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# Postnatal Stimulation: The Effects on Cholinergic Enzyme Activity in Undernourished Rats[1] (38914)

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Nutritional status and environmental conditions have both been shown to be important for physical and psychological development (1). An understanding of the interaction of nutrition and environment will most likely be necessary for determining the optimum conditions for growth and development. One area of common interest to workers in nutrition and psychology is the response of the enzyme acetylcholinesterase to both nutritional and environmental manipulation (2). The activity of cholinesterases have been suggested as indices of nutritional status (3) as well as being uniquely related to behavior (4).

Behavioral changes related to nutrition have received much attention in recent years (5). "Additional stimulation" provided to malnourished animals during early development has been shown by Levitsky and Barnes (6) to ameliorate some of the altered behaviors attributable to malnutrition. The purpose of the present study was to investigate the effects of malnutrition due to low milk availability during suckling on the cholinergic enzymes of animals raised under laboratory conditions, and those subjected to the psychological procedure called "handling" (7). The use of the procedure called "handling" as an experimental manipulation has been shown to have a variety of long lasting physiological and behavioral effects such as altered levels of plasma corticosterone (8) and increased tactual variation seeking (9). Even though this procedure may actually be stressful to the animals during the period of manipulation, it has been hypothesized to accelerate both physical and behavioral development in rats, cats and premature infants (10).

*Materials and Methods.* Pregnant Holtzman rats (210 g) were maintained on a 25% casein diet throughout gestation. At birth male offspring were randomly assigned to foster mothers and maintained in litters of 8 pups/dam throughout the period of lactation. One half of the litters were suckled to dams fed the 25% casein control diet (C) and the other half were fed a 12% casein diet in order to reduce milk availability to the pups (DL). Handling consisted of removing the pups from the nesting cage and placing them into a cage with wood shavings for 3 min. At the end of 3 min the young were placed back in the nesting cage. This manipulation was repeated each morning throughout lactation until the animals were weaned at 21 days of age. Ten litters from each nutritional treatment were handled and ten litters were non-handled. All animals were weaned to a 25% casein diet and individually caged. A complete description of diets has been published previously (11). After 4 wk of rehabilitation animals were killed and brains minus cerebellum assayed for choline acetyltransferase (ChAc) by the method of Fonnum (12) and acetylcholinesterase (AChE) by the method of Ellman (13). AChE was differentiated from the other cholinesterases by using the specific AChE inhibitor 284C51 (14). Both tissue homogenates and brain cell suspensions were prepared on each of the four treatment groups. Neuronal and glial fractions were separated on Ficoll gradients according to the method of Hemminki (15). Carbonic anhydrase was measured in the neuronal and glial cell rich fractions (16) as an index of neuronal and glial cell separation (17).

*Results.* The body and brain weights are given in Table I and indicate that the previously undernourished animals had sig-

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TABLE I. BODY AND BRAIN (MINUS CEREBELLUM) WEIGHTS AT DAY 49 OF RATS FED 25% CASEIN (C) OR 12% CASEIN (DL) DIETS, AND HANDLED (H) OR NON-HANDLED (NH) DURING THE LACTATION PERIOD. VALUES ARE PRESENTED ± STANDARD ERROR OF THE MEAN.

| Experimental group | N | Body wt. g | Brain wt. (Minus cerebellum) g |
|---|---|---|---|
| C-NH | 10 | 224.0 ± 7.7 | 1.60 ± 0.29 |
| C-H | 11 | 222.8 ± 4.9 | 1.58 ± 0.23 |
| DL-NH | 13 | 172.1 ± 4.6[a] | 1.44 ± 0.16[a] |
| DL-H | 10 | 166.9 ± 4.3[b] | 1.45 ± 0.22[b] |

[a] Significantly different from NH controls $p < 0.0005$.

[b] Significantly different from H controls $p < 0.0005$.

TABLE II. ACTIVITIES OF CHOLINE ACETYLTRANSFERASE (ChAc) AND ACETYLCHOLINESTERASE (AChE) ON DAY 49 OF RAT BRAIN HOMOGENATES. RATS FED 25% CASEIN (C) OR 12% CASEIN (DL) DIETS, AND HANDLED (H) OR NON-HANDLED (NH) DURING THE LACTATION PERIOD. VALUES ARE PRESENTED ± STANDARD ERROR OF THE MEAN.

| Enzyme | Treatment C-NH | C-H | DL-NH | DL-H |
|---|---|---|---|---|
| N | 7 | 8 | 10 | 7 |
| ChAc/g | 3.99 ±0.14 | 4.16 ±0.20 | 3.94 ±0.22 | 4.15 ±0.23 |
| ChAc/mg protein | 37.36 ±1.78 | 38.09 ±2.29 | 36.03 ±2.23 | 36.67 ±1.80 |
| AChE/g | 8.82 ±0.14 | 9.52 ±0.46 | 9.64 ±0.18[a] | 9.72 ±0.23 |
| AChE/g protein | 82.69 ±1.17 | 87.13 ±5.12 | 87.66 ±1.36[b] | 85.90 ±1.73 |

[a] Significantly different from NH controls $p < 0.005$.

[b] Significantly different from NH controls $p < 0.01$.

Enzyme activities: ChAc μmoles 14-C-acetylcholine syn/hr/g tissue, nmoles 14-C-acetylcholine syn/hr/mg protein; AChE μmoles acetylthiocholine hydrolyzed/min/g tissue, or per gram protein.

nificantly lower body and brain tissue weights than the controls. There was no effect of "handling" on body or brain tissue weight.

The activity of AChE in the brain homogenate was increased in the non-handled undernourished animals, but there was no difference between the handled undernourished and control groups (Table II). The difference between handled and nonhandled controls was not significant due to the large variability in the activity of the handled animal. Decreases in ChAc activity have been observed in the brainstem in animals undernourished and rehabilitated, but not in the forebrain (18). The combination of both regions may make it more difficult to observe differences.

Neuronal and glial cell fractions were assayed for carbonic anhydrase as an index of separation of cellular components. The ratio of carbonic anhydrase in the glial and neuronal-rich cell fractions was 5.9. This ratio fell between the previously reported values of 5.6 and 11.8 using similar separation techniques (19, 20). Examination of the cell fractions showed a decrease in ChAc in the neuronal-rich fraction of non-handled undernourished animals, compared to controls (Table III). This decrease did not occur in the handled undernourished animals. A decrease in AChE activity occurred in the glial cell fraction of the handled undernourished animals, but was absent in the non-handled undernourished animals. No differences were observed in the AChE activity of neuronal-rich fractions.

The presence of both ChAc and AChE in both neurons and glial cells has been reported by authors employing both cell separation techniques (21) and tissue culture of central nervous system cells (22). This, plus the fact that the individual components (ChAc, AChE and the cholinergic receptor) appear to be regulated during development by independent genetic control mechanisms, make the functional importance of the changes in enzyme activity unclear. A decrease in the concentration of ACh in the brains of rats given either protein or calorie restrictions for 5 wk postnatally has, however, been reported by Rajalakshmi *et al.* (23).

The relationship of the cholinergic enzymes and environment has drawn much attention. In this study we have attempted to limit differences in environment to the handling manipulation alone. This was done

TABLE III. ACETYLCHOLINESTERASE (AChE) AND CHOLINE ACETYLTRANSFERASE (ChAc) ACTIVITY IN NEURON RICH, AND GLIAL RICH CELL FRACTIONS. RATS FED 25% CASEIN (C) OR 12% CASEIN (DL) DIETS, AND HANDLED (H) OR NON-HANDLED (NH) DURING THE LACTATION PERIOD. VALUES ARE PRESENTED ± STANDARD ERROR OF THE MEAN.

| Enzyme | Treatment C-NH | C-H | DL-NH | DL-H |
|---|---|---|---|---|
| Neuron rich cell fraction | | | | |
| ChAc/mg protein | 2.63 ± 0.39 | 2.74 ± 0.31 | 0.82 ± 0.32[a] | 3.73 ± 1.73 |
| AChE/mg protein | 31.20 ± 6.99 | 38.54 ± 6.56 | 33.95 ± 11.75 | 35.19 ± 7.50 |
| Glial rich cell fraction | | | | |
| ChAc/mg protein | 2.67 ± 0.56 | 3.90 ± 0.97 | 1.39 ± 0.58 | 4.12 ± 2.13 |
| AChE/mg protein | 93.32 ± 17.62 | 104.11 ± 30.51 | 110.00 ± 15.27 | 63.84 ± 9.71[b] |

[a] Significantly different from control NH $p < 0.025$.

[b] Significantly different from DL-NH $p < 0.05$.

Enzyme activities: ChAc nmoles 14-C-acetylcholine syn/hr/mg protein, AChE nmoles acetylthiocholine hydrolyzed/min/mg protein. Each treatment used an n of 3.

by (a) cross-fostering animals at birth to randomize the genetic background within each litter-cluster during the suckling period; (b) by using ten different litters for each treatment to enable sampling of no more than two animals and in most cases one animal previously assigned to the same suckling dam. In previous experiments we have been unable to reverse the increase in activity of acetylcholinesterase in brain homogenate by 31 wk of nutritional rehabilitation (24). The results of this study suggest that when both groups are handled the increase in acetylcholinesterase activity in brain homogenate disappears. In addition, the handling manipulation resulted in an increase in the depressed ChAc activity associated with the neuronal cell-rich fraction of undernourished animals. This work and that of Levitsky and Barnes (6) suggests that the detrimental aspects to the central nervous system of undernutrition may be reversed by providing undernourished animals with environmental stimulation.

*Summary.* Rats were malnourished during the first 3 wk of life by feeding their lactating dams a low protein diet. Half of both control-fed and malnourished groups were "handled" daily during the suckling period. After 4 wk of postweaning dietary rehabilitation and individual caging they were killed and brain minus cerebellum taken for choline-acetyltransferase (ChAc) and acetylcholinesterase (AChE) determination on neuronal-rich or glial-rich cell fractions. Early postnatal malnutrition resulted in a decrease in ChAc activity in the neuronal-rich cell fraction of the non-handled rats, but no change in ChAc activity of this cell fraction was observed from rats that had been handled. This finding parallels the prior observation that malnutrition induces behavioral changes that continue after nutritional rehabilitation, but these behavioral abnormalities are minimized or abolished by handling.

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