Somatotrophin effects on fetal brain growth: Failure to replicate¹

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Offspring of pregnant rats given purified growth hormone did not differ on three behavioral tests, in brain size, or in number of brain cells from offspring of those given a placebo. These findings are contrary to previous reports.

Zamenhof, Mosley, & Schuller (1966) reported that high concentrations of growth hormone in the fetal environment of rats resulted in greater than normal brain weight, reflecting, especially, an increased density of neurons. Others (Clendinnen & Eayrs, 1961; Zamenhof, 1942) have reported similar results and have found behavioral differences, e.g., heightened maze running efficiency, favoring the treated over normal control Ss. The purpose of the present investigation was to replicate these findings so that further investigations, such as studies of the interactions between variations in brain and exposure to differing early environments, might be done. Although the procedure was modeled after that of Zamenhof et al (1966) and personal communication with Zamenhof, we found the hormone to have no effects.

Method

The parents of the Ss were hooded rats. Following mating, the pregnant females were given somatotrophin, physiological saline, or were treated as if they were injected but with no fluid actually infused. (When initial testing showed the two control groups not to differ, they were combined for all further analyses of the data). The injections were either 3 mg of somatotrophin dissolved in .5 ml of saline or a like amount of saline without the hormone. The mothers were injected subcutaneously daily from Day 7 through Day 15 following copulation (nine injections). The hormone was the highly purified crystalline (Batch No. 12) made available by the Endocrinology Study Section, NIH. It was prepared for injection by dissolving it in physiological saline under sterile conditions while adjusting the pH to approximately 7.0 with weak solutions of NaOH.

At 21 days of age, the offspring of the treated mothers (14 litters) and the controls (18 litters) were taken from their mothers, weighed, marked, and placed in sex segregated community cages.

At 51 to 53 days of age, 49 females (20 from the treated mothers and 29 from control mothers) began passive avoidance training. The passive avoidance apparatus was a 76 cm square field with a grid floor and with an 11 cm square pedestal in the middle of the field, 8 cm above the grid. Each S was placed on the pedestal once daily for three days, and latency for moving off the pedestal was measured. No shock

was given during these three days and all Ss moved from the pedestal within 4 sec. On the fourth day, the grid was electrified and S received approximately 1 sec of foot shock as soon as he was on the grid. On the fifth day, S was again placed on the pedestal and latency of stepping down from the pedestal was measured. Successful avoidance was defined as staying on the pedestal for longer than 30 sec.

At 110 and 120 days of age, 83 females (28 from the treated mothers) were selected for active avoidance training. The active avoidance apparatus was a T maze with a grid floor and a lever in each arm of the T (for details of the T maze see Nielson, McIver, & Boswell, 1965). Five sec after the door of the start box opened, a loud buzzer and foot shock came on. Both the buzzer and the shock were terminated when S pressed the lever in the correct goal box of the maze. The correct side was the arm opposite to the one S ran toward first. Ss were tested during a single session until they ran to the correct side on nine out of 10 trials without entering the other arm of the T.

At 100 to 110 days of age, 16 males (eight from each group) were selected (matched for age, weight, and number of siblings) to begin the procedures of Hebb-Williams maze testing. The Hebb-Williams maze was like the one described by Rabinovitch & Rosvold (1951), and their training procedure was followed. These 16 Ss' brains were later analyzed for the amount of DNA per gram of brain by a diphenylamine colorimetric assay modeled after Burton's (1956). DNA concentrations determined in this way are considered to index the number of brain cells (Zamenhof et al, 1964). The procedure differed from Burton's (1956) in that acetaldehyde was not added to the diphenylamine reagent, and the brain DNA content was related to a standard calf-thymus DNA.

The brains of 101 adult female Ss, nearly the entire sample of experimental and control females, were trimmed and weighed immediately following their removal from the cranium.

Results

At weaning there were no differences in Ss' body weights which could be attributed to the growth hormone ($\bar{X}$ of somatotrophin males = 47.6 g, $\bar{X}$ of placebo males = 46.2 g ($t = .1$, $df = 147$); $\bar{X}$ of somatotrophin females = 47.4 g, $\bar{X}$ of placebo females = 44.7 g ($t = .5$, $df = 109$)).

Behavioral differences between the offspring of somatotrophin treated mothers and offspring of mothers who received a placebo or pseudoinjection were slight:

(a) Ten of 20 Ss from the somatotrophin group and nine of 29 Ss from the placebo group made passive avoidance responses on the test trial ($X^2=1.08$, $df=1$); (b) Mean active avoidance trials to criterion were 14.2 for the somatotrophin Ss and 15.5 for the placebo Ss ($t=.9$, $df=81$); and (c) $\bar{X}$ errors made on the 12 Hebb-Williams maze problems were 106 for the somatotrophin Ss and 104 for the placebo Ss ($t=.1$, $df=14$).

Neither total brain weight ($\bar{X}$ of somatotrophin Ss = 1739 mg, $\bar{X}$ of placebo Ss = 1772 mg; $t=.2$, $df=99$) nor amount of DNA per gram weight of brain ($\bar{X}$ of somatotrophin Ss = 1921 μ g, $\bar{X}$ of placebo Ss = 1940 μ g; $t=.4$, $df=14$) differed between the offspring whose mothers were differentially treated. The amount of DNA found in the present study is higher than that reported by Zamenhof, Bursztyn, Rich, & Zamenhof (1964) for adult rats (approximately 700 μ g/g), probably because the brain DNA was related to a standard calf-thymus DNA, rather than the phosphorus content of DNA.

Discussion

The present methods for weighing the brains and assaying cell number are not ideal for determining absolute values for these parameters, but we believe that they were sensitive and reliable enough to have shown differences of the magnitude reported by Zamenhof et al (1966). If there were differences in brain conformation between the two groups, they were too slight to add reliably to the variance of the individual behavioral differences among Ss.

Because the present procedures were like Zamenhof's, it is unlikely that procedural differences would account for the discrepant results. Perhaps a more tenable explanation would be that the injections themselves differed. Although the growth hormone produced for distribution by the Endocrinology Study Section was highly purified, the possibility remains that slight,

subtle differences in the composition or differences from contamination during preparation were critical for the effect of accelerated neuronal growth. Zamenhof et al (1966) injected growth hormone from Batch 10. Our growth hormone was from Batch 12, and we took special precautions to keep the injections sterile.

Isolating the cause of accelerated brain growth is an important goal and may be useful in the study of brain-behavior; however, until the critical features of the procedure are made explicit, studies relating brain conformation to behavior with these procedures will not be easy.

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Notes

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