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The Effects of Early Stress and Undernutrition on the Behavior of Young Adult Rats and The Correlations Between Behavioral and Brain Parameters

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VILLESCAS, R., S. ZAMENHOF AND D. GUTHRIE. *The effects of early stress and undernutrition on the behavior of young and adult rats, and the correlations between behavioral and brain parameters.* PHYSIOL. BEHAV. 23(5) 945–954, 1979.—Control and chronic undernourished newborn rat pups were exposed to cold stress for 3 min daily or left undisturbed on Days 2–11. On Days 25–29 (preweaning) and at 90 days of age, stressed and non-stressed pups in each group were tested in a cross-maze and a T-maze, and their preference for novel and social stimulation and activity levels were measured. At 90 days only, another group of such pups was trained to learn an active avoidance response to electric shock. The results on maze performance show that prior to weaning, stressed controls (SC) and nonstressed undernourished (NSU) pups exhibited shorter response latencies, and a greater preference for social stimulation than the non-stressed controls (NSC). At 90 days, SC and NSU again exhibited shorter latencies, and higher activity levels, and also a greater preference for novel stimulation than the NSC animals. In these parameters, there were no significant differences between stressed undernourished (SU) and NSU animals. In original avoidance learning, undernourished animals exhibited a slower rate of acquisition learning than controls. However, in reversal learning, NSU animals made significantly more errors than the SU animals. There were no significant differences between the two control groups and the SU group. These results show that early cold stress alone can have the same behavioral effects as early undernutrition alone and that early stress can have a significant reversing effect on the learning performance of previously undernourished adult animals. Statistical correlations between behavioral parameters (X- and T-maze behavior and active avoidance learning) and crude brain parameters (weight, cell number (DNA), cell density and protein) were investigated in the above animals. It was found that, in general, behavioral parameters whose high values appear to be of advantage for the animal (e.g., number of entries or social time in mazes and percent correct moves in the original and reversal learning) had significant positive correlations with weight, cell number (DNA), and protein content, at least in cerebral cortex and cerebellum, and negative correlations with cortical cell density. Conversely, parameters whose high values are of disadvantage (latency in mazes, or total trials to criterion and total errors in learning) generally had significant negative correlations with brain weight, cell number, and protein content, and positive with cortical cell density. Age, stress or undernutrition had no effect on these results. It is concluded that, statistically, even these crude brain parameters may be significantly correlated with behavior. That is, animals tend to perform better if their cortical cells are further apart and if their brain weight, cell number (DNA), and protein content are higher.

Early stress Early undernutrition Learning age Brain parameters Correlations between parameters Brain and behavior

IN RECENT years, the behavioral effects of undernutrition in rats have received considerable attention. Studies have shown that previously undernourished animals exhibit slower extinction rates [6], more errors in maze learning [41] and abnormalities in emotionality and reactivity [1, 17, 42]. For the most part, it is generally accepted that these changes are irreversible. More important, however, is that recent reviews [2,16] of the area indicate that very few studies are investigating methods for intervening with the behavioral effects of early undernutrition. In a recent study, Whatson and Smart [32] suggested that while some behavioral effects of early undernutrition may be considered as primary or direct consequences, it is possible that early undernutrition may have other non-nutritional side effects (i.e., altered maternal care and social interaction) which could also influence early behavioral development. Novakova [22] has earlier demonstrated that maternal contact from a non-lactating rat "aunt" during weaning was sufficient to reduce behavioral abnor-

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malities in undernourished pups separated from their natural mother. Slob *et al.* [25] also demonstrated that undernourished pups provided with normal amounts of maternal care failed to show any behavioral abnormalities at weaning. Although there are very few studies [20, 21, 26, 27, 30] describing the relationship between maternal care and pup behavior in undernourished litters, still the results of these few studies support the hypothesis that other non-nutritional variables can have a significant influence on behavioral development.

In another area of investigation, a large number of studies (for reviews see [5,15]) have demonstrated that increased infantile stimulation such as handling or cold stress can also have significant long-lasting effects on early behavioral development. For example, it has been shown that early handled animals exhibit higher exploratory activity levels, a lower emotional response to stress and better discrimination learning as adults. The results of these studies are important for two reasons. First, a comparison of the effects of early undernutrition and early stimulation indicates that while undernutrition leads to a retardation of growth and development, early stimulation has the reverse effects. Second, based on the large number of separate nutritional and psychological studies that describe the effects of early undernutrition and early stimulation, respectively, Frankova [7] was the first to suggest the possibility of an interaction between early undernutrition and early stimulation. However, it is of interest to note that despite the suggestion by Frankova, very few studies have investigated the combined effects of both treatments. In one of the few available studies, Levitsky and Barnes [18] demonstrated that early handling plus an enriched environment were sufficient to eliminate behavioral abnormalities in animals undernourished from birth to seven weeks of age. Based on these results, it is clear that additional studies are needed to have a better description of the interactive effects of early undernutrition and early infantile stimulation (i.e., handling, cold stress, electric shock).

In the present study we have first investigated the effects of early infantile stimulation (cold stress) on the behavioral development of undernourished rats. Emphasis was placed on comparing pre- and postweaning pup behavior. Specifically, preference for tactual, novel and social stimulation and active avoidance learning were investigated in young and adult animals.

In continuation of this work, we have made a systematic study of correlations between brain parameters and behavioral parameters as described above. At present, one could have several views on the question of what in the brain structure makes the animal behaviorally superior. One view is that these structural parameters (details of wiring diagram, chemical complexity, etc.) are still too subtle to study. Another view is that although these details are important, perhaps even most important, still there may be other parameters that are accessible to study and that also influence behavioral performance. Among them are the crude or bulk parameters: the weight of the brain and its parts, their cell number or DNA, cell density and protein content. In this part we will investigate this second view.

The concept that one of these parameters, the quantity of brain tissue, is positively correlated with brain performance is, of course, quite old: the concept was based on the observation that as one proceeds from lower primates to man, neocortex weight and cell number increase (reviews in [12,23]). It could be that the improvement in fine parameters, the wiring diagram or chemical complexity, cannot work to the full advantage unless the mass of brain tissue has also increased. Within one species (rat), Lashley [14] studied the effect of removal of brain mass on behavior. His results led him to the conclusion that the capacity to learn the maze is dependent upon the amount of functional cortical tissue; the more complex the problem to be learned, the greater the retardation produced by any given extent of lesion. However, subsequently the concept of the importance of the correlation between brain weight and behavioral performance within human species fell in disrepute when it was found post mortem that some gifted individuals had small brains. Needless to say, such few observations on old age brains of individuals of non-uniform genetic background are statistically worthless.

In recent years there was a renewed interest in this problem. The comparison of brains of different species or of the refinement of functions within species suggested that cerebral cell number is intimately involved in brain performance [9,31]. A weak correlation between brain size and intelligence in man has been reported [29]. Passingham [23] claimed that "in many respects, man's neocortex does not differ from that of other primates, and, where it does, the difference can be shown to be related to the differences in total size of the neocortex." According to recent studies of Szentagothai [28], it is indeed the wiring and the mass that have improved from lower primates to man, and not the neurons themselves. Recently, evidence was presented suggestive of better performance of animals whose brains were heavier as a result of genetic selection (reviews in [24, 34, 38]) or as a response to environmental variability [10,11].

The present work is systematic study of correlations between several brain parameters of several brain parts, and several behavioral parameters, in control rats as well as in stressed or chronically undernourished or stressed and undernourished rats, using techniques essentially as described above.

Subjects and Design

The rat pups in this study were from 20 litters of Sprague-Dawley derived females bred in our colony. Ten pregnant females (Group 1) were selected from the control colony and fed ad lib (15.0 g/24 hr) with a 20.5% protein diet. Another group of 10 pregnant females (Group 2) was selected from a chronic undernourished population (F_6) and restricted to 2/3 or 10 g/24 hr of the control diet throughout gestation [40]. The pregnant females were housed in stainless steel maternity cages 22×25×22.5 cm. Each cage was enclosed at the back, sides, and top, and contained a removable tray partially filled with wood shavings.

After birth, mothers and offspring in both groups were given food and water ad lib. On Day 2 postpartum, control and prenatally undernourished litters were assigned to either a stressed or non-stressed condition resulting in four groups of animals: nonstressed controls (NSC), stressed controls (SC), nonstressed undernourished (NSU), and stressed undernourished (SU). All litters were culled to 8 pups, and those with fewer than 6 were not used in the study. Pups for the stressed condition were removed from the nest and placed inside a refrigerator in separate small cardboard compartments for 3 min on Days 2–11. After the 3 min of stress, the pups were returned to the home cage. Beginning on Day 16, the mothers and offspring in group 1 (prenatally undernourished) were again fed the restricted diet until weaning at 30 days. Postweaning, all pups were fed ad lib.

Apparatus

A refrigerator set at 4–6°C was used to impose cold stress. A cross-maze shaped in the form of a Greek cross [3] was constructed from 0.5 cm thick dark Plexiglas and was used for measuring the rat's (pups and adults) preference for constancy or variation of tactual and novel stimulation. The center area measured 22.5×22.5 cm; each of the four other compartments was 37.5 cm long×22.5 cm wide, with walls 37.5 cm high. The compartments were constructed to provide varying amounts of tactual and novel stimulation. The floor of one compartment (i.e., condition 1) was smooth Plexiglas; a second floor was covered with sandpaper of light coarseness. These two conditions provided a minimal amount of stimulation. The third floor was covered with a sandpaper of intermediate coarseness and small wood blocks and climbing bars were provided for extra novelty. In the fourth compartment, the same amount of tactual and novel stimulation was provided with white illumination from a 4-W light bulb.

A T-maze [4] made from plywood was used for measuring the rat's preference for social and novel stimulation. The walls of the maze were 37.5 cm high and each arm measured 27.5 cm×27.5 cm. The stem was 25 cm long and 27.5 cm wide. Overall, the crosspiece was 82.5 cm long. The floor and walls were painted black. A white line was drawn on the floor of the maze where the stem met the crosspiece. Another line was drawn at a right angle to the first one dividing the crosspiece into 2 areas. In the left arm, a 30 day old male rat randomly selected from the colony was placed in a wire mesh cage and used as the social stimulus. In the right arm, five wood blocks were used for novel stimuli. The blocks were cut in random shapes and were small enough for the pups to manipulate. To maintain novelty, the blocks were changed daily.

A two-chambered shuttle box constructed from steel sheets (0.3 cm thick) was used for training adult animals to learn a 2-way active avoidance response to electric shock. The walls of the box were 22.5 cm high; each chamber was 40 cm long and 15 cm wide at the top. The two side walls of each chamber were assembled in a V-shape against the middle partition and the 2 opposite end walls, resulting in a trough-shape shuttle box with a steel floor 3 cm wide. The top of the box was covered with a clear Plexiglas lid to allow a top view of the subject. A Lafayette Master Shocker (Model 82400) was the source of electric shock.

METHOD

Cross-Maze

At 25 days of age, individual pups were removed from their home cage and observed in the cross-maze for 10 min. Testing began by placing the pup in the center area. The initial latency to enter any of the 4 compartments, the time spent in each compartment, and total number of entries were recorded. The time scores were recorded with separate stop-watches. An index representing the subject's preference for level of tactual and novel stimulation was determined. Each compartment was assigned a value on a scale of 1 to 4, corresponding to the amount of novel stimulation provided in that compartment. The time spent in each condition was multiplied by its assigned value. The scores from the 4 compartments were summed to yield a rating score. A high score was interpreted to represent a preference for high levels of novel stimulation. The maximum score was 2400.

T-Maze

On Days 26–29, each pup was observed daily for 5 min in the T-maze. The pups were placed in the starting area (stem) of the maze and measured for latency to leave and for amount of time spent in the social and novel areas. A measure of general activity was determined by tabulating the number of times the subject went from one area to the other. At 30 days of age, half of the pups in each group were sacrificed for determination of brain parameters. The remaining animals were weaned to the control diet and retested in both mazes on Days 91–94.

Shuttle-Box Training

Beginning at 95 days of age, adult animals were trained to learn a two-way active avoidance response to electric shock in a shuttle box. Training was divided into acquistion and reversal learning. All training sessions were conducted in a dark room with red illumination. In acquisition learning, Trial 1 began by placing the subject in the lighted left chamber of the shuttle box. A pilot light (40 mA, 28 V) located on the back wall of each chamber provided the white illumination. After 10 sec, the white light was switched to the opposite chamber; lights:off was the conditioned stimulus (CS). The CS remained for 10 sec, during which the subject could make an avoidance response to electric shock by crossing over to the lighted chamber. If the subject did not cross over during the CS period, electrical shock (0.35 mA), the unconditioned stimulus (UCS), was delivered through the steel floor until the subject crossed over and the trial was then scored as an escape response. When the subject made an avoidance response into the safe lighted chamber, a 10-sec period was again allowed before presentation of the CS. Acquisition learning continued at 100 trials per day for 10 consecutive days or until each subject met a learning criterion of 10 consecutive avoidance responses. After each subject met criterion for acquisition learning, the CS was reversed (lights:off to lights:on) and training for reversal learning continued on the following day. The number of trials per day, the learning criterion and the level of electric shock, were the same. During each type of learning, the total trials to criterion, percentage of avoidance responses and the total number of errors were recorded for each subject. In this study, an error was scored each time the subject failed to remain in the safe chamber of the box until presentation of the CS.

Data Analysis

Pups outcomes in the cross-maze and avoidance learning were analyzed in a 2 (Pup Nutrition)×2 (Cold Stress) factorial design. T-maze data were analyzed in a 2 (Pup Nutrition)×2 (Cold Stress)×4 (Days) factorial design, with Days treated as a repeated measure. Litter means were used as data units for analysis of the cross-maze and T-maze behavior (the number of male pups per litter ranged from 2 to 6). Analysis of the shuttle-box data was based on individual scores. To compensate for the interdependence of data, latency scores in the cross-maze and T-maze scores were subjected to an arcsin transformation. The total number of entries and rating score of preference for stimulation were subjected to a square-root and reciprocal transformation, respectively. In the shuttle-box, total trials to criterion and number of errors were subjected to a reciprocal and log $(x + 1)$ transformation, respectively. Statistical tests used

for all post hoc comparisons of significant interactions were based on Tukey's *t*-test [13].

Other Methods

Dissecting methods and biochemical determinations were as described in our previous papers [32,39]. The brain parameters measured were weight, DNA [35-37] (cell number) and protein content [19] for cerebral cortex, cerebellum, stem and remainder (diencephalon) as well as the ratio cortical DNA/cortical weight (index of cell number/cortical volume, i.e., cortical cell density).

In this work we have tentatively assumed that the high value of certain behavioral parameters (e.g., number of entries or social time in mazes, and percent correct moves in learning) are of advantage for the animal. Conversely, we have tentatively assumed that the high values of some other behavioral parameters (e.g., latency in mazes, or total trials to criterion and total errors in learning) are of disadvantage to the animal. Furthermore, we have tentatively assumed that the high value of certain brain parameters (weight of brain parts, their cell number (DNA) and protein content) are of advantage (as evidenced by evolutionary trend [12,23]), whereas the high value of cortical cell density is of disadvantage because it would mean a smaller dendritic tree for each cell (again as evidenced by evolutionary trend, reviews in [12,23]). The results should reveal (a) whether the correlation coefficients have reached the level of significance, and (b) whether the signs of these correlation coefficients are in the directions expected from the above assumptions.

RESULTS

Behavior at 30 Days

The analyses on cross-maze data indicated a significant Pup Nutrition effect, $F(1,15)=5.38$, $p<0.05$, on entries. Control pups made more entries (mean=31) than undernourished pups (mean=22). There were no significant differences in initial latency to move or preference for levels of novel stimulation between groups. In T-maze performance, data analyses indicated that there was a significant Cold Stress effect on entries, $F(1,15)=28.98$, $p<0.001$, and time in the start areas, $F(1,15)=4.05$, $p<0.05$. Cold Stressed pups made more entries (mean=10) into each area of the T-maze but spent less time in the start area (mean=9%) than nonstressed pups (mean=5, and mean=17%, respectively). In addition, there was a significant Pup Nutrition×Cold Stress effect on initial latency to leave the start area, $F(1,15)=29.01$, $p<0.001$, and time in the social area, $F(1,15)=4.41$, $p<0.05$.

The results summarized in Table 1 show that stressed control (SC) pups and nonstressed undernourished (NSU) pups exhibited shorter latencies and a higher preference for social stimulation than nonstressed control (NSC) pups. The results also show that in undernourished litters (groups c and d), early cold stress did not have a significant effect on latency, but did produce a significant decrease in social time.

Behavior at 90 Days

The analysis of T-maze data indicated the presence of a significant Pup Nutrition effect, $F(1,16)=21.40$, $p<0.001$, on time spent in the start area. Control animals spent more time (mean=14%) in the start area than previously undernourished animals (mean=8%). In addition, the analyses indicated that there was a significant Pup Nutrition×Cold Stress effect on latency, $F(1,16)=3.97$, $p<0.05$, entries, $F(1,16)=24.02$, $p<0.001$, and time in the social area, $F(1,16)=13.22$, $p<0.05$, and novel area, $F(1,16)=19.07$, $p<0.001$.

As indicated in Table 2, SC and NSU animals exhibited significantly shorter latencies, a greater number of entries and more time spent in the novel area than NSC animals. In stressed litters (Groups b and d), early undernutrition produced a significant decrease in latency and time spent in the novel area, but a significant increase in social time. The analyses of cross-maze data indicated that there were no significant differences between groups as result of either early cold stress or early undernutrition.

Active Avoidance Learning at 90 Days

The analyses of shuttle-box data indicated that there was a significant Pup Nutrition effect, $F(1,42)=5.30$, $p<0.02$, on total trials required for acquisition learning, and a significant Pup Nutrition×Cold Stress effect, $F(1,42)=5.29$, $p<0.02$, on frequency of errors during reversal learning.

As indicated in Table 3, during acquisition learning, previously undernourished animals required significantly more trials to reach learning criterion than controls. There were no significant differences in the percentage of correct avoidance responses or total errors between groups.

The results in reversal learning, summarized in Table 4, show that NSU animals (Group c) made significantly more errors than the two control groups (a and b). However, SU animals (Group d) exhibited a significant reduction in total

TABLE 1

NUTRITION × STRESS EFFECT ON T-MAZE BEHAVIOR OF 30-DAY OLD MALE RATS

Behavioral* paramaters	Groups: Controls nonstressed (a)	Controls stressed (b)	Undernourished nonstressed (c)	Undernourished stressed (d)	Probability
Latency (sec)	60 ± 51	5 ± 2	25 ± 62	10 ± 15	a:b,<0.05 a:c,<0.05
Social Time (%)	50 ± 27	68 ± 13	69 ± 23	61 ± 19	a:b,<0.05 a:c,<0.05 c:d,<0.05

*Values represent group means plus standard deviations.

TABLE 2

NUTRITION × STRESS EFFECT ON T-MAZE BEHAVIOR OF 90-DAY-OLD MALE RATS

Behavioral* parameters	Groups: Controls nonstressed (a)	Controls stressed (b)	Undernourished nonstressed (c)	Undernourished stressed (d)	Probability
Latency (sec)	22.0 ± 28.0	9.0 ± 18.0	4.8 ± 2.7	3.7 ± 1.7	a:b,<0.01 a:c,<0.01 b:d,<0.05
Entries	10.0 ± 3.9	16.0 ± 6.6	18.0 ± 9.5	15.0 ± 1.7	a:b,<0.01 a:c,<0.01
Novel Time (%)	24.8 ± 15.0	44.3 ± 14.8	33.3 ± 14.5	26.3 ± 15.5	a:b,<0.01 a:c,<0.05 b:d,<0.01
Social Time (%)	58.7 ± 14.7	42.2 ± 13.2	57.6 ± 14.6	64.7 ± 16.6	a:b,<0.01 b:d,<0.01 c:d,<0.01

*Values represent group means plus standard deviations.

TABLE 3

NUTRITION EFFECT ON SHUTTLE BOX AVOIDANCE LEARNING OF 90-DAY-OLD MALE RATS

Groups*	Learning Parameters: Total Trials	% Correct	Errors
Controls	159 ± 127	36.3 ± 15.7	2.1 ± 1.7
Undernourished	228 ± 114	30.8 ± 16.5	3.0 ± 2.4
Probability	0.02	n.s.	n.s.

*Values represent group means plus standard deviations.

errors and were not significantly different from controls. There were no significant differences in total trials or percentage of correct avoidance responses in reversal learning between groups.

Body Weights

The analyses of body weight data indicated that there was a significant Pup Nutrition×Stress effect, $F(1,34)=11.21$, $p<0.005$, at weaning, and a significant Pup Nutrition effect, $F(1,49)=47.05$, $p<0.001$, at 90 days of age. The results summarized in Table 5 show that at weaning there was a significant deficit in the body weight of NSU pups relative to the weight of NSC pups.

Correlations

Correlation coefficients between cross-maze and T-maze behavioral parameters, and cerebral cortex and cerebellum parameters in 30-day-old male rats are shown in Table 6. Only those parameters found to be significantly correlated ($p<0.05$, two-sided) are shown. An asterisk denotes that the sign (+ or −) of the correlation coefficient coincides with our assumptions (see Method). Table 7 shows analogous results for 90-day-old males. Table 8 shows correlation coefficients between active avoidance learning parameters (original and reversal learning) and brain parameters in 90-day-old male rats. Cortex, cerebellum, stem, and remainder (diencephalon) are also included. Overall 75% of the statistically significant correlations are in the expected direction.

Only 7% of the correlations involving 30-day-old animals were statistically significant; 71% of those were in the expected direction. Of the correlations involving 90-day-old

TABLE 4

NUTRITION × STRESS EFFECT ON FREQUENCY OF ERRORS DURING REVERSAL LEARNING OF SHUTTLE BOX AVOIDANCE

Parameters*	Groups: Controls nonstressed (a)	Controls stressed (b)	Undernourished nonstressed (c)	Undernourished stressed (d)
Errors†	5.0 ± 4.7	4.0 ± 3.1	14.0 ± 9.7	5.0 ± 3.4

*Significance: a:c, $p<0.01$.

†Values represent group means plus standard deviations.

TABLE 5

NUTRITION EFFECTS ON BODY WEIGHTS OF 30-DAY-OLD MALE RATS

	Groups			
	Controls nonstressed (a)	Controls stressed (b)	Undernourished nonstressed (c)	Undernourished stressed (d)
Body Weight*† (g)	68.0 ± 9.4	71.3 ± 10.3	61.0 ± 10.6	43.4 ± 4.8

*Significance: a:c, $p<0.05$; b:d, $p<0.01$; c:d, $p<0.01$.
†Values represent group means plus standard deviations.

TABLE 6

CORRELATIONS BETWEEN MAZE BEHAVIORAL PARAMETERS AND BRAIN PARAMETERS IN 30-DAY-OLD MALE RATS

Parameters Correlated	Group	Correlation Coefficient	Significance
Latency (X) - Cortical DNA	CNS	−.59*	<0.05
Latency (X) - Cortical Cell Density	UNS	−.66	<0.05
Latency (X) - Cerebellar DNA	UNS	.60	<0.05
Latency (T) - Cerebellar DNA	CNS	−.71*	<0.01
Latency (T) - Cerebellar Weight	CS	−.81*	<0.01
Latency (T) - Cortical Protein	UNS	−.73	<0.01
Entries (T) - Cortical Weight	CNS	.57*	<0.05
Entries (T) - Cortical Weight	CS	.68*	<0.05
Entries (T) - Cortical Cell Density	CS	−.78*	<0.05
Entries (T) - Cortical Cell Density	US	−.92*	<0.05
Social (T) - Cerebellar DNA	CNS	.61*	<0.05
Social (T) - Cerebellar DNA	CS	.76*	<0.05
Social (T) - Cortical Cell Density	UNS	.61	<0.05
Start (T) - Cerebellar DNA	CNS	−.70*	<0.01

Mazes: X—X-maze; T—T-maze.
Groups: CNS—control nonstressed; CS—control stressed; UNS—undernourished nonstressed; US—undernourished stressed.
*—sign of correlation as expected.
Total number of correlation coefficients computed: (4 groups) × (7 brain parameters) × (7 maze behavioral parameters)=196.
Percent significant 14/196=7%.
Percent in expected direction 10/14=71%.

animals, 7.5% were statistically significant, and 76% of those were in the expected direction. There were no significant differences in the numbers or directions of significant correlations among the four groups of animals. In the absence of a suitable statistical procedure for testing whether significantly more than 50% of the correlations are in the expected direction, a simulation procedure was used, which showed that the observed percentages (71% and 76%) correspond to significance levels of approximately $p<0.15$ and suggest a possible trend.

DISCUSSION

Behavior

The results of this study have demonstrated that during preweaning development, nonstressed undernourished (NSU) pups and stressed control (SC) pups exhibited shorter response latencies and a greater preference for social stimulation than the nonstressed controls (NSC). With respect to the interaction between early undernutrition and early stress, our results showed that while there was no significant difference between the latency of stressed and nonstressed undernourished pups in the T-maze, early cold stress resulted in a significant reduction of preference for social stimulation in undernourished pups. In adults, the results again showed that NSU animals were very similar to SC animals in the T-maze performance. When compared to the NSC group, NSU and SC animals were more responsive, exhibited a higher activity level and a greater preference for novel stimulation.

Contrary to what was expected, our study demonstrated that (with the exception of social stimulation) early cold stress did not have a significant effect on the maze perform-

TABLE 7

CORRELATIONS BETWEEN MAZE BEHAVIORAL PARAMETERS AND BRAIN PARAMETERS IN 90-DAY-OLD MALE RATS

Parameters Correlated	Group	Correlation Coefficient	Significance
Latency (X) - Cerebellar Weight	US	−.59*	<0.05
Entries (X) - Cerebellar Protein	CNS	.44*	<0.05
Entries (X) - Cortical Cell Density	US	−.80*	<0.01
Preference for Stimulation (X) - Cerebellar Weight	CNS	.45*	<0.05
Start Time (T) - Cortical Protein	CS	−.55*	<0.05
Latency (T) - Cerebellar DNA	UNS	.72	<0.05
Entries (T) - Cerebellar DNA	UNS	−.73	<0.05
Start (T) - Cortical Weight	UNS	−.80*	<0.05
Start (T) - Cortical Protein	UNS	−.75*	<0.05
Start (T) - Cerebellar Weight	UNS	−.79*	<0.05
Start (T) - Cerebellar DNA	UNS	−.79*	<0.05
Start (T) - Cortical Cell Density	US	−.77	<0.01
Entries (T) - Cortical Cell Density	US	−.64*	<0.05

Symbols as in Table 6.
Total number of correlation coefficients computed as in Table 6.
Percent significant 13/196=6.6%.
Percent in expected direction 10/13=77%.

ance of undernourished animals. Furthermore, the data on maze performance indicated that there were no additive effects of early cold stress and early undernutrition (Groups c and d) on any of the behavioral parameters. Although our data suggest that early cold stress was not sufficient for intervening with the effects of early undernutrition, the important observation in the present study was that nonstressed-undernourished animals were very similar to stressed controls in maze performance prior to weaning and at adulthood.

Since very few other studies have investigated the combined effects of early stimulation and early undernutrition, it is difficult to interpret the similarity between the maze behavior of SC and NSU pups. For example, when compared to NSC pups, the shorter latencies and higher activity levels of NSU pups could reflect hyperactivity in a novel environment; however, when compared to the stressed controls, an interpretation based on early stimulation studies [5,15] would suggest lower emotionality and increased exploratory behavior. The similarity between the behavior of both groups, despite the significant body weight deficit in undernourished pups suggests the possibility that other nonnutritional environmental factors (i.e., maternal and littermate behavior) may have influenced the behavioral development of undernourished litters. For example, Smart and Preece [27] have reported that undernourished lactating mothers spend significantly less time in direct contact with the pups in the nest area than well-nourished mothers. It has also been reported [21] that undernourished litters exhibit less play and exploratory activity outside the nest area during the weaning period. Whether the maternal care and littermate behavior of NSU liters was the same as in the stressed controls was not recorded in the present study. The possibility of a heightened food drive seems to be ruled out since the control pups exhibited more activity than undernourished pups in the cross-maze before weaning.

As far as learning performance in the adult animals is concerned, our study demonstrated that on the average, undernourished animals were slower in acquisition learning than control animals. However, our data showed that during reversal learning early cold stress had a beneficial effect on the learning performance of undernourished animals. Undernourished animals exposed to early cold stress made significantly fewer errors than the nonstressed undernourished animals. This finding appears to be consistent with the interpretation of previous studies [7, 8, 18] suggesting that early stimulation (i.e., handling, cold stress) may intervene with the behavioral effects of early undernutrition. Overall, however, our data seem to suggest that learning performance of undernourished animals may reflect a specific behavioral deficit (ability to inhibit a learned response) rather than a deficit in general learning ability. This was demonstrated by the finding that nonstressed undernourished animals exhibited significantly more errors than the nonstressed controls, but yet required the same number of trials during reversal training.

In summary of this part, the present study has demonstrated that: (1) early cold stress and early undernutrition can have the same consequences on some behavioral parameters and (2) early cold stress improves the ability to inhibit a learned response in undernourished animals. Future studies should investigate the effects of other early environmental factors before interpreting the behavioral development of early undernourished animals.

Correlations

The purpose of this part was to establish: (a) whether the correlations between behavioral parameters and brain parameters can be demonstrated (i.e., whether these correlation coefficients have reached the level of significance) and (b) whether the signs of these correlation coefficients are in the directions expected from our assumptions (see Method).

TABLE 8

CORRELATIONS BETWEEN ACTIVE AVOIDANCE LEARNING PARAMETERS AND BRAIN PARAMETERS IN 90-DAY-OLD MALE RATS

Parameters Correlated		Group	Coefficient	Significance
Total Trials	(OL) - Remainder DNA	CNS	−.59*	<0.01
Total Trials	(OL) - Cerebellar Protein	CNS	−.49*	<0.05
Total Trials	(OL) - Cortical Weight	CS	−.69	<0.05
Total Trials	(OL) - Cortical Cell Density	CS	−.70	<0.05
Total Trials	(OL) - Cortical Weight	UNS	−.89*	<0.01
Total Trials	(OL) - Cortical DNA	UNS	−.90*	<0.01
Total Trials	(OL) - Cerebellar Weight	UNS	−.86*	<0.05
Total Trials	(OL) - Cerebellar Protein	UNS	−.77*	<0.05
Total Trials	(OL) - Stem Weight	UNS	−.80*	<0.05
Total Trials	(OL) - Cerebellar DNA	US	.68	<0.05
% Correct	(OL) - Remainder DNA	CNS	.51*	<0.05
% Correct	(OL) - Cerebellar Protein	CNS	.53*	<0.05
% Correct	(OL) - Cortical Cell Density	CS	.60	<0.05
% Correct	(OL) - Cerebellar Weight	UNS	.80*	<0.05
% Correct	(OL) - Cerebellar Protein	UNS	.97*	<0.01
% Correct	(OL) - Stem Weight	UNS	.85*	<0.05
% Correct	(OL) - Stem Protein	UNS	.82*	<0.05
Total Errors	(OL) - Cortical DNA	CNS	−.63*	<0.01
Total Errors	(OL) - Cortical Cell Density	CNS	−.75	<0.01
Total Errors	(OL) - Stem Protein	CS	−.60*	<0.05
Total Errors	(OL) - Stem DNA	UNS	−.90*	<0.01
Total Errors	(OL) - Cerebellar Protein	US	−.64*	<0.05
Total Errors	(OL) - Cortical Cell Density	US	.86*	<0.01
Total Trials	(RL) - Cerebellar Protein	CNS	−.46*	<0.05
% Correct	(RL) - Remainder DNA	CNS	.59*	<0.01
% Correct	(RL) - Cerebellar Protein	CNS	.55*	<0.05
Total Errors	(RL) - Cortical Cell Density	CNS	−.63	<0.01
Total Trials	(RL) - Cortical Weight	CS	.67	<0.05
% Correct	(RL) - Stem Protein	CS	.72*	<0.05

OL—Original Learning; RL—Reversal Learning.
Other symbols as in Tables 6 and 7.
Total number of correlation coefficients computed: (4 groups) × (13 brain parameters) × (7 learning parameters)=364.
Percent significant 29/364=7.9%.
Percent in expected direction 22/29=75.8%.

It can be seen from Tables 6–8 that some correlation coefficients were statistically significant, but not substantially more than would be expected by chance, and not for all parameters. Possibly, more correlation coefficients would reach significance if more animals were used, or if one could remove such sources of statistical variability as the fact that some other factors may not have been equal for all animals. For example, to be more indicative of final neuron number (rather than neuron plus glia), it might be better to determine DNA right after the end of neuron proliferation (i.e., at birth, review in [33]), but that would preclude subsequent behavioral tests.

The signs of these correlation coefficients are in the direction expected from our assumptions in 75% of the significant cases. If the assumptions are entirely haphazard, only 50% should be in the expected direction. Perhaps one or more assumptions, especially as to behavioral parameters whose high value was assumed to be of advantage, were incorrect, and if all the assumptions were correct, the percentage with signs in the expected directions would be higher than 75. One must, however, consider that, in general, behavioral parameters whose high value was assumed to be of advantage, were consistently positively correlated with each other and negatively with those assumed to be of disadvantage (see discussion of Tables 1–5). For brain parameters, the assumption that the stem and the remainder will be as well correlated as cerebral cortex and cerebellum proved to be wrong for the maze behavior (30 and 90 days old); the percentage of correlations with signs in the direction expected was no more than 50 (not entered in Tables 6 and 7). On the other hand, for brain parameters in the stem and the remainder in active avoidance learning, all correlations were in the expected direction (Table 8); we cannot offer a non-statistical explanation of this discrepancy.

It is of interest that all the correlations (Table 6–8) are essentially uninfluenced by the ages tested, undernutrition, and/or stress. Possibly this is due to the basic nature of these correlations.

If these results are accepted as indicating preliminary, but by no means final evidence of correlations between behav-

ioral and brain parameters, than the following trends emerge: (1) Higher values of weight, DNA (i.e., higher cell number [13, 15, 19]) and protein content of various brain parts are correlated with those behaviors which are of more advantage to the animal. (2) Lower values of cortical cell density (i.e., cells further apart: possibly more extensive dendritic tree) are correlated with those behaviors which are of more advantage to the animal.

One must, however, also consider the possibility that the benefit of having the brain parameters as above is not direct, but only serves to take full advantage of some other, finer brain features that are more involved in behavioral improvements.

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