is present in quantities of from 0.1 to 0.3 percent by weight.

For very dilute systems, however, the absorption due to the small number of atoms in a matrix of other atoms may appear as only a small perturbation against a large background. Researchers have increased the sensitivity of the EXAFS method by a factor of 100 to 1000 by measuring x-ray fluorescence instead of absorption. In this way, the only signal comes from the specific atoms being studied. The Bell researchers have used this technique to look at iron in a dilute solution of hemoglobin with about one iron atom for every million of other atoms. And Melvin Klein and his colleagues at the University of California, Berkeley, used the same technique to study molybdenum in the nitrogenase enzyme system. Nitrogenase enzyme is a component of the nitrifying bacteria that convert nitrogen from the atmosphere into ammonia. Few of these EXAFS studies would be practical without synchrotron radiation as a source.

A third feature of synchrotron radiation is the pulsed nature of the light. At SPEAR, for example, the pulses are 0.3 nanosecond wide and are separated by 780 nanoseconds. The pulsed nature of the radiation results because the electrons in a storage ring or synchrotron travel in bunches rather than in a continuous beam. This feature has been largely unexploited as yet, although Brown, Bachrach, and their colleagues have been able to use the pulsed time structure of the synchrotron radiation in their photoemission experiments. Nor-

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Neurochemistry: Unraveling the Mechanism of Memory

How a brain stores information has long been a puzzling problem, but in recent years advances in neurochemical techniques have provided scientists with some tools for exploring the small chemical events that occur when an individual learns something. Although the process of memory storage is still far from clear, many feel that it involves establishment of new nerve pathways or selective strengthening of old ones, probably within certain brain areas.

The field, however, is in some disarray; claims lie unproven and unrefuted, and disagreement as to the most fruitful approaches is common. Nonetheless, research into the mechanisms of memory continues to expand in scope and in importance. Because anatomical evidence is practically nonexistent and very difficult to obtain, the emphasis has been on the neurochemistry of memory. The central idea seems to be that the formation of new pathways is accomplished in part by neurochemical events, and it is the nature, location, and sequence of these events, particularly the synthesis of RNA and protein, that is the subject of many investigations.

In order to determine what changes result from learning, it is necessary to compare an animal that has learned a task to one that has not. One difficulty in making this seemingly simple comparison lies in the fact that any training experience will expose the animal to stressful situations involving such stimuli as flashing lights, shocks, handling, exercise, and frustration. Controlling for these other variables is particularly important because many of them appear to have effects on brain biochemistry similar to those believed to occur when an animal learns. Indeed, many believe that it may be impossible to design appropriate controls for these variables.

Another difficulty is that scientists do not always agree on what should be included under the term "learning." The traditional definition, modification of behavior by experience, is very vague, and might include behavior changes that may be primarily developmental. Some scientists argue that there may be different types of learning, each of which initiates different biochemical events in the nervous system.

A further problem is that the biochemistry of the brain is tremendously complex. It has recently become clear that it is very difficult, even with the techniques not available, to determine precisely what biochemical events occur during learning, and more difficult still to relate those events to memory storage. The enormous problems encountered by investigators in this field have led to the widespread belief that no easy solutions are forthcoming, but the consistent findings of many laboratories using many different kinds of learning situations suggests that progress is being made.

One of the groups that supports a broader definition of learning as almost any behavior modified by experience is a brain research group in England, including Steven Rose of Open University, Pat Bateson of Cambridge University, and Gabriel Horn of the University of Bristol. They have been investigating the biochemical changes associated with imprinting in chicks. A chick "imprints" upon, or learns to follow, its mother when it is exposed to her as a moving stimulus during a sensitive period of development within the first few days of life. Many stimuli, such as moving objects or flashing lights, can substitute for the mother, and the chick will imprint upon the substitute. Rose's group and others believe that imprinting can be considered a specific case of a more general phenomenon of learning, and thus that the neurochemical events which are triggered by exposure to an imprinting stimulus will be similar to those triggered whenever an animal learns something new.

Hypothesizing that increased RNA synthesis is associated with imprinting, Rose's group injected chicks with an isotopically labeled precursor for RNA after exposing them to an imprinting stimulus, a flashing light. Some of this labeled precursor would be used to synthesize new RNA. Later, when they extracted the RNA from the brain tissue, the amount of radioactivity incorporated into the RNA, relative to the free radioactivity, provided an estimate of how rapidly the cells were synthesizing the precursor into new RNA. The RNA from portions of the imprinted chicks' brains contained more radioactivity than that extracted either from birds exposed to plain light or from birds kept in total darkness, which suggests an increase in RNA synthesis.

Rose's group has further used this technique to investigate changes in protein metabolism. Imprinted birds incorporated more radioactive lysine into protein than did control birds, which suggests an increase in protein synthesis. This increase was observed in the same area of the brain in which the changes in RNA occurred. These results support the hypothesis that learning initiates RNA and protein synthesis.

The conclusions which can be drawn from any studies using the radioactive precursor technique are limited, however. One of the limitations is that it is very difficult to be certain that the labeled precursor is distributed evenly to all the metabolic compartments of the cells and throughout the tissue when it is injected into the animal. If the distribution is different because of the training—for example, because of changes in blood flow—then the proportion of radioactivity present in extracted fractions may be more a function of where the precursor tended to concentrate rather than where high rates of synthesis were under way. In addition, these effects might mean a change in the rate at which RNA is degraded rather than in the rate at which it

is synthesized. Despite these and other problems, investigators using this technique feel that it is sufficiently sensitive to at least suggest small changes in the rate of synthesis in portions of the brain which would go totally unnoticed if only total brain RNA were examined. Rose's group points out that other potential correlates of learning, such as changes in blood flow, which might account for the increases observed in incorporation should be no less interesting than changes in synthesis rates since they may help to explain how the animal responds to the task of remembering new information.

One way to add evidence to the hypothesis that learning initiates new RNA formation is to show, using separate biochemical techniques, that the processes which support RNA synthesis increase in activity before the changes in RNA are observed. Rose's group has found that this increase in activity is indeed the case for RNA polymerase, the enzyme used in RNA synthesis.

This group has also tried to separate the effects of learning from those of motor performance and sensory stimulation. To control for motor performance, they surgically divided the chick's brain into two hemispheres, and thus were able to use the animal as his own control. The side of the brain opposite to that eye which saw the imprinting stimulus showed increased incorporation of labeled RNA and protein compared to the "non-learning" side. These effects may have been due to the difference in sensory stimulation between the two eyes, however. One approach they are using to control for sensory stimulation is to expose chicks to the flashing lights for varying amounts of time. On the next day, all the birds were injected with radioactive precursor for RNA and exposed to the flashing light for 60 minutes. Those birds that were imprinted for short periods on the first day, and thus had the most to learn on the second day, showed the most incorporation of radioactivity into RNA. The birds that had the least to learn on the second day showed the least amount of incorporation. Although other interpretations are possible, this group believes that studies such as theirs suggest that these biochemical effects may be related to learning rather than to sensory stimulation or motor performance.

Not all scientists are convinced that imprinting is a good model for studying learning, because large developmental changes are likely to be occurring at the same time. John Wilson, Edward Glassman, and their colleagues at the University of North Carolina at Chapel Hill use a more conventional learning task, shock avoidance, in adult mice. They trained mice to avoid a foot shock by jumping onto a ledge when they were warned of the impending shock by a light and a buzzer. These mice incorporated more label into brain RNA than either untrained mice or mice that had learned the task previously, which suggests that RNA was turning over more rapidly when the learning was actually taking place.

The Chapel Hill group has also studied protein turnover rates in mice trained to avoid shock. Brain protein in trained animals showed a higher incorporation of radioactivity, which suggests that protein was also being turned over more rapidly. However, the evidence for increased protein turnover following training is not as clear as that for RNA, partly because the changes in protein are also observed in animals that are simply aroused or stressed. Although these studies indicate that a great deal of activity is going on in the brain while learning and information storage are taking place, they do not demonstrate how memories are actually stored, or how these biochemical events might alter nerve pathways.

Other Learning Tasks

Holger Hydén and his colleagues at the University of Göteborg in Sweden use both a different learning task and a different biochemical technique, and have obtained results that also implicate changes in RNA and protein metabolism. Rats generally have a preferred paw when reaching for food, but they can learn to use the other paw if it is the only way to reach the food. Hydén thus hypothesizes that learning effects should be concentrated on the side of the brain opposite to the paw that the animal is learning to use, whereas stress, sensory stimulation, and other factors should affect both sides of the brain. He compares the left side to the right side, and any differences are tentatively attributed to learning. Using an unusual microdissection technique in which the RNA content of nerve cells can be estimated, Hydén found that the cortical neurons on the "learning" side contained 40 percent more RNA than those on the other side. In further studies he concluded that the RNA from the "learning" side differed from that of the "non-learning" side in base composition. Hydén believes that this learning task probably stimulated the formation of new RNA in the side of the brain where the learning was taking place.

Several studies have attempted to determine if the biochemical changes are concentrated in specific areas of the brain. Hydén, using the task of change in paw preference, found that protein synthesis rates seemed to increase in limbic structures, such as the hippocampus, during the early stages of learning, whereas increases during the later stages were observed in the cortex, the area of the brain thought to be associated with higher intellectual processing. The hippocampus has often been implicated in long-term memory storage, partially because humans with hippocampal lesions have tremendous difficulty with long-term memory. However, there is no direct evidence that the hippocampus—or any other brain area, for that matter—is actually the site of storage.

Ivan Izquierdo and his colleagues in Sao Paulo, Brazil, are also studying the role of the hippocampus in learning and memory. They too have observed increases in the rate of RNA synthesis as well as in RNA concentration in this structure in rat brain; the increases last for about 15 minutes after training. In addition, studies done by Rose's group showed that most of the biochemical effects were concentrated in the forebrain roof of the chick brain, a structure which includes the hippocampus.

Hydén's group found a particularly large increase in a protein called S-100 which only appears in the brain. The increase appeared in parts of the hippocampus after training. Hydén reasoned that if S-100 is necessary for memory storage, injections of an antiserum for S-100 should retard or inhibit the storage process. The antiserum did indeed have an inhibiting effect on learning, but, as many scientists have pointed out, any substance that affects brain biochemistry might impair a complex behavior such as learning. A protein which is simply necessary for learning may not be related to the actual storage process. Moreover, some investigators, such as Adrian Dunn of the University of Florida, are beginning to think that it is futile to search for a single biochemical event that occurs during learning but not during stress, sensory stimulation, or arousal. Many investigators now believe that learning can probably not proceed at all without these other effects, and it may not be possible to separate them.

Dunn notes that while changes seem to be more concentrated in some areas of the brain, they are far from restricted to specific brain structures. He speculates that many of these effects might simply be due to the stress of learning and to adrenocorticotropic hormone, a pituitary hormone released in response to stress which is known to improve performance on some learning tasks. Perhaps the animal, in any novel situation, shows a gross stress response that is reflected by changes in many biochemical pathways and actually may improve the animal's ability to acquire new information rapidly. Memory storage might be located in nerve pathways that are left al-

tered after the stress response diminishes.

No research has advanced far enough yet to explain how nerve pathways might be altered by the changes which have been found to be correlated with learning. However, many scientists favor the hypothesis that pathways must be altered by changing the ability of nerve cells to transmit information to one another through effects on synapses, the connection sites between the cells. For example, the Chapel Hill group has found that there is an increase in the phosphorylation of proteins in the area of the synapses which is correlated with training. They hypothesize that this effect may be involved with rapid changes in the functioning of the synapses. Although the biochemical evidence is still sparse, William Greenough and his colleagues at the University of Illinois do have preliminary anatomical evidence that learning does indeed involve the alteration of nerve pathways. Adult rats given several weeks of training on various problems showed more branching in the outer parts of the nerve cells in the cortex compared to their littermates which were handled but given no formal training. The solution to how these structural alterations occur is clearly one of the major goals in this field.

A somewhat different approach to the problem which has many adherents involves the use of drugs to inhibit various metabolic reactions. These studies generally support the proposed hypothesis that learning initiates the formation of new RNA and protein synthesis. They have also been particularly useful in clarifying the possible timing of this sequence by showing when an injection of an inhibitor drug is capable of interfering with memory. Bernard Agranoff, of the University of Michigan, has shown that puromycin, a drug which inhibits protein synthesis, can interfere with memory storage in goldfish, but only if administered shortly after training. Goldfish injected an hour or more after training did not seem to forget the task, which involved learning to move away from a light to avoid a shock.

Samuel Barondes and his colleagues at the University of California at San Diego have used a number of drugs to inhibit memory storage by inhibiting RNA or protein synthesis. Although in general their studies support the proposed model, they believe that one of the critical variables in determining whether an inhibitor drug will affect memory storage, aside from the time of administration and the amount of the actual metabolic inhibition, is the amount of training the animal has received. A poorly learned response is very susceptible to these drugs, which suggests to these investigators that as a task is learned more pathways become involved; inhibitor drugs given after a great deal of training will have less effect. This notion is supported by studies from other laboratories in which protein synthesis inhibitors are injected directly into parts of the brain. If the injections are postponed until well after training, many areas of the brain, particularly in the cortex, must be injected in order to inhibit memory formation.

Problems with Inhibitor Studies

Studies using inhibitor drugs are open to a number of criticisms, however. Most of these drugs have diverse side effects apart from protein or RNA synthesis inhibition, and many scientists point out that it is not certain that the drugs' specific effects on synthesis account for the inhibition of memory storage. Larry Squire of the University of California at San Diego tries to circumvent this problem by using a number of different drugs, all of which inhibit the same metabolic pathway but in different ways. These drugs have very few, or at least nonoverlapping, side effects. A number of protein synthesis inhibitors that meet these qualifications are available, and this approach has further implicated protein synthesis in memory storage.

The drug inhibitor studies are also potentially very useful in demonstrating that some metabolic pathways are *not* involved in memory storage. For example, Agranoff's group has shown that the inhibition of DNA synthesis does not impair memory storage. There is some suggestion from the synthesis inhibitor studies that short-term memory may be a somewhat distinct physiological process from long-term memory. Barondes and his colleagues have shown that if a drug that inhibits protein synthesis is injected before training, the animals can remember the task for a few hours but their retention is severly impaired afterward; this suggests that the inhibition only affected long-term memory.

Some investigators have suggested that specific macromolecules may be synthesized in association with each memory, although this viewpoint is controversial. George Ungar of Baylor College of Medicine favors the possibility that information might be stored in the arrangement of amino acids in certain proteins. He trained rats to avoid the dark compartment of a two-chambered box and injected extracts of the trained rat brain into untrained rats, trying to isolate a substance which would improve their learning performance on this task. He has isolated and indeed synthesized a peptide, now called "scotophobin" (from the Greek *scotos* meaning darkness and *phobos* meaning fear); when it was injected into untrained rats, it improved their performance. Few laboratories have been able to replicate this "memory transfer" phenomenon by the injection of scotophobin, but some have reported that scotophobin isolated from rat brain even improves dark avoidance learning in mice and goldfish. However, doubt exists about whether the peptide is actually the stored memory for dark avoidance, or whether it simply improves performance by raising the arousal level of the untrained animal. It has been pointed out, for example, that part of the scotophobin chain resembles adrenocorticotrophic hormone, which improves learning performance on many tasks, perhaps by raising arousal levels.

Neurochemical analysis is only one of the ways to study memory storage, and the entrance of many critical biochemists into the field has provided a valuable conservatism about what can be discovered using neurochemical techniques. Certainly very few scientists are now willing to state unequivocally, on the basis of information obtained from biochemical studies, that learning initiates new RNA and protein synthesis. On the other hand, the large majority of recent studies has consistently supported this hypothesis, and most scientists agree that it is a very viable model.

What role these changes might play in the process of storing memory is unknown, however, and other approaches are clearly necessary. Studies of the changes in the electrical activity of single neurons and neuronal pathways, for example, may further the understanding of how and where these biochemical events might alter synaptic transmission. Rapid advances have recently been made in the study of fairly simple learning systems in invertebrates. Many neurophysiologists believe that this approach is particularly valuable because the number of nerves involved in learning is relatively small, and nerve pathways can be mapped. Experiments in which lesions are made in areas of the brain after training can help to identify specific areas that are necessary for memory formation. Electroconvulsive shock, which seems to affect both the electrical and biochemical activity of the brain, also inhibits certain types of memory formation, and many studies using this amnestic agent have contributed a great deal to the understanding of learning and memory.

Although only the first few steps have been taken, it is clear that the brain responds to the challenge of remembering new information in ways that are predictable, and that are likely to be further clarified in the near future.

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