Experience and Plasticity in the Central Nervous System

Is the nervous system modified by experience? Are such modifications involved in learning?

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The neural mechanisms involved in learning have always excited great interest, but such are the complexities which surround their study that their analysis seemed virtually impossible. In recent years, however, there has been a great increase in knowledge of the central nervous system (CNS), and many new techniques have become available for its study. These advances have generated an upsurge of research activity into the neural bases of learning, and the field has become one of the most exciting in biology.

In this article we inquire whether the morphological, physiological, and biochemical properties of the CNS are modified by experience (*1*). Since learning is a special result of experience, we go on to consider whether any neural changes have been observed which may be related exclusively to the acquisition and storage of information.

When we consider learning we restrict ourselves to the biochemical correlates, not only because our own efforts have been in this area, but also because the difficulties of interpretation are particularly well defined. We do not discuss other biochemical approaches to the study of learning, such as the use of inhibitors of protein synthesis, or attempts to transfer learning biochemically, or electrophysiological correlates of learning, since all these areas have recently been reviewed (*2, 3*). We discuss experiments that illustrate some of the problems that arise in attempting to relate a neural change to the processes responsible for particular and lasting changes in behavior and go on to consider our own work on imprinting in the light of these problems. In conclusion we discuss whether the evidence for plasticity in the CNS can provide guidelines for further analysis of learning and memory.

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General Effects of Experience on the CNS

Fibers in the optic nerve of vertebrates make highly specific connections with neurons in the optic tectum. The formation of these linkages does not appear to depend on visual function, and the connections, once established, do not appear to exhibit any capacity for functional adaptation (*4*). Are all neurons and their connections of this kind, or are some capable of being modified by experience? Attempts to answer these questions have largely been made by studying the effects on the CNS of varying the visual experience of young animals.

Effects of Varying Visual Experience

One method of modifying the visual experience of animals is to rear them in darkness. Such treatment affects the morphology of neurons in the visual pathways (*5–9*). For example, neurons in the outer layers of the visual cortex of dark-reared mice are smaller and more densely packed than those in light-reared controls (*6*). In this cortical area, there is also a reduction in the number of spines [regions of synaptic contact (*10*)] per unit length of the apical dendrites of large pyramidal cells (*7–9*) and, in cats, a reduction in the density of the neuropil (*11*). Light deprivation may not, however, be the only difference between light- and dark-reared animals; there may also be differences in the pattern and amount of locomotor activity and in general metabolism. These factors, rather than light deprivation as such, might be responsible for the histological changes in the cerebral cortex. Indeed, similar histological changes have been described in the cerebral cortex of rats thyroidectomized shortly after birth or reared on a restricted diet (*12*). These general factors cannot, however, solely be responsible for the changes described in the cortex of the dark-reared animals. This is because rearing in the dark, unlike thyroid deficiency or inanition, appears differentially to affect the visual cortex (*7, 8, 13*).

Morphological changes can also be demonstrated in dark-reared animals that have been exposed to light. The changes affect both presynaptic and postsynaptic structures. When dark-reared rats are exposed to light for as little as 3 hours at the age of 3 weeks, changes occur in the diameter of synaptic terminals in the visual cortex (*14*). Alterations in the morphology of synaptic knobs also occur in the lateral geniculate nucleus and retina of dark-reared rats exposed to light (*15*). When mice reared in darkness are exposed to light 20 days after birth, there is an increase in the number of dendritic spines per unit length of the apical dendrites of pyramidal cells in layer 5 of the cortex. Valverde (*8*) found that the frequency of spines varied with the duration of light exposure (Fig. 1). After 4 days of exposure, spine frequency was not significantly different from that of the light-reared controls.

If the lids of one eye of a kitten are sutured shortly after birth (*16*), the occluded eye becomes functionally disconnected from the cortex. During these early weeks of postnatal life, neurons in the visual cortex of the kitten are highly sensitive to characteristics of the visual environment, and their receptive field properties can be modified by the changes in this environment (*17–19*). Although the period of maximum susceptibility of many cells may be quite brief (*20*), the actual period of susceptibility of the visual cortex appears to extend beyond 3 months of age (*21*).

The visual cortex of rats raised from birth with other rats in a large cage containing toys and running tracks (en-

riched environment) differs in a number of ways from that of rats brought up alone without such playthings (impoverished environment). In the enriched environment group, the visual cortex is thicker, the cell bodies are larger, the dendrites are more branched, and the postsynaptic thickenings in the middle layers of the visual cortex are longer than in the controls. These effects, some of which also occur in the brains of adult rats, are not solely a result of differences in the amount of visual stimulation, since the visual cortex of blind rats reared in the enriched environments differs from the cortex of rats reared in the impoverished environment (*22*). The factors responsible for these changes and the mechanisms by which they are brought about are not known.

Since variations in visual experience result in variations in some morphological and functional properties of neurons in the visual system, we might also expect that biochemical changes could be detected. Rose (*23*), in a study parallel to that of Cragg (*14*), exposed dark-reared rats to light. The incorporation of [^{3}H]lysine into acid-insoluble substances in these animals was compared with that in controls that remained in the dark. After up to 3 hours of light exposure there was a transient elevation of incorporation into the visual cortex. This elevation was followed by a depression, which gradually disappeared as the length of exposure was increased to 4 days. Similar changes (Fig. 2) have been observed at the retina and lateral geniculate nucleus (*23, 24*). This biphasic response may help to explain the contradictory reports on the biochemical consequences of varying the visual experience of animals (*25*). Elevated incorporation in the first phase is confined to a cell fraction enriched in nerve cell bodies. The increased incorporation into protein is not general. Certain specific protein fractions from the retina and visual cortex are affected differently. Incorporation into fractions enriched with glial cells is not elevated (*26, 27*). These experiments suggest that the onset of visual experience results in an enhanced synthesis of specific proteins in particular cell types in the visual pathways.

The evidence for plasticity in the adult CNS is less well documented than for the developing nervous system. Nonetheless, the adult nervous system is capable of long-lasting and specific changes as a consequence of experience, inasmuch as transmission through various neural pathways gradually declines when a stimulus is repeatedly applied (*28*). This change, which has many features in common with behavioral habituation (*29*), is found in vertebrates and invertebrates; in the latter, the change in transmission may last for many hours or days and possibly longer (*30, 31*). The adult CNS also retains a capacity for morphological adaptation that, although only demonstrated in pathological conditions, is potentially of great interest for studies of the effects of the environment on the brain. Raisman (*32*) has shown that neurons in the septal nuclear complex in the forebrain of adult rats receive afferent synaptic terminals from two sources, each of which can selectively be destroyed (Fig. 3). The distribution of the terminals from the two sources is different. When one source of afferents is damaged, it appears that afferents from the other source sprout (*33*) and form synaptic terminals in the spaces previously occupied by the other afferent fibers.

Biochemical Changes Associated with Learning

There is strong evidence, then, that the CNS is modified by experience. But is there evidence that these or similar modifications underlie learning? After all, experience frequently has general or short-lived behavioral effects that would not ordinarily be attributed to learning. Broadly speaking, learning refers to the processes involved in acquisition and storage when a particular experience exerts a specific and rela-

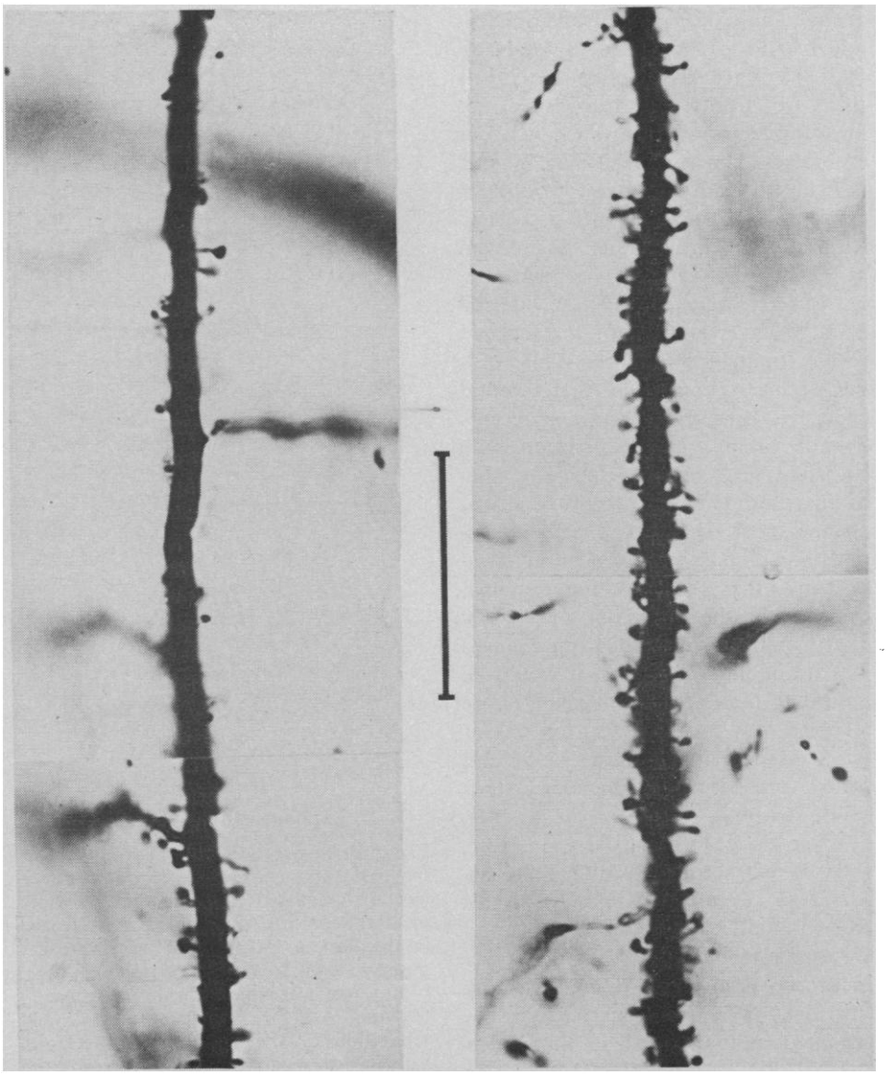

Fig. 1. Segments of apical dendrites in the visual cortex of mice raised in darkness for 20 days and then allowed to live under normal conditions for 4 days (left) and 10 days (right). Scale, 20 μm. [From Valverde (*8*)]

tively lasting effect on behavior. Admittedly, a diverse collection of phenomena—ranging from habituation to the most complex types of problem-solving—are lumped under the general category of learning (*34*). Nevertheless, without prejudging whether unity exists in mechanisms underlying learning, we can ask if any one of these phenomena is implicated when experience gives rise to changes in the CNS. In this section we discuss the problems of interpreting experiments relating biochemical changes in the nervous system to learning procedures and changes in behavior (*35*). We do so because it has often been assumed without adequate evidence that such biochemical changes are an exclusive part of the acquisition or storage mechanisms. We then describe our own work and indicate how we have attempted to deal with some of these problems.

The difficulties of finding a direct and exclusive link between a neural change and a learning process are formidable. Procedures used to train animals may have a variety of side effects that are themselves responsible for measurable neural changes (*3, 36*). For example, a shock avoidance technique is likely to lead to massive changes in concentrations of hormones associated with stress. Furthermore, a problem arises because learning processes are not directly observed; they are inferred from the behavioral changes associated with a training procedure. While it is first necessary to establish that a neural change is part of the nexus of events directly linking training conditions with a lasting and specific change of behavior, the relations between the neural change and learning may not be exclusive. The change may also be involved in many other processes. For example, even though the acquisition of a visual discrimination is dependent on changes in state of photoreceptors, it would be absurd to argue that such changes are involved only in learning. Problems of this kind are raised in more subtle and varied forms by all reductionist studies of learning.

While the goals of analysis are clear, they are extraordinarily difficult to attain. For example, Kerkut and his collaborators (*37*) have been conducting extensive biochemical studies on a cockroach preparation first devised by Horridge (*38*). The cockroach is decapitated and arranged so that when one of the metathoracic legs hangs down it dips into saline, completes an electrical circuit, and the preparation receives a shock. The preparations are highly variable, but in many the leg is eventually retracted for a sustained period so that it no longer dips into the saline and therefore avoids further shocks. Descriptively, the adaptive behavior of the leg is very similar to avoidance conditioning in intact animals, since yoked controls, which receive shock at the same time as the experimentals regardless of leg position, do not retract their legs for sustained periods. Kerkut and his colleagues have found numerous biochemical differences between the ganglia of experimentals, yoked controls, and undisturbed preparations, and suggest that some of these differences might be the basis of memory. However, the differences between experimental and yoked control preparations may lie in the extent to which the metathoracic leg was retracted. Hoyle (*39*) found that after the preparation was trained, the coxal adductor muscles, which keep the leg in a retracted position, showed a continuous discharge at a high frequency. The possibility that the biochemical measures are related to the maintained discharge in the coxal adductors is strengthened by the observation that acetylcholinesterase activity, which fell as the leg received fewer shocks, began to rise as the effects of training wore off and the muscles relaxed. At present it would be unwise to assume that the biochemical differences between experimentals and yoked (*40*) cockroach preparations are specifically related to the acquisition process.

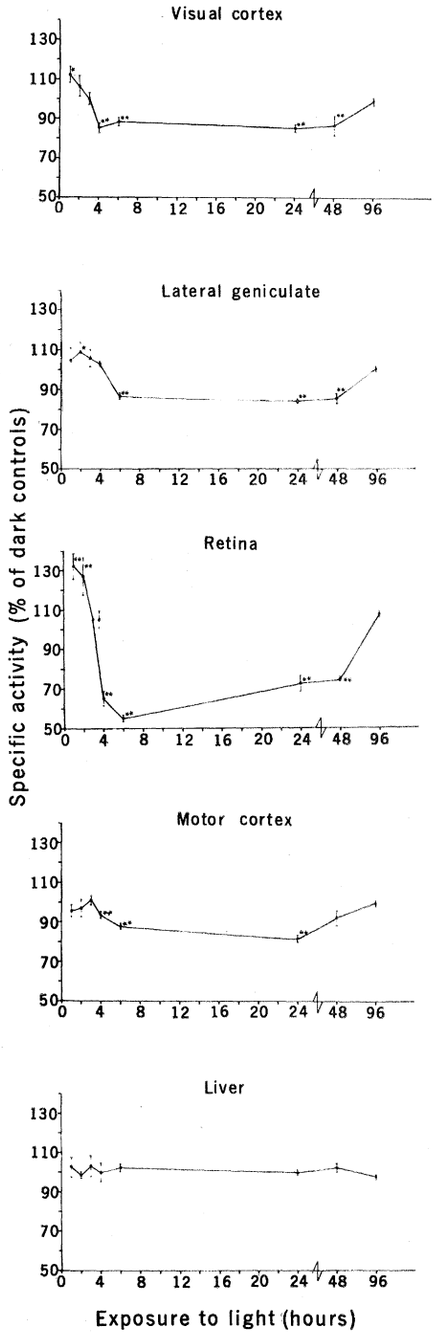

Fig. 2. Incorporation of [^{3}H]lysine following first exposure to light. Specific activities are expressed as percentage of those of dark controls. Littermate rats, reared in the dark to 50 days of age, were exposed to light for varying periods of time. [^{3}H]Lysine was injected intraperitoneally 60 minutes before killing of animals and separation of the various brain regions. Values are mean ± standard error from between 5 and 14 pairs at each time point. Significant differences compared to dark controls: *, $P < .01$; **, $P < .001$.

Comparable problems are raised by the well-known work of Hydén and Lange and their co-workers (*41, 42*). In recent years they have used rats that prefer to use one forepaw rather than the other when reaching for food pellets in a tube. In the training situation, the tube containing the food was placed against a wall so that each rat was forced to use its nonpreferred forepaw if it was to reach the food. Hydén and his collaborators concluded that in one region of the hippocampus, synthesis of S100 protein (an acidic, brain-specific protein of molecular weight 21,000) increased during initial training. Synthesis remained high when training was resumed 2 weeks afterward, but returned to the level of the control group at subsequent training after 4 weeks. Such experiments suggest that enhanced protein synthesis is correlated with acquisition rather than with maintained performance of the activity. Furthermore, injection of an antiserum specific to S100 protein 4 days after the beginning of training blocks further acquisition. The number of reaches that rats made with the nonpreferred paw leveled off, whereas

a control group continued to improve their performance. While these results strongly implicate the hippocampus and S100 protein in the processes involved in acquiring a new skill with the forepaw, the question remains: Are these processes exclusively involved in learning a new skill? May they not also be involved in many other situations that require, say, the animal's focused attention, but do not involve learning? For example, would a rat that is forced by suitable penalties or rewards to wait for a particular signal before responding—and, hence, forced to maintain a high level of vigilance—show high rates of synthesis during performance as well as during acquisition? Nothing that has been done so far provides a clear answer on this point.

Biochemical Changes during Imprinting

In our work in this area (*43–48*), we have examined the sequence of biochemical changes that occur during imprinting of young chicks. The recently hatched chick will quickly form a social attachment to a conspicuous object as a result of being exposed to it (*49*). This learning process is called imprinting and involves the first significant visual experience for the birds. We argued that the resulting cellular changes were likely to be greater than those produced by comparable visual experience later in life.

At the stage of development when learning occurs most readily, the birds show an astonishing responsiveness to conspicuous objects. They will attempt to approach for hours on end, even though they receive no additional reward for doing so. For example, in one experiment, day-old chicks were placed in running wheels from which they could see a rotating, flashing light. The chicks were positioned in this way for 12 sessions of 20 minutes interspersed with 20-minute rest periods in the dark. Even after 4 hours of training, their readiness to approach showed no signs of diminishing (Fig. 4). The birds' continued responsiveness is useful, because it is maintained long after they have learned the characteristics of the stimulus to which they are exposed. Some of the general behavioral changes, such as increased attentiveness and motor activity, that are frequently confounded with learning can thus be dissociated from the processes involved in acquisition. The recently hatched chick has an additional advantage for in vivo work in that the blood-brain barrier has not yet fully developed (*50*).

In the first series of experiments, the incorporation of [^{3}H]lysine into acid-insoluble substances was studied. Eggs were incubated and hatched in the dark. Chicks from the early part of the hatch were maintained in the dark until the start of behavioral procedures, 14 to 19 hours later. Chicks from each batch were divided into three groups. One group of chicks was maintained in the dark (dark controls), another group was exposed to overhead illumination (light controls), and another group (experimentals) was also exposed to a flashing orange light known (*51*) to be highly effective as an imprinting stimulus. The period of exposure was 105 minutes. Each chick received an injection in the heart region of 20 microcuries of [^{3}H]lysine 90 minutes before death.

At the end of the experiment, each chick was killed, and the brain was

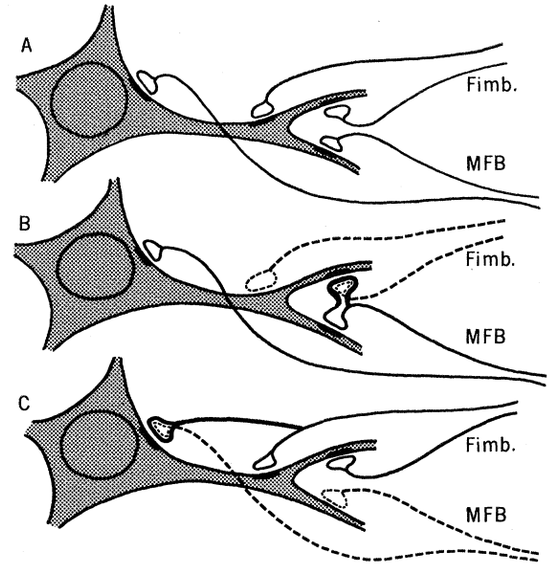

Fig. 3. Plastic changes after a lesion in the forebrain of adult rats. (A) In the normal situation, afferent fibers from the medial forebrain bundle (*MFB*) terminate in boutons on the cell soma and on dendrites, while the fimbrial fibers (*Fimb.*) are restricted in termination to the dendrites. (B) Several weeks after a lesion of the fimbria, the medial forebrain bundle fiber terminals extend across from their own sites to occupy the vacated sites, thus forming double synapses; degenerated connections, discontinuous line; presumed plastic changes, heavy black line. (C) Several weeks after a lesion of the medial forebrain bundle, the fimbrial fibers now give rise to terminals occupying somatic sites, which are presumably those vacated as a result of the former lesion. [After Raisman (*32*]

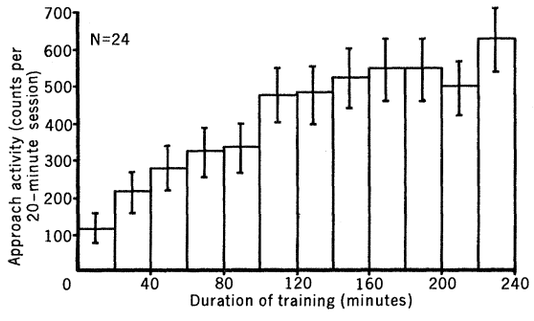

Fig. 4. Chick approach activity (mean ± S.E.) in successive 20-minute training procedures. Each day-old chick was placed for 20-minute periods in an activity wheel 50 centimeters from a flashing rotating light. Successive training sessions were separated by 20 minutes in the dark. Four counts were obtained for each complete revolution of the wheel, and 400 counts is equivalent in distance to movement of approximately 100 meters; *N*, number of animals.

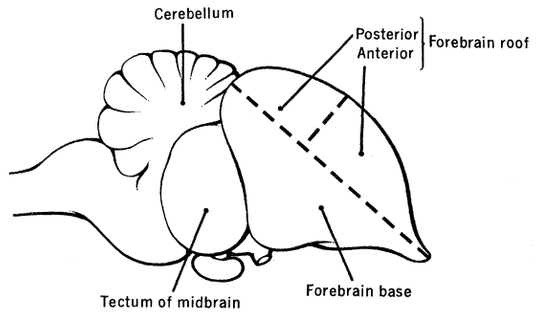

Fig. 5. Lateral view of chick brain. The forebrain was divided as shown into two the plane indicated by the longer broken line. In some experiments the roof was divided as shown into two parts. The midbrain was separated from the forebrain and from the hindbrain by vertical incisions immediately anterior and posterior to the tectum. The cerebellum and hindbrain were discarded.

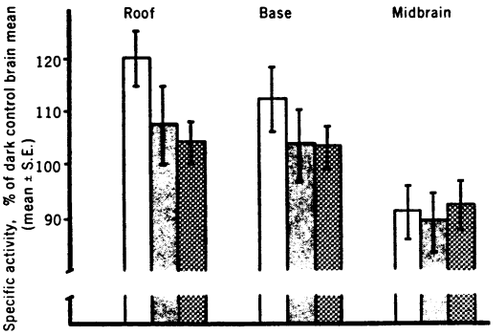

Fig. 6. Standardized specific activities for radioactive lysine incorporated into acid-insoluble substances in the forebrain roof, forebrain base, and midbrain. Open bars refer to experimental chicks ($N = 17$): light stippled bars, to light controls ($N = 18$); and heavy stippled bars, to dark controls ($N = 17$). Incorporation into the forebrain roof was significantly higher ($P < .05$) in the experimentals than in the dark controls. [From Bateson *et al.* (*43*)]

divided into three regions—the forebrain roof, the forebrain base, and the midbrain, which includes the optic tectum (Fig. 5). The brain regions were frozen on Dry Ice until they were assayed for acid-insoluble radioactivity. The specific activity measure (disintegrations per minute of acid-insoluble radioactivity per milligram of protein) was standardized for body weight and normalized between experiments. This measure is referred to as the standardized specific activity (SSA). The acid-insoluble together with the acid-soluble fractions are referred to as the "pool." All assays were done without knowledge of the behavioral treatment the chick had received.

A significant elevation of incorporation in the experimentals compared with the dark control birds occurred in the forebrain roof region; incorporation in this region for the light control group was intermediate between values for the other two groups (Fig. 6). No significant differences in incorporation were found in the other brain regions, and there were no significant differences in pool values for any region among the three groups of chicks.

If these results reflect changes of incorporation into protein, we might reasonably expect a change in the incorporation of a labeled base into RNA. If this occurred, it would not only enhance the confidence with which we might regard the results with lysine, but it might also prove a more sensitive measure of change of biochemical activity, because the rate of incorporation of RNA precursor into acid-insoluble material was much lower. After 90 minutes, 60 to 70 percent of the [^{3}H]lysine was in the acid-insoluble fraction and hence, presumably, bound to macromolecules, whereas only 4 to 6 percent of the [^{3}H]uracil was bound in this way.

In another series of experiments, chicks were again divided into dark control, light control, and experimental groups; each chick received an injection of 20 μc of [^{3}H]uracil 150 minutes before death. The periods of exposure of the experimental and light controls were 38 minutes, 76 minutes, and, in a separate experiment, 160 minutes. The SSA's were expressed as a percentage of the mean for the dark controls. The earliest significant change (Fig. 7) was an elevated incorporation into the forebrain roof of the experimental chicks after 76 minutes of exposure to the flashing light. After 160 minutes of exposure, incorporation was elevated in all brain regions of both experimental and light controls. The largest change, occurring in the experimentals, was in the midbrain. This region contains the optic tectum, in which prolonged activation by photic stimulation may, for example, create a greater demand for transmitter substances than in other regions of the brain.

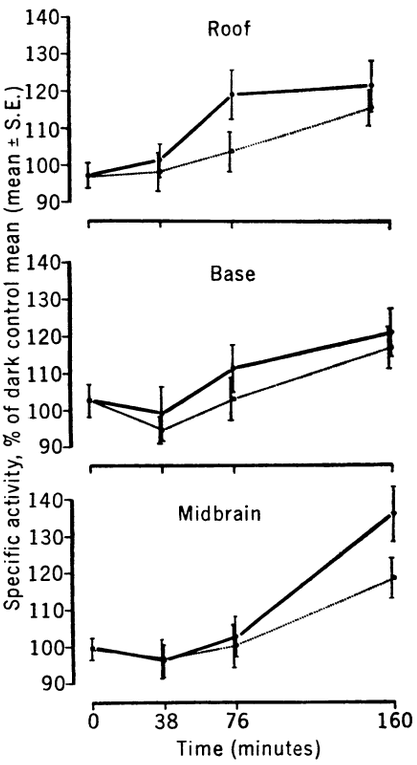

Fig. 7. Standardized specific activities (mean ± S.E.) of presumed RNA from the forebrain roofs, bases, and midbrains of differently treated chicks. The experimental birds (heavy line) were exposed to a flashing light, and the light controls (dotted line), to a continuous light. Twelve experimentals and 12 light controls received 38 minutes of exposure, 19 experimentals and 19 light controls received 76 minutes of exposure, and 18 experimental and 18 light controls received 160 minutes of exposure; 35 chicks were dark controls. [From Rose *et al.* (*45*)]

Two effects can therefore be distinguished. One of these occurred in all brain regions after prolonged exposure to light and may be a nonspecific consequence of stimulation; the other appeared in the forebrain roof after 76 minutes of exposure to the imprinting stimulus.

The enzyme RNA polymerase is necessary for the synthesis of RNA, so an enhanced level of activity of this enzyme might be expected before the enhancement of incorporation into RNA. The RNA polymerase activity in the forebrain roof of chicks exposed to a flashing light for only 30 minutes was 34 percent higher than that of the dark controls (*52*). The differences between groups for the other regions were not significant. No differences were found after 45 minutes of stimulation. This effect is obtained on an enzyme system that can be assayed in vitro and has the advantage of eliminating problems associated with fluctuations of pool size in incorporation studies in vivo, as discussed below. The activity of the enzyme adenylate cyclase was also measured in vitro. This is a predominantly membrane-bound enzyme necessary for the synthesis of adenosine 3′,5′-monophosphate (cyclic AMP). The activity of adenylate cyclase was increased in the forebrain roof of experimental chicks compared with either light or dark controls after 60 minutes of exposure to the respective conditions (*53*).

Some Biochemical Ambiguities of Interpretation

Before looking in more detail at the behavioral implications of these data, it is necessary to consider their biochemical significance. Although in the battery of changes that we have observed the change in each variable lends support to the others, there is

an ambiguity of interpretation which is central to the incorporation data that we (and others) have presented. This rests in the neat elision by which incorporation of labeled precursor tends to be equated with "net synthesis." At best, incorporation of precursor is a measure of turnover, and not of net synthesis. Another difficulty is that the actual precursor to the macromolecule is intracellular, whereas the label is injected into the bloodstream, the peritoneal cavity, or the cerebrospinal fluid. Variations in regional blood flow [which is affected by sensory stimulation (*54*)], in uptake mechanisms across cell membranes, and in the pool of the unlabeled precursor (*55*) all complicate interpretation. In an attempt to avoid these difficulties, it has become the practice to normalize the results by calculating "relative activity," that is, the ratio of bound radioactivity to total radioactivity, bound and unbound, or of bound radioactivity to unbound radioactivity. Indeed, in some cases, such as in the transfer of handedness experiments (*42*), the effect of training is only apparent when this procedure is used (*56*).

Another example of the use of the relative activity measure is provided in the work of Glassman and Wilson and their colleagues (*57*). In the experimental situation that they have most commonly used, mice were presented with a light and sound for 3 seconds before an electric shock was delivered through the metal grid floor of their cage. The experimental mice continued to receive shocks until they jumped onto a ledge. Yoked control mice were presented with the same sequence of stimuli as the experimental animals, but were unable to escape from the shock. Among other things, the incorporation of isotopically labeled uridine into nuclear and ribosomal RNA was higher in the brains of experimental animals compared to the yoked controls, especially in the diencephalon and hippocampus. More recent studies (*58*) under slightly different experimental conditions, including precursor injection by the subcutaneous route rather than the intracranial route, have led to a reassessment of these results. They are now interpreted not in terms of changes in the synthesis of macromolecules, but in terms of a reduction in the labeling of uridine monophosphate, the precursor used as the "normalizing" factor in the earlier work. The results of a further study, in which significantly increased phosphorylation of nonacid extractable nuclear proteins was found in brains of experimentals but not of yoked controls, would not appear to be affected by these complications, although other difficulties of interpretation remain (*40*).

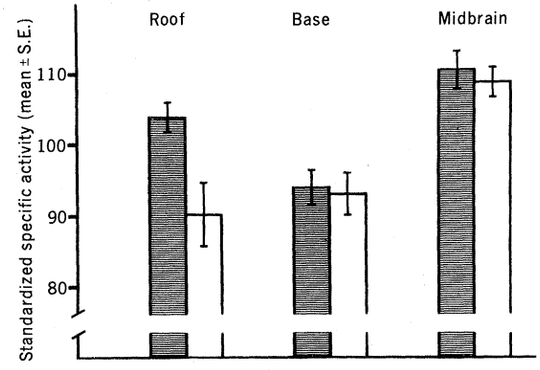

Fig. 8. Standardized specific activities (mean ± S.E.) of presumed RNA from trained sides (hatched bars) and untrained sides (open bars) of forebrain roofs, forebrain bases, and midbrains of split-brain chicks. The trained side was contralateral to the exposed eye. [From Horn *et al.* (*46*)]

Even when the uncertainty over the precursor is resolved, the relative activity measure is still only partially adequate. This is because it is calculated at a single point in time, whereas incorporation studies measure the sum of events occurring over a period during which there may be considerable fluctuation in pool between experimental and control animals. The interpretation of the ratio becomes even more difficult when the relation between bound and unbound radioactivity is complex (*44*).

It is not only uptake that may change. There may also be changes in metabolism of the precursor along other pathways. Thus, it has recently become apparent that when tritiated leucine, lysine, uracil, or uridine are used as precursors, within 1 hour of injection up to 50 percent of the free radioactivity may be found not in the precursor, but in other small molecules or in water (*59*). Provided that the rate of dissociation is not affected by the training procedure, this phenomenon cannot account for quantitative differences in incorporation between experimental and control groups. However, in double labeling experiments in which one group receives ^{14}C-labeled precursors and the other receives ^{3}H-labeled precursors, differences between experimental and control groups could result from differences in rates of dissociation of the isotopes from the precursors (*60*). All experiments with ^{3}H-labeled precursors are open to question: If the dissociated tritium exchanges with hydrogen in other precursors, the radioactivity may appear in many acid-insoluble molecules (such as protein, RNA, or DNA). As a result, the identity of the macromolecules containing the isotope will be in doubt unless collateral evidence is available.

Yet another complicating factor may be introduced by changes in low-molecular-weight precursors other than the labeled one. Thus, in the dark-reared rat there are changes in the concentrations of a number of amino acids in the free pool which might conceivably affect protein synthesis rates (*55*). Unequivocal interpretation of incorporation data would require continuous monitoring of intracellular pool sizes and specific activities, a criterion that has not been achieved in any of the experiments reviewed here. Many of these objections disappear, of course, if differential labeling between protein or RNA fractions is observed, for it is difficult to interpret such effects as being caused by changes at the precursor level. The fact that such differential labeling has been found for proteins of the retina and visual cortex in the dark-reared rat (*27*) lends confidence to the other data. Similarly, changes in enzyme activity are not open to this criticism although they are subject to others, such as interpretation in terms of activation or induction. Changed enzyme activities represent a snapshot of the situation at a particular time; incorporation measures the sum of a cumulative sequence.

Some Behavioral Ambiguities of Interpretation

If, with appropriate caution, the biochemical data are accepted at face value, we still have to consider their relation to processes responsible for a change in behavior. The imprinting experiments we have reported so far do not go very far to meet the criticism outlined above concerning the nonspecific effects of the training procedure on brain biochemistry. Many fac-

tors could account for the biochemical differences between the experimental group exposed for 76 minutes and the other chicks. The groups may, for example, have differed in the amount of motor activity and in the levels of stress to which they were subjected. Protein and RNA metabolism in the central nervous system may be affected by motor activity (*61*), by stress (*62*), and by exogenous adrenocorticotrophic hormone (*63*) or corticosterone (*64*). The experiments with exogenous hormones suggest that any procedure that modifies the endogenous concentration of these substances may have repercussions on RNA and protein synthesis in the brain. Such general effects can largely be allowed for by effectively restricting input to one side of the brain during the training procedures. If any biochemical differences exist between the "trained" and "untrained" sides of the brain, it is reasonable to ascribe them to differences in visual experience. They are unlikely to result from general changes in hormonal levels as a consequence of stress, from differences in nonvisual sensory stimulation, or from differences in motor activity between the corresponding sides of the body. The supraoptic commissure of 12 chicks was divided shortly after hatching (*46, 47*). After they had recovered from the operation, each chick had one eye covered with a patch and was exposed to a flashing yellow light for 60 minutes. The chick was then given two choice tests between the familiar flashing yellow light and an unfamiliar flashing red light, first with its trained eye exposed and then with its untrained eye uncovered. All of the chicks approached the familiar light with the originally trained eye uncovered, but not with the other eye uncovered. The incorporation of [^{3}H]-uracil into acid-insoluble substances was higher in the trained side of the forebrain roof than in the untrained side. No other regional differences between trained and untrained sides were observed (Fig. 8). No significant regional differences in pool size were found between the two sides of the brain. This rules out the possibility that incorporation of the labeled base into macromolecules can be ascribed to asymmetric changes in pool size—resulting, for example, from differences in cerebral blood flow, which can be affected by patterned visual stimulation (*54*). There are good grounds for supposing that both sides of the brain of the intact chick are trained when input is restricted to one side (*65*). We therefore expected and found no differences in incorporation of [^{3}H]-uracil between the two sides of the brain of intact chicks after monocular exposure to a flashing light (*47*).

We concluded from the "split-brain" studies that the effects of our imprinting procedure on the incorporation of uracil cannot be attributed to some of the general consequences of training. The split-brain preparation does not, however, eliminate all such nonspecific effects (*36*), and it remains possible that the biochemical changes are caused by sensory stimulation as such and are not the exclusive effects of training. In the next series of experiments (*48*), we attempted to examine this possibility.

Chicks were trained for 60 minutes on the second day after hatching, after having been trained for 20, 60, 120, or 240 minutes on the first day. We reasoned that if incorporation in some regions was specifically related to learning, birds that had been exposed for a longer period on the first day and had learned more of the characteristics of the stimulus object would show a lower rate of incorporation in those regions on the second day. In this hypothesis it is assumed that the extent to which further learning takes place diminishes as the length of training increases. We found, in the anterior part of the forebrain roof, that as the length of exposure on the previous day increased, incorporation of [^{3}H]uracil into acid-insoluble substances decreased [figure 2 of (*48*)]. No such relationship was found in any other region of the brain. As with other studies, it could be argued that the biochemical changes in the forebrain roof are correlated with vigilance on the part of the young birds as they learn. This view would have some plausibility if the chicks trained for longer periods on the first day after hatching were less responsive than other chicks to the familiar stimulus on the second day. However, if anything they approached more vigorously than the other chicks; therefore, it does not seem likely that the lower rate of incorporation in the anterior roof region of their forebrains can be explained in terms of reduced attentiveness. While these results confirmed our expectations, the data can also be interpreted in terms of a general effect on the rate of neural development (*48*).

Taken together, the results of all our experiments are consistent with the view that the rapidly occurring biochemical changes in the forebrain roof are specifically related to the training procedure, although the evidence is not yet conclusive. By degrees, we have been able to rule out a number of strong alternatives that might have explained the biochemical results. We are optimistic, therefore, about the possibility of discovering whether the biochemical changes are necessary for the development of a preference and are exclusively related to the training procedure.

Conclusions

Strong evidence indicates that the morphological and functional organization of parts of the CNS in many species can be modified by changes in internal and external environments. Many biochemical variables are also affected; in particular, the connectivity of neurons can be altered by experience. The evidence that any of these changes is intimately involved in learning is suggestive but remains inconclusive. Does the analysis of learning pose altogether new and different neurobiological problems, or has the investigation of the general neural effects of experience offered models for the further pursuit of learning? The answer lies, in part, in the relations at the behavioral level between learning and other processes with less specific outcomes.

In defining learning at the behavioral level, various distinctions are drawn to separate the effects of learning from those of other processes. Probably the most important criterion is behavioral specificity. Consider exposure of a mammal to an intense sound that destroyed part of the basilar membrane. If, as a result, no behavior patterns could subsequently be elicited by sounds of that pitch, these nonspecific effects on behavior would not usually be explained in terms of learning. A more subtle example of an effect of experience on behavior which would not normally be attributed to learning is that of exposing young kittens with both eyes open to vertical lines. Shortly after exposure, such kittens are said to be unresponsive to lines placed at right angles to the familiar orientation (*18*). Consequently, all behavior patterns dependent on the detection of lines with unfamiliar orientation would presumably no longer occur (*66*). This result differs from the effects of imprinting young birds, even though the

procedures are rather similar. Whereas the chicks cease to respond socially to objects that differ in certain characteristics from those to which they were exposed, these chicks have no difficulty in detecting unfamiliar conspicuous objects, which they actively avoid (*49*).

The criterion of specificity of effect on behavior, used for identifying learning, is easy enough to apply when classifying extreme cases, but intermediates may pose considerable problems; the distinctions may be entirely arbitrary. Consequently, it is by no means obvious that sharp discontinuities exist between behavioral changes supposedly dependent on learning and behavioral changes that are accompanied by lasting changes in other behavior patterns.

The possibility of continuities at the behavioral level raises the question of whether the neural mechanisms underlying behavioral change at one end of this spectrum can be related to those at the other end. Changes occur in the morphological and functional properties of neurons during ontogeny. The direction of these changes is such that neurons progressively lose their plasticity. The factors responsible for the termination of plasticity may be local (*67*); may be remote but internal, as with changes in hormone levels (*68*); or may be external, such as visual experience (*16–18, 69*). Spinelli *et al.* (*21*) found that once the receptive field of a neuron in the visual cortex of a kitten had been modified, the receptive field was not subsequently changed by other visual experiences. Despite the diverse ways that changes are brought about, the end result is that the functional and morphological properties of the affected neurons become rigorously defined, with a concomitant loss of plasticity.

Could similar changes underlie learning? A neuron may be functionally connected to many others, but the number of connections may become restricted as a consequence of synaptic activity initiated by the training procedure. The combinations of synaptic inputs necessary to terminate connectional plasticity might be expected to have varying grades of complexity. This view is based on studies in the mammalian visual and auditory systems, in which the features of external stimuli necessary to fire a cell are more complex as recordings are made successively from first-order to fifth-order sensory neurons. Some neurons concerned in storage would have the necessary combinations of synaptic inputs provided by relatively simple external stimuli, so these neurons would cease to be plastic quite early in life. For other modifiable neurons, the necessary combinations of input may have such a low likelihood of occurring that the connections remain plastic for a large part of the animal's life cycle.

At the biochemical level, similarities may be found between the effects of hormones on target organs (*70*) and the effects of experience on specific changes in behavior. Impulses generated in the CNS by stimuli falling on receptor surfaces during a training procedure change the membrane potential of many neurons. At modifiable neurons, such electrical changes in the membrane may lead to a changed phosphorylation of nuclear proteins (*3, 71*), to the methylation of DNA bases (*72*), or to both. With either change, modification of gene expression would occur and would lead to activation of RNA polymerase. This would be followed by enhanced synthesis of messenger RNA, polysome formation, and synthesis of particular proteins. These proteins may be involved in the modification of connectivity either by affecting synapses directly or by affecting them indirectly through enzyme action on other cell constituents, such as lipids or transmitter molecules.

If the training procedures modify the connectivity of neurons in those parts of the CNS which are necessary for the analysis of common features of the environment, such as lines and angles, the sensory capacities of the animal would be restricted. As a result the animal would not be able to discriminate certain stimuli, and all behavior patterns dependent on the detection of these stimuli would be affected. The results of this experience would be long lasting but nonspecific. If the neural changes occur in parts of the CNS which are not used to extract common features of the environment, there might be no change in the sensory capacities of the animal. In this case, the effects of the experience would be much more like the effects of training in a conventional learning situation. In behavioral terms, the consequences of a procedure having a general effect and of one having a specific effect are different. The cellular changes might, however, be identical in the two situations, although the sites of the changes in the CNS would have to differ.

Nevertheless, it is unwise to assume that one cellular mechanism underlies storage in all learning situations; there is no reason to suppose that the constraints on the storage of acquired information are nearly so limited as those on the storage of genetic information. Within one animal, storage could take place by different means. For example, storage may be represented by a growth of synaptic terminals (*73*), a change in the number of receptor sites on the postsynaptic membrane (*74*), the inactivation of synaptic transmission (*30, 75*), and so on. If the capacity to learn has evolved independently in a number of taxonomic groups (for example, cephalopods and vertebrates) and meets a variety of biological needs, it may be that storage takes place in diverse ways throughout the animal kingdom.

References and Notes

1. The terms "experience" and "plasticity" will not be defined rigorously. We use "experience" in the general sense used by T. C. Schneirla [*L'Instinct dans le Comportment des Animaux et de l'Homme* (Fondation Singer-Polignac, Masson et Cie, Paris, 1956)]: the effects of extrinsic stimulation on development and behavior. The term "plasticity" is used to refer to relatively long-lasting changes in the organization of the nervous system associated with experience or physiological stimulation.
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