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Effects of Neonatal Cerebral Ventricular Injection of ACTH 4-9 and Subsequent Adult Injections on Learning in Male and Female Albino Rats

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CHAMPNEY, T. F., T. L. SAHLEY AND C. A. SANDMAN. *Effects of neonatal cerebral ventricular injection of ACTH 4–9 and subsequent adult injections on learning in male and female albino rats.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 3–9, 1976. An investigation of permanent developmental effects of a potent, long acting ACTH/MSH 4–10 analogue (Organon 2766) on adult passive avoidance performance and of subsequent peripheral adult injections of the same substance on visual (black and white) discrimination learning and reversal in a Thompson-Bryant box was conducted. A subproblem analysis of visual and position preferences during reversal was performed. No differences in passive avoidance performance or in original discrimination learning were obtained. Both infant and adult treatments enhanced reversal learning and visual orientation (proportion of responses to the previously positive stimulus). Infant treatment suppressed position orientation in males and enhanced position orientation in females. These effects were interpreted as indicating that ACTH-like peptides enhance attention to the relevant stimulus by a direct effect on the brain.

Neuropeptides Attention Sex differences Adrenocorticotropic hormone Melanocyte-stimulating hormone ACTH analogues Discrimination learning Intracranial injections

CLASSICAL opinion suggested that adrenocorticotrophic hormone (ACTH) was active in the stress response of most animals and that melanocyte-stimulating hormone (MSH) changed the pigmentation of lower vertebrates and was merely vestigeal in mammals. However, recent evidence indicates that ACTH, MSH and the centrally active amino acid sequence that these two molecules share (MSH/ACTH 4–10) have observable behavioral consequences. Administration of these substances to adult rats has resulted in delayed extinction of active [3,23] and passive avoidance learning [17,32], delayed extinction of an appetitive task [31] and enhanced reversal learning of a brightness discrimination task [27,33]. The results have been interpreted as indicating that the heptapeptide influences emotion or fear [2,23], memory [3], motivation [8,34], arousal [1], and attention [27,29].

In a series of recent studies, infant rats (age 2–7 days) were given intraperitoneal injections of MSH and tested as adults with several behavioral tasks [30]. Early postnatal treatment of rats with MSH resulted in improved efficiency on a DRL-20 task, facilitated reversal learning, improved performance of the changed dimension of an extra-dimensional shift in a visual discrimination problem, and enhanced acquisition and hastened extinction of an active avoidance response.

The results of the developmental studies also raised the possibility that the behavioral effects of these neuropeptides were sex-dependent. The enhanced reversal learning appeared to be restricted to male animals. In another developmental study, increased gregariousness was most evident in females treated with MSH [30].

The current experiments were designed to explore further the sex and task dependency of early exposure to peptide hormone analogues. A longer acting derivative (ACTH 4–9 analogue; Met-40_2, 8 D-Lys, 9 Phe-ACTH 4–9, Organon 2766) purported to be many times more potent than ACTH, MSH or their other analogues was employed. To maximize the probability that the injections would effect the learning process by direct CNS action, infant rats were injected intraventricularly. In addition, Experiment 2 was designed to assess the possibility that rats pretreated with the ACTH 4–9 analogue as infants would be sensitized to administration of this peptide derivative as adults.

EXPERIMENT 1

METHOD

Animals

Ten litters of rats delivered by polyparous mothers of the Sprague-Dawley Holtzman albino strain were housed with their mothers for the first three weeks postpartum in plastic maternity cages. At approximately 21 days of age the pups were weaned and housed in double metal cages in groups of 3 or 4. Cage mates were kept homogeneous with respect to litter, experimental group, and sex. Twenty-four hour constant diffuse lighting was used throughout the experiment. Seventy-seven rat pups were included in the experiment.

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Surgery

At approximately 24 hr postpartem, pups were anesthetized in a small jar with gauze soaked in Metophane (methoxyflurane) for approximately 2 min, until they were no longer responsive to pain. Pups were retained in modeling clay mounted on a wooden block firmly secured in a stereotaxic apparatus with head elevated about five degrees from horizontal. A 10 μl Hamilton microliter syringe was mounted on the stereotaxic carrier. Injection coordinates were unilateral and 1.0 mm anterior to bregma, 1.0 mm lateral from the longitudinal suture and 2.0 mm ventral from the surface of the skull. The locus of the injection was the lateral ventricle near its confluence with the third ventricle. Coordinates were varified by injecting india ink into the brain of one pilot pup. Pups were injected with 10 μg in 0.5 μl of a structural modification of ACTH 4 9 (4-O_2-Met, 8 D-Lys, 9 Phe-ACTH 4–9, Organon 2766). This compound which is a longer acting pharmacologic modification of the ACTH 4 9 molecule, is putatively an ACTH 4 10 like substance in its behavioral actions. The rats were injected with the ACTH 4–9 analogue dissolved in .01 normal acetic acid and 0.85% NaCl in aqueous solution or with the control vehicle. The syringe needle was left in place in the brain for about two minutes following injection to allow hormone to diffuse into the ventricular system. The pups were marked with a code so that the experimenter remained blind with respect to treatment group membership throughout the study. About 90% of the operated pups survived through the time of testing. Their viability did not differ from unoperated controls.

Apparatus

A passive avoidance box was used in the experiment. It consisted of a wire mesh runway extending out over the edge of a table, a sliding doorway and a goal compartment. The goal compartment was 25.4 cm high, 30.5 cm wide and 34.3 cm long, the doorway 10.2 cm square and the runway 10.2 cm wide by 24.5 cm long. The box was constructed with transparent red Plexiglas and the runway wire mesh. The grid floor consisted of 0.5 cm brass bars spaced 1.3 cm apart. Scrambled DC current, 0.6 mA was delivered to the grids via a Scientific Prototype model No. 4027JM power source and Grason-Stadler model No. E1100DA shock generator and scrambler and timed with a Testan Universal timer. The apparatus was located in a walk-in sound attenuated compartment with a 300 W incandescent light above the runway.

Procedure

The procedure was a double blind one-trial passive avoidance learning situation. Treatment groups consisted of 15 male and 19 female ACTH 4 9 analogue treated rats, 12 male and 15 female operated controls, and 9 male and 7 female unoperated controls. Animals were 90 to 120 days old at the time of testing. Animals were handled routinely just prior to testing for 1–2 min and were tested at about the same time every day. On Day 1 animals were placed on the runway facing away from the goal box, latencies to enter the box were recorded and animals were allowed to remain in the box for five minutes following entry. On Day 2 animals were placed on the runway and latencies recorded, the doorway was closed and a 3 sec 0.6 mA footshock delivered to the grid immediately following entry. Animals were immediately removed and returned to their home cage. On Days 3, 4, 5 and 9 animals were placed on the runway, latencies recorded, and animals were returned to their home cage following entry into the box or after 300 sec expired without a response.

RESULTS

Since rats treated as infants with ACTH/MSH 4 10-like substances had never been tested in a passive avoidance paradigm, it was expected that effects on performance would resemble those previously reported for both infant and adult treated animals [10,32]. This expectation was not confirmed by the present experiment. The latency data was analyzed by analysis of variance (Balanova) with treatment, sex and days as factors. There were no significant differences between unoperated and operated control animals, therefore control animals were pooled. Other than an effect of days, the only significant differences between groups emerged as a three-way interaction, $F(5,365) = 2.83$, p 0.025 for latencies). Simple effects tests performed for avoidance latencies within each day indicated that only the treatment by sex interaction was significant 48 hr after shock, $F(1,73) = 6.82$, $p<0.02$. It is apparent from Fig. 1 that males treated with the ACTH 4 9 analogue showed an enhanced passive avoidance latency 48 hr after footshock and from Fig. 2 that females treated with the ACTH 4 9 analogue showed a decreased latency 48 hr after footshock. Latency data were transformed logarithmically in order to better conform to the analysis of variance assumptions and no differences in avoidance latencies related to treatment or sex were significant.

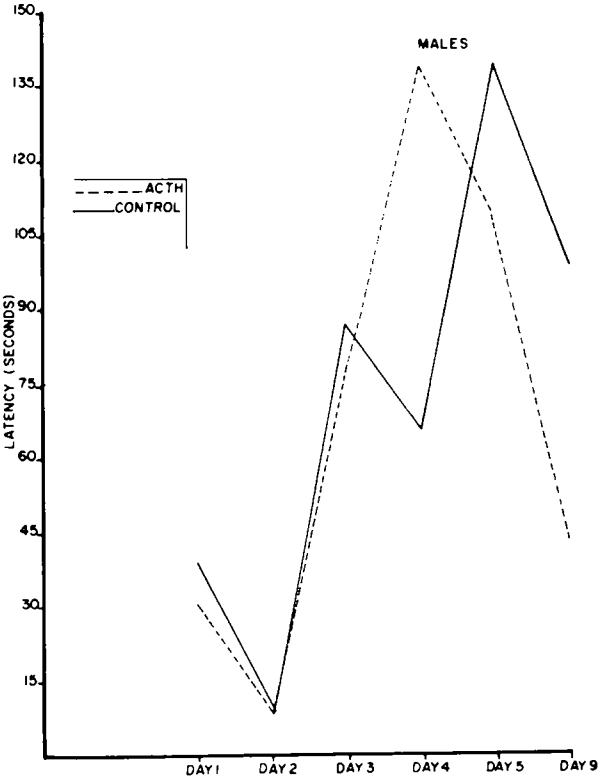

FIG. 1. Latencies for males to place four feet in the goal box in a passive avoidance response (PAR) situation represented as a function of day of testing.

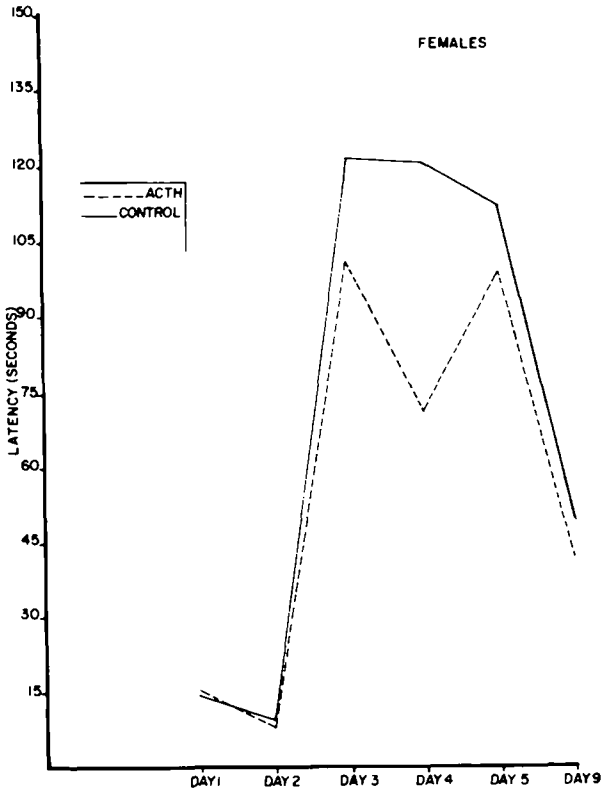

FIG. 2. Latencies for females to place four feet in the goal box represented as a function of day of testing. Animals received a shock on Day 2.

EXPERIMENT 2

METHOD

Animals

The same rats as in Experiment 1 were used in this experiment except for 1 male operated and 3 male unoperated controls for a total of 73 animals.

Apparatus

The test apparatus was a Thompson-Bryant discrimination box consisting of a start compartment, a choice compartment and a goal box. The start compartment was separated from the choice compartment by a guillotine door. Black and white discriminanada doors were inserted into 8.6 cm square openings separating the choice compartment from the goal compartment–one of these could be barred shut from behind. The distance from the start compartment to the goal compartment was 52.0 cm. The discriminanda were 4.4 cm apart with a 6.7 cm partition protruding between them. The walls of the box were constructed of black Plexiglas 17.8 cm in height and the top was clear. One 3 W light was attached to the top above the start compartment and one above each of the discriminanda. The floor of the goal box was black Plexiglas. The same grid, shock apparatus and sound attenuated chamber as in Experiment 1 were used.

Procedure

The procedure was a double blind visual discrimination learning and reversal situation. In order to test the possibility that infant injection may have sensitized rats to later injections, the treatment groups from Experiment 1 were each divided in half–half of each group received a subcutaneous injection of 4-O_2-Met, 8-D Lys, 9 Phe-ACTH 4–9 (Organon 2766) and half the vehicle solution 5 min prior to testing during every day of original learning and reversal. Table 1 represents the experimental design. The dosage of the ACTH 4–9 analogue was 10 μg in 0.5 cc of 0.01 normal acetic acid and 0.85% NaCl in aqueous solution. One half of one cc of the vehicle was administered to controls. On the day prior to the beginning of the experiment each animal was adapted to the Thompson box for 15 min with all doors open. Animals were tested at about the same time every day. On each trial animals were placed in the start box and the door was opened throughout the rest of the procedure. Animals were tested in squads of 6, alternating with the other animals in the squad so that trials were spaced. Goal box discriminanda doors were kept clean and free of extraneous odor using a cleaning solution of 95% ethyl alcohol and diluted acetic acid -doors were cleaned at least once during every trial. Animals were tested 25 trials per day or until a criteria was reached and then returned to the home cage. Reversal training did not begin until about 7 days after the beginning of original learning. Three days intervened between tasks. One experimenter uninformed of group membership tested all of the animals throughout the procedure. One-half second of 0.6 mA footshock was used as negative reinforcement.

TABLE 1

THE DESIGN OF EXPERIMENT 2: THE NUMBER OF ANIMALS IN EACH TREATMENT GROUP. EACH ANIMAL RECEIVED TREATMENTS BOTH AS AN INFANT AND AS AN ADULT

Infant Treatment:	ACTH 4-9	ACTH	Control	Control
Adult Treatment:	ACTH 4-9	Control	ACTH	Control
Males	N = 7	N = 8	N = 8 (op. = 6) (unop. = 2)	N = 9 (op. = 5) (unop. = 4)
Females	N = 9	N = 10	N = 11 (op. = 8) (unop. = 3)	N = 11 (op. = 7) (unop. = 4)

Pretraining. During the shaping procedure animals received continuous bursts of half second shock for hesitation of more than 5 sec on movement toward the start box until forward progress was resumed. Black and white diagonally striped doors were used during shaping. On trial one the doors were placed against the back of the goal box. On trial two they were halfway closed. On trial three they were three-quarters closed. And for the remainder the doors were completely closed. Criterion was 5 consecutive correct responses of knocking down a goal box door when all the way closed. Trials to criterion during shaping were recorded.

Original learning. Animals were trained to run to an unlocked white door to enter the goal box to a criterion of 9 out of 10 correct responses. The black door was locked.

The positive discriminanda—the white door was alternated between left and right according to a Gellerman sequence. An error was recorded and the animals received a shock if any part of their body (usually the nose) came in contact with the negative discriminanda—the black door. During the first 15 trials animals also received a shock for lack of forward progress exceeding 5 sec but an error was not recorded for this. All recorded errors and correct responses also reflected a left or right choice.

Reversal learning. The procedure was exactly the same as for original learning except that the black door was positive and the white door, negative. Because the criterion for errors was exclusively for discriminative as opposed to hesitational errors, the number of trials during reversal spent responding to the previously positive visual discriminanda (visual orientation) to an 8 out of 10 criteria or better was determined. The number of reversal trials spent responding to a left or right position preference (position orientation), to an 8 out of 10 criteria was also tabulated. Visual orientation was evaluated as an extinction score, initiated on the first reversal trial and terminated when choices of the previously positive stimulus deteriorated to 7 out of 10. The number of visual orientation responses was divided by the total reversal score to yield a proportion independent of total trials to criteria. The number of position response trials was then determined. Trials included in the visual orientation scores were not counted. First the correct-error scores in the raw data were converted to left-right responses using the Gellerman sequence as a key. Preferred position responses were defined as left or right responses occurring in sequence that included eight or more responses out of 10 in the same direction. The last ten trials of reversal learning were never included. If an animal shifted from one position preference to the other and met the 8 out of 10 criteria in both cases, both sets of trials counted in the socre (only one male and one female performed in this manner). The proportion of the total trials to criterion spent responding to a position preference was tabulated and reported.

RESULTS

During original learning males reached criterion in significantly fewer trials than females, $F(1,65) = 5.03$, $p<0.05$. However, no effect of hormone treatment was observed during original learning. The operated and unoperated controls did not differ in performance and were therefore pooled. Reversal learning performance was significantly enhanced by injections of the ACTH 4–9 analogue. This was in agreement with previous studies in which ACTH, MSH or their analogues were administered to adult rats [27,33] and to rats pretreated as infants [30].

Trials to criterion and reversal subproblem data were analyzed with a completely crossed infant treatment by adult treatment by sex analysis of variance. Trials to criterion scores served as the dependent variables for shaping, original learning and reversal. The proportions of total trials to criterion spent responding to the previously positive visual stimulus and to a position preference served as dependent variables for the reversal subproblem analysis. Simple effects tests were computed independently for each sex. Due to the heuristic and suggestive nature of the subproblem analysis, marginally significant interactions $p<0.10$ were accepted as a justification for performing simple effects tests.

Reversal learning was significantly enhanced after both infant, $F(1,65) = 8.25$, $p<0.005$, and adult, $F(1,65) = 11.18$, $p<0.001$, injections of the ACTH 4–9 analogue. It is apparent from Figs. 3 and 4 that the ACTH 4–9 analogue exerted an identical effect in males and females, which resulted in a decrease in the number of trials to criterion for reversal learning. Animals treated as infants, as adults or both learned reversal much faster than animals given saline both as infants and as adults.

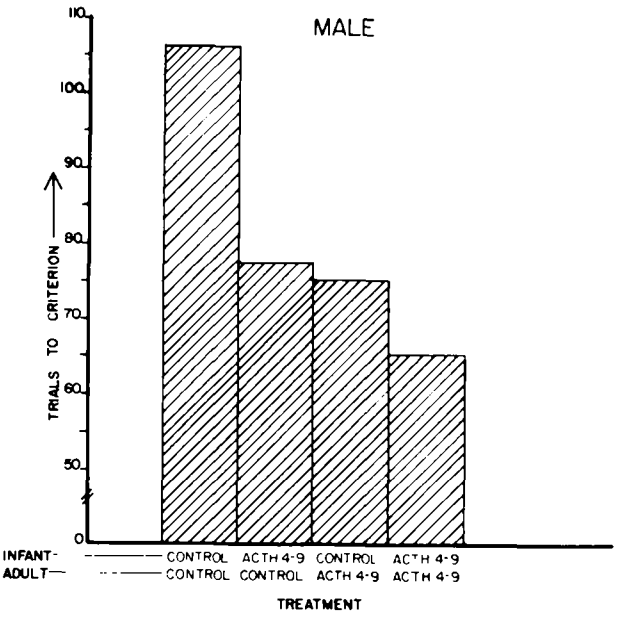

FIG. 3. Trials to a nine out of ten criterion represented as a function of treatment group for males during reversal learning.

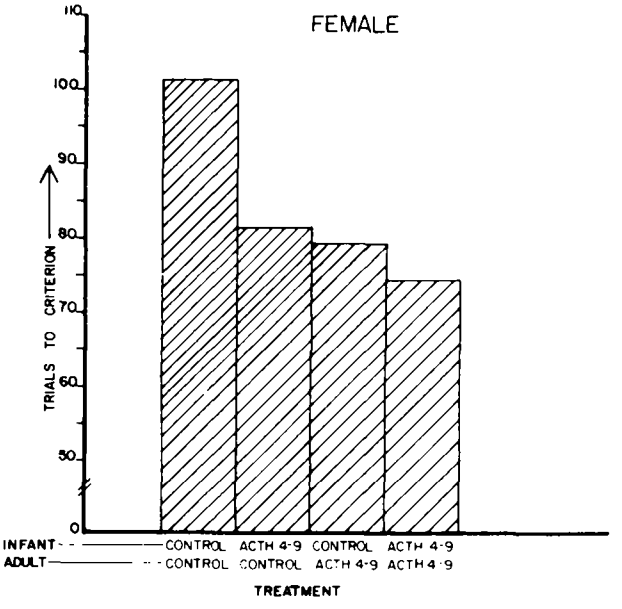

FIG. 4. Trials to a nine out of ten criterion represented as a function of treatment for females during reversal learning.

Simple effects within each sex indicated that the difference between performance of males treated as infants with the ACTH 4–9 analogue and control animals was significant, $F(1,28) = 8.02$, $p<0.01$ while the effect was nonsignificant for females. Treatment of adults with the ACTH 4–9 analogue significantly enhanced reversal performance in males, $F(1,28) = 9.82$, $p<0.005$, but the enhancement was nonsignificant in females, $F(1,37) = 3.65$, $p>0.05$. The effects of ACTH/MSH 4 10-like substances on reversal learning obtain in both male and female rats but appear to be much more robust in males than in females.

During acquisition of reversal learning in the Thompson Bryant box, rats were typically observed to elaborate a sequence of behaviors possibly related to attempts at problem solving, coping or minimizing punishment. At first the sequence involved responding to the previously positive visual stimulus. In the second phase reversion to a position preference was observed and finally, acquisition of the reversal discrimination was accomplished. During the intervening trials responding was observed to vary between strictly position choices and unclassifiable although probably not random choices. Position oriented rats tended to acquire reversal by persevering in the position habit and shifting to the new positive stimulus when it was discrepant with the position choice just prior to committing an error. They thus avoided punishment and solved the reversal problem in a go-no go [11] fashion. Visually oriented rats were observed to move to a point close to but equidistant from the two discriminanda and shift gaze between the stimuli prior to making a choice. Figure 5 illustrates the profiles of orientation response patterning in each of the treatment groups for males and Fig. 7 for females. The mean proportions represented were derived by the subproblem analysis of reversal learning previously described. The unclassified proportions presented are merely equal to one minus the sum of the other two means.

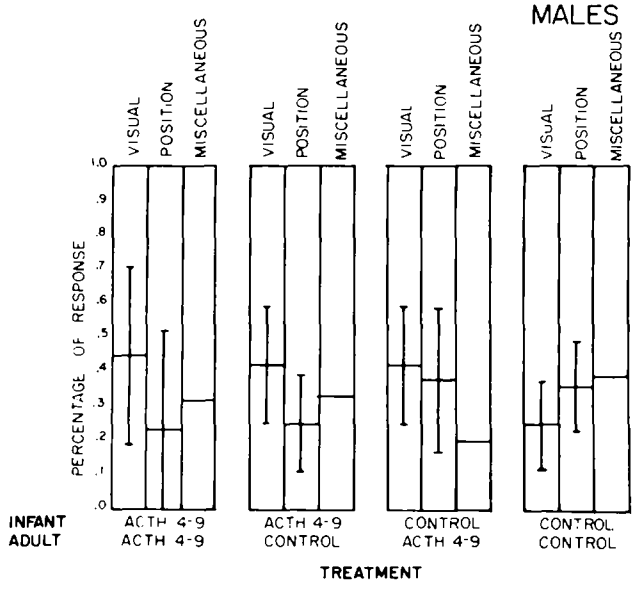

FIG. 5. The percentage of responses to the previously positive stimulus (visual), to a position preference and unclassifiable responses (miscellaneous) during reversal learning represented as a function of treatment group. The $t_{.95}$ confidence intervals also appear.

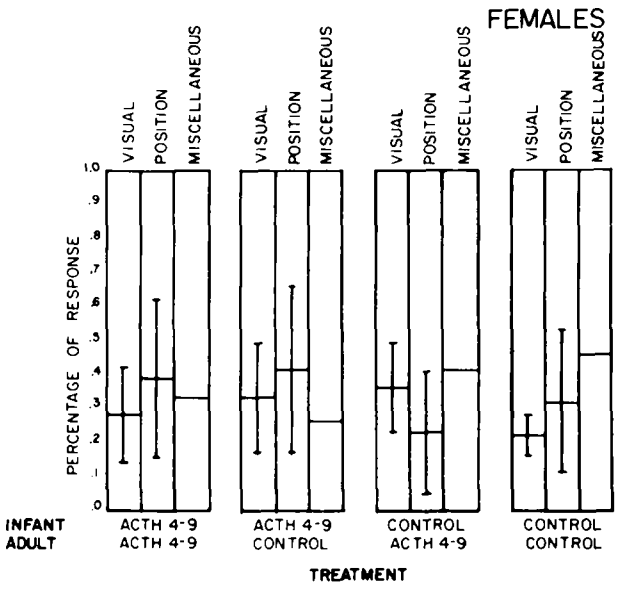

FIG. 6. The percentage of responses to the previously positive stimulus (visual), to a position preference (position) and unclassifiable responses (miscellaneous) during reversal learning represented as a function of treatment group. The $t_{.95}$ confidence intervals also appear.

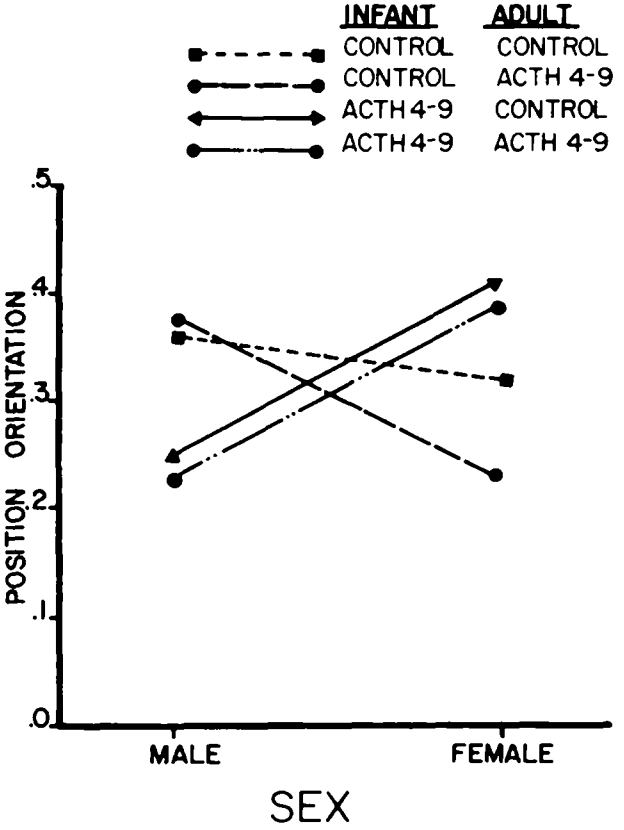

FIG. 7. The proportion of position responses during reversal learning presented as a function of sex. Solid and dashed lines represent changes in position orientation within treatment groups as a function of sex. The interaction of infant treatment and sex was significant at $p<0.05$.

Infant treatment with the ACTH 4–9 analogue significantly enhanced visual orientation in animals given the control solution as adults, $F(1,34) = 5.52$, $p<0.025$. Animals given the control solution as infants, but treated as adults with the ACTH 4–9 analogue showed significant enhancement of visual orientation, $F(1,35) = 8.01$, $p<0.01$. Figure 5 indicates that enhanced visual orientation was obtained in male rats treated with the ACTH 4–9 analogue either as infants, as adults or both. Figure 6 indicates that while adult injections of the ACTH 4–9 analogue enhanced visual orientation in females, infant injections of the ACTH 4–9 analogue produced a decrement in this effect.

Only infant injections of the ACTH 4–9 analogue appeared to effect position orientation. The infant treatment by sex interaction was significant $F(1,65) = 3.97$, $p<0.05$, and is illustrated in Fig. 7. The figure illustrates that infant treatments with ACTH/MSH 4–10-like peptides suppress position orientation in males and enhance position orientation in females. Also, control males were less position oriented than control females.

DISCUSSION

Primarily this study was conceived as a further test of the applicability of an attentional model [19, 20, 36] for explaining the effects of ACTH 4-10-like substances. It was surmised that an injection of a potent, long acting compound directly into the brains of 1-day-old rat pups followed by a daily regimen of injections as adults during the discrimination learning and reversal procedure would serve to maximize behavioral effects and thus enable a more definitive resolution of the behavioral actions of these peptides.

The results of Experiment 1 indicated that infant treatment with the ACTH 4–9 analogue did not effect avoidance learning. In Experiment 2 the same animals were tested but were further divided into groups receiving daily injections of the ACTH 4–9 analogue or saline-acetic acid vehicle solution. An enhancement of visual discrimination reversal learning was obtained as a result of both infant and adult treatments with the ACTH 4–9 analogue. These results provide strong support to the contention that ACTH 4-10-like substances enhance processes in the central nervous system related to attention. If the effect of these peptides was merely to prolong extinction performance rather than enhance attention to the relevant stimulus category a decrement in reversal learning would have been expected as a result of the peptide. If the effect of these peptides was merely to heighten arousal levels a decrement in passive avoidance performance would have been expected.

The effects of early injections of ACTH/MSH 4–10-like substances have been investigated in several other recent studies in our laboratory. For instance, neonatal IP injections of MSH on Days 2–7 (1) produced a significant enhancement of efficiency ratios on a DRL 20 schedule in male albino rats tested as juveniles, (2) enhanced performance of visual discrimination learning, reversal, and extradimensional shift paradigms in males but not in females; and (3) a significant enhancement of gregariousness (rats spent more time in contact) was observed in preestrous females and in males as juveniles and as adults [30]. The effects of early injections of ACTH 4–10-like substances can be accounted for in terms of enhancement of a single process, attention. An enhanced ability to direct the focus of attention, to maintain focus on relevant unchanged stimulus conditions and to switch focus when the changed stimulus conditions are motivationally significant, account best for the present results. The effect of injections of ACTH 4–10-like substances is proposed to be obtainable by either infant or adult injections and in both males and females. The effect may require a more potent substance (or possibly a higher dose of a weaker substance), a more effective route of administration, and different measurements in order to be detected in females.

Sex differences obtained in the present study were consistent with fairly well known sex differences reported to occur in human learning [18], and those obtained in other studies with ACTH 4–10-like peptides [30]. It was reported [18] that visuospatial and quantitative abilities in boys are greater while haptic and proprioceptive abilities are greater in girls. The female rats would thus be expected to show less visual orientation than males so that the reversal habit would extinguish faster regardless of treatment when a position response was the correct solution. The trends observed in the present study, indicating greater visual orientation in males and greater position orientation in untreated females are consistent with these observations in humans.

A study of visual discrimination reversal learning in cats following neonatal hippocampectomy obtained findings similar to the present results [11,24]. Reversal learning was observed to encompass three phases–extinction of the previously learned response, reversion to a position response pattern, and acquisition of the reversal discrimination. Hippocampectomized cats responded to the reversal problem in a go-no go serial decision making fashion as did position oriented rats in the present study. Infant injections of ACTH 4–9 suppressed position orientation in males and enhanced position orientation in females although the effects were more subtle than those reported with hippocampectomized cats. Female rats treated as infants with the ACTH 4–9 analogue behaved like neonatally hippocampectomized cats; they appeared hyperactive and solved the discrimination problem in a go-no go fashion.

Several lines of evidence indicate that the activity of the hippocampus is effected by ACTH/MSH 4–10-like substances. Radioactive labeling studies indicate that this action may be indirect, via target sites in other regions of the brain such as the occipital cortex or the brain stem [14]. It has been found [32] that changes in EEG associated with MSH injections resembled hippocampal theta activity. It has also been reported [6,26] that neonatal administration of corticosterone impaired uptake of 3H-corticosterone by the hippocampus in adult rats and that this was correlated with increased activity and suppression of passive avoidance learning. Furthermore, other evidence [21] has demonstrated that single units in the hippocampus can be inhibited by peripheral injections of corticosterone and facilitated by injections of ACTH.

It has been suggested [5], among other possibilities, that the hippocampus may be involved in attentional processes. The hippocampus may subserve selective attention by inhibiting on-going activity subserving processing of information within irrelevant categories leaving areas in the brain within the focus of attention unaffected. Both infant and adult injections of the ACTH 4–9 analogue enhanced visual orientation during reversal learning. This effect may have been mediated by alterations in hippocampal activity. Effects of ACTH/MSH 4–10-like substances on position

and visual orientation may result from suppression of other, less relevant sensory-motor integrative processes and may be mediated by target tissue sites that in turn influence the activity of the hippocampus.

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