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HANDLING, BODY WEIGHT GAIN AND PITUITARY GROWTH HORMONE CONTENT IN YOUNG RATS[1]

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ABSTRACT. Experimental rats were handled for the first 21 days of life while controls were not disturbed. At 25 days of age, animals were weighed, killed by decapitation and pituitary glands removed. Pituitary growth hormone content was measured by densitometric comparison of disc gel column electrophoresed pituitary homogenates with similarly treated growth hormone standard. Analysis of the data indicated that handling did not have a significant effect upon either weaning weight or pituitary growth hormone content.

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INTRODUCTION

A number of studies have shown that neonatal handling of rats accelerates maturational processes in rats (Altmann et al. 1968, Daly 1973). Other investigators have used overall body weight gain as an index of growth and have found neonatally handled animals to be heavier either at weaning (Cines and Winick 1979, Levine 1968) or later in life (Denenberg and Karas 1961, Sobel et al. 1979, Williams et al. 1975). The age at which handling results in a difference in weight may depend upon whether the animals were stroked or fondled (gentled) as done by Cines and Winick (1979), or merely removed from the mother for a short period according to the scheme of Denenberg and Karas (1961).

In an early review, Levine (1960) suggested that the more vigorous rate of growth observed in handled animals may result from increased growth hormone synthesis and secretion by the anterior pituitary gland. Handling is known to accelerate the maturation of a number of neural parameters (Denenberg 1975), so we hypothesized that this treatment might influence mechanisms controlling growth hormone production. To test this hypothesis, experimental rats were handled for the first 21 days of life, while controls were not disturbed. At 25 days of age, animals were weighed, killed, and their pituitaries assayed for growth hormone concentration.

METHODS AND MATERIALS

Nulliparous female and sexually naive male Sprague-Dawley rats weighing 150–174 g were obtained from Spartan Research Animals (Haslett, MI). Upon arrival, the animals were allowed to acclimate to the new laboratory conditions for a period of 2–4 weeks as suggested by Denenberg (1977). The rats were housed in a temperature-controlled room (20C ±1C) with 12 hr of artificial lighting from 0700 to 1900 hr and were fed Purina Lab Chow and tap water ad lib. Females were mated upon reaching a body weight of 220–240 g. Successful matings were determined by the presence of spermatozoa in the vaginal lavage, at which time females were isolated and caged singly.

On the morning a litter was found (day 1), it was reduced to 8 pups by carefully removing young in excess of this number. No effort was made to normalize the sex ratio since the difference in body weights of male and female rats is not significant before 4 weeks of age (Slob and van der Werff ten Bosch 1975), and since it was desired to minimize disturbance of the mothers and littermates. After reduction, litters were randomly assigned to the nonhandled control group or the handled experimental group. Nonhandled control litters were not further disturbed until day 25.

The handling procedure used was similar to that described by Denenberg and Karas (1961) with minor modifications. Briefly, the mother was initially removed to a clean cage to prevent her manipulation of the remaining pups as individuals

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were being handled. Each pup was then placed singly for 3 min into a plastic 4-L container with wood shavings as bedding, then returned to the original cage. The mother was returned to the litter once all pups had been handled. Pups were not stroked or otherwise manipulated during the 3-min handling period. Handling was done once a day for the first 21 days of life.

On day 25, body weights were determined to the nearest 0.01 g, and all pups were killed by decapitation. Pituitary glands were rapidly excised and weighed to the nearest 0.01 mg. Pituitaries were stored frozen at −20C until subsequent analysis of growth hormone was made.

Upon thawing, individual pituitaries were homogenized in 40% sucrose. Pituitary growth hormone was separated from other proteins in the homogenate by polyacrylamide disc gel electrophoresis methods developed by Davis (1964) and Ornstein (1964) as modified by Lewis et al. (1965). Electrophoresed gel columns were analyzed densitometrically, and concentration of pituitary growth hormone was quantified by comparison of area under the curves for unknowns and rat growth hormone standard (NIAMDD-Rat GH-B5).

Pituitary growth hormone content and body weight were analyzed statistically using a hierarchical analysis of variance (ANOVA, Myers 1972) which permits assessment and control of litter effects. While this process reduces the total number of observations per group from 40 for individual pups to 5 for litters, it gives a more realistic estimation of group variance (Abbey and Howard 1973).

RESULTS

Since no significant differences in body weights between the sexes were found, all statistical analyses considered the litter as the unit for error terms. Thus there were 10 litters, 5 handled and 5 nonhandled controls with 8 pups in each litter. Statistical analysis of the data failed to discern significant differences ($P > 0.05$) between any of the dependent variables measured in handled and nonhandled animals. Handled animals did not have an increased body weight at 25 days of age when compared to their nonhandled counterparts of the same age (table 1). Furthermore, pituitary growth hormone content was not found to differ between handled and nonhandled rats. However, eye opening consistently occurred a day earlier in handled animals (day 13 vs. day 14), and adrenal weights were slightly, but not significantly, depressed by handling (24.5 vs. 25.8 mg/100 g body wt).

TABLE 1

Comparison of body weight, pituitary weight and pituitary growth hormone concentrations of handled and nonhandled rats.

	Handled	Nonhandled
Body weight (g)	60.25 ± 0.72*	59.35 ± 0.93
Pituitary weight (mg)	2.5 ± 0.1	2.5 ± 0.1
Growth hormone concentration (μg/mg tissue)	17.2 ± 2.0	19.7 ± 2.1

*Values are expressed as means ±SE.

DISCUSSION

Several investigators have examined the effects of handling on various physiological and behavioral endpoints in young rodents, but none has reported measuring either pituitary or serum growth hormone levels. In the present study we found that postnatal handling on days 1–21 did not alter significantly the pituitary growth hormone content of 25-day-old pups. While some investigators have found appreciably higher pituitary growth hormone content in rats of the same age using radioimmunoassay (RIA) (Sinha et al. 1973), levels reported in the present study are comparable with others found using polyacrylamide gel electrophoresis (Geel and Timiras 1970) and with those of more recent reports using RIA (Walker et al. 1977, Columbe et al. 1980). Perhaps the difference in RIA results derive from improvements in immunoassay techniques over the past decade. The similarity of pituitary growth hormone content in handled and nonhandled animals does not warrant the assumption that hormone synthesis and secretion are similar, since glandular hormone content is the result of dynamic hormonal synthesis, release, and activitation (Kraicer et al. 1977). Nonetheless, the similarity of final body weight and of pituitary growth hormone content is suggestive of similarity in hormone synthesis and secretion.

The present study found handling to

have an insignificant effect on body weight at 25 days as well. Although growth control is a complex process involving many molecular mechanisms, absence of differential body weight gain may be reflective of the absence of a handling effect on pituitary growth hormone levels. Many investigators separate the pups from the mother (i.e., wean) at 21 days. Since the young in this study were left with the mother through 25 days of age, the additional maternal input may have been influential in altering their final body weight. However, Williams et al. (1975) found that handling pups weaned earlier than normal had no influence on body weight at 25 days of age. Thus, it would appear that presence or absence of the mother for a few days before or after 21 days of age plays no major role in the weight gain of pups in response to handling.

The accelerating effect of handling on some aspects of maturation is a well-accepted notion. Indeed, handled animals in the present study demonstrated slightly accelerated eye openings and slightly depressed adrenal gland weight. However, the use of differential body weight gain as an overall measure of accelerated growth stimulated by the handling procedure appears to be subject to more confounding variables. Among these are litter effects (King 1969), and the handling procedure itself. Statistical analysis in the present study was done by hierarchical ANOVA (Myers 1972) to ensure that litter effects were controlled. This may explain why our results do not corroborate those of earlier studies which found handling to accelerate postnatal growth (Levine 1968). However, in more recent studies, the method of handling may have produced accelerated body weight gain which is significant at the time of weaning. For example, Cines and Winick (1979) reported elevated body weight at weaning in pups handled on postnatal days 1–21, using a much more vigorous handling process than that employed in the present study. These investigators removed pups from the home cage for 20 min daily and stroked the animals for 2 min of this time. Such "gentling" procedures were strictly avoided during the present study. Pups were removed from the nest for only 3 min daily and were not manipulated during this period as described by Denenberg and Karas (1961). Indeed, the latter technique performed on days 1–21 has been reported to reduce weaning weight in some cases (Denenberg and Karas 1961, Thoman and Levine 1970), whereas body weight was elevated later in life (Denenberg and Karas 1961). Given that this handling scheme may not increase body weight at weaning but may have its influences later in life (Denenberg and Karas 1961, Denenberg 1975, Levine 1957), it would be of interest to determine whether patterns of growth hormone synthesis and release are altered in older animals that have been handled this way in infancy. Further, measurement of these parameters in gentled animals would be worthwhile.

LITERATURE CITED

Abbey, H. and E. Howard 1973 Statistical procedure in development studies on species with multiple offspring. Develop. Psychobiol. 6: 329–335.

Altmann, J., G. D. Das and W. J. Anderson 1968 Effects of infantile handling on morphological development of the rat brain: An exploratory study. Develop. Psychobiol. 1: 10–20.

Cines, B. M. and M. Winick 1979 Behavioral and physiological effects of early handling and early malnutrition in rats. Develop. Psychobiol. 12: 381–389.

Coulombe, P., J. Ruel and J. H. Dussault 1980 Effects of neonatal hypo- and hyperthyroidism on pituitary growth hormone in the rat. Endocrinol. 107: 2027–2033.

Daly, M. 1973 Early stimulation of rodents: A critical review of present interpretations. Brit. J. Psychol. 64: 435–460.

Davis, B. J. 1964 Disc electrophoresis. II. Method and application of human serum proteins. Ann. New York Acad. Sci. 121: 404–427.

Denenberg, V. H. 1975 Effects of exposure to stressors in early life upon later behavioral and biological processes, p. 269–281. *In:* L. Levi (ed.), Society, stress, and disease: Childhood and adolescence (Vol. 2), Oxford Univ. Press, NY.

——— 1977 Assessing the effects of early experience, p. 127–247. In: R. D. Myers (ed.), Methods in psychobiology (Vol. 13), Academic Press, NY.

——— and G. G. Karas 1961 Interactive ef-

fects of infantile and adult experiences upon weight gain and mortality in the rat. J. Comp. Physiol. Psychol. 54: 685–689.

Geel, S. E. and P. S. Timiras 1970 Influence of growth hormone on cerebral cortical RNA in immature hypothyroid rats. Brain Res. 22: 63–72.

King, D. L. 1969 The effect of early experience and litter on some weight and maturational variables. Develop. Psychobiol. 1: 576–584.

Kraicer, J., G. Beraud and D. W. Lywood 1977 Pars intermedia ACTH and MSH content: Effect of adrenalectomy, gonadectomy and a neurotropic (noise) stress. Neuroendocrinol. 23: 352–367.

Levine, S. 1957 Infantile experience and consummatory behavior in adulthood. J. Comp. Physiol. Psychol. 50: 609–612.

——— 1960 Stimulation in infancy. Sci. Amer. 202: 80–86.

——— 1968 Influence of infantile stimulation on the response to stress during preweaning development. Develop. Psychobiol. 1: 67–70.

Lewis, U. J., E. V. Cheever and W. P. Vander Laan 1965 Studies on the growth hormone of normal and dwarf mice. Endocrinol. 76: 210–215.

Myers, J. L. 1972 Fundamentals of experimental design (2nd ed.). Allyn and Bacon, Boston, MA.

Ornstein, L. 1964 Disc electrophoresis. I. Background and theory. Ann. New York Acad. Sci. 121: 321–349.

Sinha, Y. N., J. N. Wilkins, F. Selby and W. P. Vander Laan 1973 Pituitary and serum growth hormone during undernutrition and catch-up growth in rats. Endocrinol. 92: 1768–1771.

Slob, A. K. and J. J. van der Werff ten Bosch 1975 Sex differences in body growth in the rat. Physiol. Behav. 14: 353–361.

Sobel, E. H., K. A. Zuppinger and E. E. Joss 1979 Effects of isolation, handling and restraint on growth of rats. Growth 43: 1–6.

Thoman, E. B. and S. Levine 1970 Hormonal and behavioral changes in the rat mother as a function of early experience treatments of the offspring. Physiol. Behav. 5: 1417–1421.

Walker, P. J., H. Dussault, G. Alvarado-Urbina and A. Dupont 1977 The development of the hypothalamo-pituitary axis in the neonatal rat: Hypothalamic somatostatin and pituitary and serum growth hormone concentrations. Endocrinol. 101: 782–787.

Williams, D. I., G. B. Bailey and M. H. S. Lee 1975 Handling rat pups after early weaning. Behav. Biol. 13: 505–509.