited by T. Crook and S. Gershon. New Canaan, CT: Mark Powley Assoc., Inc., 1981, pp. 155–171.
7. Bohus, B., W. H. Gispen and D. deWied. Effect of lysine vasopressin and $ACTH_{4-10}$ on conditioned avoidance behavior of hypophysectomized rats. *Neuroendocrinology* **11:** 137–142, 1973.
8. Bohus, B., H. H. L. Hendricks, A. A. van Kolfschoten and T. G. Krediet. Effect of $ACTH_{4-10}$ on copulatory and sexually motivated approach behavior in the male rat. In: *Sexual Behavior: Pharmacology and Biochemistry,* edited by M. Sandler and L. Gessa. New York: Raven Press, 1977, pp. 269–275.
9. Branconnier, R. T., J. O. Cole and G. Gardos. $ACTH_{4-10}$ in the amelioration of neuropsychological symptomatology associated with senile organic brain syndrome. *Psychopharmacology (Berlin)* **61:** 161–165, 1979.
10. Butter, H., Y. Lapierre, K. Leprade, P. Firestone, A. Cote and F. Pierre-Louis. A comparative study of the efficacy of $ACTH_{4-9}$ analogue (Org 2766) and methylphenidate on hyperkinetic behavior. Report to Organon, 1979.
11. Champney, T. F., T. L. Sahley and C. A. Sandman. Effects of neonatal cerebroventricular injection of $ACTH_{4-9}$ and subsequent adult injections on learning in male and female albino rats. *Pharmacol Biochem Behav* **5:** 3–9, 1976.
12. de Wied, D. Effect of peptide hormones on behavior. In: *Frontiers in Neuroendocrinology,* edited by W. F. Ganong and L. Martini. New York: Oxford Univ. Press, 1969, pp. 97–140.
13. deWied, D. Behavioral effects of neuropeptides related to ACTH, MSH and β-LPH. In: *ACTH and Related Peptides: Structure Regulation and Action,* edited by D. T. Krieger and W. F. Ganong. *Annals of the New York Academy of Science, Vol 297,* 263–274, 1977.
14. deWied, D., T. J. B. van Wimersma Greidanus and B. Bohus. Pituitary peptides on motivational, learning and memory processes. In: *Psychotropic Action of Hormones,* edited by T. M. Itil, G. Laudahn and W. M. Herrmann. New York: Halstead Press, 1976, pp. 183–194.
15. Ferris, S. H., G. Sathananthan, S. Gershon, C. Clark and J. Moshinsky. Cognitive effects of $ACTH_{4-10}$ in the elderly. *Pharmacol Biochem Behav* **5:** Suppl 1, 173–178, 1976.
16. Gispen, W. H., J. Buitelaar, V. M. Wiegant, L. Terenius and D. deWied. Interaction between ACTH fragments, brain opiate receptors, and morphine induced analgesia. *Eur J Pharmacol* **39:** 393–397, 1976.
17. Gold, P. E. and R. van Buskirk. Effects of post-trial hormone injections on memory processes. *Horm Behav* **1:** 509–517, 1976.
18. Gold, P. E. and R. van Buskirk. Enhancement and impairment of memory processes with post-trial injections of adrenocorticotropic hormone. *Behav Biol* **16:** 387–400, 1976.
19. Jouhaneau-Bowers, M. and J. LeMagnen. ACTH self-administration in rats. *Pharmacol Biochem Behav* **10:** 325–328, 1979.
20. Lichtensteiger, W. and F. Monnet. Differential response of dopamine neurons to α-melanotropin and analogs in relation to their endocrine and behavioral potency. *Life Sci* **25:** 2079–2087, 1979.
21. Lynch, B. and H. Killackey. Neuroanatomical techniques for neurobehavioral research. In: *The Neurobiology of Aggression,* edited by R. E. Whalen. New York: Plenum Press, 1974, pp. 99–123.
22. McGivern, R. F., C. Berka, G. G. Bernston, J. M. Walker and C. A. Sandman. Effect of naloxone on analgesia induced by food deprivation. *Life Sci* **25:** 885–888, 1979.

23. Murphy, J. V. and R. E. Miller. The effect of adrenocorticotropin (ACTH) on avoidance conditioning in the rat. *J Comp Physiol Psychol* **48:** 47–49, 1955.
24. Nyakas, C., G. Levay, J. Viltsek and E. Endroczi. Effects of neonatal $ACTH_{4-10}$ administration of adult adaptive behavior and brain tyrosine hydroxylase activity. *Dev Neurosci* **4:** 225–232, 1981.
25. Olton, D. S., J. A. Walker and F. A. Gage. Hippocampal connection and spatial discrimination. *Brain Res* **139:** 295–308, 1978.
26. Pigache, R. M. and H. Rigter. Effect of peptides related to ACTH on mood and vigilance in man. *Frontiers in Hormone Research* **8:** 193–207, 1981.
27. Rappaport, J. L., P. O. Quinn, A. P. Copeland and C. Burg. $ACTH_{4-10}$: Cognitive and behavioral effects in hyperactive learning disabled children. *Neuropsychobiology* **2:** 291–296, 1976.
28. Sandman, C. A., B. B. Walker and C. A. Lawton. An analog of $MSH/ACTH_{4-9}$ enhances interpersonal and environmental awareness in mentally retarded adults. *Peptides* **1:** 109–114, 1980.
29. Sandman, C. A., B. B. Walker and C. Berka. Influence of afferent cardiovascular feedback on behavior and the cortical evoked potential. In: *Perspectives in Cardiovascular Psychophysiology*, edited by J. T. Cacioppo and R. E. Petty. New York: Guilford Press, 1982, pp. 189–222.
30. Sands, S. F. and A. A. Wright. Enhancement and disruption of retention performance by ACTH in a choice task. *Behav Neural Biol* **27:** 413–422, 1979.
31. Schotman, P. and J. Allaart. Biphasic modulation by ACTH-like peptides on protein synthesis in a cell-free system from rat brain. *J Neurochem* **37:** 1349–1352, 1981.
32. Smith, C. M. and F. L. Strand. Neuromuscular response of the immature rat to $ACTH/MSH_{4-10}$. *Peptides* **2:** 197–206, 1981.
33. Strand, F. L., A. Cayer, E. Gonzalez and H. Stobey. Peptide enhancement of neuromuscular function: Animal and clinical studies. *Pharmacol Biochem Behav* **5:** Suppl 1, 179–187, 1976.
34. Strand, F. L. and C. M. Smith. LPH, ACTH, MSH and motor systems. *J Pharmacol Exp Ther* **11:** 509–533, 1980.
35. Thompson, R. and J. H. Bryant. Memory as affected by activity of the relevant receptor. *Psychol Rep* **1:** 393–400, 1955.
36. Urban, I., F. H. Lopes de Silva, W. Storm van Leeuwen and D. deWied. A frequency shift in the hippocampal theta activity: An electrical correlate on central action of ACTH analogs in the dog. *Brain Res* **69:** 361–365, 1974.
37. Urban, I. and D. deWied. Changes in excitability of the theta activity generating substrate by $ACTH_{4-10}$ in the rat. *Exp Brain Res* **24:** 325–344, 1976.
38. van der Helm-Hylkema, H. and D. deWied. Effect of neonatally injected ACTH and ACTH analogues on eye-opening of the rat. *Life Sci* **18:** 1099–1104, 1976.
39. Veith, J. L., C. A. Sandman, W. M. Walker, D. H. Coy and A. J. Kastin. Endorphins: Systematic administration selectively alters open field behavior of rats. *Physiol Behav* **20:** 539–542, 1978.
40. Verhoeff, J., A. Witter and D. deWied. Specific uptake of behaviorally potent $(^3H)ACTH_{4-9}$ analog in the septal area after intraventricular injection in rats. *Brain Res* **131:** 117–128, 1977.
41. Versteeg, D. H. G. and R. J. Wurtman. Effect of $ACTH_{4-10}$ on the rate of synthesis of (^3H)catecholamines in the brains of intact hypophysectomized and adrenalectomized rats. *Brain Res* **93:** 552–557, 1975.
42. Walker, J. M., G. G. Berntson, C. A. Sandman, A. J. Kastin and H. Akil. Induction of analagesia by centeral administration of Org 2766, an analog of $ACTH_{4-9}$. *Eur J Pharmacol* **69:** 71–79, 1981.
43. Wiegant, V. M., J. Jolles, D. L. Colbern, E. Zimmermann and W. H. Gispen. Intracerebroventricular ACTH activates the pituitary-adrenal system: Dissociation from a behavioral response. *Life Sci* **25:** 1791–1796, 1979.