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EFFECTS OF ENVIRONMENTAL COMPLEXITY AND TRAINING ON BRAIN CHEMISTRY AND ANATOMY: A REPLICATION AND EXTENSION[1]

MARK R. ROSENZWEIG, DAVID KRECH, EDWARD L. BENNETT, AND MARIAN C. DIAMOND
University of California, Berkeley

In a recent paper (Krech, Rosenzweig, & Bennett, 1960) we summarized reasons for predicting that increasing an animal's experience should lead to a rise in activity of the enzyme cholinesterase (ChE) in the brain. We reported there that the brains of rats exposed to the condition of environmental complexity and training (ECT) did differ from those of littermates kept as isolated controls (IC), but the differences were not entirely as predicted: in the cerebral cortex, the ECT animals had significantly *less* ChE activity per unit of weight of tissue; in the rest of the brain, the ECT animals had significantly *more* ChE activity per unit of weight. We also indicated that a study was then in progress to attempt to specify more precisely the loci of these enzymic changes—both in the cerebral cortex and in the rest of the brain. In the present paper we report first on a replication of the original experiment; secondly, on new findings that help explain why ChE activity of ECT animals decreased in the cortex while increasing in the rest of the brain, and thirdly, on attempts to specify further the sites of enzymic changes.

METHODS

Subjects

Our previous experiment used 75 littermate pairs, 150 animals from six different strains, one rat of each pair being randomly assigned to the ECT condition and its littermate being assigned to the IC condition. Because the chemical analysis in the present experiment was to be much more extensive than before, the limitations of our facilities made it necessary to use fewer animals. We therefore selected for the replication four of the six strains—S_1, S_3, K, and RCH—by the following considerations: (*a*) the S_1 strain showed the ECT effects strongly and clearly in the original experiment, while the S_3 strain, which had been selectively bred from the same parental stock as had the S_1 strain, showed smaller and less consistent effects. (*b*) The K strain, descendants of a cross between the S_1 and S_3 strains, had also shown small and rather inconsistent ECT effects originally. Since we are interested in determining the generality of the ECT effects, it was felt desirable to replicate work on this strain. (*c*) The RCH strain (originally developed in our laboratories for high cortical ChE by Roderick, 1960) had shown pronounced and consistent ECT effects. However, only seven pairs of animals had been used, and we therefore thought it desirable to replicate the experiment with this strain. (*d*) The two strains omitted from the replication are the RDH and RDL strains—both of which had shown strong ECT effects previously and which had been represented by a total of 19 pairs of animals. All the animals of the original and replication experiments to be compared were males. (In the original experiment one group of S_1 females had also been tested.)

Procedure

Behavioral treatment. The behavioral procedure was an exact duplicate of that described in detail in our previous report (Krech et al., 1960) and is therefore summarized only briefly here: At weaning on approximately Day 25 after birth, one animal of each pair of littermates was assigned at random to the ECT condition and the other to the IC condition. The ECT condition included living in a group of 10 animals in a large cage provided with "toys," daily handling by *E*, daily exploration in the Hebb-Williams maze, and some formal training in the Lashley III maze, the Dashiell maze, and the Krech Hypothesis Apparatus. The IC animals lived in individual cages, under reduced illumination, and without contact or sight of other animals. They had a minimal amount of handling by the *E*s (for weighing) and no opportunity for exploration or formal training. Both the ECT and IC animals had free access to unlimited supplies of food and water. The ECT and IC conditions were maintained until the animals were sacrificed at about 105 days of age.

Chemical analysis. The rats were delivered to our enzyme assay laboratory under code numbers that

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did not reveal their behavioral group. The animals were sacrificed by decapitation following a prearranged order in which littermates were taken consecutively but with the sequence randomized as between the ECT and IC member of each pair. The brain was exposed and dissected into a number of parts for chemical analysis. For strains S_1 and S_3 the brains were dissected into 15 different parts and for strains K and RCH, 5 different parts.

For the S_1 and S_3 animals the cortex was divided into 4 sections and the subcortex into 11 sections. The cortical sections were as follows: A sample of about 25 to 30 mg. of tissue was removed from each of the visual areas (V) of the two hemispheres. A sample of about 20 to 25 mg. was removed from each of the somesthetic areas (S) of both hemispheres. The location of these samples is shown in Figure 1, left. After the removal of the V and S sections, the remaining dorsal cortex was removed, this area extending laterally to beneath the temporal ridge of the skull, medially to the corpus callosum, posteriorly to the cerebellum, and anteriorly to the attachment of the olfactory bulbs. The ventral cortex comprised all the remaining cortical and contiguous tissue, including such areas as cortex piriformis, amygdaloid nuclei, hippocampus, dentate gyrus, cortex entorhinalis, and corpus callosum (see Fig. 1, right).

The subcortical sections for the S_1 and S_3 animals were as follows: The olfactory bulbs were transected at their attachment to the cerebral cortex. The olfactory

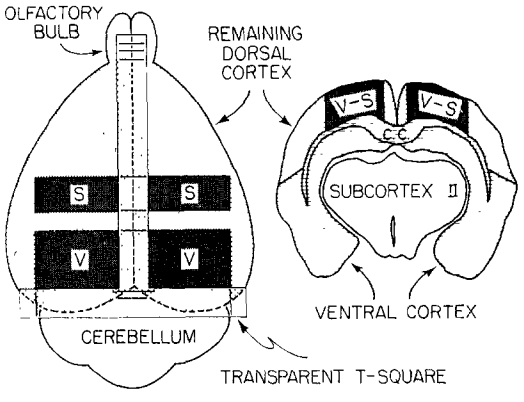

FIG. 1. *Left* a diagram of the dorsal aspect of the rat brain, showing how the samples of the visual area (V) and of the somesthetic area (S) are dissected, guided by a small transparent T square. (The V and S samples together make up what is labeled "sensory cortex" in this paper.) *Right*, a diagramatic representation of a sagittal section of the rat brain. (Total dorsal cortex is made up of the V and S sections—telescoped together in this diagram—plus the remaining dorsal cortex. Total cortex is made up of total dorsal cortex plus ventral cortex. Subcortex II equals the complete brain —including the cerebellum—minus total cortex. Subcortex I equals the complete brain (including the cerebellum) minus total dorsal cortex; otherwise stated, subcortex I includes both subcortex II and ventral cortex.)

tubercles were outlined by the olfactory tracts laterally, the median forebrain bundle dorsally and the midline medially. The hypothalamus was dissected along the optic tract laterally, the anterior commissure decussation anteriorly, the posterior aspect of the mammillary body posteriorly; the dorsal boundary was cut on a level with the anterior commissure decussation anteriorly, and continued posteriorly to intersect the perpendicular cut behind the mammillary body. The superior colliculi and inferior colliculi were represented by tissue removed from their dorsal surfaces to a depth of 1 mm. (about 6 to 7 and 5 to 6 mg., respectively). After the removal of the rest of the superior colliculus, a 2-mm. cylinder was bored beneath its former position in order to obtain a midbrain sample of the reticular formation (about 7 mg.). Following the removal of the cerebral cortex, the paired caudate nuclei were removed, cutting along the medial and ventral surface of the corpus callosum, the dorsal surface of the median forebrain bundle, the internal capsule posteriorly and medially, the septal area marking the medial border. Thalamic tissue was represented by a sample weighing about 7 to 9 mg. taken midway between the anterior and posterior thalamus, ventral to the *habenula*, *n. paraventricularis thalami*, and *n. parataenialis*. The cerebellum was taken dorsal to all three cerebellar brachii. The medulla and pons, taken as a single sample, was dissected at the posterior aspect of the midbrain anteriorly, and the junction of the medulla with the spinal cord posteriorly. The sample referred to as the "remainder of subcortex" consisted of all brain tissue not represented in the samples described above; it amounted to about 13% of the entire brain, by weight. When the brain was divided into 15 sections, there was a loss of weight due to evaporation that amounted to 5 to 8%, depending upon the ambient humidity. Since the brains of littermates were always dissected consecutively and in the same way, no systematic difference between ECT and IC animals could have arisen from this source of variation.

For the K and RCH animals, the four cortical sections were taken as described above. The rest of the brain was analyzed as a single unit which will henceforth be referred to as "subcortex II" (see Fig. 1, right) to distinguish it from the more inclusive subcortical sample of our previous report, which will be referred to as "subcortex I."

The sections for all four strains were chosen so as to enable us to obtain measures comparable to those taken in the previous experiment as well as new measures. Thus, our original cortical samples, the V and S areas of the cerebral cortex, were dissected exactly as before. Our original "subcortex" (subcortex I), the brain minus the dorsal cortex, could be precisely reconstituted from samples described above.

Immediately after dissection the weight of each part was determined, accurate to 0.1 mg., with a "semimicro" direct-reading analytical balance (*Sartorius Selecta*). The tissue was frozen quickly on dry ice and stored at −20° C. For no tissue sample did more than 30 min. elapse between decapitation and freezing of the brain section.

The samples were assayed for ChE activity within 2 mo. after their removal; extended storage at −20° C.

does not seem to affect ChE activity. The analytical procedures, using an automatic titrator, have been reported previously (Rosenzweig, Krech, & Bennett, 1958b). The ChE activity is reported either as *total* activity or as activity per unit of tissue weight (*specific* activity). Total activity is given in terms of moles acetylcholine (ACh) $\times 10^8$ hydrolyzed per minute. Specific activity is total activity divided by the weight of tissue sample; that is, moles ACh $\times 10^{10}$ hydrolyzed per minute per milligram of tissue. Previously we have used only this specific measure.

Results

Replication

ChE Values, ECT versus IC Groups

The mean values of ChE activity per unit of weight for the ECT and IC groups of each strain are presented in Table 1. Values are given for the sensory cortex (average of the V and S areas), subcortex I, and for the ratio of this cortical to this subcortical measure—the CS ratio. Each of these measures was determined as in the previous experiment. (In that report, the sensory cortex was called "dorsal cortex" and subcortex I was called "subcortical brain.") In every case the original findings are replicated: Each ECT group shows *lower* cortical ChE activity than the corresponding IC group; each ECT group shows *higher* subcortical ChE activity than the IC group; and the ECT groups are lower than the IC groups in the cortical/subcortical (CS) ratio. The differences between the ECT and IC groups were found, by analyses of variance on paired littermates, to be significant in the sensory cortex at .05 level, in subcortex I at the .01 level, and in the CS ratio at the .001 level. (The respective F ratios were 6.92, 10.38, and 37.49, $df = 1$ and 38 in all three instances.) Differences among strains were also significant for all three ChE measures at better than the .001 level of confidence. (Strain differences have appeared consistently in our work.) No significant interaction between experimental treatment and strain was found for any of the three measures. Thus, while the strains differ in absolute levels of specific activity of ChE, they all show the ECT effects. Combining the results of the original and the replication experiments, we see that the ECT effects have occurred without exception in each of the six strains and in each of the 11 groups tested.

Figure 2, which presents the combined results for the CS ratio, permits a graphic comparison of the original and replication experiments, strain by strain. Each rectangle in the figure represents the difference in CS value between an ECT rat and its IC littermate. For the original experiment on the S_1, S_3, K, and RCH strains, the IC value was higher than the ECT value in 37 of 47 cases (79%) and in the present replication in 35 of 42 cases (83%). For both experiments combined, including now the strains not replicated, the IC value was higher

TABLE 1

Mean Values of Cholinesterase Activity per Unit of Weight for Experimental and Control Groups

Strain	N (Pairs)[a]	Sensory Cortex[b]		Subcortex I[c]		CS Ratio ($\times 10^3$)	
		ECT	IC	ECT	IC	ECT	IC
S_1	11	62.1	64.1	158	155	394	413
S_3	9	57.5	57.8	158	155	365	372
K	12	57.9	60.2	172	166	337	363
RCH	10	67.9	69.6	173	170	392	410
All	42	61.3	62.9	166	162	371	389

Note.—All ChE values are in moles ACh $\times 10^{10}$ hydrolyzed per minute per milligram of tissue.

[a] For S_1, S_3, and K animals, only one pair of rats was taken from any one litter. For RCH animals, two litters contributed two pairs each, while the other pairs were drawn from separate litters. Thus the 42 pairs represent 40 different litters.

[b] ChE activity of the sensory cortex was obtained by averaging the values obtained for the samples of the visual and somesthetic regions of the cortex. This sensory cortex was referred to as "dorsal cortex" in our original article.

[c] Subcortex I is the brain minus the dorsal cortex. It was referred to as "subcortical brain" in our original article.

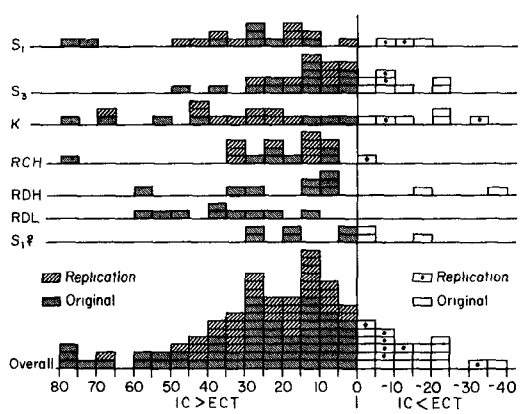

Fig. 2. Differences in cortical/subcortical ratio ($\times 10^3$) of specific cholinesterase activity between IC and ECT littermates, for the original and replication experiments.

than the ECT value in 94 of 117 pairs of animals (80%).

Our more extensive chemical analysis with the present replication permitted us to measure specific ChE activity in more inclusive cortical regions than the sensory cortex. Two additional cortical measures were made: (*a*) *Total dorsal cortex*, this sample comprising the samples from the visual and somesthetic areas plus remaining dorsal cortex, as described above. (*b*) *Total cortex*, including both total dorsal cortex and ventral cortex. For these new measures, the cortex was not "sampled," but *all* the tissue falling under the definition was assayed. In total dorsal cortex the ECT animals had a mean value of ChE per unit of weight of 68 and the IC animals, of 69; in total cortex, the respective means were 85 and 87. Both differences between groups amounted to approximately 2% and neither was significant.

When total dorsal cortex is removed, the rest of the brain is what we have called subcortex I. When total cortex is removed, the remainder of the brain is subcortex II. We have already seen that in subcortex I the ECT animals have significantly greater ChE activity than the IC animals ($p < .01$); the difference is about 2%. In subcortex II, the ECT animals average 184 and the IC, 178. The difference, about 3%, is highly significant ($F = 21.86$; $df = 1, 38$; $p < .001$). Thus, subcortex II, which excludes all cortical tissue, provided the more sensitive subcortical index of the ECT effect on specific ChE activity.

While the only cortical measure to give a change that is in itself significant is the sensory cortex, cortical/subcortical ratios involving *any* of the cortical or subcortical measures differentiate significantly (at the .001 level) between the ECT and IC groups. Thus, again we see the power of cortical/subcortical ratios in discriminating between ECT and IC animals.

Changes in Cortical Weight and Total ChE Activity

Up to now we have considered measures of specific activity of ChE (determined for any sample by dividing its *total* ChE activity by its weight). In this section we will be concerned with total ChE activity for any given sample. Two unexpected results emerged from this new analysis: (*a*) The cerebral cortex is significantly heavier in the ECT animals than in their IC littermates. (*b*) While as noted above, the ECT animals have lower specific ChE activity in the cortex than do the IC, the ECT generally have greater total ChE activity in the cortex than do the IC.

Weight changes. Total brain weight reveals practically no differences (less than 1%) between ECT and IC groups. This result replicates the finding of our original study. However, comparisons of weights of subsections of the brain tell a different story (Table 2). For each of the three cortical measures—sensory cortex (the sum of samples from the visual and somesthetic regions), total dorsal cortex, and total cortex—the ECT animals are about 4% heavier than the IC animals. Analyses of variance for littermate pairs show that each of these differences is significant at better than the .01 level (sensory cortex, $F = 8.33$; total dorsal cortex, $F = 10.80$; total cortex, $F = 14.51$; throughout, $df = 1, 38$). For the subcortical measures the ECT show a slight and statistically insignificant drop in weight in comparison with the IC.

Each analysis of variance for cortical and

TABLE 2

MEAN BRAIN WEIGHTS OF EXPERIMENTAL AND CONTROL GROUPS (IN MILLIGRAMS)

Strain	*N* (Pair)	Sensory Cortex		Total Dorsal Cortex		Total Cortex		Subcortex I		Subcortex II		Total Brain	
		ECT	IC	ECT	IC	ECT	IC	ECT	IC	ECT	IC	ECT	IC
S_1	11	90	82	335	320	663	626	1222	1202	894	896	1557	1522
S_3	9	93	93	399	395	738	731	1374	1385	1035	1048	1773	1779
K	12	104	100	431	405	707	688	1310	1338	1035	1055	1741	1743
RCH	10	106	103	457	443	809	774	1390	1385	1037	1053	1847	1828
All	42	98	94	405	390	726	701	1320	1324	999	1011	1725	1713

Note.—All brain samples are defined in Fig. 1.

subcortical weight measures shows significant differences among strains. The comparison of ECT and IC groups must therefore be made strain by strain since the *absolute* weight of the cortex was determined to a greater degree by the strain of the animal than by the condition under which it was raised. Thus, for example, for any cortical measure, the S_1 ECT group has a *lower* weight than the IC groups of the other three strains. Nevertheless, as we have seen, the ECT rats have significantly *higher* cortical weights than their IC littermates. Furthermore, since no significant interaction effects were found between experimental treatment and strain, the increase of cortical weight as a consequence of environmental complexity and training is general.

The data of the previous experiment were then reanalyzed to determine whether similar changes in cortical weight had occurred in the original S_1, S_3, K, and RCH groups. Since ventral cortex had not been taken separately at that time, only two measures could be considered—sensory cortex, and total dorsal cortex. Sensory cortex was heavier by 5% in the ECT rats ($F = 15.58$; $df = 1, 43$; $p < .001$). Total dorsal cortex was heavier in the ECT group by 2% ($F = 2.70$; $df = 1, 43$; not significant). Thus, in both the original and replication groups the ECT animals had heavier cortices than the IC animals.

In both experiments the subcortex remained unaffected by the environmental conditions, the ECT groups having slightly but nonsignificantly lower weights in both cases.

Changes in total cholinesterase activity. Table 3 presents the data on total ChE activity for six brain regions. These data indicate that total ChE activity is greater for the ECT than for the IC groups in almost every comparison. The only reversals in the table are seen in the sensory cortex for the S_3 and K strains. Based on all four strains, total ChE of the ECT group exceeds that of the IC group by about 1% in the sensory cortex and by about 2% in total dorsal cortex and in total cortex. None of these cortical differences is statistically significant as determined by analyses of variance. The values for either subcortex I or II are about 2% greater in the ECT than in the IC group, and these differences are at the .01 and .05 levels, respectively. (The F ratio for subcortex I was 7.71, and for subcortex II, 5.38; $df = 1, 38$.) The values for total brain are greater for ECT than for IC in the case of every strain, and the over-all difference of 2% is significant at the .01 level ($F = 8.06$; $df = 1, 38$). Significant differences among strains are found for every measure of total ChE activity. Despite these strain differences, the total enzyme activity is seen to be greater for the ECT than for the IC animals, throughout the brain.

The data of the previous experiment were then scrutinized in this regard. Only two comparisons could be made, since only sensory cortex and subcortex I had been analyzed chemically in that experiment. The ECT group was found to have somewhat higher values than the IC group in both measures, but the differences were only 1.5% for the sensory cortex and 0.3% for subcortex I, and neither was significant.

Explorations at Additional Brain Loci

The present experiment, in addition to replication of the original, had as a further objective the more precise specification of the sites of enzymic changes in the brain related to

TABLE 3

MEAN VALUES OF TOTAL CHOLINESTERASE ACTIVITY OF EXPERIMENTAL AND CONTROL GROUPS

Strain	*N* (Pairs)	Sensory Cortex		Total Dorsal Cortex		Total Cortex		Subcortex I		Subcortex II		Total Brain	
		ECT	IC	ECT	IC	ECT	IC	ECT	IC	ETC	IC	ECT	IC
S_1	11	55	52	227	222	568	554	1926	1866	1585	1533	2153	2088
S_3	9	53	54	257	251	619	609	2165	2151	1803	1794	2422	2402
K	12	59	60	281	272	594	585	2260	2222	1946	1909	2541	2494
RCH	10	71	71	337	332	713	696	2405	2351	2028	1986	2741	2683
All	42	60	59	275	269	621	608	2187	2144	1840	1804	2462	2413

Note.—Cholinesterase activity is given in terms of moles acetylcholine $\times 10^8$ hydrolyzed per minute.

differences in environmental complexity and training among animals.

The specification can be done at increasing degrees of anatomical precision: (*a*) Separate analyses of organs of the brain, e.g., caudate nucleus, cerebellum; (*b*) analyses of distinct subdivisions of organs of the brain that can be dissected separately, e.g., regions of the cerebral cortex, different thalamic nuclei; (*c*) quantitative histochemical investigations of different brain section, e.g., different layers of the cerebral cortex. Techniques for histochemical analyses (*c* above) are now being elaborated in our laboratories.[2] Results on certain subdivisions of the cerebral cortex (*b* above) have already been presented in the preceding pages. The results to be presented in the following section are mostly of analyses of separate organs (*a* above).

Changes in Subdivisions of Subcortex

In the attempt to localize the subcortical ECT effects, the ECT and IC animals were compared at 11 different parts of the subcortex. These parts, it will be remembered, were the olfactory bulbs, olfactory tubercles, caudate nuclei, thalamus, hypothalamus, superior colliculi, inferior colliculi, reticular formation, medulla, cerebellum, and a section which consisted of the remaining subcortical brain tissue. This extensive chemical analysis involving the ChE analysis of a total of 440 samples of brain tissue was made only for the S_1 and S_3 strains. For reasons which will soon become apparent, we decided against continuing such a detailed analysis for the K and RCH strains.

Two general conclusions can be drawn from the data on the subdivision of the subcortex:

1. The patterns of specific ChE activity levels for the 11 subcortical regions were almost identical for the S_1 and S_3 strains. The order of ChE activity, from highest to lowest, was the following: olfactory tubercles, caudate nuclei, superior colliculi, remainder of subcortex, reticular formation, thalamus, medulla, hypothalamus, inferior colliculi, olfactory bulbs and cerebellum. The consistency in pattern despite the difference between the two strains in absolute ChE activity levels suggests the possibility that a ChE "mapping" of the subcortex will give us as highly generalizable results as the ChE mapping of the cortex. For the cortex we have found (Rosenzweig, Krech, & Bennett, 1958a, pp. 380–382) a caudal-rostral gradient of ChE activity for every strain of rat studied. We are now completing in our laboratory an extensive ChE mapping of the subcortex for six strains. These data will be reported separately.

2. The second finding was essentially a negative one. The ECT animals of both strains showed slightly higher specific ChE activity than the IC animals for almost all 11 subdivisions of the subcortex, and only in the case of the cerebellum did any one subdivision, *taken by itself*, show a significant ECT-IC difference for both strains. For the S_1 strain 9 of the 11 ECT rats showed a higher cerebellar ChE activity level than their IC littermates. For the S_3 strain, the corresponding numbers were 8 of 9. The mean ChE values for the ECT and IC animals were 47.2 vs. 46.0 for the S_1 and 47.3 vs. 44.6 for the S_3. An analysis of variance demonstrated the difference to be significant ($F = 17.55$; $df = 1, 18$; $p < .01$). Inspection of weight and total ChE activity of the cerebellum showed that the ECT animals were about 2% lower in weight and about 2% higher in total enzyme activity than the IC groups. Neither difference was significant. For the other 10 subdivisions as well, differences between ECT and IC in weight and total ChE were not significant.

Since the extensive analysis of the subcortex of the S_1 and S_3 animals gave little indication of specific localization of the ECT effect in the parts studied, it was decided not to proceed with this time-consuming analysis for the K and RCH animals.

Discussion

We stated in the Results that the ECT effects on specific activity of ChE had been found without exception in each of the 11 groups tested so far. Account should be taken here of one additional group—a preliminary group reported in the 1958 Pittsburgh Symposium (Rosenzweig, Krech, & Bennett, 1961)—which appears to be a partial exception. For

[2] A first report on ChE activity at the different layers of the cerebral cortex for the S_1, S_3, RDH, and RDL strains has recently been presented (Diamond, Diamond, Bennett, Krech, & Rosenzweig, 1961).

this group of eight pairs of S_1 males, the ECT animals showed *higher cortical* as well as higher subcortical ChE values than their controls. These ECT animals had been compared, however, with control littermates which had not been isolated but had lived three to a cage and had been exposed to the normal stimulation of the animal colony. This social control (SC) condition we have since found to produce values of specific ChE generally intermediate between the ECT and IC conditions (see Table 3, Krech et al., 1960). While in the preliminary group of eight pairs the cortical values were higher for the ECT than for the SC rats, the relative difference was not as great as for the subcortical values, and consequently the CS ratio was again lower for the ECT than for the SC animals. A further analysis of the data of this preliminary group shows that the ECT animals had higher values than the social controls in both weight and total ChE activity of sensory cortex. Thus, in spite of the fact that differences in environmental complexity were not great between ECT and SC groups, all the usual ECT effects were found with the exception of a decline in specific ChE at the cortex.

We had pointed out in our original report (Krech et al., 1960) that we did not then have any reasonable hypothesis to account for the fall in ChE activity in the sensory cortex as a consequence of environmental complexity and training. Indeed, for the reasons enumerated (Krech et al., 1960, pp. 509–510) we had entered into the experiment with the expectation that ChE activity would show a rise both in the subcortical area (which it did) and in the cortical area (which it did not). That puzzling set of results has now been replicated in every detail in the present experiment. However, with the new data on changes in cortical weight and total ChE activity, a solution to the puzzle is suggested.

It will be recalled that the cortical weight of the ECT animals showed a significant increase (about 4%) over that of the IC rats. The total ChE activity of the ECT rats also showed a gain over their IC littermates, but the gain in total ChE activity was slight—about 1% or 2% depending upon the cortical area analyzed. This means that the observed fall in cortical specific activity of ChE (ChE activity per unit weight) in the ECT rats was due—at least in part—to the fact that their cortical weights were increasing at a faster rate than their total ChE activity.

Thus, the prediction made in our original paper that environmental stimulation will *increase* ChE activity in the brain (cortex as well as subcortex) is now confirmed if we use total ChE as our measure. The finding that cortical weight increases as a consequence of environmental complexity was not anticipated. These two findings, taken together, suggest that environmental complexity and training results in: (*a*) *A differential morphological* change in the animal's brain—increase in cortical weight and relative decrease, although slight, in subcortical weight. The fact that this effect is differential as between cortex and subcortex makes it clear that the ECT effect cannot be ascribed to over-all acceleration of brain growth. (*b*) *A differential biochemical* change in the animal's brain—increase in total ChE activity, but with the ChE activity in the subcortex increased whether expressed in terms of activity per unit weight or total activity, whereas in the cortex only the latter measure showed an increase. More recent experiments from our laboratory confirm these findings (Rosenzweig, Krech, Bennett, & Zolman, in press; Zolman & Morimoto, 1962).

Our original measure, the CS ratio (cortical/subcortical ratio of specific activity of ChE) can thus be seen as a reflection of both these effects. It should be pointed out that neither primary effect (change in cortical weight or change in total ChE activity) correlates very highly with the CS ratio, nor does either differentiate as well between ECT and IC groups as does the CS ratio. For another thing, the CS ratio shows a higher correlation with error scores in problem-solving than do the two primary effects (Krech, Rosenzweig, and Bennett, 1962). We therefore intend to use all three measures until a more parsimonious resolution is possible.

The unexpected finding of a morphological change as consequence of environmental complexity and training is as intriguing as our predicted finding of a biochemical change. It is probable that the observed difference in wet weight of cortical tissue reflects a change in the volume rather than in the density of the

tissue since we had found previously that protein, expressed as a percentage of wet weight, does not differ between ECT and IC animals either in cortex or in subcortex (Krech, Rosenzweig, Bennett & Longueil, 1959). Consequently, ChE activities of the ECT and IC groups bore the same relation whether ChE was expressed per unit of wet weight or per milligram of protein. It will now be necessary to determine what more intimate changes are involved in the change of cortical weight. Possibilities include increases in the volume of neural cell bodies, of neural cell processes, or of glial cells, or increases in myelin or in the vascularization of the tissue. We are now subjecting ECT and IC groups to various morphological and histological analyses in an attempt to find answers to these new problems.

In showing the significant increase in cortical weight of the ECT animals over their IC littermates, we are not implying that *absolute* cortical weight can be taken as a correlate of experience or of ability to learn. In this regard it is worth pointing out that the S_1 animals, which are superior to the S_3 animals in several tests of learning ability (Rosenzweig, Krech, & Bennett, 1960), are significantly lighter in all brain-weight measures than the S_3 animals. Our analyses are of *changes* in cortical weight, here estimated as against littermate controls. While the S_1 animals are inferior to the S_3 animals in absolute cortical weight, it should be noted that in the ratio of weight of total cortex to subcortex II, the S_1 animals are superior to the S_3 by 2%. This cortical/subcortical ratio also differentiates significantly between the ECT and IC groups of all four strains, as does the CS ratio of specific activity of ChE—for weight of total cortex divided by weight of subcortex II, $F = 13.37$; $df = 1, 38$; $p < .001$. Thus, it may be that a correlate of learning ability or experience will be found to be an index of "corticalization"—the relative distribution of brain tissue and enzymic activity as between cortex and subcortex.

In our previous study, we found that the differences in specific ChE attributable to experimental treatment were several times smaller than the differences due to genetic factors (strain differences). This finding is replicated here (see Table 1). At the sensory cortex the greatest difference, 4%, between paired ECT and IC groups occurred in the K strains. The greatest difference between strains, 18%, occurred between the S_3 and RCH strains. Similarly, at subcortex I, the greatest difference due to treatment amounted to 2%, while the greatest difference due to genetic factors amounted to 9%. But again, as in our original study, the preponderance of the genetic over the environmental factors is considerably reduced when we use the CS ratio as the index. Here the greatest difference between strains was 15% (S_1 vs. K), while the greatest difference due to treatment amounted to 8% (ECT and IC groups of the K strain).

Our generalization can now be extended to brain weight (Table 2) and to total ChE (Table 3). For each weight measure in Table 2, the maximum difference due to treatment is less than the maximum difference between strains. The predominance of the genetic factor is least apparent in the case of the sensory cortex, where the maximum difference between strains is only about twice as great as the maximum difference due to treatment. With the more inclusive and less arbitrarily defined cortical areas, the predominance of the genetic factor is more marked. For total dorsal cortex and total cortex, the genetic factor is responsible for weight differences seven and five times as great, respectively, as those related to experimental treatment. In the case of total ChE (Table 3) as well, the genetic factor is considerably stronger than the experimental treatment in producing differences. For each of the brain regions measured, the maximum difference between strains is at least six times as great as the maximum difference between ECT and IC groups of a single strain.

Thus, the results of our original experiment and the replication strongly support these two general conclusions: (*a*) Manipulating the environment of animals during the 80 days after weaning can alter significantly the weight of the cerebral cortex, the total ChE activity of the brain, and the cortical/subcortical distributions of specific activity of ChE and of tissue weight. (*b*) Similar but much greater alterations in the brains of the animals can be accomplished by a program of genetic selection carried out over a few generations.

Summary

In 42 littermate pairs of male rats of four strains, one member of each pair was kept in

isolation while the other was raised under conditions of environmental complexity and training. These conditions were maintained from weaning until the animals were sacrificed at about 105 days of age. The experimental animals, in comparison with their littermate isolated controls, showed several significant changes in cholinesterase (ChE) activity per unit of tissue weight (replicating in detail the results of the original experiment). The experimental animals had lower specific activity of ChE in the cerebral cortex, higher ChE activity in the subcortex, and a lowered cortical/subcortical ratio of specific ChE activity. For two strains, the subcortex was divided into 11 functional regions in an attempt to localize further the sites of change, but only 1 subdivision taken by itself, the cerebellum, showed a significant experimental effect. In the present experiment we also obtained two additional measures for all brain tissue, weight and total activity of ChE. The experimental animals, compared with their littermate controls, showed significantly greater weight of cerebral cortex and significantly greater total ChE activity in the subcortex and in the whole brain. The increase in cortical weight was greater than the increase in cortical ChE activity.

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