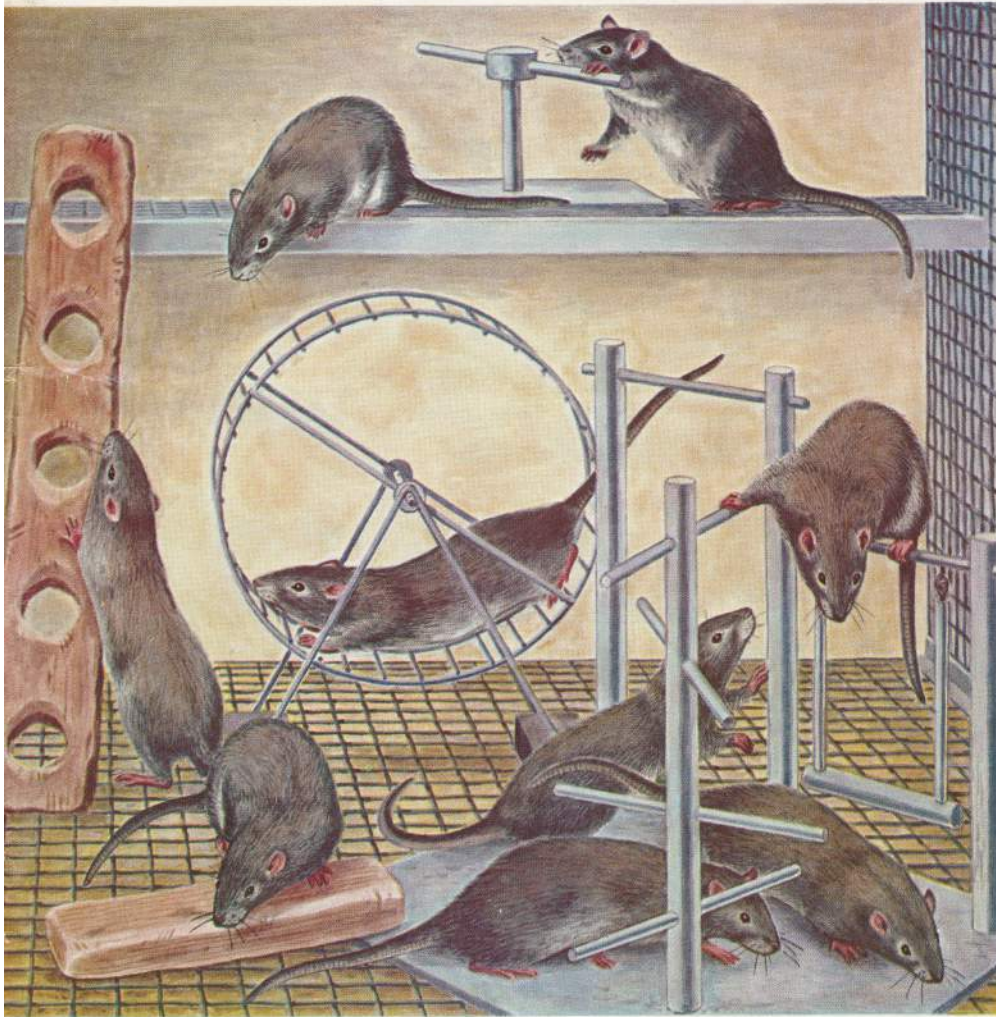


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EXPERIENCE AND THE BRAIN

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Brain Changes in Response to Experience

Rats kept in a lively environment for 30 days show distinct changes in brain anatomy and chemistry compared with animals kept in a dull environment. The implications of these effects for man are assessed

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Does experience produce any observable change in the brain? The hypothesis that changes occur in brain anatomy as a result of experience is an old one, but convincing evidence of such changes has been found only in the past decade. It has now been shown that placing an experimental animal in enriched or impoverished environments causes measurable changes in brain anatomy and chemistry. How these changes are related to learning and memory mechanisms is currently being studied by an interdisciplinary approach that involves neurochemical, neuroanatomical and behavioral techniques.

The earliest scientific account of brain changes as a result of experience that we have been able to find was written in the 1780's by an Italian anatomist, Michele Gaetano Malacarne. His experimental design is worth describing briefly, since it resembles the one we are using in our laboratory at the University of California at Berkeley. He worked with two dogs from the same litter and with two parrots, two goldfinches and two blackbirds, each pair of birds from the same clutch of eggs. He trained one member of each pair for a long period; the other member of the pair was left untrained. He then killed the animals and examined their brains. He reported that there were more folds in the cerebellum of the trained animals than in that of the untrained ones. Although his study was noted by some of his contemporaries, we have not found any evidence that others attempted to carry out similar experiments. Knowledge of Malacarne's experiment quickly faded away.

During the 19th century there was considerable interest in the relation between the size of the human head and intellectual ability and training. In the 1870's Paul Broca, a famous French phy-

sician and anthropologist, compared the head circumference of medical students and male nurses and found that the students had larger heads. Since he believed the two sets of young men were equal in ability, he concluded that the differences in head size must have been due to the differences in training. Clearly Broca's logic was not impeccable, and there are other possible explanations for the differences he found. His critics pointed to the lack of correspondence between skull size and brain volume, the important roles of age and body size in determining brain size and the relative stability of the size of the brain in comparison with the size of most other organs. By the beginning of the 20th century not only had experimenters failed to prove that training resulted in changes in the gross anatomy of the brain but also a consensus had developed that such changes could not be detected, and so the search was generally abandoned.

With the development of new biochemical tools and techniques in the 1950's, some investigators began to ask if chemical changes in the brain following training could be detected. They looked for changes at the synapses that transmit impulses from one nerve cell to another or for changes in the nucleic acids (RNA and DNA) of nerve cells. The techniques used to find chemical or anatomical changes in the brain following experience are not difficult in principle but they must be carried out with precision because many of the changes that occur are not large. Here is how a basic experiment is conducted with laboratory rats of a given strain. (In our experiments we have worked with several strains of rats and with laboratory mice and gerbils; we have observed similar effects in all these animals.) At a given age, often at weaning, sets of three males are taken from each litter. Usually

a dozen sets of three males are used in an experiment. This yields stabler and more reliable results than working with a single set, as Malacarne did.

The use of rodents for these studies is convenient for several reasons. Brain dissection is simpler in rodents than it is in carnivores or primates because the cerebral cortex of rodents is smooth and not convoluted like the cortex of higher mammals. The gray cortex can be stripped away from the underlying white matter more readily in rodents than it can in higher mammals. Rodents are small, inexpensive and bear large litters, so that littermates with the same genetic background can be assigned to different conditions. In addition, geneticists have developed inbred lines of rats and mice, and working with these inbred lines gives us further control over the genetic background.

The three male rats from each litter are assigned at random so that one rat remains in the standard laboratory colony cage, one rat is placed in an enriched environment and the third is put in an impoverished environment. It should be noted that "enriched" and "impoverished" are not used in an absolute sense but only in relation to the standard laboratory colony environment that is the usual baseline for studies in anatomy, biochemistry, physiology, nutrition and behavior.

In the standard laboratory conditions a few rats live in a cage of adequate size with food and water always present [see illustration on opposite page]. In the enriched environment several rats live in a large cage furnished with a variety of objects they can play with. A new set of playthings, drawn out of a pool of 25 objects, is placed in the cage every day. In the impoverished environment each rat lives alone in a cage. Originally the

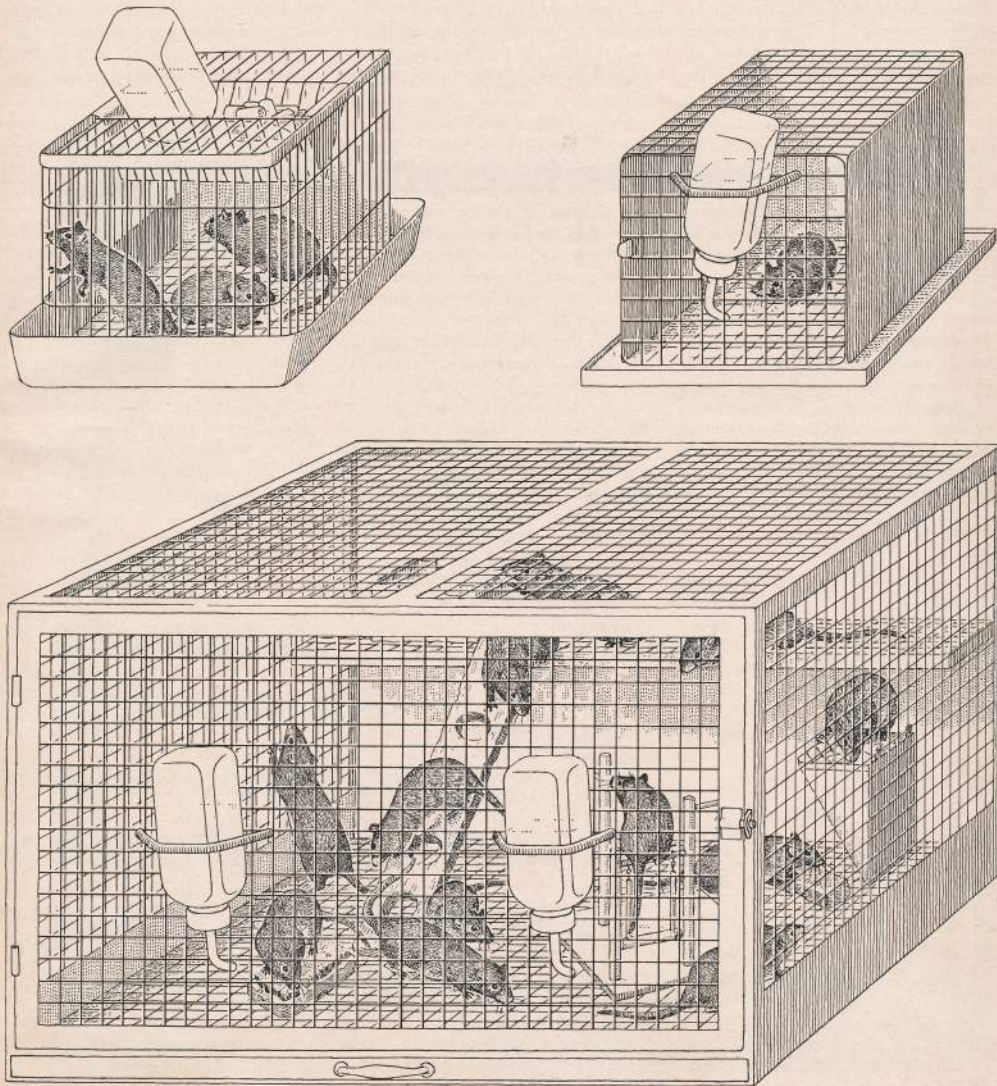
isolated rats were kept in a separate quiet room, but this turned out to be unnecessary.

At the end of a predetermined experimental period, which can be from a few days to several months, the rats are sacrificed and their brains are removed. The brain dissection and analysis of each set of three littermates are done in immediate succession but in a random order and

identified only by code number so that the person doing the dissection does not know which cage the rat comes from. With practice a skillful worker can do dissections with considerable precision and reliability. To delineate the various cortical regions a small plastic calibrated T square is used [see illustration on page 25]. Samples removed from a cortical region are weighed to the nearest tenth

of a milligram and then placed on dry ice. The samples are kept frozen until chemical analysis is performed to determine the activity of the neurotransmitter enzymes in them.

If the rat brains are to be used for anatomical studies, the animal is anesthetized and perfused with a fixative solution. Later sections of the brain are prepared for microscopy.



THREE LABORATORY ENVIRONMENTS that produce differences in brain anatomy of littermate rats are depicted. In the standard laboratory colony there are usually three rats in a cage (*upper left*). In the impoverished environment (*upper right*) a rat is kept

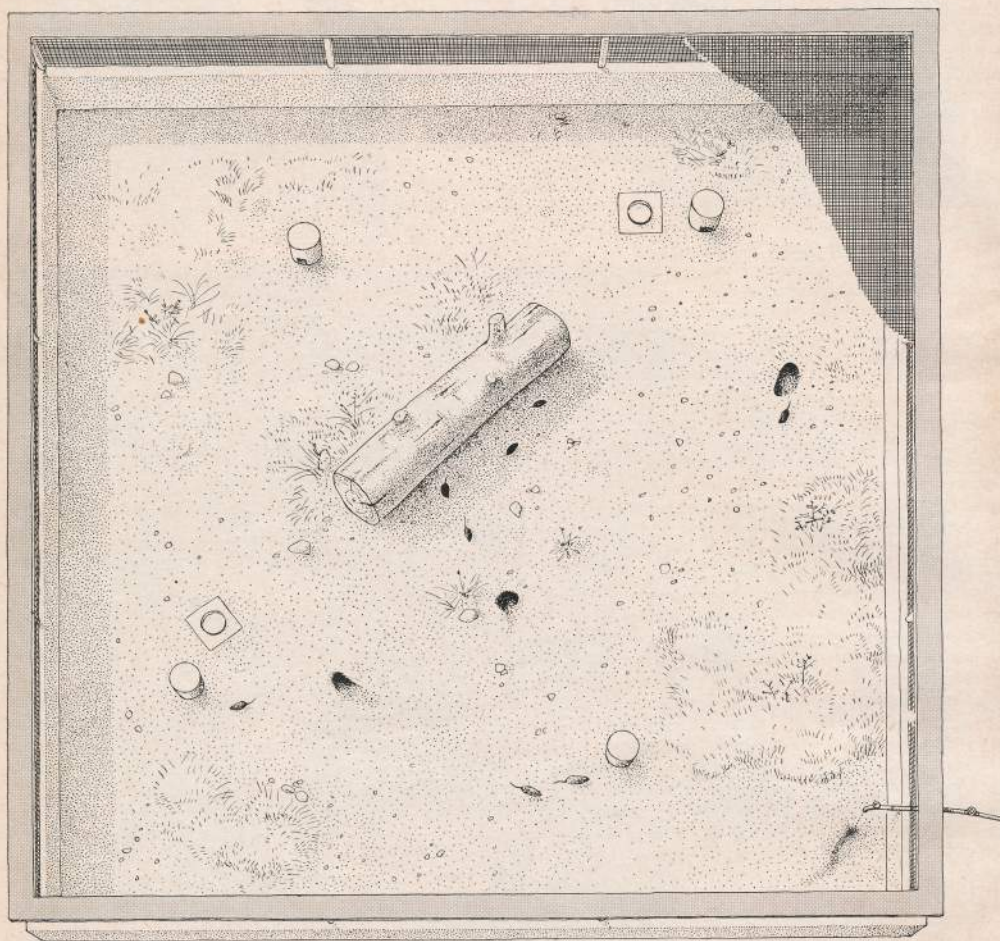
alone in a cage. In the enriched environment 12 rats live together in a large cage furnished with playthings that are changed daily. Food and water are freely available in all three environments. The rats typically remain in the same environment for 30 days or more.

In the 1950's we had been attempting to relate individual differences in the problem-solving behavior of rats to individual differences in the amount of the enzyme acetylcholinesterase in the brain. (At the time and until 1966 the psychologist David Krech was a member of the research group.) The enzyme rapidly breaks down acetylcholine, a substance that acts as a transmitter between nerve cells. The excess transmitter must be neutralized quickly because nerve impulses can follow each other at a rate of hundreds per second. This enzymatic activity is often measured in terms of tissue weight, and so in our early experi-

ments we recorded the weight of each sample of brain tissue we took for chemical analysis. We found indications that the level of brain acetylcholinesterase was altered by problem-solving tests, and this led us to look for effects of more extensive experience. To our surprise we found that different experiences not only affected the enzymatic activity but also altered the weight of the brain samples.

By 1964 we had found that rats that had spent from four to 10 weeks in the enriched or the impoverished environments differed in the following ways: rats with enriched experience had a

greater weight of cerebral cortex, a greater thickness of cortex and a greater total activity of acetylcholinesterase but less activity of the enzyme per unit of tissue weight. Moreover, rats with enriched experience had considerably greater activity of another enzyme: cholinesterase, which is found in the glial cells and blood capillaries that surround the nerve cells. Glial cells (named from the Greek word for "glue") perform a variety of functions, including transportation of materials between capillaries and nerve cells, formation of the fatty insulating sheath around the neural axons and removal of dead neural tissue.



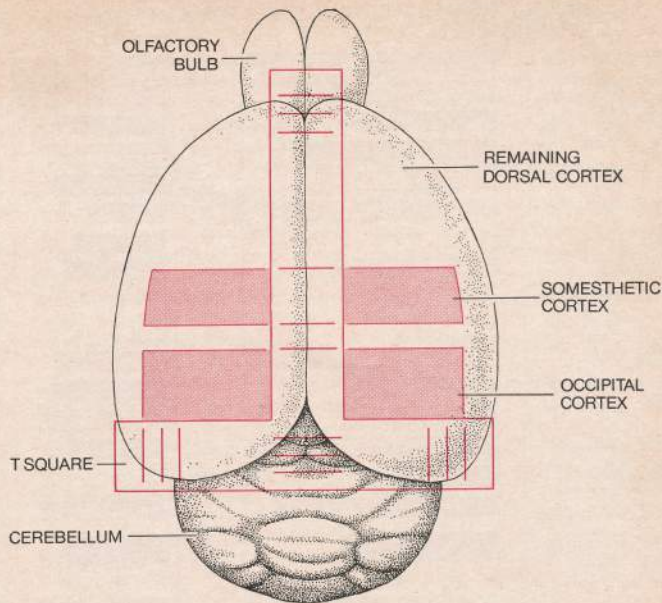
SEMINATURAL ENVIRONMENT for studying the effects of experience on the brain is provided by outdoor enclosures at the Field Station for Research in Animal Behavior at the University of California at Berkeley. The enclosures have a concrete base 30 feet

by 30 feet with a screen over the top. Inbred laboratory rats thrive in the outdoor setting when food and water are provided. The rats revert to burrowing, something that their ancestors, which had lived in laboratory cages, had not done for more than 100 generations.

We later found that there were more glial cells in rats from the enriched environment than there were in rats from the impoverished one, and this may account for the increased activity of cholinesterase. Although differences in experience did not change the number of nerve cells per unit of tissue, the enriched environment produced larger cell bodies and nuclei. These larger cell bodies indicate higher metabolic activity. Further chemical measures involving RNA and DNA pointed in the same direction. The amount of DNA per milligram of tissue decreased, presumably because the bulk of the cortex increased as the number of neurons, whose nuclei contain a fixed amount of DNA, remained relatively constant. The amount of RNA per milligram remained virtually unchanged, yielding a significant increase in the ratio of RNA to DNA, and this suggests a higher metabolic activity. In most of the experiments the greatest differences between enriched and impoverished experience were found in the occipital cortex, which is roughly the rear third of the cortical surface.

We do not know why the occipital region of the cortex is affected by enriched experience more than other regions. At first we thought that differences in visual stimulation might be responsible, but when we used blinded rats, the occipital cortex still showed significant differences between littermates from the enriched and the impoverished environments. We found the same effects when normal rats were placed in the different environments and kept in darkness for the entire period. This is not to say that deprivation of vision did not have an effect on the anatomy and chemistry of the brain. The occipital cortex of rats that were blinded or kept totally in the dark gained less weight than the occipital cortex of littermates that were raised in standard colony conditions with a normal light-dark cycle, but this did not prevent the occurrence of the enrichment-impoverishment effect.

Although the brain differences induced by environment are not large, we are confident that they are genuine. When the experiments are replicated, the same pattern of differences is found repeatedly. For example, in 16 replications between 1960 and 1969 of the basic enriched-environment-v.-impoverished-environment experiment, using the same strain of rat exposed to the experimental conditions from the age of 25 to 105 days, each experiment resulted in a greater occipital-cortex weight for the rats in the enriched environment. Twelve



CORTICAL AREAS of a rat brain are located for dissection with the aid of a calibrated plastic T square to ensure uniform samples. The desired sections are removed, weighed and stored on dry ice. The remaining cortex and the subcortex also are weighed and frozen.

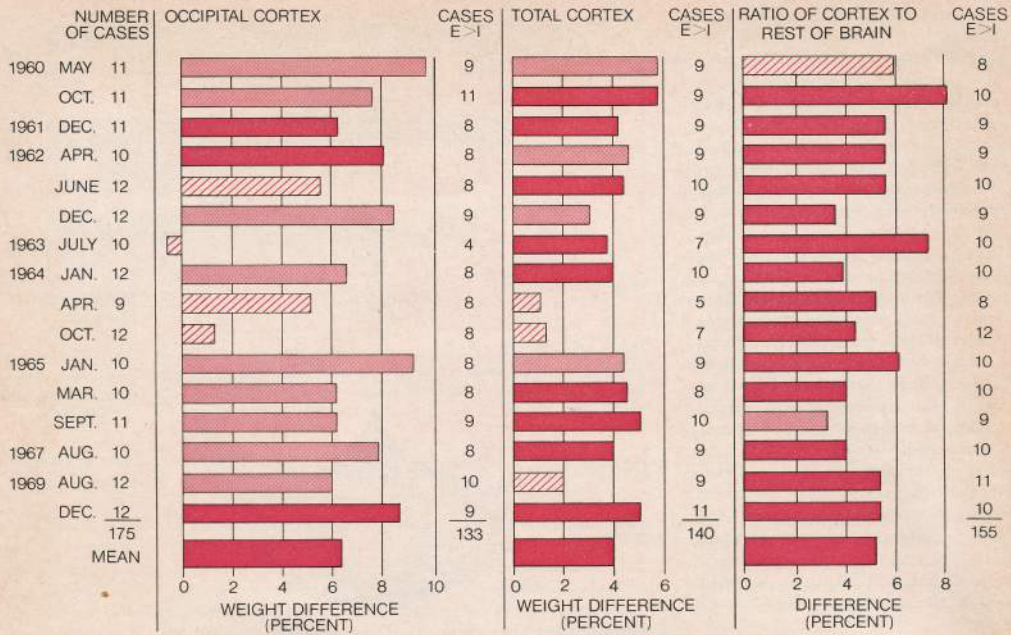
of the 16 replications were statistically at better than the .05 level, that is, for each of the 12 experiments there was less than one chance in 20 that the difference was due simply to chance or biological variability. For weight of the total cortex, 13 of the 16 experiments showed significant differences [see top illustration on next page].

The most consistent effect of experience on the brain that we found was the ratio of the weight of the cortex to the weight of the rest of the brain: the subcortex. It appears that the cortex increases in weight quite readily in response to an enriched environment, whereas the weight of the rest of the brain changes little. Moreover, since rats with larger bodies tend to have both a heavier cortex and a heavier subcortex than smaller rats, the ratio of the cortex to the rest of the brain tends to cancel the influence of body weight. For animals of a given strain, sex, age and environment the cortex/subcortex ratio tends to be the same even if the animals differ in body weight. When the environment is such that the cortex grows, the cortex/subcortex ratio shows the change very clearly and reliably. On this measure 14 of the 16 experiments were significant at the .01 level.

One of the major problems for mea-

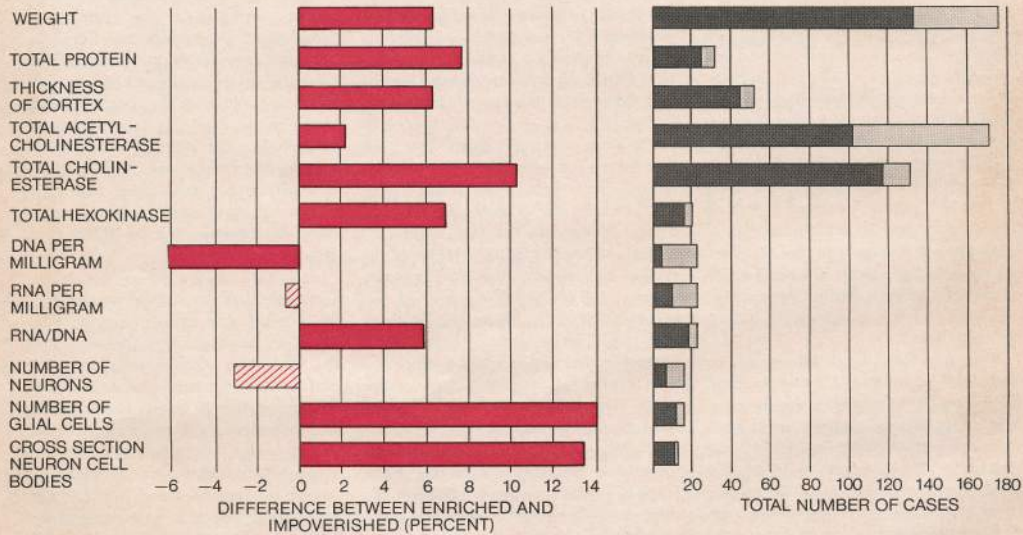
suring the effects of experience on the brain is finding an appropriate baseline. Initially we took the standard laboratory colony condition as the baseline, as most other investigators have. The cortex/subcortex-weight ratio in rats from the enriched environment is greater than the ratio in rats from the standard colony environment, and this ratio in turn is greater than the ratio in rats from the impoverished environment. Where thickness of cortex is concerned, both environmental enrichment and impoverishment are effective but on different regions of the cortex.

Suppose that the natural environment in which the animals evolved were taken as the baseline. Compared with the laboratory environments, even the enriched one, a natural environment may be much richer in learning experiences. For inbred laboratory animals, however, it is no longer clear what the natural environment is. Laboratory rats and mice have been kept for more than 100 generations in protected environments, and inbreeding has made their gene pool different from the natural one. For this reason we have begun to study wild deer mice (*Peromyscus*). The mice are trapped in the San Francisco area and brought to our laboratory; some are kept in almost



BRAIN-WEIGHT DIFFERENCES between rats from enriched environments and their littermates from impoverished environments were replicated in 16 successive experiments between 1960 and 1969 involving an 80-day period and the same strain of rat. For the occipital cortex, weight differences in three of the replications were

significant at the probability level of .01 or better (dark colored bars), nine were significant at the .05 level (light colored bars) and four were not significant (hatched bars). The ratio of the weight of the cortex to the rest of the brain proved to be the most reliable measure, with 14 of the 16 replications significant at the .01 level.



OCCIPITAL CORTEX of rats kept in enriched or impoverished environments from 25 to 105 days showed the effects of the different experiences. The occipital cortex of rats from the enriched environment, compared with that of rats from the impoverished one, was 6.4 percent heavier. This was significant at the .01 level or bet-

ter, as were most other measures (dark colored bars). Only two measures were not significant (hatched bars). The dark gray bars on the right show the number of cases in which the rat from the enriched environment exceeded its littermate from the impoverished environment in each of the measures that are listed.

natural conditions at an outdoor station and others are put into laboratory cages. The work with deer mice is still in progress, but we have also placed laboratory rats in the outdoor setting. We found that when food is provided, laboratory rats can thrive in an outdoor enclosure even in a wet winter when the temperature drops to the freezing point. When the ground was not too wet, the rats dug burrows, something their ancestors had not done for more than 100 generations. In each of eight experiments the rats kept for one month in the outdoor setting showed a greater brain development than their littermates that had been kept in enriched laboratory cages. This indicates that even the enriched laboratory environment is indeed impoverished in comparison with a natural environment.

It is possible that the brain changes we found are not the result of learning and memory but are due to other aspects of the experimental situation, such as the amount of handling and stress, or perhaps an altered rate of maturation. For example, simply handling rats, particularly young ones, is known to increase the weight of their adrenal glands. Rats in the enriched environment are handled each day when they are removed from their cage while their playthings are being changed, whereas rats in the impoverished environment are handled only once a week for weighing. We tested the effects of handling on brain changes some years ago. Some rats were handled for several minutes a day for either 30 or 60 days; their littermates were never handled. There were no differences between the handled rats and the nonhandled ones in brain weight or brain-enzyme activity. More recently rats from both the enriched and the impoverished environments were handled once a day and the usual brain differences developed.

Stress was another possible cause of the cerebral effects. Rats from the impoverished environment might have suffered from "isolation stress" and rats from the enriched environment may have been stressed by "information overload." To test this notion Walter H. Riege subjected rats to a daily routine of stress. The rats were briefly tumbled in a revolving drum or given a mild electric shock. The stress produced a significant increase in the weight of the adrenal glands but did not give rise to changes in the brain measures that we use. It seems clear that stress is not responsible for the cerebral changes we have found.

It was also possible, since some of the brain changes we have found go in the same direction as changes that occur in

normal maturation, that enriched experience simply accelerates maturation or that isolation retards it. Changes in the depth of the cerebral cortex and certain other changes resulting from an enriched environment go in the opposite direction to what is found in normal growth. The cortical thickness of standard colony rats reaches a maximum at 25 days after birth and then decreases slightly with age, whereas enriched experience causes cortical thickness to increase even in year-old rats. In fact, Riege has found that an enriched environment will produce as great an increase in brain weight in fully mature rats as it does in young rats, although the adult rats require a longer period of environmental stimulation to show the maximum effect.

The effect of enriched environment on very young rats has been tested by Dennis Malkasian. He puts sets of three litters of six-day-old rat pups and their mother either into an unfurnished cage or into a cage containing play objects. Brains were taken for anatomical analysis at 14, 19 and 28 days of age. At each age pups from the enriched environment showed a greater thickness of cerebral cortex, and in some parts of the cortex the differences were larger than those found in experiments with rats examined after weaning.

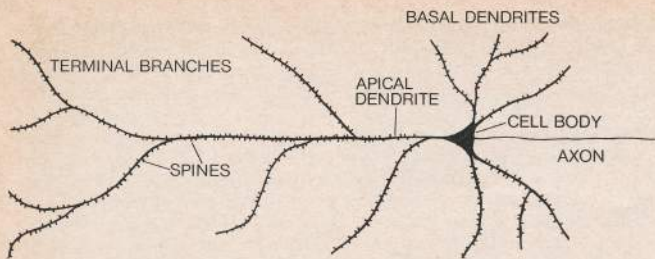
When we first reported our results other investigators were understandably skeptical, since the effect of experience on the brain had not been previously demonstrated. After our findings had been replicated, some investigators began to think that the brain may be so plastic that almost any treatment can modify it, for example merely placing a rat for 15 minutes a day in any apparatus other than its home cage. This does not happen; although cerebral changes are easier to induce than we had supposed at first, a moderate amount of experience is still necessary. We recently demonstrated that two hours of daily enriched experience over a 30-day period is sufficient to produce the typical changes in brain weight. On the other hand, placing a group of 12 rats in a large unfurnished cage for two hours a day for 30 days did not bring about significant changes in our usual brain measures. Moreover, putting rats alone in large cages with play objects for two hours a day is not very effective, probably because a single rat does not play with the objects much and tends to rest or to groom itself. The enriched environment will produce cerebral changes in a single rat if the rat is stimulated to interact with the objects. This can be done by

giving the rat a moderate dose of an excitant drug or by putting it into the enriched environment during the dark part of its daily cycle (rats are nocturnal animals). A recent experiment indicates that cerebral changes can also be achieved by putting the rat into the enriched environment after several hours of food deprivation and placing tiny pellets of food on and in the play objects.

There can now be no doubt that many aspects of brain anatomy and brain chemistry are changed by experience. Some of our most recent efforts have been directed toward determining the changes that occur at the synaptic level in the occipital cortex, a region of the brain that shows relatively large changes with experience in enriched environments. Over the past few years Albert Globus of the University of California at Irvine has been counting the number of dendritic spines in brain sections from rats that have been exposed to an enriched environment or an impoverished one in our laboratory. Most of the synaptic contacts between nerve cells in the cortex are made on the branchlike dendrites of the receiving cell or on the dendritic spines, which are small projections from the dendrites. Globus made his counts on the cortical neuron called a pyramidal cell [see top illustration on next page]. He found more spines, particularly on the basal dendrites, in rats exposed to an enriched environment than in littermates from the impoverished environment.

An even more detailed view of changes in the synaptic junctions has come out of a study we have done in collaboration with Kjeld Møllgaard of the University of Copenhagen, who spent a year in our laboratory. He prepared electron micrographs of brain sections from the third layer of the occipital cortex of rats. Measurement of the synaptic junctions revealed that rats from enriched environments had junctions that averaged approximately 50 percent larger in cross section than similar junctions in littermates from impoverished environments. The latter, however, had more synapses per unit area [see illustration on page 29].

William T. Greenough, Roger West and T. Blaise Fleischmann of the University of Illinois have also found that there is increased synaptic contact in enriched-experience rats. Some other workers have reported that increased size of synapse is associated with a decreased number of synapses, whereas decreased size of synapse is associated with an increased number. It seems that memory



DENDRITIC SPINES, tiny "thorns," or projections from the dendrites of a nerve cell, serve as receivers in many of the synaptic contacts between neurons. The drawing is of a type of cortical neuron known as the pyramidal cell. Rats from an enriched environment have more spines on these cells than their littermates from an impoverished environment.

or learning may be encoded in the brain either by the selective addition of contacts between nerve cells or by the selective removal of contacts, and that both processes may go on at the same time.

Does an enriched environment or an impoverished environment alter learning ability? Although some studies suggest that experience in an enriched environment usually improves subsequent learning, the effects are often short-lived. The result depends on many factors, for example the measure of learning that is used, the age at which the enriched experience is provided and the type of task that is learned. Early enrichment may improve subsequent learning of one task, have no effect on another task and actually impair learning in a third. Perhaps we should not expect much transfer of

capacity among entirely different kinds of behavior. Nor should we expect experience in an enriched environment to lead to an increase in "general ability"; every environment is specific and so are abilities. Harry F. Harlow of the University of Wisconsin has shown that early problem-solving in monkeys may have the deleterious effect of fixating infantile behavior patterns; such monkeys may never reach the efficient adult performance that they would have attained without the early training. Again, this result is specific and should be generalized only with caution.

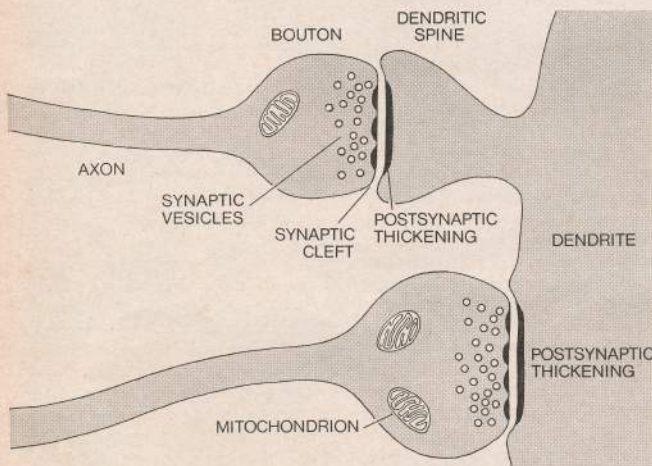
Formal training of rats, such as teaching them to press a lever in response to a signal or to run a maze, produces changes in brain anatomy and chemistry, but the type of training seems to deter-

mine the kind of changes. Victor Fedorov and his associates at the Pavlov Institute of Physiology near Leningrad found changes in brain weight and in the activity of acetylcholinesterase and cholinesterase after prolonged training of rats, but the pattern of changes is different from what we found with enriched and impoverished environments. In our laboratory we have given rats daily formal training in either operant-conditioning devices or in a series of mazes for a month or more and have found changes in brain weight and brain enzymes. These changes, however, were rather small and also had a pattern different from the changes induced by environmental experience. This is clearly a problem that requires more research.

The effect of experimental environments on the brains of animals has sometimes been cited as bearing on problems of human education. We should like to sound a cautionary note in this regard. It is difficult to extrapolate from an experiment with rats under one set of conditions to the behavior of rats under another set of conditions, and it is much riskier to extrapolate from a rat to a mouse to a monkey to a human. We have found generally similar brain changes as a result of experience in several species of rodents, and this appears to have fostered the assumption that similar results may be found with carnivores and with primates, including man. Only further research will show whether or not this is so. Animal research raises questions and allows us to test concepts and techniques, some of which may later prove useful in research with human subjects.

If this research leads to knowledge of how memories are stored in the brain, it will have obvious implications for the study of conditions that favor learning and memory and also of conditions that impair learning and the laying down of memories. Among the unfavorable conditions that are of great social concern are mental retardation and senile decline in ability to form new memories. Clues to the prevention or amelioration of these conditions could be of great social value. Let us also consider two other areas into which such research on brain plasticity may extend.

One of these areas concerns the effects of malnutrition on the development of the brain and of intelligence. Some investigators, such as R. H. Barnes and David A. Levitsky of the Cornell University Graduate School of Nutrition, have proposed that certain effects of malnutrition may actually be secondary effects of environmental impoverishment. That is,

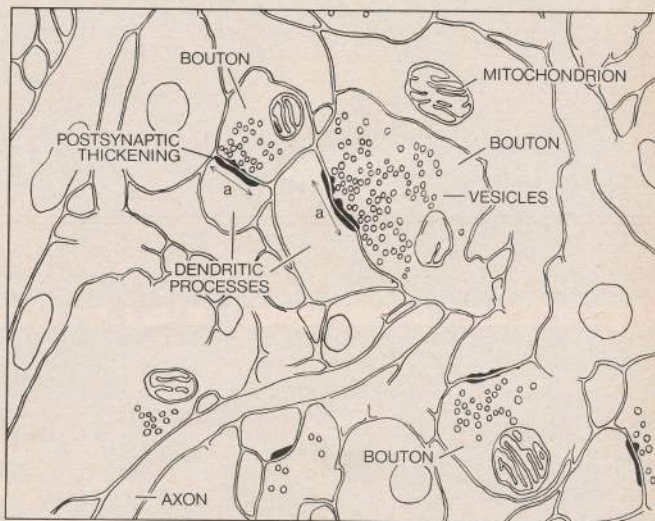


SYNAPTIC JUNCTIONS between nerve cells can be between axon and dendritic spine or between axon and the dendrite itself. The vesicles contain a chemical transmitter that is released when an electrical signal from the axon reaches the end bouton. The transmitter moves across the synaptic cleft and stimulates the postsynaptic receptor sites in the dendrite. The size of the postsynaptic membrane is thought to be an indicator of synaptic activity.

since a prominent effect of malnutrition is to make the person or animal apathetic and unresponsive to the environment, the individual then suffers from lack of stimulation, and this may be the direct cause of some of the symptoms usually associated with malnutrition. Current research suggests that some of the effects of malnutrition may be offset by programs of environmental stimulation or increased by environmental impoverishment.

Another possibly beneficial result of our research findings would be to stimulate a resurgence of attempts to determine relations between experience and brain anatomy in man. This was a topic of some interest late in the 19th century, and a number of reports were published. For example, in 1892 there was a publication on the postmortem examination of the brain of a blind deaf-mute, Laura Bridgman. It was found that the parts of her cortex that were involved in vision and hearing were thin and lacked the pattern of folding found in the normal human brain. In contrast, the region of her cortex devoted to touch had a normal appearance. It would be of interest to see if such results could be generalized by a large-scale modern postmortem study of brains of people who had been deprived of one or more senses. It would be even more interesting to find out if heightened employment of a sense leads to supranormal development of the associated brain region. Would musicians as a group, for example, show an enhanced development of the auditory cortex?

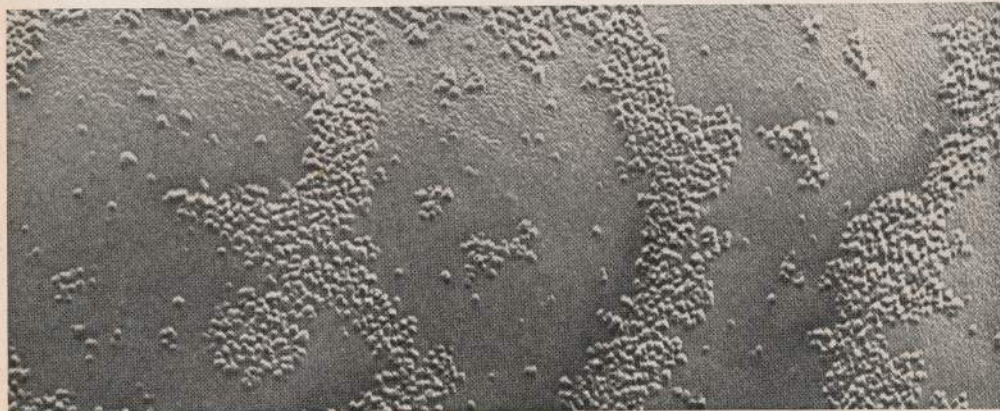
The human brain, because of the specialization of the two cerebral hemispheres, is more likely to provide answers to such questions than animal brains. Spoken words are analyzed in the auditory region of the left cerebral hemisphere, whereas music is analyzed in the auditory region of the right hemisphere. (These hemispheric functions are reversed in a few people.) The relative development of different regions in the same brain could be measured, so that the subjects would be their own control. In recent investigations Norman Geschwind and Walter Levitsky of the Harvard Medical School have found that 65 percent of the human brains they examined showed a greater anatomical development of the auditory area in the left hemisphere, 11 percent showed a greater auditory development in the right hemisphere and 24 percent showed equal development on the two sides. On the other hand, behavioral and physiological tests indicate that 96 percent of the people tested have left-hemisphere speech dom-



BRAIN SECTION from the occipital cortex of a rat is enlarged 37,000 times in this electron micrograph by Kjeld Møllgaard. The map identifies some of the components. Measurement of the postsynaptic thickening is shown in the map of the section by the arrow (a). The number of synaptic junctions was also counted. It was found that rats reared in an enriched environment had junctions approximately 50 percent larger than littermates from an impoverished environment, and the latter had more junctions, although smaller ones, per unit area.

inance and presumably have a greater development of the auditory area on that side. Is it possible that people with musical training account for most of the cases in which size of the right auditory area equals or exceeds the size of the left? In order to find out investigators will have to measure sufficient numbers of brains of individuals whose major abilities and disabilities are known. In

fact, such a program was proposed 100 years ago by Broca, but the techniques available then were not adequate to carrying out the project. Today the results of our animal studies can serve as a guide, and investigators can look more penetratingly for the anatomical and chemical changes in the human brain that are correlated with experience and learning.



EVIDENCE FOR PROTEINS within the bilayer structure of cell membranes is provided by freeze-etch electron microscopy. A suspension of membranes in water is frozen and then fractured with a sharp blade. The fracture will often split a membrane in the middle along a plane parallel to the surface. After platinum and carbon vapors are deposited along the fracture surface the specimen can be studied in the electron microscope. The micrograph at the top shows many particles 50 to 85 angstroms in diameter embedded in

a fractured membrane from rabbit red blood cells. The other two views show how the number of particles is greatly reduced if the membrane is first treated with a proteolytic enzyme that digests 45 percent (*middle*) or 70 percent (*bottom*) of the original membrane protein. The missing particles have presumably been digested by the enzyme. The membrane preparations are enlarged some 95,000 diameters in these micrographs made by L. H. Engstrom in Daniel Branton's laboratory at the University of California at Berkeley.