EXPERIMENTAL NEUROLOGY 22, 493–503 (1968)

Effect of Differential Environmental Enrichment on Brain Weight and on Acetylcholinesterase and Cholinesterase Activities in Mice

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Received July 16, 1968

Two inbred strains of mice were used in a replication of the Berkeley rat studies on the cerebral effects of differential environmental enrichment. Those raised in a complex environment and given extensive maze training showed significantly heavier brain weights and higher total AChE and ChE activities than mice raised in isolation. The enriched animals also showed a greater ratio of cortical to subcortical weight. These results corroborated the rat experiments. Significant strain differences were also found. A/Crgl mice showed heavier weights and higher total enzyme activities in the subcortex and ventral cortex than did C57BL/Crgl mice, while the opposite was true in some other regions of the cortex. Specific AChE and ChE activities were greater for the A/Crgl mice throughout the brain.

Introduction

One of the most fundamental and controversial issues in the study of brain function is the extent to which morphologic changes can be induced directly in nervous tissue by environmental influences. Despite its long-standing importance, however, this problem has only recently been subjected to the detailed and systematic experimentation that it both deserves and requires. The most extensive investigation of environmentally produced cerebral changes to date has been conducted by Rosenzweig *et al.* (6). Their experiments have consistently demonstrated increases in the depth, weight, glial cell frequency, and total acetylcholinesterase (AChE)

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and cholinesterase (ChE) activities of the rat cerebral cortex as a result of rearing in an enriched environment.

Although these effects have proved repeatable and have been observed in several strains of rats, similar experiments with other species have been lacking. For this reason, the biological generality and reliability of the studies are still in question. The present experiment attempts to answer the need for a broader biological approach by a replication of some of this work using two inbred strains of mice. Brain weights and AChE and ChE activities are reported for environmentally enriched and impoverished mice in brain regions closely comparable to those studied in the rat experiments. Cortical depth and neuroglial cell differences will be the subject of a later report.

Method

Since this experiment was intended as a replication of the rat studies in principle rather than in fact, a few details of the rearing environments and training procedures were changed as convenience and the differing demands of rat and mouse maintenance required. However, all procedures relating to brain dissection and chemical analysis closely duplicated those used in the rat studies (1, 6).

Subjects. The subjects were 24 male mice of the A/Crgl strain and 24 of the C57BL/Crgl strain that were 5–6 weeks old at the start of the experiment. Within each strain the animals were matched according to body weight and assigned randomly to either an enriched or an impoverished environmental condition.

Environmental Conditions. Animals in the impoverished control (IC) condition were individually housed in compartments made of hardware cloth measuring 6.3 × 12.6 × 9.5 cm. Two sheet-metal partitions spaced 2.5 cm apart separated adjacent cages so that social interactions were minimized. Burlap screens prevented visual stimulation from the laboratory room on all sides. Each mouse had constant access to food and water. The IC mice were never removed from these cages or handled until killing, and every attempt was made to protect them from environmental disturbances of all kinds.

Mice in the environmental complexity and training (ECT) condition were housed in two hardware-cloth cages measuring 65 × 65 × 38 cm, in the same laboratory room as the IC cages. Animals of the two strains were separated, with 12 animals of one strain in each cage. The cages were furnished with numerous "toys," some of which were permanent and others that were exchanged between the cages or presented for a limited time only. The toys were designed to provide a maximum variety of visual, tactual, olfactory, auditory, and kinesthetic stimulation. Some examples

were: a ticking clock with a luminous dial, a running wheel, perfumed cotton, an ice cube, and various swings, climbing ramps, and platforms. The arrangement in each cage was changed daily, and novel objects were added frequently. Occasionally the home cages were reversed so that the mice of each strain spent about half of the time in each cage.

For approximately 1 hour a day, all of the ECT animals were removed from their cages and placed alternately on either a 1 × 1-meter clear Plexiglas open field positioned over a differently patterned base each day and with a variety of objects on top, or in a 76 × 91-cm modified Hebb-Williams maze in which the barriers were rearranged daily.

On day 31, formal training was begun in a Lashley III maze. The mice were first pretrained in a straight runway for 5 days with a small amount of peanut butter (about 50 mg) serving as a reward. The animals were not deprived of food. The same amount of peanut butter was given to the IC mice each day as a control for dietary factors. After 10 days of training, one trial per day, the goal and start positions in the maze were reversed for 4 days of reversal training.

Next the animals were given 10 days of training on the Dashiell checkerboard maze. As before, they were given one trial per day for a peanut butter reward. The last 12 days of formal training involved two trials per day on a black-white visual discrimination problem in a Y-maze.

Brain Dissection. The unanesthetized animals were killed by decapitation 75 days after the start of the experiment. In order to control for possible experimenter practice effects, the decapitation and brain dissection were carried out in a scrambled, prearranged order which assured that the mice of neither environmental condition or strain came more frequently earlier in the series than those of the other condition or strain. Each mouse was identified only by a code number so that its environmental background was unknown until the end of the study.

All brains were dissected into five bilaterally equal sections prior to weighing and freezing: (a) visual cortex; (b) somatosensory cortex; (c) remaining dorsal cortex; (d) ventral cortex (including the corpus callosum and the hippocampus); and (e) the subcortex and cerebellum, which constituted the remainder of the brain after the four cortical samples were removed. All of these sections corresponded closely to the samples selected in the rat studies (1, 6). According to Krieg (4) and Rose (5), the mouse and rat brain are quite comparable with respect to these divisions.

The dissection was carried out with a fine scalpel using a clear plastic T-square as a guide. Grooves on the T-square identified the boundaries of the cortical sections as illustrated in Fig. 1. Immediately after removal, each sample was weighted with a Mettler micro projection balance and

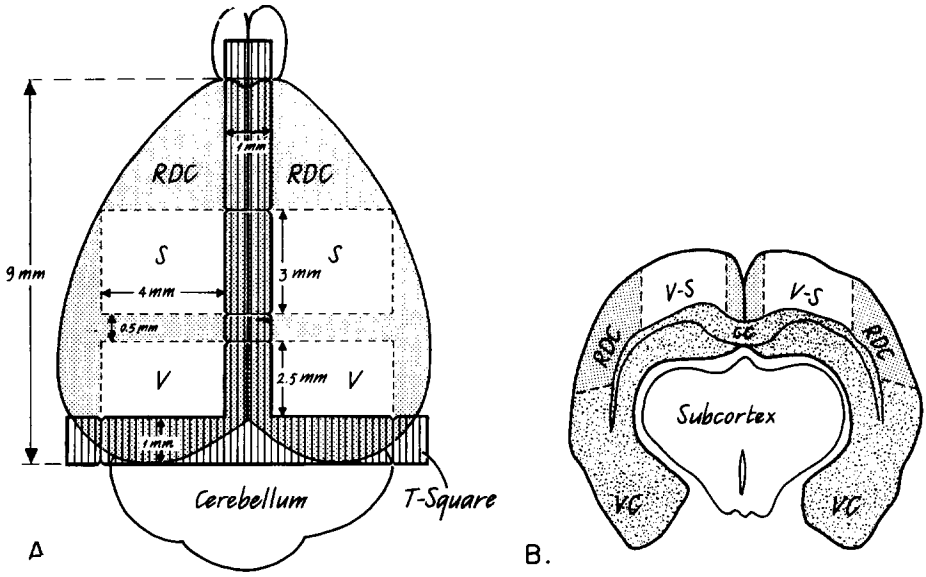

FIG. 1. A. Diagram of the dorsal aspect of a mouse brain showing how the boundaries of the visual (V), somatosensory (S), and remaining dorsal cortical (RDC) regions were delimited for dissection with the aid of a transparent plastic T-square. B. Diagram of a frontal section through a mouse brain illustrating the boundaries of the V, S, RDC, ventral cortical (VC), and subcortical samples and the corpus callosum (CC).

frozen quickly on dry ice. Total time from decapitation until the five brain samples were all weighed and frozen was approximately 15 min for each animal. The tissues were then stored in a freezer at −20 C until the chemical analyses were begun about 1 month later.

Chemical Analyses. The AChE and ChE activities were determined by a colorimetric method adapted from Ellman *et al.* (3) using a Beckman DU spectrophotometer. For the AChE assays, acetylthiocholine (ASCh) was used as the substrate, and promethazine (2.5×10^{-5} M solution) was added as a selective ChE inhibtor. ChE activity was measured against butyrlthiocholine (BSCh) with AChE inactivated by a highly specific inhibitor, BW 284C51J.[2]

In both the AChE and the ChE analyses, each brain sample was assayed separately in two independent runs. The resulting values were then averaged. In some cases, when the two values differed excessively (more than 3%), a third independent run was made.

[2] Supplied by Burroughs Wellcome & Co., Tuckahoe, New York.

Results and Discussion

In general, the results for both strains of mice in this experiment support the findings for the rat that differential environmental enrichment can produce significant changes in both brain weight and chemistry. Significances of differences between ECT and IC mice in tissue weight, total AChE, specific AChE (activity calculated per unit wet weight of tissue), and total and specific ChE activities were determined for each of the five brain regions for each strain by analyses of variance.

Tissue Weight. Table 1 presents the mean wet weight of each of the five brain regions for ECT and IC mice and the percentage differences between the groups for both the A/Crgl and C57BL/Crgl strain. The means calculated for two combined measures, total cortex and total brain, and for terminal body weight (weight of animal at time of killing) are also given.

For the A strain, all of the ECT cortical regions were significantly heavier than the corresponding IC regions at beyond the .01 level of confidence. The C57 strain showed similar results, but the visual and remaining dorsal cortical differences did not reach significance. The total cortex was significantly heavier for the ECT mice at better than the .001 level of confidence for both strains. There was no significant difference between

TABLE 1

WEIGHTS OF BRAIN REGIONS IN MILLIGRAMS FROM C57BL/Crgl AND A/Crgl MICE EXPOSED TO THE ECT OR IC CONDITIONS

	A/Crgl strain				C57BL/Crgl strain			
Brain region	ECT	IC	% Diff.[a]	p	ECT	IC	% Diff.[a]	p
Cortex								
Visual	14.5	12.1	19.6	$< .01$	13.8	13.1	5.8	NS
Somato-sensory	17.4	16.2	7.4	$< .01$	18.8	17.5	7.2	$< .05$
Remaining dorsal	54.5	49.7	9.6	$< .01$	58.8	56.1	4.8	NS
Ventral	73.5	66.4	10.7	$< .01$	72.5	64.8	11.8	$< .01$
Total	160.0	144.5	10.7	$< .001$	164.0	151.6	8.2	$< .001$
Subcortex and cerebellum	243.1	236.8	2.7	NS	227.8	223.3	2.0	NS
Total brain	403.1	381.2	5.7	$< .01$	391.8	374.9	4.5	$< .01$
% Cortex/ subcortex	65.8	61.2	7.6	$< .01$	72.0	68.0	6.0	$< .01$
Terminal body weight	23.7	25.1	−5.5	NS	23.4	23.5	−0.4	NS

[a] $100 \times (\overline{X}\ \text{ECT} - \overline{X}\ \text{IC})/\overline{X}\ \text{IC}$.

ECT and IC animals of either strain in the subcortex and cerebellum or in terminal body weight.

In the rat experiments (1, 6), ECT effects on the brain have frequently been considered in terms of a ratio of total cortex to subcortex and cerebellum (% cortex/subcortex). Differences in this measure were also significant in both strains of mice ($p < .01$) with the ECT animals having the higher values, as in the rat.

AChE Activity. The results for total AChE activity are presented in Table 2. None of the individual cortical differences reached significance for the C57 strain. For the A strain, however, the ECT mean significantly exceeded the IC mean in the visual, somatosensory, and dorsal cortical sections ($p < .05$). The difference between ECT and IC mice in the subcortex and cerebellum was small, and it did not reach significance for the C57 strain.

Looking at the brain as a whole, the ECT animals within each strain clearly showed greater total AChE activity than the IC animals. The ECT-IC difference for total brain was significant at beyond the .001 level of confidence for the A strain and beyond the .01 level for the C57 strain. Thus it may be concluded that in the mouse, as in the rat, a relatively complex rearing environment is associated with greater total activity of AChE in the brain.

TABLE 2

MEAN TOTAL AChE ACTIVITY IN BRAIN REGIONS FROM A/Crgl AND C57BL/Crgl MICE EXPOSED TO THE ECT OR IC CONDITION [a]

	A/Crgl strain				C57BL/Crgl strain			
Brain region	ECT	IC	% Diff.[b]	p	ECT	IC	% Diff.[b]	p
Cortex								
Visual	996	896	11.1	$< .05$	896	894	.2	NS
Somato-sensory	1403	1332	5.3	$< .05$	1466	1421	3.2	NS
Remaining dorsal	5486	5078	8.0	$< .05$	5639	5654	−.3	NS
Ventral	10230	9864	3.7	NS	8839	7832	12.9	NS
Total	18115	17172	5.5	NS	16840	15801	6.6	NS
Subcortex and cerebellum	58790	56763	3.6	$< .05$	52714	51332	2.7	NS
Total brain	76905	73935	4.0	$< .001$	69554	67133	3.6	$< .01$
% Cortex/ subcortex	30.9	30.4	1.8	NS	32.0	30.8	3.8	NS

[a] Expressed in moles $\times 10^8$ of ASCh hydrolyzed per minute.

[b] $100 \times (\bar{X}$ ECT $- \bar{X}$ IC$)/\bar{X}$ IC.

In specific AChE activity (AChE per unit wet weight of tissue), it was the IC mice that had the higher values in every cortical area, again confirming the results for the rat. However, this difference was significant only in the visual region for the A mice and in the dorsal cortex for the C57 mice (Table 3).

ChE Activity. Differences between ECT and IC animals in total ChE activity were insignificant in all but the ventral cortex and total cortex for both strains. In these regions, the ECT mean significantly exceeded the IC mean at beyond the .01 level of confidence (Table 4). There was also a significant difference between ECT and IC mice of both strains in the cortical/subcortical ratio of ChE activity. The ECT cortex showed greater ChE activity relative to the subcortex and cerebellum than did the IC cortex.

The only significant difference found in specific ChE activity (Table 5) was in the visual cortex for the A strain ($p < .01$). Here it was the IC group that had the higher mean value. This finding contrasts with the results for the rat in that specific ChE activity has usually been found to be greater in the environmentally enriched animals. Thus, in the mouse, the changes in ChE activity with environmental enrichment generally appear to parallel the changes in AChE activity, whereas this trend is not apparent in the rat.

TABLE 3

MEAN AChE ACTIVITY PER MILLIGRAM OF TISSUE IN BRAIN REGIONS FROM A/Crgl AND C57BL/Crgl MICE EXPOSED TO THE ECT OR IC CONDITION [a]

	A/Crgl strain				C57BL/Crgl strain			
Brain region	ECT	IC	% Diff.[b]	p	ECT	IC	% Diff.[b]	p
Cortex								
Visual	68.5	74.3	−7.8	< .01	64.8	68.2	−5.0	NS
Somato-sensory	80.4	82.0	−2.0	NS	78.0	81.1	−3.9	NS
Remaining dorsal	100.6	102.1	−1.4	NS	95.6	100.5	−4.8	< .05
Ventral	138.5	148.8	−7.0	NS	121.8	120.9	0.7	NS
Total	113.0	119.0	−5.1	NS	102.6	104.2	−1.5	NS
Subcortex and cerebellum	242.0	239.9	0.9	NS	231.5	230.0	0.6	NS
Total brain	190.9	194.1	−1.6	NS	177.6	179.1	−0.9	NS
% Cortex/ subcortex	48.8	49.6	−5.6	NS	44.4	45.3	−2.1	NS

[a] Expressed in moles × 10^{10} of ASCh hydrolyzed per minute per milligram of brain tissue.

[b] $100 \times (\bar{X}\ \text{ECT} - \bar{X}\ \text{IC})/\bar{X}\ \text{IC}$.

TABLE 4

MEAN TOTAL ChE ACTIVITY IN BRAIN REGIONS FROM A/Crgl AND C57BL/Crgl MICE EXPOSED TO THE ECT OR IC CONDITION [a]

	A/Crgl strain				C57BL/Crgl strain			
Brain region	ECT	IC	% Diff.[b]	p	ECT	IC	% Diff.[b]	p
Cortex								
Visual	44.9	43.5	3.2	NS	41.4	39.1	5.7	NS
Somato-sensory	53.3	48.6	9.6	NS	50.9	48.6	4.6	NS
Remaining dorsal	187	173	7.9	NS	200	192	4.1	NS
Ventral	412	369	11.7	$<.001$	349	297	17.6	$<.01$
Total	698	635	9.9	$<.01$	641	577	11.2	$<.01$
Subcortex and cerebellum	2238	2186	2.4	NS	1580	1598	−1.2	NS
Total brain	2936	2821	4.1	NS	2221	2175	2.1	NS
% Cortex/ subcortex	31.2	29.1	7.2	$<.001$	40.7	36.4	12.0	$<.05$

[a] Expressed in moles $\times 10^8$ of BSCh hydrolyzed per minute.

[b] $100 \times (\overline{X}\ \text{ECT} - \overline{X}\ \text{IC})/\overline{X}\ \text{IC}$.

TABLE 5

MEAN ChE ACTIVITY PER MILLIGRAM OF TISSUE IN BRAIN REGIONS FROM A/Crgl AND C57BL/Crgl MICE EXPOSED TO THE ECT OR IC CONDITION [a]

	A/Crgl strain				C57BL/Crgl strain			
Brain region	ECT	IC	% Diff.[b]	p	ECT	IC	% Diff.[b]	p
Cortex								
Visual	3.095	3.620	−14.5	$<.01$	2.984	3.020	−1.2	NS
Somato-sensory	3.047	2.982	2.2	NS	2.706	2.777	−2.6	NS
Remaining dorsal	3.420	3.478	−1.7	NS	3.397	3.425	−0.8	NS
Ventral	5.618	5.570	0.9	NS	4.819	4.585	5.1	NS
Total	4.361	4.395	−0.8	NS	3.909	3.807	2.7	NS
Subcortex and cerebellum	9.199	9.230	−0.3	NS	6.931	7.160	−3.2	NS
Total brain	7.279	7.395	−1.6	NS	5.669	5.800	−2.3	NS
% Cortex/ subcortex	47.4	47.6	−0.4	NS	56.7	53.7	5.3	NS

[a] Expressed in moles $\times 10^{10}$ of BSCh hydrolyzed per minute per milligram of brain tissue.

[b] $100 \times (\overline{X}\ \text{ECT} - \overline{X}\ \text{IC})/\overline{X}\ \text{IC}$.

Strain Differences. Differences between A/Crgl and C57BL/Crgl mice were pronounced in both brain weight and chemistry. Table 6 shows the mean and significances (based on analyses of variance) for all measures for the two strains with the ECT and IC values combined. The terminal body weights did not differ significantly. The mean for the A mice was 24.4 g, while that for the C57 mice was 23.4 g.

In the cortex, the C57 animals showed heavier weights in all but the ventral region, and these differences were highly significant in the somatosensory and dorsal cortex ($p < .001$). In the subcortex and cerebellum, however, it was the A strain that showed the heavier mean weight ($p < .001$).

In general, strain differences in total enzyme activities tended to parallel the differences in weight. There was a tendency for the somatosensory and remaining dorsal cortical areas to show higher total activities for the C57 mice, while the ventral cortex and subcortex showed higher activities for the A mice.

This pattern did not appear in the results for specific enzyme activities, however. For both AChE and ChE, and A animals showed greater specific activities in every brain region.

Although the statistical analyses did not reveal a significant interaction between environmental treatment and strain for any individual brain region, the pattern of effects across brain regions did appear to differ for the two strains in at least one important respect. For the A strain, the visual cortex showed greater ECT effects in both weight and AChE activity than any other brain region. Changes in the visual cortex for the C57 strain, however, were small (relative to the cortex as a whole) and insignificant. For the C57 mice, the largest changes were found in the ventral cortex. Rats have consistently been found to show a pattern similar to the A mouse strain in this respect, with the largest differences occurring in the visual cortex and relatively smaller differences in the ventral cortex (1).

It is clear from the previous rat experiments and the present mouse study that consistent cerebral differences can be measured between the brains of environmentally enriched and impoverished animals. However, these results are open to a variety of interpretations. Whether the effects are a direct result of environmental stimulation on the brain or a by-product of some hormonal or other mediating influence is a question that can be answered only after much more extensive experimental investigation. Thyroid weight and protein-bound iodine in the blood are being measured in our laboratory in relation to environmental complexity in an attempt to explore one possible hormonal mediating factor. Measurements of adrenal weights in enriched and impoverished rats have already been

TABLE 6

STRAIN DIFFERENCES IN BRAIN ANATOMY AND CHEMISTRY FOR A/Crgl AND C57BL/Crgl MICE (ECT AND IC CONDITIONS COMBINED)

	Mean wt. (mg)	Mean tot. AChE [a]	Mean spec. AChE [b]	Mean tot. ChE [c]	Mean spec. ChE [d]
Visual cortex					
A/Crgl	13.30	946	71.4	44.2	3.357
C57BL/Crgl	13.45	895	66.5	40.2	3.002
p	NS	NS	$< .001$	NS	$< .05$
Somatosensory Cortex					
A/Crgl	16.84	1367	81.20	51.0	3.014
C57BL/Crgl	18.15	1443	79.55	49.8	2.741
p	$< .001$	$< .01$	NS	NS	NS
Remaining dorsal cortex					
A/Crgl	52.10	5282	101.35	180.0	3.449
C57BL/Crgl	57.45	5646	98.05	196.0	3.411
p	$< .001$	NS	$< .05$	$< .01$	NS
Ventral cortex					
A/Crgl	69.95	10047	143.65	390.5	5.594
C57BL/Crgl	68.65	8335	121.35	323.0	4.702
p	NS	NS	$< .001$	$< .001$	$< .001$
Total cortex					
A/Crgl	152.25	17643	116.00	666.5	4.378
C57BL/Crgl	157.80	16320	103.40	609.0	3.858
p	$< .05$	$< .05$	$< .001$	$< .001$	$< .001$
Subcortex and cerebellum					
A/Crgl	239.95	57776	240.95	2212.0	9.214
C57BL/Crgl	225.55	52023	230.75	1589.0	7.045
p	$< .001$	$< .001$	$< .001$	$< .001$	$< .001$
Total brain					
A/Crgl	392.15	75420	192.10	2878.5	7.337
C57BL/Crgl	383.35	68333	178.35	2198.0	5.734
p	NS	$< .001$	$< .001$	$< .001$	$< .001$
% Total cortex/ subcortex					
A/Crgl	63.49	30.64	49.20	30.15	47.53
C57BL/Crgl	70.00	31.44	44.86	38.54	54.92
p	$< .001$	NS	$< .01$	$< .001$	$< .001$

[a] Expressed in moles $\times 10^8$ of ASCh hydrolyzed per minute.

[b] Expressed in moles $\times 10^{10}$ of ASCh hydrolyzed per minute per milligram of tissue.

[c] Expressed in moles $\times 10^8$ of BSCh hydrolyzed per minute.

[d] Expressed in moles $\times 10^{10}$ of BSCh hydrolyzed per minute per milligram of tissue.

completed and have shown no significant effect of environmental complexity when the effect of body weight is controlled (7).

The significance of the particular cerebral changes observed in response to environmental enrichment and the light they shed on mechanisms of learning and memory is another important and as yet unanswered question. Some of the changes probably reflect the over-all adaptation of the brain to the increased sensory input imposed on it by the enriched environment. Other differences, however, may be more directly related to the cerebral learning mechanism and may provide a clue as to how—and where—memories are stored in the brain. These issues can begin to be resolved when the effects of cerebral exercise in general have been distinguished from the effects of particular kinds of learning experiences. At present, the only conclusion warranted is that cerebral tissue does respond measurably to sustained environmental pressure, and that the cholinergic enzyme system is involved in that response.

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