relative mobilities and the numbers of the different carriers.

7. The usual test of the quality of a crystal is its residual resistance ratio, that is, the ratio of the resistivity at room temperature to the resistivity at 4.2°K. Although at present there is no way to relate the resistance ratio and purity of one metal to the resistance ratio and purity of another, the ratio is a reliable test between different samples of the same metal. The resistance ratios of the best samples currently available of some of the metals discussed here are, Cu, 8000; Zn, 60,000; Sn, 100,000; Fe, 300; Ni, 2700; Mo, 4000; W, 80,000.
8. Yu. Gaidukov, Sov. Phys. JETP (English Transl.) 10, 913 (1960); J. R. Klauder and
J. E. Kunzler, The Fermi Surface, W. A. Harrison and M. B. Webb, Eds. (Wiley, New York, 1960), p. 125.
9. A. B. Pippard, Phil. Trans. Roy. Soc. London A250, 325 (1957).
10. A description of these experimental techniques for determining the shape of the Fermi surface is given by A. B. Pippard, Rept. Progr. Phys. 23, 176 (1960); their application to copper is given in several papers in The Fermi Surface, W. A. Harrison and M. B. Webb, Eds. (Wiley, New York, 1960).
11. For studies of tin, see N. E. Alekeseevskii, Yu. P. Gaidukov, I. M. Lifshitz, V. G. Peschanskii, Sov. Phys. JETP (English Transl.) 12, 837 (1961); for studies of lead, see N. E.
Alekeseevskii and Yu. P. Gaidukov, ibid. 14, 256 (1962); for gallium, see W. A. Reed and J A. Marcus, Phys. Rev. 126, 1298 (1962); for zinc, see W. A. Reed and G. F. Brennert, ibid. 130, 565 (1963); for magnesium, see R. W. Stark, T. G. Eck, W. L. Gordon, ibid., in press.
12. E. Fawcett, Phys. Rev. 128, 154 (1962).
13. —— and W. A. Reed, ibid. 134, A723 (1964).
14. W. A. Reed and E. Fawcett, Bull. Am. Phys. Soc. 7, 478 (1962).
15. E. Fawcett and W. A. Reed, Phys. Rev. Letters 9, 336 (1962).
16. H. Ehrenreich, J. R. Philipp, D. J. Olechna, Phys. Rev. 131, 2469 (1963); J. C. Phillips, ibid. 133, A1020 (1964).

Chemical and Anatomical Plasticity of Brain

Changes in brain through experience, demanded by learning theories, are found in experiments with rats.

Edward L. Bennett, Marian C. Diamond,

David Krech, Mark R. Rosenzweig

Here it may be asked whether the organs [of the brain] increase by exercise? This may certainly happen in the brain as well as in the muscles; nay, it seems more than probable, because the blood is carried in greater abundance to the parts which are excited, and nutrition is performed by the blood. In order however, to be able to answer this question positively, we ought to observe the same persons when exercised and when not exercised; or at least observe many persons who are, and many others who are not, exercised during all periods of life.—J. G. SPURZHEIM, 1815 (1).

I have shown that the brains of domestic rabbits are considerably reduced in bulk, in comparison with those of the wild rabbit or hare; and this may be attributed to their having been closely confined during many generations, so that they have exerted their intellect, instincts, senses and voluntary movements but little.—CHARLES DARWIN, 1874 (2).

One might suppose that cerebral exercise, since it cannot produce new cells (neural cells do not multiply as do muscular cells) carries further than usual the development of protoplasmic expansions and neural collaterals, forcing the establishment of new and more extended intercortical connections.—S. RAMÓN Y CAJAL, 1895 (3).

The question is not whether neural events change the status of the tissue in which they occur. The only question which may still be debated is: whether such changes as do undoubtedly occur have the permanence and those other properties which we must attribute to memory-traces. According to our present knowledge the primary effect which nerve impulses produce in ganglionic layers is chemical activity . . .—WOLFGANG KÖHLER, 1938 (4).

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 [acetylcholinesterase] activity of the brain, and the cortical/subcortical distributions of the 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.—M. R. ROSENZWEIG, D. KRECH, E. L. BENNETT, and M. C. DIAMOND, 1962 (5).

As these quotations show, it has long been speculated that the use of the brain might lead to changes in its size, in the interconnections of its cells, and in its chemical composition. Speculation led

to research, and in the last century measurements of the size and weight of brains of men were made in an effort to discover differences that might relate to the degree of intellectual attainment. The first results were encouraging, since men of distinction were usually found to have larger brains than those of inferior intellect. Gradually it was realized, however, that men of different stations in life often differed in health and nutrition as well as in intellect, and that the former factors might affect brain weight. There were also striking exceptions to the general relation—idiots with large brains and geniuses with small brains. The hypothesis of an intrinsic relation between brain size and cerebral exercise or ability was therefore generally abandoned. In its place there were suggestions of more subtle factors involving neural interconnections or chemical changes in the brain. The difficulty of working with such factors discouraged research, and the problem largely reverted to the speculative realm.

In spite of their speculative nature, physical or chemical residuals of experience in the brain continued to be incorporated in most physiological theories of learning. It was generally supposed that changes must occur in the brain in order to account for memory—the registration, the storage, and the retrieval of information. No other hypothesis seemed tenable. So certain were the theoreticians about this that they long ago gave names to these hypothesized changes—they called them "memory traces" or "brain engrams." But, unfortunately, the brain physiologists and anatomists were singularly unsuccessful in finding any solid evidence to justify

The authors are associated with the University of California, Berkeley. Dr. Bennett is research biochemist and associate director of the Laboratory of Chemical Biodynamics. Dr. Diamond is lecturer and research associate anatomist. Dr. Krech and Dr. Rosenzweig are professors of psychology.

the certainty of their colleagues. In time an alternative hypothesis was developed—the hypothesis that memory might be stored purely in the form of reverberating neural impulses. However, experiments in the late 1940's and early 1950's indicated that electrical shock could disrupt impulses and that low temperature could drastically reduce their number, but that in both of these cases memories formed before the treatment were still available afterwards. The reverberating impulse hypothesis of long-term memory was therefore abandoned, and the residual change hypothesis was revived. In fact most workers had never left it. Now it became even more important to find some evidence for physiological residuals of experience in the brain. Indeed, evidence for *brain plasticity* would seem to be propaedeutic and essential for the sound development of research on many major questions of brain-behavior relations.

We took up the search for responses of the brain to experience about 6 years ago in the context of a larger project dealing with relations of intelligent behavior in animals to brain chemistry. This project was started in 1953 by the two psychologists and the chemist of our group. It could not have begun nor continued without the interest and material support of Melvin Calvin, who has long espoused interdisciplinary research in chemistry and biological sciences. We have also benefited from the counsel of Everett Dempster on research involving genetic selection for brain chemicals. When we found evidence 4 years ago that experience could modify not only the chemistry but also the anatomy of the brain, a neuroanatomist joined the group in order to investigate both gross and microscopic anatomical changes in detail. What we have to report, therefore, is the result of several years of cooperative interdisciplinary effort on one research problem (6).

Prediction of Chemical Change with Training

Transmission of neural impules across the synapses or junctions between neurons requires a chemical step. When the impulse reaches the end of a neuron, it causes the release of a chemical mediator which diffuses across the narrow synaptic gap and combines with receptor sites on the post-synaptic membrane. This alters the post-synaptic potential. If this potential reaches the threshold value, a nerve impulse is initiated. Acetylcholine was the first synaptic transmitter to be studied in the peripheral nervous system, and evidence for its role in the brain has been accumulating steadily. Once acetylcholine is liberated at a synapse, it is rapidly inactivated by the enzyme acetylcholinesterase, so that the synapse is restored to its resting state.

In earlier studies, we had found evidence that the ratio of cerebral acetylcholine concentration to acetylcholinesterase activity is positively related to problem-solving ability in rats (7); that is, the higher the ratio, the better the learning scores. We then hypothesized that differential experience would lead to quantitative changes in this synaptic transmitter system. Specifically, we predicted (8) that enhanced stimulation and training would increase the rate of liberation of acetylcholine, and that this in turn would lead to an increased rate of synthesis of acetylcholinesterase. We did not at the outset intend to look for changes in the size or weight of the brain, because the idea that "cerebral exercise" can increase the size of the brain had been pretty generally abandoned by the 20th century. Indeed, we had completed several experiments and published an article on effects of training on brain chemistry (8) before further examination of the data suggested that there are anatomical changes as well.

Design of Behavioral Treatments

In designing an experimental approach to the problem, we followed the second proposal of Spurzheim by varying the amount of experience given to different groups of subjects. We incorporated several features in the design to reduce random variation among subjects and thus to increase the reliability of any results that we might obtain. All comparisons were made among animals alike in lineage, parentage, sex (male) and age, and differing systematically only in amount of experience. To test the generality of the effects, different lines of rats were employed in several of the experiments.

What type of experience might be important in producing measurable cerebral effects could not be foretold, so we combined both informal and formal training. The control animals are kept under colony conditions, housed three in a cage and exposed to ongoing activity in the room, but with no special treatment; this we term our Social Condition (SC). For enhanced experience, animals are given Environmental Complexity and Training (ECT). The ECT animals are housed in groups of 10 to 12 in large cages provided with "toys." (Animals in an ECT cage are shown in Fig. 1.)

Every day they are placed for 30 minutes in a square field 90 cm on a side where the pattern of barriers is changed daily. In the home cage and
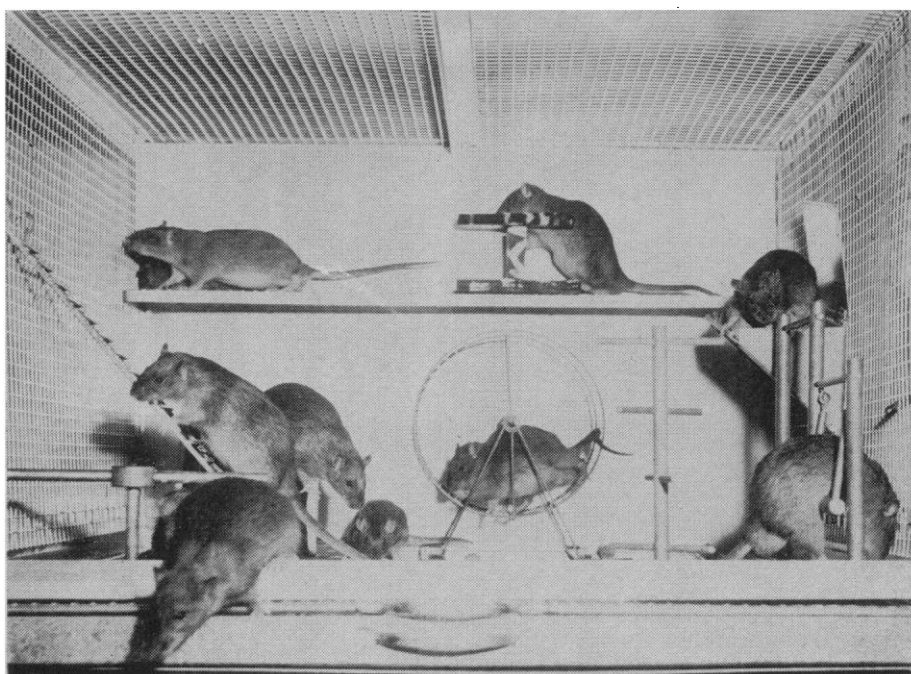

Fig. 1. Animals in an Environmental Complexity and Training cage.

in the open field, the rats play and explore as actively as kittens. After some weeks they are also given one or two trials a day in various standardized mazes for sugar pellet rewards. For the third condition—reduced experience—animals are caged singly in a dimly lit and quiet room where they cannot see or touch another animal (although they can hear and smell them); this is the Isolated Condition (IC). Animals in all three conditions have food and water available at all times, and all are weighed on the average of once in 2 weeks. In most of our experiments, animals are kept under the experimental conditions for 80 days and then are killed for analysis of the brain (9).
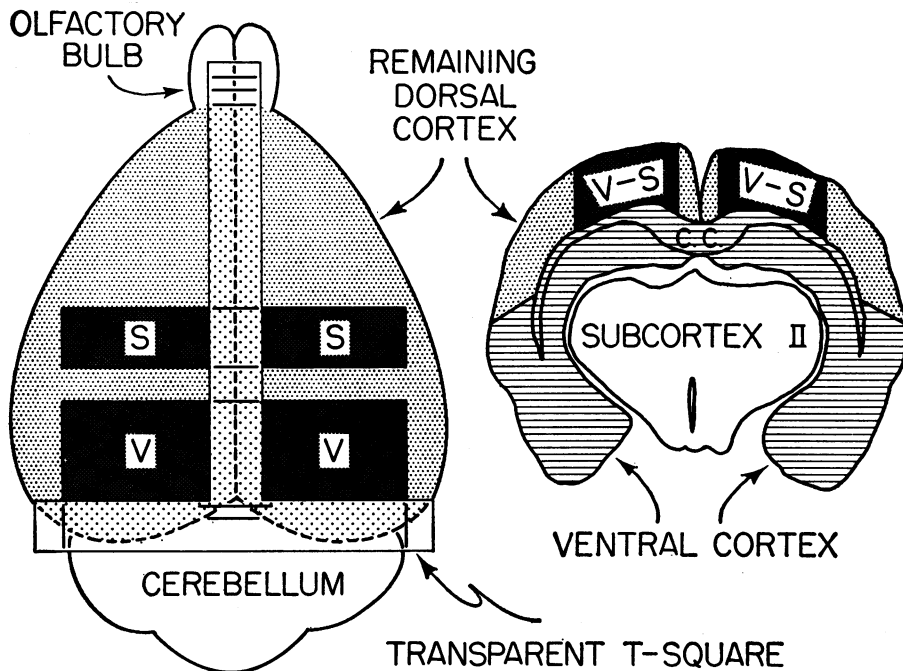

Fig. 2. To the left, the dorsal aspect of the rat brain, showing how samples of the visual cortex (V) and of the somesthetic cortex (S) are dissected, guided by a small transparent T-square. To the right, a transverse section of the rat brain. Total cortex is made up of four samples: the V and S sections—telescoped together in this diagram—plus the remaining dorsal cortex, plus the ventral cortex. The rest of the brain—labelled Subcortex II here—includes the olfactory bulbs and the cerebellum. [Reprinted with permission from J. Comp. Physiol. Psychol.]
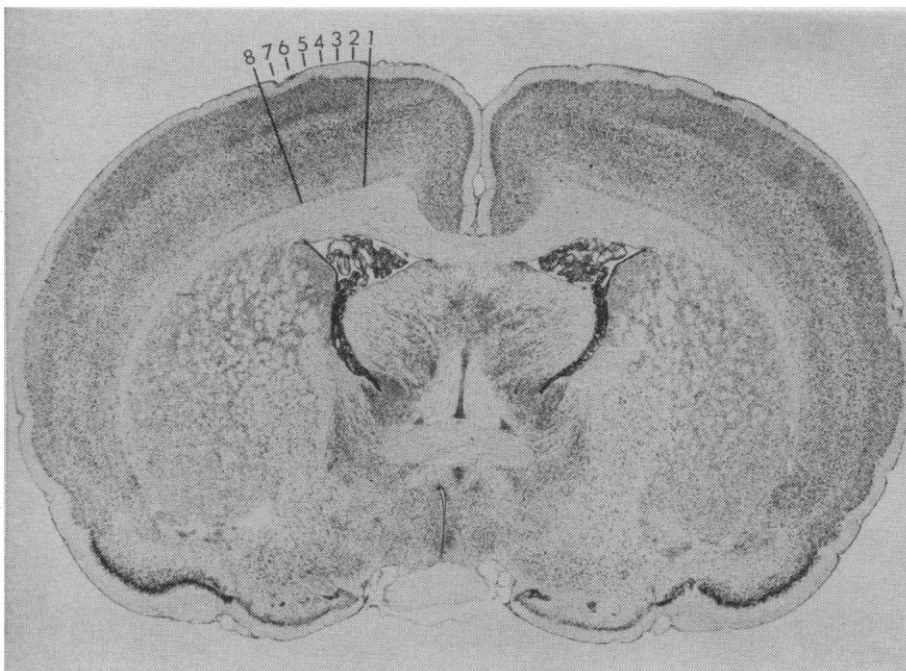

Fig. 3. A transverse section of rat brain showing somesthetic cortex. The decussation of the anterior commissure was used as the subcortical landmark to identify this region. The numbered lines through the cortex indicate where micrometer readings of cortical depth were taken. [Reprinted with permission from M. C. Diamond, D. Krech, M. R. Rosenzweig, Fig. 1, J. Comp. Neurol., 123, 112 (1964)]

Removal of Brain Samples and Chemical Analysis

At the end of the behavioral phase of an experiment, the animals are delivered to the chemists under code numbers that do not reveal their group membership. The animals are killed by decapitation, members of a litter being taken in immediate succession but with randomized order for treatment within litters. In most experiments the brain is then removed and divided by gross dissection into the following five samples: (i) sample of visual cortex, (ii) sample of somesthetic cortex, (iii) remaining dorsal cortex, (iv) ventral cortex and adjacent tissue including corpus callosum, hippocampus, and amygdala, and (v) the remaining brain or subcortex. The first four samples will be referred to collectively as "total cortex."

The visual and somesthetic samples are demarcated with a miniature plastic T-square, as Fig. 2 shows. In defining the regions from which the visual and somesthetic samples were selected, we were guided by earlier ablation and electrophysiological mapping studies of the rat cortex (10). Once these regions are circumscribed, they can be peeled cleanly from the underlying white matter, since in the rat brain there is a clear mechanical gradient between these two kinds of tissue. Figure 3 indicates the sharp histological boundary between the cortex and the white matter of the rat— far more distinct than the boundaries in man, cat, or dog. The absence of folds in rat cortex also facilitates stripping off the cortex. As each sample is removed, it is weighed and then frozen on a block of dry ice. About 10 minutes elapse between decapitation and the weighing of the last sample for a rat. The samples are then stored at −20°C until they can be analyzed chemically.

Until the last few years we analyzed acetylcholinesterase activity by means of an automatic titrator or "pH-stat." Recently, we have speeded the analyses without sacrificing reliability by using

a colorimetric method adapted from that of Ellman, Courtney, Andres, and Featherstone (*11*).

While reliability of chemical results *within* any experiment is high, there are some variations of absolute values from one experiment to another. We therefore found it prudent, when investigating the effects of new variables, to include our standard groups (ECT and IC) within each experiment. This has led to the replication of the main conditions in many successive experiments. While the replication is costly, the consistency of the results over many experiments provides great assurance about the existence of the effects which we will now describe.

Effects of Experience on Weight of Brain Tissue

In seven successive experiments, we have used animals of the Berkeley S₁ line, putting them into the enriched and impoverished environments at weaning (about 25 days of age) and maintaining them there for about 80 days. Three of these experiments also included Social Control (SC) groups, as we will discuss later.

A highly consistent difference between the brains of littermates given enriched or restricted experience occurs in the weight of the cerebral cortex. The left column of data in Table 1 gives the mean weight of total cortex for each group in each of the seven experiments. It also gives the number of pairs in which the cortical weight of the ECT animal surpassed that of its IC littermate and the amount by which the ECT mean exceeded the IC mean, expressed as a percentage of the IC mean. As can be seen from Table 1, a highly consistent set of results was obtained in experiments performed over a 4-year period, sampling a number of different generations of the S₁ line, and with some experiments falling in each season of the year.

Several points should be noted with regard to this greater growth of cortex among the enriched-experience rats.

1) It does not reflect overall growth of the whole brain. As the right-hand column of Table 1 shows, outside of the cortex there is actually a slight decrease in weight of the brain of enriched-experience animals (1.2 percent, $p < .05$).

2) It does not reflect an increase in body weight. The enriched-experience animals end the experiment weighing

about 7 percent *less* than their rather inactive isolated littermates. If we had calculated the results in terms of brain weight per unit of body weight rather than in terms of absolute brain weight, the relative difference between groups would have been much larger.

3) It does not occur equally throughout the cortex. Instead, as Table 2 shows, there is a pronounced regional distribution, the mean difference being greatest in the visual region (6.2 percent, $p < .001$) and least in the somesthetic region of the cortex (2.7 percent, $p < .05$).

4) It can be measured in other terms than wet weight of tissue, and completely comparable results are obtained. In two further experiments, we sectioned the brains for anatomical measurement instead of consuming them in chemical analysis (*12*). The depth of the cortex was measured from the pial surface down to the underlying white matter, using an optical micrometer. Figure 3 shows the location of lines along which we measured the depth. Subcortical landmarks were used to locate comparable cortical regions to be measured in each animal. In the visual region, the cortex of enriched-experience rats was 6.2 percent thicker than that of their littermates ($p < .001$), while in the somesthetic region the difference was only 3.8 percent thicker ($p < .01$). The anatomists making these measurements did not, of course, know which experimental treatment any animal had received.

5) Finally, the increases in weight and depth of the cortex with experience do not mean that absolute weight of cortex can be used as a correlate of experience or ability. The absolute weight of the cortex is determined not only by experience but also by other factors such as heredity. For example, the Berkeley S₃ line [descendents of Tryon's maze-dull line (*13*)] has about one-tenth greater weight of cortical tissue than the S₁ line (descendents of Tryon's maze-brights). We have found the S₃ animals to be inferior to the S₁ animals in solving several standardized mazes (*7, 14*). Within each line, the enriched-experience animals develop significantly heavier cortices than their isolated littermates (*15*), but the ECT's of the S₁ line nevertheless have lighter cortices than the IC's of the S₃ line. It must be borne in mind that our experiments are all confined to the question, "How does experience transform the brain from what it would have been without that experience?"

Effects of Experience on Acetylcholinesterase Activity

Activity of acetylcholinesterase (Table 3) was measured in the same seven experiments for which we have just considered the results on brain weight. Total activity of the enzyme was greater for the enriched-experience than for the isolated animals by 2.7 percent in total cortex ($p < .01$) and by 2.1 percent in the rest of the brain ($p < .001$). As was the case with tissue weight, the increase in total activity is greatest in the visual region of the cortex—3.6 percent—and least in the somesthetic region. Note that in each cortical region the relative increase in total acetylcholinesterase activity is less than the increase in tissue weight, as can be seen by comparing Tables 2 and 3. In the rest of the brain, however, the enzy-

Table 1. Brain weights of littermate S₁ rats exposed to Environmental Complexity and Training (ECT) or Isolation Condition (IC) from 25 to 105 days of age; seven replication experiments.

Experiment: Termination date, N(pairs)	Statistic	Weight (mg)	
		Total cortex	Rest of brain
May 1960, 11	ECT $\bar{X}$	663	894
	IC $\bar{X}$	626	896
	ECT > IC*	9/11	5/11
	% Diff.†	5.8	−0.1
Oct. 1960, 11	ECT $\bar{X}$	682	971
	IC $\bar{X}$	645	992
	ECT > IC*	9/11	5/11
	% Diff.†	5.8	−2.1
Dec. 1961, 11	ECT $\bar{X}$	702	912
	IC $\bar{X}$	672	922
	ECT > IC*	9/11	5/11
	% Diff.†	4.4	−1.1
Mar. 1962, 10	ECT $\bar{X}$	705	916
	IC $\bar{X}$	673	923
	ECT > IC*	9/10	5/10
	% Diff.†	4.8	−0.7
June 1962, 12	ECT $\bar{X}$	740	977
	IC $\bar{X}$	708	988
	ECT > IC*	10/12	5/12
	% Diff.†	4.5	−1.1
Dec. 1962, 12	ECT $\bar{X}$	710	980
	IC $\bar{X}$	688	982
	ECT > IC*	9/12	6/12
	% Diff.†	3.2	−0.2
July 1963, 10	ECT $\bar{X}$	694	912
	IC $\bar{X}$	667	942
	ECT > IC*	7/10	2/10
	% Diff.†	3.9	−3.2
1960–1963, 77	ECT $\bar{X}$	700	939
	IC $\bar{X}$	669	951
	ECT > IC*	62/77	33/77
	% Diff.†	4.6	−1.2
	p‡	< .001	< .05

* Number of littermate pairs in which the ECT value exceeds the IC value. † Percentage by which the ECT mean exceeds the IC mean. ‡ Probability values were determined by analyses of variance.

Table 2. Weights of brain regions from 77 littermate pairs of S_1 rats in ECT and IC conditions (seven experiments combined).

| Statistic | Weight (mg) | | | | | | |
| | Cortex | | | | | Rest of brain | Total brain |
	Visual sample	Somes-thetic sample	Remain-ing dorsal	Ventral	Total		
ECT $\overline{X}$	63.0	48.7	282	306	700	939	1639
IC $\overline{X}$	59.3	47.4	268	294	669	951	1620
ECT > IC*	57/77	48/77	57/77	51/77	62/77	33/77	51/77
% Diff.†	6.2	2.7	5.2	4.0	4.6	−1.2	1.2
p‡	<.001	<.05	<.001	<.01	<.001	<.05	<.05

* Number of littermate pairs in which the ECT value exceeds the IC value. † Percentage by which the ECT mean exceeds the IC mean. ‡ Probability values were determined by analyses of variance.

matic activity goes up with enriched experience although weight decreases slightly. (These relations for both cortex and subcortex are shown in Fig. 4.) If enzymatic activity is measured *per unit of tissue weight,* as is often done, the enriched-experience rats have lower values than the restricted animals in the cerebral cortex but higher values in the rest of the brain. The possible significance of this peculiar pattern of changes will be discussed later.

Protein may provide a better base than wet weight of tissue for measuring enzymatic activity. Total protein was determined in experiments 4, 5, and 6 of Table 1 and was found to vary directly with tissue weight, as Fig. 4 shows; therefore the measures of enzymatic activity were quite comparable whichever base was used. If anything, we have found measures *per unit of protein* to be somewhat more stable, so that the statistical significance of differences tended to be greater for this measure than for activity per unit of weight.

These differences in enzymatic activity are reliable, as is shown both by

statistical analyses and by their consistency from replication to replication. The value for total acetylcholinesterase activity is greater for the ECT than for the IC groups in each of the seven experiments for total cortex (53 of 74 littermate pairs) and in six of the seven experiments for the rest of the brain (50 of 76 pairs). The chemists doing the analysis did not know to which group any animal belonged.

There still remain certain questions of validity: Is it certain that the changes we measure reliably are changes of acetylcholinesterase activity and that they occur in brain tissue rather than in the blood content of the brain? Certain possible interpretations that call the validity of our measures into question have been ruled out by further tests that we have made:

1) Acetylcholine can be split in the brain not only by the specific enzyme acetylcholinesterase, but also by a less specific enzyme, cholinesterase (16). Since our usual measure includes all enzymatic activity that hydrolyzes acetylcholine, the effects so far reported

might be due in whole or in part to changes in cholinesterase rather than acetylcholinesterase activity. In recent experiments with S_1 rats we have explicity investigated this question. The results demonstrate that with our current methods, which employ acetylthiocholine as the substrate, at least 95 percent of the enzymatic activity measured in the rat brain is due to acetylcholinesterase and at least 90 percent of the changes reported here are due to acetylcholinesterase.

2) Our anatomical investigation has shown that the mean diameter of capillaries is greater in the cortices of enriched-experience animals than in those of their littermates (12). Since red blood cells contain acetylcholinesterase and blood serum contains cholinesterase, this raises the possibility that the increases in activities of the two enzymes in the cortex might only reflect increased blood content. We have investigated this possibility and have found that the activities per unit weight of these enzymes in blood are on the order of one-sixth of the values for cortical tissue. Thus, even if the entire increase in cortical weight were to be attributed to increased blood volume, the enzymes in the blood could not account for the observed changes.

Are the Effects Due to Variables Other Than Enriched Experience?

Let us now consider whether it is indeed enriched experience that is responsible for the changes in cerebral weight and acetylcholinesterase activity or whether other aspects of the behavioral conditions may bring about these effects. Three possible alternative explanations have been subjected to experimental tests:

1) Since the animals in the condition of environmental complexity and training receive more handling and are more active than the animals in isolation, can these components alone produce cerebral effects similar to those we have described?

2) May the differences between the groups be attributed chiefly to the isolation to which the restricted animals are subjected?

3) May the cerebral differences be due simply to alterations induced in the rate of early development by differential experience?

These questions will be taken up in turn in the next three sections.

Table 3. Total acetylcholinesterase activity of brain regions from S_1 rats in ECT and IC conditions (seven experiments combined). Activity is expressed in terms of moles of acetylcholine hydrolyzed per minute under our standard assay conditions.

| Statistic | Activity (10^{-8} mole/min) | | | | | | |
| | Cortex | | | | | Rest of brain | Total brain |
	Visual sample	Somes-thetic sample	Remain-ing dorsal	Ventral	Total		
ECT $\overline{X}$	38.6	36.5	212	344	631	1825	2455
IC $\overline{X}$	37.2	35.8	206	336	614	1787	2401
ECT > IC*	50/76	43/77	48/76	46/76	53/74	50/76	52/73
% Diff.†	3.6	1.8	3.2	2.6	2.7	2.1	2.2
p‡	<.01	N.S.§	<.01	<.10	<.01	<.001	<.001

* Number of littermate pairs in which the ECT value exceeds the IC value. † Percentage by which the ECT mean exceeds the IC mean. ‡ Probability values were determined by analyses of variance. § N.S., not significant.

Possible Cerebral Effects of
Handling or Differential Locomotion

Since handling has been shown to affect both physiological variables and later learning in the rat, we designed an experiment to look for possible cerebral effects of handling. Twenty-four animals were taken from their cages and handled daily, 12 for 30 days and 12 for 60 days, while littermates were never removed from their cages. This treatment started at weaning in order to provide a control for our previous studies. The results yielded no indication of cerebral changes with handling.

To control for differential locomotion, we ran two experiments in each of which some rats had free access to activity wheels while their littermates did not. No consistent differences were found between the active and inactive groups.

Recently, we ran another experiment in which rats of one group were handled daily and also had free access to activity wheels, while their littermates had neither of these forms of stimulation. Again, 80 days of this differential treatment did not produce significant cerebral differences between the groups. It is clear that neither differential handling, nor locomotion, nor the combination of both treatments can produce the cerebral changes we are considering.

Is Isolation Stress the
Cause of Our Effects?

Isolation has been reported to be stressful for rats, making them so aggressive that they cannot be handled with bare hands, producing caudal dermatitis, and making them much more susceptible to a toxic agent, isoprenaline (17). It is, therefore, possible that the differences we observed were due primarily to the deleterious effects of isolation stress on the restricted group. We doubt this interpretation for the following two reasons.

First, animals of our lines do not show the obvious signs of isolation stress described above. When isolated for 80 or even 160 days, they can still be picked up with bare hands for their occasional weighings, and they do not develop caudal dermatitis. The adrenals were weighed in some experiments, and those of the isolates were not enlarged. It may be that some strains are stressed

severely by isolation while others are not.

Second, evidence comes from the last three of the seven experiments with S_1 rats; in these experiments we also included a colony or Social Control (SC) group in which animals were housed three to a cage. The results permit us to measure the effects of increasing or restricting experience, using the SC condition as a baseline (see Fig. 5). On every measure except weight of subcortex, the ECT group differed significantly from the SC group and differed further from them than did the IC group. Thus the bulk of the effects on cerebral weight and acetylcholinesterase activity is due to enriching rather than to restricting the experience of our colony animals (18). Similar findings for older animals will be reported in the next section.

Are Cerebral Changes
Confined to Young Animals?

The experiments described to this point have all been done with animals put into the differential environments at weaning, when they were about 25 days old, and maintained there until the age of about 105 days. During this period, colony animals show an increase

of about 30 percent in total brain weight. To test the possibility that the complex environment and training simply accelerated the development of the normally rapidly growing young brain, we ran two successive experiments with older S_1 rats, employing 12 sets of male triplets in each experiment (19). One animal in each litter was assigned to the ECT condition, one to the SC condition, and one to the isolation condition. The animals were taken from the colony at about 105 days of age, the age at which the younger animals had been killed. At about 70 days rats are sexually mature, and during the 80-day period following 105 days of age, growth of total brain weight of rats in the colony is only about 6 percent—as contrasted with 30 percent in the preceding 80-day period.

The results for older animals, shown in Fig. 6, are seen to be rather similar to those for younger animals (Fig. 5). In Fig. 6, the ECT animals surpass the colony controls significantly on every measure, while the IC animals do not differ significantly from the colony controls on any measure. The regional differentiation of effect among the adults, presented in Tables 4 and 5, is even sharper than that among the young animals (Tables 2 and 3). Among the adults the increases of total acetylcho-
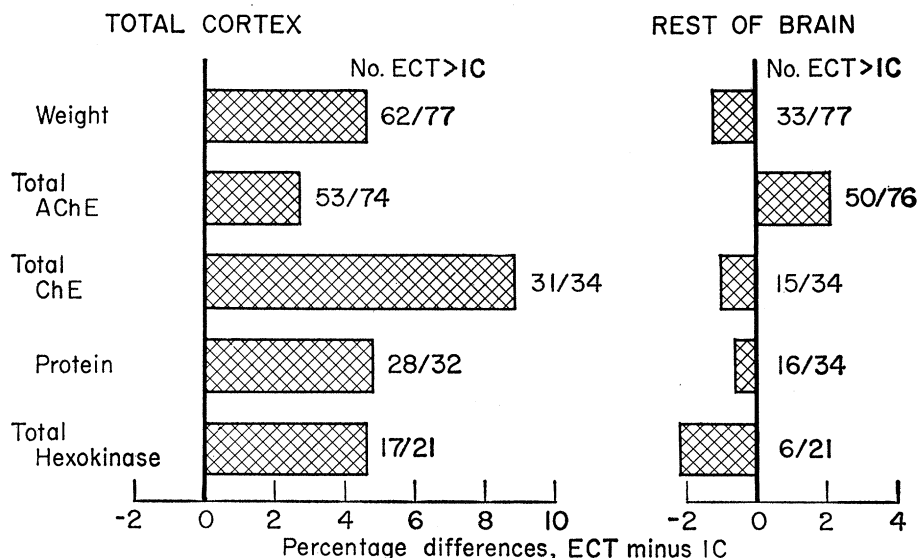

Fig. 4. Relative differences between enriched-experience (ECT) and restricted (IC) groups on several cerebral measures. Each difference is expressed as a percentage of the IC value. For each measure, the number of littermate pairs in which the ECT value exceeded the IC value is also given. In the cortex, the ECT-IC difference in acetylcholinesterase is less than that for tissue weight, the difference in cholinesterase is greater, and the differences in protein and hexokinase are almost identical to the weight difference. In the rest of the brain, only acetylcholinesterase is greater for the enriched-experience than for the restricted animals; all other measures show the ECT animals lower than the IC animals, and by roughly equal percentages. The figure is based entirely upon males of the Berkeley S_1 line.

Table 4. Weights of brain regions from 24 littermate pairs of S_1 rats in ECT and IC conditions from 105 to 185 days of age (two experiments combined).

Statistic	Weight (mg)						
	Cortex					Rest of brain	Total brain
	Visual sample	Somes-thetic sample	Remain-ing dorsal	Ventral	Total		
ECT $\overline{X}$	72.6	52.9	280	338	744	1019	1763
IC $\overline{X}$	65.6	51.7	266	319	702	995	1697
ECT > IC*	20.5/24	12/24	17/24	18/24	22/24	17/24	17/24
% Diff.†	10.7	2.3	5.4	6.0	5.9	2.4	3.9
p‡	<.001	N.S.	<.005	<.001	<.001	<.01	<.001

* Number of littermate pairs in which the ECT value exceeds the IC value. † Percentage by which the ECT mean exceeds the IC mean. ‡ Probability values were determined by Duncan's new multiple range test, since the analysis of variance included the SC group.

linesterase approach more nearly the increases of cortical weight than was true among the young animals. At the subcortex, the adult ECT animals gain in tissue weight almost as much as they gain in total acetylcholinesterase activity, whereas the young ECT animals did not gain at all in subcortical weight.

We conclude that the adult brain shows increases in cortical weight and total acetylcholinesterase activity as readily as the young brain. The occurrence of such cerebral effects among adults supports the argument that these effects, rather than being consequences of accelerated early development, are residuals of experience.

Effects on Other Chemical Measures

The pattern of change of acetylcholinesterase activity with experience differs from those of the four other chemicals that we have measured so far: cholinesterase, protein, hexokinase, and serotonin.

Cholinesterase activity was determined in the last three experiments completed with S_1 rats, and the results are given in Table 6. Total cholinesterase activity in total cortex was 8.9 percent greater in the ECT than in the IC groups. As Fig. 5 (based on the same animals) indicates, cortical weight was greater by 3.9 percent and total acetylcholinesterase activity by 3.2 percent. Thus, in terms of activity per unit of weight, cholinesterase increased in the cortex while acetylcholinesterase decreased. In the rest of the brain, however, cholinesterase activity remained essentially unchanged, while acetylcholinesterase activity rose significantly. The different patterns of change for tissue weight and total enzymatic activity are presented graphically in Fig. 4. With total cholinesterase activity, in contrast to the case for acetylcholinesterase activity, the major differences are found between the isolated and colony groups, rather than between the ECT and colony groups (see Table 6).

Certain other chemical measures show changes similar to those of tissue weight. We have already seen that this is true of protein. We have also measured hexokinase activity in experiment

4 of Table 1 and in one other experiment. This enzyme is important in the general metabolism of cells but it does not appear to have a different function in the nervous system from its function in other organs. Hexokinase activity per unit of weight did not alter with differential experience in either cortex or subcortex. A substance that has attracted a good deal of study recently with respect to brain activity is serotonin, which some investigators believe to be a central synaptic-transmitter agent. Gordon T. Pryor in our laboratories has assayed serotonin in S_1 littermates exposed to the ECT or IC conditions. While Pryor found the usual modifications of cortical weight and of acetylcholinesterase and cholinesterase activities, he was unable to find a significant difference between groups in the concentration of serotonin (see 20).

In summary, when we compare the extreme experiential groups—ECT versus IC—the results show a clearly differentiated pattern among chemicals in the cortex. Acetylcholinesterase changes less with experience than does the weight of the tissue; cholinesterase changes more; and the others—protein, hexokinase, and serotonin—vary with tissue weight. The decrease in acetylcholinesterase activity per unit of cortical weight suggests that the growth of cortex with use involves especially elements low in acetylcholinesterase, such as glia, noncholinergic neurons, and blood vessels and blood volume. (Our finding of increased diameter of cortical blood vessels in ECT animals has already been cited.) The increase in cholinesterase activity per unit of cortical weight may be another indication of the growth of glia cells, since these are relatively high in cholinesterase activity. The lack of change in protein, hexokinase, and serotonin per unit of weight indicates that the additionally formed tissue is normal in its chemical endowment as far as these constituents are concerned.

Table 5. Total acetylcholinesterase activity of brain regions from S_1 rats in ECT and IC conditions from 105 to 185 days of age (two experiments combined). Activity is expressed in moles of acetylcholine hydrolyzed per minute under our standard assay conditions.

Statistic	Activity (10^{-8} mole/min)						
	Cortex					Rest of brain	Total brain
	Visual sample	Somes-thetic sample	Remain-ing dorsal	Ventral	Total		
ECT $\overline{X}$	42.2	37.1	195	361	635	1828	2463
IC $\overline{X}$	38.5	37.0	189	349	614	1779	2392
ECT > IC*	16/24	12/24	15/24	19/24	19/24	19/24	19/24
% Diff.†	9.6	0.3	2.9	3.4	3.4	2.8	3.0
p‡	<.001	N.S.	N.S.	N.S.	<.05	<.005	<.001

* Number of littermate pairs in which the ECT value exceeds the IC value. † Percentage by which the ECT mean exceeds the IC mean. ‡ Probability values were determined by Duncan's new multiple range test.

Generality of Effects over Lines

In addition to the S_1 animals, five other lines have been exposed to our standard ECT and IC conditions, and all lines have shown cerebral effects in the same directions but varying in magnitude (5, 8). The S_1 line was one of two lines showing relatively large effects, while the S_3 line was one of those

showing relatively small effects. In order to determine the reliability of such genetic differences, we have performed three replications in each of which S_3 groups were run and analyzed simultaneously with S_1 groups. In all three cases, the S_1 animals showed larger effects on all measures than the S_3 animals (15). We conclude that while all lines show cerebral plasticity in response to environmental pressure, some lines are more readily modifiable than others.

Blindness and Light Deprivation

Further evidence of plasticity of the brain comes from experiments in which animals were blinded or totally deprived of light at about 25 days of age. The experiments continued for about 80 days. In two experiments (21) blinded animals, when compared with sighted littermates kept in the same enriched condition, showed a 5 percent loss in weight of the visual cortex and an 8

percent loss in the superior colliculi (midbrain visual reflex centers). Total acetylcholinesterase activity surprisingly increased by 4 percent in the visual cortex of the blinded rats and decreased by 21 percent in the superior colliculi. In three experiments we have found that deprivation of light in sighted animals produced anatomical and chemical effects similar in direction but generally smaller in magnitude than those following blinding (22). The results of these experiments suggest that modifying the amount of experience in one sensory modality can affect rather specifically the brain regions serving that modality. Further results suggest that impairment of one sensory channel leads to greater use of other modalities and thus to greater cerebral development ("compensation") in the corresponding brain areas. Specifically, if blinded or light-deprived animals are raised in a complex environment, the somesthetic area of the cortex shows increases in weight and total acetylcholinesterase activity, when compared with light-stimulated littermates raised in otherwise comparable environments. Results on this effect of blinding were published in 1963 (21). Since then, we have accumulated further confirmatory results on blinding, and Eleanor Saffran in our laboratories has found similar results in experiments with light deprivation.

Selective Breeding

Darwin, as we have seen, offered evidence for brain plasticity through comparison of domestic and wild animals. It seems reasonable to suppose that the cumulative effects of generations of selection could produce large changes in the brain. In fact, a program of selection for cortical acetylcholinesterase carried out by T. H. Roderick in our laboratories has demonstrated relatively large changes in brain chemistry after six generations (23). Two foundation stocks (Castle and Dempster) were used for a simultaneous replication, and lines with both high and low acetylcholinesterase activity were selected from each stock. The Roderick Castle high and low lines differed by 34 percent ($p < .0005$) in acetylcholinesterase activity per unit of cortical weight, and the Roderick Dempster lines differed by 25 percent ($p < .0005$). These differences are several times larger than those that we have been able to produce by differential experience.
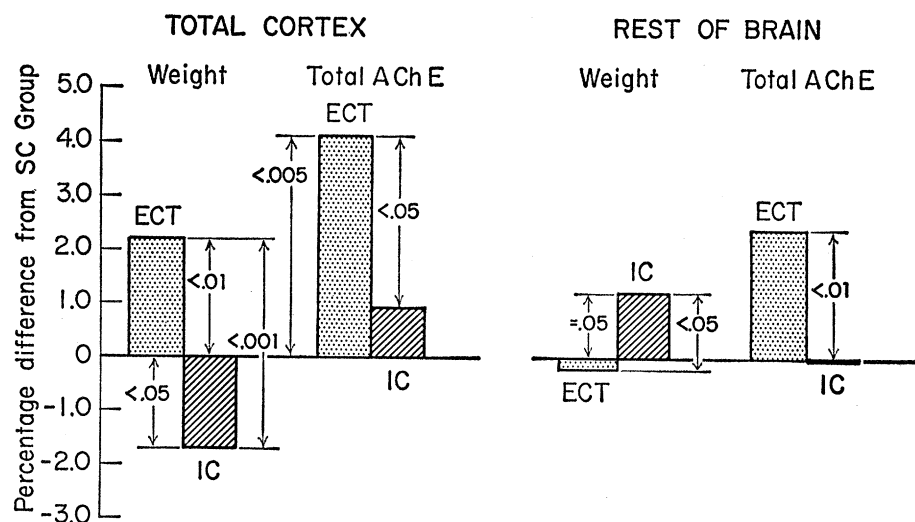

Fig. 5. Cerebral values of enriched-environment (ECT) and isolated (IC) groups, given in relation to the baseline of the standard colony (SC) group. In each case, the difference from the SC value is expressed as a percentage of the SC value. Where differences are significant, the p values are shown by the figures on the arrows. Thus, in weight of total cortex, the ECT animals were 2.2 percent heavier than the SC animals ($p < .01$), and the IC animals were 1.6 percent lighter than SC baseline ($p < .05$). The data were obtained in three experiments involving 34 sets of male triplets of the S_1 line. The rats were in the differential environments from the age of 25 days to the age of 105 days.
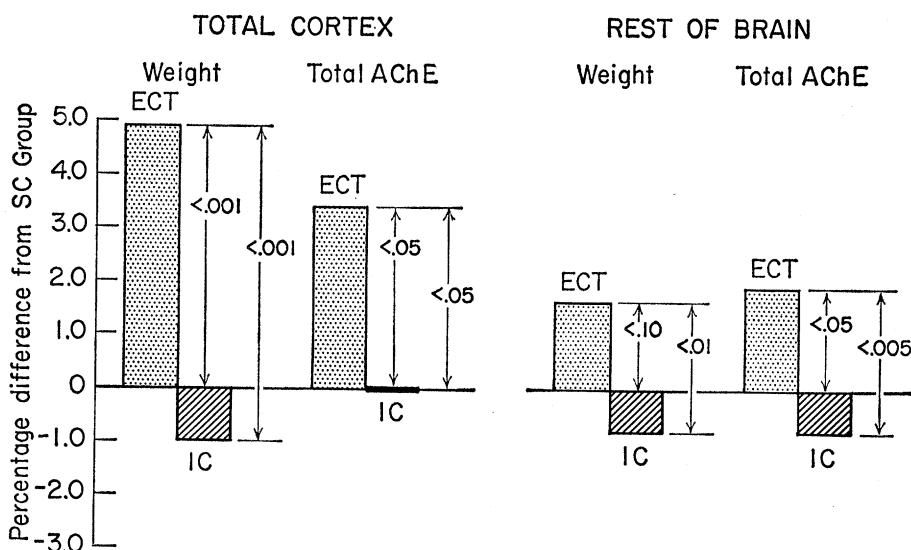

Fig. 6. Comparisons among brain values of littermate rats maintained in the ECT, SC, or IC conditions from 105 to 185 days of age. The same conventions are followed as in Fig. 5. Thus, in weight of total cortex, the ECT animals were 4.9 percent heavier than the SC group ($p < .001$) and the IC animals were 1.0 percent lighter than the SC animals (nonsignificant). The data were obtained in two experiments including 24 sets of male triplets of the S_1 line.

Possible Functional Significance

We believe it essential now to extend our knowledge of cerebral changes with experience in order to determine what significance they may have for physiological theories of learning and memory. Further characterization of the effects is being pursued both chemically and anatomically. Ribonucleic acids, various brain lipids, the synaptic-transmitter acetylcholine, and other possible neural transmitters and their related enzymes will be assayed to determine whether their concentrations or activities are affected by differential experience. Anatomically, we are extending our measures to include ramification of neuronal processes, vascularization, skull volume and dimensions, the sizes of cell bodies and of nuclei of both glia and neurons, and the ratio of glial to neuronal number. We do not want to overlook the possible participation of glial cells in cerebral changes because of our preoccupation with neural cells.

It may seem premature to attempt to assess the possible functional significance of cerebral modifications already observed while we are still attempting to extend our knowledge of them, but neither we nor our readers can avoid making at least a preliminary assessment.

For purposes of discussion let us consider whether our findings are compatible with the hypothesis that long-term memory storage involves the formation of new synaptic connections among

neurons. This was Ramón y Cajal's suggestion in the 1890's. It would appear from a number of considerations that the magnitude and nature of the observed effects are such as to accommodate a substantial increase in synaptic connections. Synaptic sites are known to be especially rich in acetylcholinesterase. An increase in number of functional synapses could, however, very well be many times greater than the concurrent increases in acetylcholinesterase activity and tissue weight. Thus, for example, Eayrs (24) reports that the number of interconnections per cortical neuron in the rat increases by about 400 percent between the ages of 15 and 36 days; meanwhile the cortex, according to data from our animals, is increasing in weight by only about 50 percent and in total acetylcholinesterase activity by only about 100 percent. These relations suggest the possibility that a 5-percent change in total acetylcholinesterase activity may reflect a 20-percent change in the number of synapses. A more direct test of this possibility may come from our attempts to measure neuronal ramifications.

Whatever the cerebral residuals of experience are, it is unlikely that they will involve large changes of either gross anatomy or chemistry. It is characteristic of the brain that its variability is extremely low. Weight of the brain varies less from individual to individual of a species than the weight of almost any other organ, and we have found the coefficients of variation for acetylcho-

linesterase activity to be almost as small as those for weight (25). Brain values can be modified by genetic selection, as we have seen, and drastic treatments such as thyroidectomy or prolonged undernourishment can alter the brain substantially. Short of such interfering factors, the enzymes and the weight of the brain are kept within tight limits. It can therefore be seen that even modifications that seem small in absolute terms may be large in terms of ordinary variability and may have functional consequences.

Conclusions

Our observations demonstrate that rats given enriched experience develop, in comparison to restricted littermates, greater weight and thickness of cortical tissue and an increase in total acetylcholinesterase activity of the cortex, the gain in weight being relatively larger than the increase in enzymatic activity. In the rest of the brain, acetylcholinesterase activity also increases, even though tissue weight decreases with enriched experience. These changes have been produced consistently in many replications; they have appeared in each line of rats tested to date, and they are found in adult as well as young animals. Control experiments have demonstrated that these changes cannot be attributed primarily to differential handling or locomotor activity, to isolation stress of restricted animals, or to altering the rate of development of the rapidly growing young brain. In a smaller number of experiments we have also found cholinesterase activity to change more than does tissue weight in the cortex but not in the rest of the brain. Protein, hexokinase activity, and serotonin follow closely the changes in weights of brain regions.

We wish to make clear that finding these changes in the brain consequent upon experience does not prove that they have anything to do with storage of memory. The demonstration of such changes merely helps to establish the fact that the brain is responsive to environmental pressure—a fact demanded by physiological theories of learning and memory. The best present conjecture, based both on the research reported here and on that of others, is that when the final story of the role of the brain in learning and memory is written, it will be written in terms of both chemistry and anatomy.

Table 6. Total cholinesterase activity of brain regions from 34 sets of triplet S_1 rats in ECT, SC, and IC conditions from 25 to 105 days of age (three experiments combined); activity expressed in moles of butyrylthiocholine hydrolyzed per minute under our standard assay conditions.

Statistic	Activity (10^{-8} mole/min)						
	Cortex					Rest of brain	Total brain
	Visual sample	Somesthetic sample	Remaining dorsal	Ventral	Total		
ECT $\overline{X}$	2.28	1.82	8.34	8.52	21.0	51.4	72.4
SC $\overline{X}$	2.23	1.76	8.18	8.42	20.6	51.1	71.7
IC $\overline{X}$	2.04	1.65	7.56	8.00	19.3	51.9	71.2
ECT > IC*	26/34	27/34	30/34	23/34	31/34	15/34	20/34
ECT > SC*	18/34	18/34	21/34	18/34	21/34	22/34	21/34
SC > IC*	24/34	23/34	25.5/34	24/34	28/34	13/34	19/34
% Diff., ECT-IC†	11.8	10.3	10.3	6.5	8.9	−1.0	1.7
% Diff., ECT-SC†	2.2	3.4	2.0	1.2	1.8	0.5	0.9
% Diff., SC-IC†	9.3	6.7	8.2	5.2	6.9	−1.5	0.8
p_{ECT-IC}‡	<.001	<.001	<.001	<.005	<.001	N.S.	=.05
p_{ECT-SC}‡	N.S.	<.10	N.S.	N.S.	=.10	N.S.	N.S.
p_{SC-IC}‡	<.01	<.005	<.001	<.05	<.001	N.S.	N.S.

* Number of cases in which the value of the littermate in the first condition exceeded that of the littermate in the second; for example, for the visual sample, the ECT value was greater than the IC value in 26 of the 34 littermate pairs.   † Percentage difference between means in the first and second conditions; for example, for the visual sample, the ECT mean exceeded the IC mean by 11.8 percent.   ‡ Probability values were determined by Duncan's multiple range test after analyses of variance.

An Invitation

Because we believe that our findings demonstrate the feasibility of research on the effects of experience on the brain, and because we believe that such research offers many challenges and a wide field for investigation, we hope to see it taken up in other laboratories. To this purpose we offer qualified investigators animals from our special lines and complete information about our behavioral, biochemical, and anatomical procedures, either through written descriptions or by direct demonstration.

References and Notes

1. J. G. Spurzheim, *The Physiognomical System of Drs. Gall and Spurzheim* (Baldwin, Cradock, and Joy, London, ed. 2, 1815), pp. 554–555.
2. C. Darwin, *The Descent of Man* (Rand, McNally, Chicago, ed. 2, 1874), p. 53.
3. S. Ramón y Cajal, *Les Nouvelles Idées sur la Structure du Système Nerveux chez l'Homme et chez les Vertébrés*, edition française revue et augmentée par l'auteur, L. Azoulay, trans. (Reinwald, Paris, 1895), p. 78.
4. W. Köhler, *The Place of Value in a World of Facts* (Liveright, New York, 1938), p. 239.
5. M. R. Rosenzweig, D. Krech, E. L. Bennett, M. C. Diamond, *J. Comp. Physiol. Psychol.* **55**, 429 (1962).
6. The collection and analysis of the data reported in this paper required the aid of chemists, anatomists, statisticians, and behavioral technicians. Among the chemists were Hiromi Morimoto, Marie Hebert, and Barbara Olton; among the anatomists, Fay Law and Helen Rhodes; among the statisticians, Carol Saslow, Bea Markowitz, and Peter Varkonyi; among the behavioral technicians, Frank Harris and Carol Poe. Roberta Robbins has been our secretary. The research has been supported by grants from the U.S. Public Health Service, the National Science Foundation, and the Surgeon General's Office. It has also been aided by the U.S. Atomic Energy Commission.
7. M. R. Rosenzweig, D. Krech, E. L. Bennett, *Psychol. Bull.* **57**, 476 (1960).
8. D. Krech, M. R. Rosenzweig, E. L. Bennett, *J. Comp. Physiol. Psychol.* **53**, 509 (1960).
9. The three conditions are described more fully in several of our reports: (8) and M. R. Rosenzweig, D. Krech, E. L. Bennett, in *Current Trends in Psychological Theory* (Univ. of Pittsburgh Press, Pittsburgh, 1961), p. 87.
10. I. Krechevsky, *J. Comp. Psychol.* **19**, 425 (1935); C. N. Woolsey and D. H. Le Messurier, *Federation Proc.* **7**, 137 (1948); J. P. Zubek, *J. Comp. Physiol. Psychol.* **44**, 339 (1951).
11. The automatic titrator method is described by M. R. Rosenzweig, D. Krech, E. L. Bennett, in a Ciba Foundation Symposium, *Neurological Basis of Behaviour* (Churchill, London, 1958), p. 337. The colorimetric method was adapted from G. L. Ellman, K. D. Courtney, V. N. Andres, Jr., R. M. Featherstone, *Biochem. Pharmacol.* **7**, 88 (1961).
12. M. C. Diamond, D. Krech, M. R. Rosenzweig, *J. Comp. Neurol.* **123**, 111 (1964).
13. R. C. Tryon, *Yearbook Natl. Soc. Stud. Educ.* **39**, part I, 111 (1940).
14. M. R. Rosenzweig, *Kansas Studies in Educ.* **14**, No. 3, 3 (1964).
15. M. R. Rosenzweig, E. L. Bennett, D. Krech, *Federation Proc.* **23**, 255 (1964), abstr.
16. In the past we and other investigators have called both enzymes "cholinesterase," but we are now employing the common terms recognized in the *Report of the Commission on Enzymes*, International Union of Biochemistry (Pergamon, New York, 1961).
17. T. Balazs, J. B. Murphy, H. C. Grice, *J. Pharm. Pharmacol.* **14**, 750 (1962); A. Hatch, T. Balazs, G. S. Wiberg, H. C. Grice, *Science* **142**, 507 (1963).
18. While the cerebral differences in weight and acetylcholinesterase activity occurred principally between the ECT and SC groups, these groups were almost identical in terminal body weights. The IC group, which was close to the SC group in cerebral measures, exceeded the SC group by about one-tenth in body weight.
19. M. R. Rosenzweig, E. L. Bennett, D. Krech, *J. Comp. Physiol. Psychol.* **57**, 438 (1964).
20. G. T. Pryor, unpublished doctoral dissertation (Univ. of Calif., 1964); *Univ. of Calif. Radiation Lab. Rept. No. 1179* (1964).
21. D. Krech, M. R. Rosenzweig, E. L. Bennett, *Arch. Neurol.* **8**, 403 (1963).
22. E. L. Bennett, M. R. Rosenzweig, D. Krech, *Federation Proc.* **23**, 384 (1964), abstr.
23. T. H. Roderick, *Genetics* **45**, 1123 (1960).
24. J. T. Eayrs, in *Structure and Function of the Cerebral Cortex*, D. B. Tower and J. P. Schadé, Eds. (Elsevier, Amsterdam, 1960), p. 43.
25. E. L. Bennett, M. R. Rosenzweig, D. Krech, H. Karlsson, N. Dye, A. Ohlander, *J. Neurochem.* **3**, 144 (1958).

University Organization for Geophysics Education

University of Miami evolves new pattern for graduate education in earth and planetary sciences.

Werner A. Baum

Most faculty members prefer to consider the problems of university organization as inconsequential and certainly of no interest. This is wishful thinking in our age of big science and big universities, as those of us in "interdisciplinary" or "multidisciplinary" subject fields know very well. Geo-

Dr. Baum is Vice President for Academic Affairs, Dean of the Faculties, and professor of meteorology at the University of Miami, Coral Gables, Florida. This article is based on a talk given at the American Geophysical Union's Symposium on Geophysics Education, held in Washington, D.C., in April 1964.

physics is in this category, involving as it does many specialties of physics, of chemistry, and even of biology.

Philip H. Abelson focused clearly on the problem in the Third Annual Klopsteg Lecture (*1*):

In this résumé of important trends in research it is apparent that almost all active fields involve multidisciplinary effort. Opportunities in some older disciplines seem limited. With the fast-shifting nature of research frontiers, it is apparent that the young student is faced with a difficult problem in preparing for research. If he specializes too early and too completely he may find that much of his knowledge is obsolescent even before he finishes graduate school. The situation calls for flexibility, and for a mastery of the fundamentals of two or more disciplines. . . .

The universities have a special responsibility. They must ask themselves whether they are preparing students for the 1980's or for the 1940's. Many schools are training their students for the 1940's. The curricula call for far too much specialized training. The student is overloaded with required courses in his specialty. He is given neither opportunity nor guidance to train himself broadly. Indeed, some departments consider a student disloyal and rather undesirable if he indicates a wish to take too many courses elsewhere. Moreover, the prejudice is usually amply conveyed.

As long as universities are organized in departments along disciplinary lines such narrow viewpoints are certain to come to the surface. To meet the new challenges will require a complete recasting of the administrative structure or at least the formation of interdepartmental arrangements designed to help the student, not to preserve the vested interests of the faculty.

The Academic Department

I believe this criticism to be basically valid. Departments do tend to be a serious impediment to change within a university. Of course, not all change