The effects of neonatal injections of α -MSH on the open-field behavior of juvenile and adult rats

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Male and female infant albino rats, between the ages of 2 and 7 days, were treated with either MSH or the vehicle solution and then tested in an open-field apparatus when they were 45 days old and again when they were 120 days old. Treatment with α -MSH resulted in greater body contact of adult males both at 45 and at 120 days of age. Females treated with α -MSH exhibited greater body contact when tested at 45 days of age, but did not display increased body contact when tested at 120 days of age. These results are indicative of a sex difference in response to neonatal administration of α -MSH.

Melanocyte-stimulating hormone (MSH) is known to have pigmentary functions in lower vertebrates, but the exact role of MSH in mammals is obscure. Indeed, recent reviews have concluded that the effects of MSH within mammals are vestigial (Brown & Barker, 1966; Lewin, 1973). However, behavioral evidence indicates that MSH may have adaptive utility. Adult male rats treated with MSH have been shown to demonstrate resistance to extinction of active (DeWied, 1965, 1966; DeWied & Bohus, 1966) and passive avoidance responses (Sandman, Kastin, & Schally, 1971), resistance to extinction of an appetitive T-maze running task (Sandman, Kastin, & Schally, 1969), facilitated reversal of a brightness discrimination task (Sandman, Alexander, & Kastin, 1973; Sandman, Miller, Kastin, & Schally, 1972), and facilitated learning of the changed dimension in a two-stage discrimination problem (Sandman, Beckwith, Gittis, & Kastin, 1974).

Levine and Mullins (1966) suggested that, in addition to activational effects found in adult organisms, hormones may also be active in the organization of the developing mammalian nervous system by influencing both the behavioral and endocrinological systems of the adult organism. Recent studies have demonstrated that neonatal injections of α -MSH facilitated efficient leverpress performance on a differential reinforcement of low rates schedule of reinforcement, improved acquisition and extinction of a discriminative two-way active avoidance response, and facilitated discriminative shift training of male rats (Beckwith, Sandman, Hothersall, & Kastin, 1977). The potential importance of possible neonatal influences of MSH on the developing brain is suggested by Kastin, Gennser, Arimura, Miller, and Schally's (1968) finding that MSH was present in human fetal pituitaries.

Although the effects of steroid hormones on sexually related behaviors have been extensively investigated (see Levine & Mullins, 1966, and Young, Goy & Phoenix, 1964, for reviews), few studies have examined the actions of hormones on nonsexual social behaviors. Levine (1972) proposed that social

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behaviors affect endocrine function by changing hormonal secretions within the individual organism. An interaction of social behavior with hormones is also suggested by the population limitation theory of Christian and Davis (1964).

The effects of MSH on social behavior have not previously been studied systematically. However, informal observations made during the developmental studies of young animals treated with MSH led to the hypothesis that there were differences in social behavior between these animals and controls treated with placebo. The purpose of the present study was to investigate systematically the actions of neonatal injections of α -MSH on juvenile and adult social behavior as assessed by means of the open-field methodology developed by Latané (1969).

METHOD

Subjects

Eighty-four rats obtained from 13 litters of Holtzman albino rats delivered by multiparous mothers served as subjects in the present experiment. The animals were housed under conditions of constant indirect illumination and maintained by means of an ad-lib watering and feeding schedule throughout the experiment.

Apparatus

The testing apparatus was a circular wooden open field (Latané, 1969), 4 ft in diameter, with an 18-in. surrounding wall. Several studies have demonstrated the sensitivity of this apparatus to various independent variables (Barrett & Ray, 1970; Livesey & Egger, 1970; Valle, 1971). The field was painted black, and the floor was marked with white lines into 49 numbered sections of equal area and approximately equivalent shape. The field was illuminated by a 60-W bulb placed directly above the center; during the testing period, this was the only light in the experimental room.

Procedure

Animals were randomly assigned to either the control group or the experimental group. For each day, from age 2 days to age 7 days, animals in the experimental group received IP injections of 0.10 ml of α -MSH ($1 \times 10^7 \mu\text{g}/\text{mg}$ in 0.01 ml acetic acid—0.4% sodium chloride); animals in the control group received an IP injection of 0.10 ml of the diluent. Solutions were coded so that experimenters did not know which animals were injected with the peptide. The code was not revealed until after the data were analyzed. In order to identify the groups, each day after the injection one group was marked on the back with a solution of Bismark Brown dye and 50% formic acid (an 88% solution of formic acid was used after the animals developed hair). Both groups were handled for an equal amount of time. Except for occasional remarking, the animals were left undisturbed until weaning (21 days of age).

At weaning, the pups were separated by sex and housed in Plexiglas cages in groups of eight animals per cage. The animals were then left undisturbed until the age of 45 days, when the first test period began. Immediately before the first day of testing, each animal was given an identifying mark on the tail with permanent ink. The marks indicated the home cage and rat number. After the completion of the first testing sessions, the animals were again left undisturbed (except to renew markings) in their home cages until the age of 120 days; the second identical test session was undertaken at this time.

Testing

Sessions consisted of a 5-min period of observation once a day for 5 consecutive days. The animals were transported to the testing room in their home cages and were removed from the cage only during the 5-min observation. The animals were tested with similarly sexed partners. Rats injected with MSH were tested with other animals injected with α -MSH; control animals were tested with other control animals. Animals tested together were taken from different cages and each rat was tested with the same partner each day during the testing period of 5 days.

Each pair of rats was placed in the center of the field tail-to-tail and released simultaneously. The animals were allowed to run about freely for 5 min. During this time, one observer recorded the position (as indicated by the number of the square upon which the animal was standing) of each animal at 10-sec intervals. Another observer recorded (using a stopwatch) the amount of time the rats spent in direct body contact (tail-to-tail, tail-to-body, and vibrissae contacts were not recorded). The observers' tasks were alternated between two experimenters for animals and days. At the end of the 5-min testing period, the animals were removed from the apparatus and the apparatus was thoroughly cleaned with a mild vinegar solution to eliminate odors before the next pair of animals was tested.

The same pairs of animals were tested with the same procedure when they reached 120 days of age. Four animals (one female control, one female treated with α -MSH, and two male controls) died between the two testing periods.

RESULTS

All data were initially analyzed by a 2 (sex) by 2 (drug) by 5 (day) analysis of variance with repeated measures of the last factor.

Body Contact

Analysis of the data (see Figure 1) for the animals at the 45-day testing period demonstrated that there were no main effects for drug, $F < 1.00$, or sex, $F(1,28) = 1.78$, $p < .2$. A significant change over testing days was indicated, $F(4,12) = 6.18$, $p = .01$). There were no first-order interactions; however, a significant second-order interaction was found between sex, drug, and day of testing, $F(4,112) = 2.97$, $p = .02$.

Further analyses of simple effects were done by the Scheffé method. As illustrated in Figure 1, the significant three-way interaction is due to the greater time in contact for females on all test days but the first (the largest mean difference came on Day 3), whereas males treated with α -MSH spent more time in contact on all days except Day 3 (the largest mean difference came on Day 1). Statistical confirmation of this effect was demonstrated by contrasts which indicated that males treated with α -MSH showed a trend toward spending more time in contact on Day 1 than females treated with α -MSH, $F(1,112) = 2.87$, $p < .1$, while females treated with α -MSH spent more time in contrast than males treated with α -MSH, $F(1,112) = 5.02$, $p < .05$, on Test Day 3.

Figure 1. Mean percent time in contact between animals at 45 days of age.

Although the function is not simple, these data indicate that neonatal treatment with α -MSH increases time in contact of rats at 45 days of age when compared with rats treated with the vehicle. The data further indicate that neonatal injections of α -MSH differentially affected males and females.

Analysis of the data for the animals at the 120-day testing period demonstrated that there were no main effects for drug, $F(1,24) = 1.54$, $p > .20$; sex, $F < 1.00$; or day of testing, $F < 1.00$. There were no significant interactions. Planned comparisons were done to assess the influences of hormonal and sexual variables. As illustrated in Figure 2, males given a placebo injection spent significantly less time in contact than males treated with α -MSH on Testing Days 2, $F(1,96) = 6.97$, $p < .05$, and 5, $F(1,96) = 10.74$, $p < .01$, and spent marginally significantly less time in contact than males given MSH on Testing Days 3, $F(1,96) = 3.07$, $p < .10$, and 4, $F(1,96) = 3.67$, $p < .10$. The comparisons also revealed a similar pattern of results for the differences between the male control group and both of the female groups.

These results suggest that neonatal treatment with α -MSH produced a long-lasting increase in time spent in body contact for males but had no long term effects on time in contact for females. Treatment with the neuropeptide increased male body contact to a level similar to that of untreated females.

Immobility

Immobility was defined as the number of consecutive 10-sec intervals of the 5-min testing period in which the rat remained in the same location. The measure was computed individually for each member of the testing pair.

Analysis of the data for the animals at 45 days of age revealed that females were marginally significantly more active than males, $F(1,60) = 3.03$, $p < .09$, and, as can be seen in Figure 3, all animals became less active as the number of testing days increased, $F(4,240) = 8.95$, $p < .01$. None of the interactions were significant.

Analysis of the data for the animals at 120 days of age demonstrated that females were significantly more active than males, $F(1,52) = 13.45$, $p = .01$. Animals treated with α -MSH did not differ in activity from controls, $F < 1.00$. The effect for day of testing was also significant, $F(4,208) = 2.71$, $p < .03$, indicating, as can be seen in Figure 4, that all animals became less active over the testing period.

Figure 2. Mean percent time in contact between animals at 120 days of age.

Figure 3. Mean number of consecutive 10-sec intervals of immobility for animals tested at 45 days of age.

Figure 4. Mean number of consecutive 10-sec intervals of immobility for animals tested at 120 days of age.

The Drug by Day interaction was also significant, $F(4,208) = 2.85$, $P = .02$. Post hoc (Scheffé) analysis of the Drug by Day interaction demonstrated that animals treated with α -MSH were more immobile on Day 2, $F(4,135) = 12.76$, $p < .05$, and demonstrated a trend toward more activity on Day 4, $F(4,135) = 8.47$, $p < .10$, than controls. Within-group comparisons among the means showed a decline in immobility from Day 2 to Day 4, $F(4,135) = 17.92$, $p < .01$, for animals treated with MSH and a rise in immobility from Day 3 to Day 5 for controls, $F(4,135) = 10.94$, $p < .05$.

DISCUSSION

The results of this study indicate that neonatal injections of α -MSH influenced the organization of later social behavior, possibly by actions upon the developing nervous system. Early treatment with α -MSH resulted in greater gregariousness in adult males at both 45 and 120 days of age. Additionally, neonatal injection of α -MSH induced an increase in time spent in body contact for females which was demonstrated at age 45 but was absent by the age of 120 days. Adult females demonstrated more open-field body contact than males regardless of early treatment. Male rats treated with α -MSH equaled the time in contact of untreated females.

The present results extend earlier reports that neonatal treatment with MSH influenced the later behavioral capacities of rats. Beckwith et al. (1977) have demonstrated that neonatal injections of α -MSH facilitate the performance of juvenile rats on an operant differential reinforcement of low rates schedule of reinforcement, enhance acquisition and

extinction of a two-way active avoidance response, and augment performance on a black-white discrimination problem learned by the animals when they were adults. The results of the present study also substantiate the long-term nature of the actions of neonatal treatment with α -MSH and suggest a possible domination of neonatal treatments with peptides over several behavioral systems.

Furthermore, these findings corroborate the results of Beckwith et al. (1977), that neonatal injections of α -MSH had a long-lasting influence on males but had minimal actions on females. In a recent study, Champney, Sahley, and Sandman (1976) demonstrated that interventricular injections of MSH/ACTH 4-9 into the brains of infant rat pups differentially influenced the performance of the rats when tested as adults on a visual discrimination problem. Taken together, these studies all strongly indicate that early treatment with the neuropeptide MSH/ACTH has a direct influence on the developing mammalian brain. Other investigators have showed that males and females respond differently to steroids (Bronson & Deshardins, 1968; Sparber, 1974), to drugs (Gray, 1971), and to the same behavioral task (Beatty, 1972). Further investigation of early treatment with neuropeptides is needed to clarify the mechanisms underlying their behavioral influences. In addition, it is strongly suggested that future investigations focus upon the possible effects of sex differences in response to neuropeptide treatment of adult organisms.

Few studies have investigated the effects of steroids on social behavior in the open field. Quadagno, Shryne, Anderson, and Gosk (1972) and Sloan and Latané (1974) demonstrated that neither neonatal castration nor hormonally induced female receptivity influenced open-field behavior. On the other hand, Joy and Latané (1971) demonstrated that adult injections of epinephrine increased social contact in the open field. The current findings were the first demonstration that a naturally occurring short-chain polypeptide influences social behavior. Although the neural mechanisms governing these actions remain to be elaborated, the finding that the endocrine system mediates social contact is very suggestive of a neglected interface between physiology and behavior.

The finding that MSH did not influence activity supports early studies of adrenocorticotrophic hormone (ACTH), a related polypeptide, which has been found to have no influence on activity level (Bohus & DeWied, 1966; DeWied, Bohus, & Greven, 1967; Tamasy, Koranyi, Lissak, & Jandala, 1973). Two of these studies (Bohus & DeWied, 1966; DeWied et al., 1967) used only a single test in the open field, a procedure which may not be very sensitive to changes in activity (Whimbey & Denenberg, 1967). The other

study (Tamasy et al., 1973) used a more sensitive within-subjects index of activity measured across 4 days. Their result indicated that all animals became more active with increasing exposure to the apparatus. The results of the present experiment suggest that activity decreases as a function of repeated days of testing. The reason for this discrepancy is not apparent.

In conclusion, these results add to the growing behavioral data indicating that early treatment with short-chain polypeptides may induce reorganization of the central nervous system which alters the interactions between the treated organism and its environment. The findings also suggest the potential important interaction of sexual and social variables and treatment with MSH/ACTH analogues.

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