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and Marian C. Diamond

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Effects of Environmental Complexity and Training on Brain Chemistry and Anatomy: A Replication and Extension[1]

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We reported recently (Krech, Rosenzweig, Bennett, 1960) that varying both the complexity of the rat's environment and its training leads to changes in the cortical-subcortical distribution of the enzyme cholinesterase (ChE) in the brain. These ChE effects of Environmental Complexity and Training we labelled the "ECT effects." We further reported at that time, that a study was in progress 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 of changes in the anatomy as well as in the chemistry of the brain, and thirdly, on attempts to specify further the sites of enzymic changes.

METHODS

Subjects

In our previous experiment 150 animals from six different strains were used. The animals were 75 littermate pairs, one rat of each pair being randomly assigned to the ECT condition and its littermate being assigned to the Isolated Control 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. This selection was determined 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.)

Behavioral Treatment

The behavioral procedure was an exact duplicate of that described in detail in our previous report (Krech et al., 1960). It will therefore be summarized only briefly here: At weaning (approximately the 25th day after birth) one animal of each pair of littermates was assigned at random to the ECT condition and the other to the Isolated Control (IC) condition. The ECT condition included living in a group of ten animals in a large cage provided with "toys", daily handling by the experimenter, 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 experimenters (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 did not reveal their behavioral group. The animals were sacrificed by decapitation following a pre-arranged 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 two of the strains (S_1 and S_3) the brains were dissected into 15 different parts. For the other two strains (K and RCH) the brains were dissected into only five different parts.

For the S_1 and S_3 animals the cortex was divided into four sections and the subcortex into 11 sections. The cortical sections were as follows: A sample of about 25-30 milligrams of tissue was removed from each of the Visual areas (V) of the two hemispheres. A sample of about 20-25 milligrams was removed from each of the Somesthetic areas (S) of both hemispheres. The location of these samples is shown in Fig. 1A. After the removal of the V and S sections, the Remaining Dorsal Cortex was removed. This area of the cortex extends 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. 1B).

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 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 mamillary 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 mamillary body. The Superior Colliculi and Inferior Colliculi were represented by tissue removed from their dorsal surfaces to a depth of one millimeter (about six to seven and five to six milligrams, respectively). After the removal of the rest of the superior colliculus, a two millimeter cylinder was bored beneath its former position in order to obtain a midbrain sample of the Reticular Formation (about seven milligrams). 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 seven to nine milligrams 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 per cent 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 five to eight per cent, depending upon the ambient humidity. Since the brains of littermates were always dissected consecutively and in the same way, no systematic difference between EC 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. 1B) 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 milligram with a "semi-micro" 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 minutes elapse between decapitation and freezing of the brain section.

The samples were assayed for ChE activity within two months 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 and Bennett, 1958b).

The ChE activity is reported either as total activity or as specific activity. Total activity is given in terms of moles acetylcholine (ACh) x 10^8 hydrolyzed per minute. Specific activity is total activity divided by the weight of tissue sample; that is, moles ACh x 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 specific ChE activity 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 ($F = 6.92$; $df = 1, 38$; $p < .05$), in the Subcortex I ($F = 10.38$; $df = 1, 38$; $p < .01$), and especially in the CS ratio ($F = 37.49$; $df = 1, 38$; $p < .001$). 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 ChE activity, 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 the IC value was higher than the ECT value in 37 out of 47 cases, or in about 76 per cent, and in the present replication in 35 out of 42 cases, or in about 83 per cent. For both experiments combined, including now the groups not replicated, the IC value was higher than the ECT value in 94 out of 117 pairs of animals, or about 80 per cent.

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: (1) Total Dorsal Cortex. This sample comprises the samples from the visual and somesthetic areas, plus Remaining Dorsal Cortex, as described above. (2) Total Cortex. This area includes 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 specific ChE value of about 68 and the IC, of about 69; in Total Cortex, the respective means were about 85 and 87. Both differences between groups amounted to approximately two per cent and neither was significant. Thus the Sensory Cortex, which showed a three per cent change (significant at the .05 level), provides the most sensitive cortical index of the ECT effect on

specific ChE activity.

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 two per cent. In Subcortex II, the ECT animals average 184 and the IC, 178. The difference is about three per cent, and it is highly significant ($F = 21.86$; $d\,f = 1, 38$; $p < .001$). Thus, Subcortex II, which excludes all cortical tissue, provides 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 <u>any</u> 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 <u>specific</u> ChE activity (determined for any sample by dividing its <u>total</u> ChE activity by its weight). In this section we will be concerned with <u>total</u> 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 <u>specific</u> ChE activity in the cortex than do the IC, the ECT generally have greater <u>total</u> ChE activity in the cortex than do the IC.

<u>Weight Changes</u>

Total brain weight reveals practically no differences (less than

one per cent) between EC 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. The brain weight data are shown in 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 EC animals are about four per cent 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, $F = 8.33$; $df = 1, 38$; Total Dorsal Cortex, $F = 10.80$; $df = 1, 38$; Total Cortex, $F = 14.51$; $df = 1, 38$). For the subcortical measures the EC show a slight and statistically insignificant drop in weight in comparison with the IC.

Each of the analyses of variance for cortical and subcortical weight measures shows significant differences among strains. The comparison of EC 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 of the cortical measures, the S_1 EC group has a lower weight than the IC groups of the other three strains. Nevertheless, as we have seen, the EC 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 re-analyzed to determine whether similar changes in 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 three weight measures could be considered--

Sensory Cortex, Total Dorsal Cortex, and Subcortex I. Sensory Cortex was heavier by five per cent in the ECT rats ($F = 15.58$; $d\,f = 1,43$; $p < .001$). Total Dorsal Cortex was heavier in the ECT group by two per cent ($F=2.70$; $d\,f = 1, 43$; not significant). Subcortex I was lighter in the ECT by two per cent ($F = 1.89$; $d\,f = 1, 43$; not significant). Thus the original and replication groups, where comparisons are possible, showed generally similar weight changes, the ECT animals in both studies having heavier cortices than the IC group (although the Dorsal Cortex failed to reach statistical significance in the original experiment) and somewhat lighter subcortical regions.

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 one per cent in the Sensory Cortex and by about two per cent in Total Dorsal Cortex and in Total Cortex. None of these differences is statistically significant, as determined by analyses of variance. The values for either Subcortex I or II are about two per cent greater in the ECT than in the IC group, and these differences are statistically significant (Subcortex I, $F = 7.71$, $d\,f = 1,38$, $p < .01$; Subcortex II, $F = 5.38$, $d\,f = 1, 38$, $p < .05$). The values for Total Brain are greater for ECT than for IC in the case of every strain, and the overall difference of two per cent is significant at the .01 level ($F = 8.06$; $d\,f = 1,38$). 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 per cent for the Sensory Cortex and 0.3 per cent for Subcortex I. While these differences were in the same direction as in the present experiment, 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 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 sections, 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 this extensive 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 every one of the 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 nine 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 eight out of nine. 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 ($F = 17.55$; $d\,f = 1, 18$; and $p < .01$). Inspection of weight and total ChE activity of the cerebellum showed that the ECT animals were about two per cent lower in weight and about two per cent higher in total enzyme activity than the IC groups. Neither of these differences was significant. For the other ten 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 ChE activity 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 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 however, had been compared with control littermates which had not been isolated, but which 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 EC'T 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 EC'T than for the SC animals. A reanalysis of the data of this preliminary group shows that the EC'T 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 environment complexity were not great between EC'T and SC groups, all the usual EC'T 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 specific ChE activity in the Sensory Cortex as a consequence of environmental complexity and training. Indeed, we had entered into the experiment with the expectation that specific 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 EC'T animals showed a significant increase (about four per cent) over that of the IC rats The total ChE activity of the EC'T rats also showed a gain over their IC littermates, but the gain in total ChE activity was slight--about one or two per cent depending upon the cortical area analyzed. This means that the observed fall in cortical specific ChE activity (ChE activity per unit weight) in the EC'T 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) seems now to be 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 EGT effect cannot be ascribed to overall 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.) Two additional experiments recently completed in our laboratory offer strong confirmation of these findings. These experiments, involving differing EGT conditions, will be reported later.

Our original measure, the CS ratio (cortical-subcortical ratio of specific ChE activity), can thus be seen as a reflection of both these effects. It should be pointed out that neither of the two primary effects (change in cortical weight or change in total ChE activity) correlates very highly with the CS ratio, nor does either differentiate as well between EGT and IC groups as does the CS ratio. For another thing, the CS ratio (as will be reported in a later study) shows a different pattern of correlation with error scores in problem-solving than do the two primary effects. 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. We are now faced with many new questions. The measured difference is in the wet weight of tissue. We cannot tell from these data whether this means a change in volume or in density of the tissue (or in both). It will also be necessary to determine what more intimate changes are involved in the change of 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 at present preparing EC and IC groups whose brains will be subjected to various morphological and histological analyses in an initial attempt to find answers in this new direction of our research.

In showing the significant increase in cortical weight of the EC 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.

In our previous study, we found that the differences in specific ChE attributable to experimental treatment were smaller, by several times, than the differences due to genetic factors (strain differences). This finding is replicated here (see Table 1): At the Sensory Cortex the greatest difference between paired EC and IC groups occurred in the K strain; it amounted to about four per cent. The greatest difference between strains occurred between the S_3 and RCH strains; it amounted to about 18 per cent. Similarly, at Subcortex I, the greatest difference due

to treatment amounted to about two per cent, while the greatest difference due to genetic factors amounted to about nine per cent. 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 about 15 per cent (S_1 *vs.* K), while the greatest difference due to treatment amounts to about eight per cent (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 of the weight measures 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 pattern of specific ChE activity (CS ratio). (b) Similar

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Footnotes

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

Table 1

Mean Specific Cholinesterase Values of

Experimental and Control Groups[a]

Strain	N (Pairs)[b]	Sensory Cortex[c] ECT	Sensory Cortex[c] IC	Subcortex I[d] ECT	Subcortex I[d] IC	CS Ratio (x 10^3) ECT	CS Ratio (x 10^3) 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

[a]All ChE values are in moles ACh x 10^{10} hydrolyzed per minute per milligram of tissue.

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

[c]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.

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

Table 2

Mean Brain Weights of Experimental and Control Groups (in Milligrams)[a]

Strain	N (Pair)	Sensory Cortex ECT	Sensory Cortex IC	Total Dorsal Cortex ECT	Total Dorsal Cortex IC	Total Cortex ECT	Total Cortex IC	Subcortex I ECT	Subcortex I IC	Subcortex II ECT	Subcortex II IC	Total Brain ECT	Total Brain 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

[a]All brain samples are defined in Fig. 1.

Table 3

Mean Values of Total Cholinesterase Activity of Experimental and Control Groups[a]

Strain	N (Pairs)	Sensory Cortex ECT	Sensory Cortex IC	Total Dorsal Cortex ECT	Total Dorsal Cortex IC	Total Cortex ECT	Total Cortex IC	Subcortex I ECT	Subcortex I IC	Subcortex II ECT	Subcortex II IC	Total Brain ECT	Total Brain 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

[a]Cholinesterase activity is given in terms of moles acetylcholine x 10^8 hydrolyzed per minute.

Legend for Figure 1A

Fig. 1A. 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 labelled "Sensory Cortex" in this paper.

Legend for Figure 1B

Fig. 1B. A diagrammatic representation of a saggital 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.

Legend for Figure 2

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