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IN HYPOPHYSECTOMIZED RATS

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CEREBRAL EFFECTS OF DIFFERENTIAL EXPERIENCE IN HYPOPHYSECTOMIZED RATS¹

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Enriched and impoverished environments have been demonstrated to affect significantly brain chemistry and brain weights in rats. To test whether these effects might be mediated hormonally, three experiments were done on rats hypophysectomized at about 30 days and on normal controls. The 78 hypophysectomized rats retained were those with complete operations as verified by inspection of target organs and sella turcica. Environmental treatments lasted about 35-65 days of age. Among operates as among controls the enriched-environment rats showed significantly greater cortical weight, decreased cortical AChE/weight, and increased ChE/AChE. Thus these effects of differential environment do not require mediation by hormones of the pituitary or those regulated by the pituitary. Hypophysectomized rats had significantly lower brain weights, lower cortical AChE/weight, and greater cortical ChE/AChE than did controls.

Although it has been clearly established that prolonged commerce with environments differing in richness of stimulation affects brain weights, brain enzymes and nucleic acids, and brain anatomy in rodents (Diamond, 1967; Rosenzweig & Bennett, 1969; Rosenzweig, Bennett, & Diamond, 1972; Rosenzweig, Bennett, Diamond, Wu, Slagle, & Saffran, 1969), the mechanisms by which these changes are produced have not yet been determined. The effects may result rather directly from use or disuse of neural cells. On the other hand, the differential experience might lead to alterations of metabolic or hormonal functions which could, in turn, affect the nervous system. A number of investigators have shown that brain development is influenced by hormones, especially in early life (e.g., Eayrs, 1968; Ford, 1971; Timiras, Vernadakis, & Sherwood, 1968).

This paper reports tests of the hypothesis that the effects of environmental experience on cerebral measures are mediated by mod-

ified outputs of certain endocrine glands. In the earlier experiments, this was tested by taking weights of the adrenal glands or the thyroid glands, in addition to brain measures, in rats exposed either to our standard enriched condition (EC) or to the impoverished condition (IC). These experiments will be reported rather briefly here. In three later experiments, hypophysectomized as well as normal rats were exposed to the experimental environments in order to determine whether the cerebral changes would occur when secretion of certain endocrines was diminished and no longer subject to environmental influence.

EXPERIMENTS ON ENDOCRINE WEIGHTS

Adrenal Glands

In our first attempts to determine whether endocrine organs are specifically involved in the EC-IC effects, we asked whether weights of certain glands would develop differentially in EC and IC groups. This was done originally with regard to the adrenals, partly because we wondered whether either the EC or IC situations might be stressful and might thus involve adrenal mediation. We, therefore, included measures of adrenal weights in standard EC-IC experiments, and we also tested effects of overt stress on both cerebral measures and adrenal weights. Adrenal weight was used as a convenient, although admit-

¹This investigation was supported by Grant GB-8011 from the National Science Foundation and also received support from the United States Atomic Energy Commission. A report of this research was presented at the 1970 meeting of the Federation of American Societies of Experimental Biology (Rosenzweig, Bennett, & Diamond, 1970).

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tedly crude, index of stress; we have found it to yield clear effects whenever we have employed overt stressors.

Method

Behavioral conditions. In the EC condition, animals are housed in a same-sex group of 10-12 in a large cage (70 × 70 × 46 cm.), and the cage is provided with stimulus objects ("toys") from a standard pool. Each day there are six or more toys in the EC cage; some may be in new positions from the previous day and some are newly taken from the pool. Rats in an EC cage are shown in Figure 1 of Bennett, Diamond, Krech, and Rosenzweig (1964); the pool of objects is shown in Figure 1 of Rosenzweig and Bennett (1969). Each day the EC animals are placed for 30 min. in the field of a Hebb-Williams apparatus (75 × 75 cm.) where the pattern of barriers is changed daily. During this time the selection and arrangement of objects in the EC cage are changed. In the IC condition, animals are housed in individual cages similar in size to standard colony cages. The IC cages have solid sidewalls so that the animals cannot see each other, and they are placed in a separate, quiet, and dimly illuminated room. In these special IC cages, the floors are made of wire bars and pans of shavings below the cages can be changed without touching the animals. All animals—EC and IC—have laboratory pellets and water available ad lib. All animals are weighed regularly, usually at weekly intervals.

Littermates were assigned semirandomly among the number of groups required. Assignment was semirandom in that, if necessary, exchanges were made to insure that the distributions of body weights among groups were similar. Runts and animals whose weight departed more than 15% from other littermates were excluded. The groups so chosen were then assigned at random to the experimental conditions. Animals were usually assigned to conditions at about 25 days of age and removed for sacrifice at about 55 days of age.

Shock stress. Except during the experimental periods, the animals were housed individually in colony cages. The shock subjects received intermittent unavoidable electrical shock in daily 12-min. sessions; the shocks were delivered through the floor bars of a special experimental box. During the shock sessions, littermate controls were placed in similar boxes but with no shock and in a different room, so they received neither shock nor stimulation from the shocked animals. Shock treatment was given over 2-wk. periods in 1959 to rats of the S₁ and K strains (each experiment including 8 littermate pairs) and for a 4-wk. period in 1966 to the RCH and RDH strains (11 and 12 littermate pairs, respectively).

Weighing adrenals and brain samples. The rats were brought to sacrifice under code numbers that did not reveal to the analysts the behavioral condition of any individual; littermates were sacrificed in immediate succession, the EC rat (or the shock

rat) being first in some pairs and the IC (or non-shock) being first in others. Brain samples were dissected and weighed according to our standard procedures (Rosenzweig, Krech, Bennett, & Diamond, 1962). The adrenals were removed rapidly, cleared of any adhering tissue, and weighed to the nearest .1 mg.

Results

We reported briefly in 1964 (Bennett et al.) that adrenal weight did not differ between EC and IC groups. A more complete statement is that, while the IC adrenals do weigh somewhat more, this is only in proportion to the greater body weight of IC rats. In six experiments run in 1964 with a total of 64 littermate pairs, adrenal weight as a percentage of body weight did not differ significantly between the EC and IC groups. In the same experiments, cerebral cortical weights were, as usual, significantly greater in EC than in IC. In recent experiments involving hypophysectomized and control rats, there is again no consistent EC-IC difference in adrenal weight/body weight of either controls or hypophysectomized animals (see Table 4).

Opposite results were obtained in the four shock experiments (39 pairs). That is, the animals that received footshock for 12 min. daily developed significantly greater ratios of adrenal weight to body weight (14%, $p < .001$) than did the nonshocked controls, but there were no differences between shocked and control animals in our usual measures of brain weights and brain chemistry. Thus, there was clear evidence of adrenal involvement in the shock experiments but not in the EC-IC experiments. Recently Riege and Morimoto (1970) ran three experiments with 30-day EC and IC groups, both with and without the stress of being tumbled daily in a revolving drum. They confirmed both that stress increases adrenal weight but does not affect our usual brain measures, and that the differential environmental conditions did not lead to significant differences in adrenal weight/body weight. More severe stress was then employed in two additional experiments.³ Here the stress included both 3-5 min. daily

³These experiments were also performed by Riege and W. H. Morimoto in our laboratories.

tumbling and 3-5 min. daily footshock. Once again, stress increased the adrenal weights but did not affect brain weights, whereas the differential environments altered brain weight but not adrenal weights.

Thyroid Gland

We also asked whether the weight of the thyroid might differ between EC and IC and thus suggest a possible thyroid involvement in production of the brain differences. Thyroid secretion has been shown to influence brain development in the rat, although it has been reported that such effects can be induced only during the first few postnatal days and cannot be produced thereafter (Eayrs, 1968). In 1968 we took thyroid weights in two experiments (T_1 and T_2) in which S_1 rats were placed in standard EC or IC situations (as described above) at 25 days of age and were sacrificed at 55 days. The results of these experiments have not been reported previously.

The results of the two thyroid experiments were closely similar in regard to both brain weights and body weights, but they were opposite in regard to thyroid weights, as can be seen in Table 1. Both experiments showed the usual significant increases in cortical weights with EC, and especially in the occipital area; both showed a highly significant increase in the ratio of cortical weight to that of the rest of the brain. In both cases, EC was significantly

lower than IC in body weight, although having the greater brain weights. When we come to thyroid weights, the similarity of results ends. In T_1 , thyroid weight/body weight was significantly greater in the EC than in the IC group ($p < .01$), but in T_2 there was a statistically significant effect in the reverse direction ($p < .05$). Since typical and significant cerebral EC-IC differences were found in both of these experiments, there was no consistent or necessary relation between thyroid weight and cerebral effects. It may be noted that either set of thyroid/body weight results, by itself, could have been taken as evidence for a significant relation between thyroid and cerebral effects—positive in one case and negative in the other. Only the attempt to replicate, and its failure, stood in the way of such an erroneous conclusion. In the more recent hypophysectomy experiments (Table 4), there was again no consistent EC-IC difference in thyroid weights.

To obtain a more functional measure of thyroid activity, protein-bound iodine was analyzed in two further 25-55 day experiments with S_1 rats.⁴ Values were obtained for 16 littermate pairs. The EC rats were lower in iodine in the first experiment but higher than IC in the second; overall the difference was -6.9% (*ns*).

CEREBRAL EC-IC EFFECTS IN HYPOPHYSECTOMIZED AND NORMAL RATS

Another design to test the possibility of hormonal mediation of the cerebral effects of experience is to abolish secretion of a gland or to make it immune from effects of environmental stimulation. This type of design was employed by Adair, Wilson, and Glassman (1968) when they removed the adrenals of mice and found that avoidance conditioning nevertheless produced changes in brain RNA and protein in the operates as well as in controls. One could proceed in this fashion to perform separate experiments on the possible roles of a series of endocrine glands. Rather than to do so, we decided to employ hypophysectomy in the hope of ruling out a number of endocrines

TABLE 1
PERCENTAGE DIFFERENCES BETWEEN EC AND IC GROUPS IN WEIGHTS OF BRAIN, BODY, AND THYROID GLAND (MALE S_1 RATS)

Weight measure	Experiment T_1 ($n = 9$ pairs)	Experiment T_2 ($n = 11$ pairs)
Occipital cortex	7.6*	13.8**
Total cortex	4.8***	6.2***
Rest of brain	1.2	1.4
Total brain	2.8*	3.5***
Cortex/Rest	3.6**	4.8***
Total body	-11.8**	-7.6**
Thyroid gland ^a	3.3	-15.0**
Thyroid/Body ^a	15.6**	-8.4*

^a $n =$ Eight pairs in each experiment.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

⁴ These experiments were performed by Leon Dorosz in our laboratories.

at the same time; if this prevented the appearance of cerebral EC-IC effects, then it would be necessary to backtrack and study the effects of inactivating single glands or combinations of glands. The pituitary secretes growth hormone and also trophic hormones for the thyroid, the adrenal cortex, and the gonads; its removal leads to regression of these target organs. Failure to find EC-IC effects after hypophysectomy might also be interpreted to mean that lack of sufficient basal level of one or more of these hormones interfered with brain metabolism even if the EC-IC effects were not mediated by alteration of hormonal output. This hypothesis could be tested by removing a gland and maintaining a fixed level of replacement hormone. If, however, EC-IC effects appeared in the hypophysectomized rats, then this would indicate that neither a normal resting level of secretion nor alteration of rate of secretion are necessary, insofar as the pituitary, the thyroid, the adrenal cortex, and the gonads are concerned.

Method

Subjects. Three successive experiments were run, H₁ and H₂ with male rats of the inbred Fischer strain (Simonsen Laboratory, Gilroy, California) and H₃ with Long-Evans rats from the colony of the Physiology-Anatomy Department, University of California, Berkeley. The Fischer rats were weighed 1 or 2 days after weaning (which occurred at about 25 days of age) and were divided into groups of four that were closely matched in weight. From each foursome, one rat was assigned at random to each of the following experimental groups: (a) hypophysectomized-EC (H-EC), (b) hypophysectomized-IC (H-IC), (c) control-EC (C-EC), and (d) control-IC (C-IC). The operations were then done under anesthesia by Jacqueline Ehlert, using a parapharyngeal approach. In H₃, litters with four or more male weanlings were used. All but two males from each litter were hypophysectomized. In all litters where at least two operates survived, two operates and the two controls were assigned among the four conditions listed above. In Experiments H₂ and H₃, unoperated animals of the same strain, sex, and age were sacrificed at the time of hypophysectomy to provide base-line measures.

Behavioral conditions. After operation, the hypophysectomized rats were housed several in a cage in a room kept at about 27° C. for a recovery period. This period lasted for 10 days in Experiment 1. Since most of the deaths in H₁ occurred soon after the operation, the recovery period was reduced to 5 days in H₂ and H₃. During this period

the controls were housed similarly and in the same room as the operates. The animals were then placed in their preassigned experimental conditions, and 30 days of standard EC and IC treatments were given. Some observations of activity were made, both in the EC cages and in the Hebb-Williams apparatus.

Postmortem verification of hypophysectomy. At sacrifice, completeness of hypophysectomy was verified both by examination of the sella turcica under a dissecting microscope and by taking the weights of the adrenals, and, in many cases, also the thyroid, the testes, and the seminal vesicles. Data were not used for any animal that showed a remnant of the pituitary or that did not demonstrate marked stunting of body growth and regression of those target organs that were taken. The target organs were also weighed in all of the control rats in Experiment H₁ and in a sample of the controls in H₂ and H₃.

Final groups for analysis. The rather severe operative mortality, especially among the Fischer strain, and the incompleteness of some operations reduced sharply the number of original foursomes that survived for analysis of effects. Some new foursomes were therefore constituted after the fact by putting together survivors that were closely similar in body weight at the outset of the experiment. The magnitudes of effects were not altered by this procedure, but the increased number of cases allowed a more critical testing of the hypothesis. Table 2 gives the number of original foursomes in each experiment, the sources of loss, the number of original foursomes remaining at the end, and the number of reconstituted sets of four. Since the Long-Evans rats showed much less operative mortality than the Fischers, there was less selection due to mortality in Experiment H₃, and therefore most weight should probably be given to the results of this experiment.

Brain enzyme analyses. For the spectrophoto-

TABLE 2
ASSIGNMENT OF ANIMALS IN HYPOPHYSECTOMY
EXPERIMENTS, LOSSES, AND MAKE-UP OF
FINAL SETS

Assignment and fates of animals	Experiment 1 (Fischer)	Experiment 2 (Fischer)	Experiment 3 (Long-Evans)
Initial sets of four (Fischer) or litters (Long-Evans)	18	35	16
Number operated	36	70	36
Deaths	16	35	4
Operative mortality	44%	50%	11%
Incomplete operations	4	4	2
Original sets of four surviving and with complete operations	6	5	10
Reconstituted sets of four	2	6	5
Final <i>n</i> per group	8	11	15

metric analysis of brain acetylcholinesterase (AChE), acetylthiocholine was the substrate and promethazine was used to inhibit cholinesterase (ChE) activity. For analysis of ChE, butyrylthiocholine was the substrate and AChE was inhibited with BW284C51 (1,5-bis[4-allyldimethylammoniumphenyl]pentan-3-one diiodide). Analyses were made in duplicate; two AChE values usually agreed within 2% and ChE values within 3%.

Results

Brain weight effects. Hypophysectomy checked the growth of body and of brain, but significant EC-IC effects occurred in both brain weights and brain chemistry. The main brain weight effects are shown in Figure 1. For economy of presentation, the

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FIG. 1. Brain weights of hypophysectomized animals taken as proportions of the preoperative base lines. (The left half of the figure is based on the combined results of Experiments H₁ and H₂, run with rats of the Fischer strain; the right half is based on Experiment H₃, run with Long-Evans rats. The upper blocks on each side show weight results for all cortical sections combined, and the lower blocks show the results for the rest of the brain. Both hypophysectomized animals [hollow symbols] and controls [solid symbols] show effects of differential environments on the brain weights, and there are also effects of hypophysectomy within both environmental conditions.)

data of the two Fischer experiments were combined. Looking at results for weight of total cortex (in the two upper blocks), it will be seen that for both strains, control rats gained more in comparison with the base-line value than did operated rats, and EC rats gained more than did IC rats. Both the effects of the operation and the effects of the environmental treatment were significant. For cortical weight in both strains, EC-H was closely similar to IC-C; in a sense, the enriched condition compensated for the effect of hypophysectomy on cortical weight. In the rest of the brain, both strains showed significantly greater growth on the part of controls than of operated rats. In both experiments with the Fischer strain, IC-H lagged behind EC-H in subcortical weight, but this was not true of the Long-Evans rats.

More detailed weight results are given in Table 3. The initial Fischer experiment showed no significant EC-IC brain weight differences among the hypophysectomized animals, but the control animals in this experiment also had relatively small weight effects, except in the occipital cortex. In the replication Fischer experiment the hypophysectomized animals yielded larger EC-IC effects than did the controls, except for the cortical/subcortical ratio, and even here the effect was statistically significant. When results of the two Fischer experiments were combined, the EC-IC percentage difference was as large among the operates as among the controls for somesthetic cortex, remaining dorsal cortex, ventral cortex, total cortex, and total brain. Furthermore, all of these EC-IC effects were statistically significant among the operates. In the Long-Evans experiment, the EC-IC percentage effects were larger among the hypophysectomized than among the control rats in most brain regions, and they were statistically significant for occipital cortex, remaining dorsal cortex, ventral cortex, total cortex, and for the cortical/subcortical ratio.

Body weights. The relation between EC and IC animals in body weight was found to differ between hypophysectomized and controls. Normally, EC animals weigh about

one-tenth less than ICs at the end of a 30-day experiment, and this was found among the controls in these three experiments. In the case of the hypophysectomized rats, however, the ECs weighed slightly more than the ICs in the Fischer experiments, and only 1.3% less in the Long-Evans experiment. It should not be supposed that this contributed importantly to the EC-IC brain weight effects, for we have found only slight influence of body weight on brain weight in such experiments. Note that in the first experiment where EC-H had the greatest body weight in relation to IC-H, there were the smallest EC-IC brain weight effects.

Hypophysectomy effects on weights of brain, body, and endocrines. In all three experiments, hypophysectomy slowed the growth of the brain, significantly in most regions, as can be seen in the right-hand columns of Table 3. This effect was less pronounced in the cortex than in the rest of the brain, so the ratio of weight of cortex to that of the rest of the brain was higher in all cases among hypophysectomized than among control animals, and significantly so in five of the six cases. Thus the operation, done in postweaning rats, not only checked brain growth but also altered its proportions. It should be emphasized that although the cerebral effects of hypophysectomy were consistent and significant, the brain is nevertheless relatively immune to the operation. Total brain weights were changed by less than 10%, whereas body weights were 60% lower in the operates than in the controls. Target organ weights were severely affected by hypophysectomy, as can be seen in Table 4. Whereas brain and body weights of the operates rose somewhat above the weanling base-line values (see Table 3), adrenal and thyroid weights regressed from the base-line levels. This occurred while the endocrine weights of the controls were doubling during the same period.

Effects on Brain Enzymes

We have reported that in experiments starting at 25 days of age and lasting 80 days, the following EC-IC differences in enzymatic activities result: (a) Activity of

TABLE 3
WEIGHT RESULTS IN HYPOPHYSECTOMY EXPERIMENTS

Experiment	Mean weights (in mg. for brain and in gm. for body)					% EC-IC differences ^a		% H-C differences ^b	
	Base line	H-EC	H-IC	C-EC	C-IC	Among H	Among C	Among EC	Among IC
Experiment H ₁ (Fischer)									
Occipital cortex	—	68.2	70.4	79.8	72.3	-3.1	10.4*	-14.5**	-2.6
Total cortex	—	735	712	781	761	3.4	2.7	-5.8**	-6.5**
Rest of brain	—	880	859	961	976	2.5	-1.5	-8.4***	-12.0***
Total brain	—	1,616	1,571	1,742	1,737	2.9	.3	-7.3***	-9.6***
Cortex/Rest	—	.835	.829	.812	.780	.8	4.2**	2.8	6.3***
Body weight	—	76.5	71.8	202	222	6.6	-9.2**	-62.1***	-67.7***
n	—	8	8	8	8				
Experiment H ₂ (Fischer)									
Occipital cortex	68.5	77.4	71.0	78.8	75.0	8.9**	5.0	-1.9	-5.3
Total cortex	688	754	707	781	749	6.6***	4.4**	-3.5**	-5.6***
Rest of brain	781	883	844	941	938	4.6**	.3	-6.2***	-11.1***
Total brain	1,469	1,636	1,551	1,722	1,687	5.5***	2.1	-5.0***	-9.1***
Cortex/Rest	.882	.854	.838	.830	.798	1.9*	4.0***	2.9**	4.9**
Body weight	61.3	74.2	70.4	186	207	5.4	-11.4***	-61.1***	-66.1***
n	11	11	11	11	11				
Experiment H ₃ (Long-Evans)									
Occipital cortex	73.6	82.9	76.1	88.6	78.7	8.9***	12.5***	-6.4***	-3.3
Total cortex	748	801	758	831	802	5.6**	3.0*	-3.7*	-5.5***
Rest of brain	833	921	919	999	1,010	.2	-1.1	-7.8***	-9.0***
Total brain	1,580	1,722	1,678	1,830	1,813	2.6	1.0	-5.9***	-7.5***
Cortex/Rest	.898	.869	.826	.833	.794	5.3***	4.8***	4.4***	3.9***
Body weight	76.8	88.9	90.1	256	282	-1.3	-9.1***	-65.3***	-68.0***
n	9	15	15	15	15				

^a Environmental effect = 100(EC-IC)/IC.

^b Hypophysectomy effect = 100(H-C)/C.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

TABLE 4
MEAN ENDOCRINE WEIGHTS (IN MG.) IN HYPOPHYSECTOMY EXPERIMENTS

Group	Adrenal						Thyroid					
	Experiment H ₁		Experiment H ₂		Experiment H ₃		Experiment H ₁		Experiment H ₂		Experiment H ₃	
	M	n	M	n	M	n	M	n	M	n	M	n
Hypophysectomized												
EC	6.2	8	7.2	11	8.7	15	3.9	8	3.6	4	3.9	8
IC	6.8	8	6.8	11	7.5	15	3.9	8	3.3	4	3.4	7
Control												
EC	31.3	8	25.6	8	36.6	5	12.0	8	10.6	4	17.0	5
IC	30.6	8	28.9	8	43.6	5	13.8	8	14.2	4	17.7	5
Base line	—	—	16.9	11	18.3	4	—	—	4.2	11	7.3	4

AChE per unit of cortical weight is significantly lower in EC than in IC; the rest of the brain shows little change in AChE/weight, so the cortical/subcortical ratio is lower in EC than in IC. (b) ChE activity per unit of cortical weight is significantly greater in EC than in IC, and so is total activity of ChE. (c) The ratio of ChE to AChE activity (a purely chemical measure in which tissue weight does not enter) is greater in EC cortex than in IC cortex (Rosenzweig, 1968; Rosenzweig et al., 1972). More recent work has shown that the AChE

effects can also be produced in experiments of 30-day duration but that it requires more than 30 days to produce the full ChE effects (Rosenzweig et al., 1972).

Chemical data from the present 30-day experiments are given in Table 5 in the form of percentage environmental effects (EC-IC) and percentage hypophysectomy effects (H-C). Data from the two experiments with Fischer rats have been combined for economy of presentation. Considering first the EC-IC effects, we see that AChE/weight is reduced in the cortex of

TABLE 5
CHEMICAL RESULTS IN HYPOPHYSECTOMY EXPERIMENTS

Experiment	% EC-IC differences ^a						% H-C differences ^b					
	AChE/Weight		ChE/Weight		ChE/AChE		AChE/Weight		ChE/Weight		ChE/AChE	
	H	C	H	C	H	C	EC	IC	EC	IC	EC	IC
Experiments H ₁ and H ₂ (Fischer)												
Occipital cortex	-2.6**	-5.2***	.2	-1.7	3.1*	3.8**	-1.9*	-4.5***	3.2*	1.2	5.2***	5.9***
Total cortex	-2.3*	-2.4*	.2	1.0	2.7*	3.5**	-1.1	-1.2	2.6*	3.4**	3.8**	4.6***
Rest of brain	-1.1	.2	-1.4	.8	-.2	.6	1.5	2.8**	.9	3.1**	-.5	.3
Total brain	-1.7*	-1.2	-1.0	.4	.8	1.7*	.2	.8	1.2	2.6**	1.0	1.9*
Cortex/Rest	-1.2	-2.5**	1.6	.3	2.9**	3.0**	-2.6**	-3.8***	1.6	.3	4.3***	4.3***
Experiment H ₃ (Long-Evans)												
Occipital cortex	-1.8	-3.1	1.3	-.2	3.4	3.5	-6.3**	-7.6**	-.8	-2.3	6.3	6.4
Total cortex	-2.1	-2.2	4.3	-.3	7.1*	2.5	-5.6***	-5.7***	1.9	-2.6	8.2*	3.6
Rest of brain	.7	.8	1.4	1.5	.3	.9	.4	.4	-1.7	-1.6	-2.2	-1.7
Total brain	-1.1	-1.0	1.6	.3	2.5	1.5	-2.0	-1.9	-1.2	-2.4	.8	-.2
Cortex/Rest	-2.9*	-3.0*	2.9	-1.7	6.1*	1.3	-6.0***	-6.1***	3.7	-1.0	10.4***	5.4*

Note.—n = 19 per group for Experiments H₁ and H₂; n = 15 per group for Experiment H₃.

^a Environmental effect = 100(EC-IC)/IC.

^b Hypophysectomy effect = 100(H-C)/C.

* p < .05.

** p < .01.

*** p < .001.

EC rats, whether or not they have been hypophysectomized; the rest of the brain shows no significant effect. The ChE/weight tends to be slightly greater in EC than in IC cortex, but, as in other 30-day experiments, there are no significant effects for either H or C rats. The ChE/AChE ratio is greater in the cortex of EC than of IC animals, and this effect is significant and as large for hypophysectomized as for control rats.

Hypophysectomy Effects on Brain Enzyme Activities

Whereas hypophysectomy reduced brain weights and EC raised them, the two treatments yielded brain enzyme effects that were usually in the same direction. This can be seen by comparing values in the right half of Table 5 (H-C effects) with the corresponding values in the left half of the table (EC-IC effects). In AChE/weight, hypophysectomy produced somewhat larger drops than did EC in the cortex and in the cortical/subcortical ratio; this was especially the case for the Long-Evans strain. In ChE/weight, hypophysectomy produced some significant increases in the Fischer rats, whereas there were no significant EC-IC differences on this measure. In ChE/AChE, the pattern of H-C effects was similar to that of EC-IC effects, but the magnitude tended to be greater for H-C, especially for the Fischer rats.

GENERAL DISCUSSION

In advance of the experiments we were concerned that the hypophysectomized rats might behave differently from the controls in the EC situation and that this might complicate or vitiate comparisons of brain effects. Observations indicated, however, that the hypophysectomized rats were just as active as the normals, at least during the daylight hours when we observed them. The gait and appearance of the operates were different from those of normal rats, but the hypophysectomized animals appeared to interact as much with the environment as did the controls.

If the operates had failed to show EC-IC effects, a possible interpretation would have

been that they lacked adequate circulating levels of endocrines necessary for normal cerebral function, rather than that environmental modulation of endocrine output is necessary to the EC-IC effects. To test this, further experiments could have been run with hypophysectomized rats receiving fixed levels of one or more hormones. Since, however, the effects occurred without any replacement therapy, we conclude that, for pituitary hormones and those regulated by the pituitary, neither normal resting levels nor environmental modulation of endocrine activity are necessary to produce cerebral effects of differential experience.

Our results testify to the relative stability of the brain of the weanling rat to hypophysectomy, while at the same time showing that it is not totally immune to effects of the operation. The relative stability is shown in the fact that brain weight continued to increase at about half the normal rate after hypophysectomy. It was within 7% of the brain weight of controls at the end of the experiment. Of course, growth of brain is already well advanced in the rat at weaning, so that even if the operation had halted brain development completely, the brain weights of the operates would have been only 14% below that of the controls. In the case of body weight, on the other hand, the normal animals more than tripled their weight during the experimental period, whereas the operates gained only 15%-20%. The case of the adrenal and thyroid glands is even more striking, since the controls showed a doubling of their weights, whereas in the operates these organs shrunk to about half their weight at operation.

In spite of the relative stability of the brain and its continued growth after hypophysectomy, our results demonstrate that its growth is significantly retarded, that the cortex is less affected in weight than is the rest of the brain; that AChE/weight is reduced by hypophysectomy, especially in the cortex; and that the ChE/AChE ratio is raised, especially in the cortex. In certain other studies, no significant effects of hypophysectomy were found on the brain of the rat, but these studies are not directly comparable with the present one, either because

different measures were employed or because subjects were taken at different ages. Thus Diamond, Johnson, Ingham, and Stone (1969) studied the depth of cortex in Long-Evans rats hypophysectomized at 28 days of age and sacrificed at 77 days. The results showed stability of this brain measure, with no difference in cortical thickness appearing between operates and controls. Under other circumstances we have shown that differences in cortical depth and cortical weight correlate positively (Rosenzweig et al., 1972). In this case, however, it is doubtful that differences between operates and controls in depth would correlate with those in cortical weight, because hypophysectomy results in a shorter but wider skull (Asling & Frank, 1963). The cortex is therefore more compact in the anterior-posterior direction but has additional space laterally. Thus the lack of effect of hypophysectomy on thickness of cortex may be due to redistribution of cortex and does not necessarily contradict the effects reported here on cortical weight and enzymatic activity. Gregory and Diamond (1968) reported on brain measures used in the present study, but with hypophysectomy carried out at 6 days of age and sacrifice at 33-35 days. Weights of cortex and of total brain were less in the operates (by 3% in both cases), but these differences fell short of statistical significance. The AChE/weight was significantly lower in the cortex of the young operates than of their controls, and the ChE/AChE ratio was greater in the operates; both of these effects correspond to ones that we have reported here. Thus, in spite of the differences of ages and sex of the subjects, the results of Gregory and Diamond provide support to the results of the present study.

This research is part of a series of studies in which we have examined possible physiological mechanisms that might produce the cerebral effects of differential experience in EC and IC environments. Evidence has been offered that the cerebral effects are due neither to stress (Riege & Morimoto, 1970; Rosenzweig et al., 1972; Rosenzweig, Krech, Bennett, & Diamond, 1968) nor to altered rates of maturation (Rosenzweig et al.,

1972; Rosenzweig et al., 1968). The present findings support the conclusion that, for hormones of the pituitary gland and for those regulated by the pituitary, neither normal resting levels nor environmental modulation of endocrine activity are necessary to produce cerebral effects of differential experience.

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