From Genes to Cognition

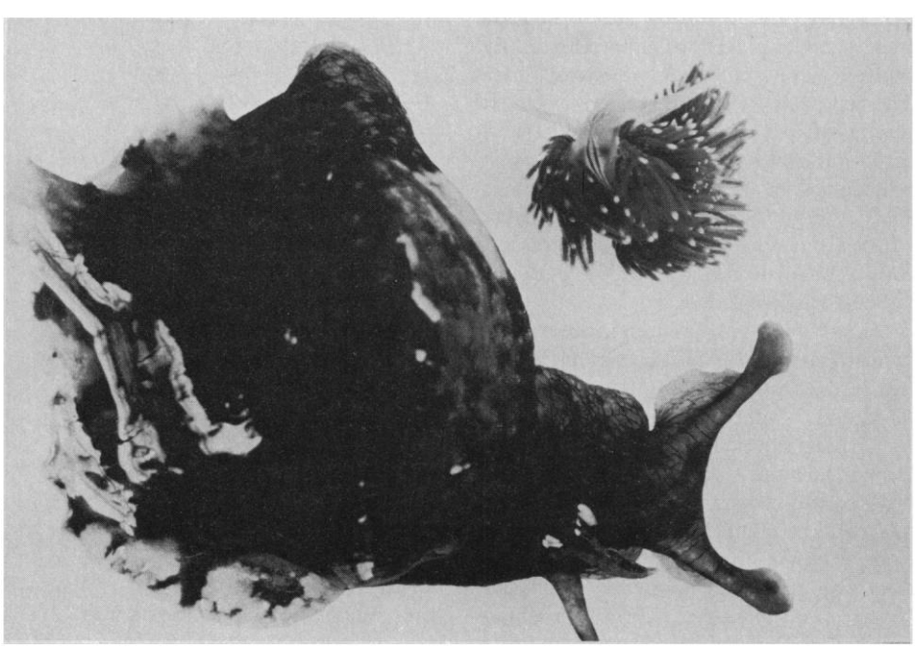
Neuronal activity triggers rapid changes that somehow lead to stable modifications necessary for permanent memory

One of the remarkable properties of the brain is that it takes a fraction of a second to learn, but the memory can last forever. Just by reading these words, you are making use of your ability to learn and remember. How does it happen? Will you remember tomorrow parts of what you read today? How is your brain changing, in temporary and permanent ways, as you learn and remember?

These were the kinds of questions that participants considered at a recent Dahlem meeting in West Berlin.* There was a good deal of debate about suitable definitions of learning and memory, which demonstrated that these basic issues in neuroscience are still a long way from being resolved. Nevertheless, the conference produced a unique synthesis of information about the bases of learning, inspired to a great extent by new approaches and new results in the field.

Regardless of debates about general definitions, researchers agree that neuronal activity is fundamental to the learning process, and that it induces transient and permanent changes at many different levels within the nervous system. One can view these levels as a hierarchy of increasing complexity. At the most basic level, molecular changes occur within single neurons. Next, there is communication among neurons at synapses. Higher still are circuits of interconnected neurons. And at the top is activity within whole assemblies of neurons, which might control complex patterns of behavior.

Dahlem participants divided themselves into four groups, each of which focused on just one level in the hierarchy. From their discussions it became clear that, thanks to the development of certain model systems for learning, changes in individual nerve cells and at synapses are beginning to be understood. The study of circuits is advancing too, with many important contributions from developmental neurobiology. But getting a firm grip on neuronal assemblies and their functions is extremely difficult, and it remains largely a theoretical field.



Dan McCoy/Rainbow

Two marine snails, *Aplysia* (left) and *Hermissenda* (right), *are providing valuable information about fundamental cellular changes involved in learning and memory.*

At the level of changes within a single cell, there is now a good deal of support for the suggestion that protein synthesis is modified by activity, which either modulates already active genes, or switches on inactive genes.

For instance, Jean-Pierre Changeux and his colleagues at the Pasteur Institute in Paris have been able to show an increase in the synthesis of acetylcholine receptor protein and its messenger RNA in muscle when electrical activity is blocked. In addition, Terje Lømo and his co-workers at the Institute of Neurophysiology in Oslo demonstrated that the pattern of direct electrical stimulation in muscle influences what type of myosin (contractile protein) it makes. Both model systems in muscle tissue involve changes in gene expression.

Now, neuroscientists are showing that changes in gene regulation also occur in nerve cells when patterns of activity vary. For instance, neuronal activity regulates the production of an enzyme, tyrosine hydroxylase, that controls the synthesis of catecholamine transmitters. According to Hans Thoenen, of the Max-Planck-Institute for Psychiatry in Planegg-Martinsried, West Germany, the key signals for making more enzyme are activation of acetylcholine receptors and a bulk influx of sodium ions. Recently, Jacques Mallet and his colleagues at the National Center for Scientific Research in Gif-sur-Yvette, France, cloned the gene for tyrosine hydroxylase, making it possible to study molecular controls over its expression.

But the closest correlation between learning and changes in protein synthesis comes from Eric Kandel and his colleagues of the Howard Hughes Medical Institute at Columbia. In the past few months they have found that protein synthesis may be required for long-term learning in the marine snail, *Aplysia*. Samuel Schacher and his colleagues, Philip Goelet and Piergiorgio Montarolo, collaborated with Kandel and have

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shown that messenger RNA or protein synthesis inhibitors block long-term, but not short-term, changes associated with learning in cultured *Aplysia* neurons.

Protein synthesis can be modified by a number of mechanisms, depending upon whether transcription or translation processes are primarily affected. For example, if activity alters how binding proteins interact with DNA, the changes in messenger RNA and protein synthesis might be stable enough to account for long-term learning.

Studies at the next level of change concern how individual nerve cells communicate with each other. Researchers who specialize in synaptic transmission are developing model systems and use both invertebrates and vertebrates for their experiments. Four of these models, in particular, are providing new information about changes in ion channels and second messenger substances that occur when activity increases to a threshold level.

Some of the best correlations between learned behaviors and changes in neuronal communication come from the marine invertebrates, *Aplysia* and *Hermissenda*. Sensory neurons of both molluscs receive input from the environment and other neurons, and provide output to motor neurons controlling the animal's behavior.

Researchers do not usually emphasize similarities between *Aplysia* and *Hermissenda*, but some parallels are obvious. For example, Kandel's group at Columbia, Thomas Carew and his colleagues at Yale University, and John Byrne and his coworkers at the University of Texas Medical School in Houston, study different forms of learning in *Aplysia*. In each case a neurotransmitter mediates specific changes—closure of certain potassium ion channels, increased levels of intracellular calcium, and enhanced transmitter release.

Daniel Alkon of the National Institute of Neurological and Communicative Disorders and Stroke and the Marine Biological Laboratory in Woods Hole, Massachusetts, and his colleagues study short- and long-term learning in *Hermissenda*. They find that light and neuronal stimulation trigger calcium entry into photoreceptor cells, and that two populations of potassium channels close.

While some investigators are capitalizing on relatively simple invertebrate models for learning, other researchers study changes in the mammalian brain. Richard Thompson, of Stanford University, and Theodore Berger, of the University of Pittsburgh, showed that synaptic communication in the rat hippocampus increases when animals are trained. According to Timothy Bliss, of the National Institute for Medical Research in London, initiating this phenomenon, called long-term potentiation, probably requires more transmitter release from presynaptic neurons together with changes in postsynaptic cells.

Recently, Masao Ito and his colleagues at the University of Tokyo found a decrease, instead of an increase, in synaptic transmission when rabbits undergo classical conditioning. The change is termed long-term depression and it occurs in the cerebellar region of the brain. Apparently, the large Purkinje cells of the cerebellum become less sensitive to an excitatory neurotransmitter, and their responses are depressed.

In all four models for learning, increased calcium influx triggers changes in synaptic transmission. In *Aplysia*, *Hermissenda*, and the hippocampus, neurotransmitters stimulate second messenger systems, protein phosphorylation, and ion channel closure. Dahlem conferees proposed that these transient ionic and molecular events might lead to long-lasting changes in protein synthesis, gene expression, and neuronal structure.

Researchers who study changes during learning at the level of neuronal circuitry are seeking clues from developmental neurobiology. According to Pasko Rakic of Yale University, "learning during development has a very big influence on neuronal circuitry. There are probably more circuitry changes during development than during adulthood."

Recent data from Rakic's lab indicate that major changes in synapse density are genetically determined. During early postnatal development in mammals, there is a sharp rise in the number of synapses in various areas of the cerebral cortex. The density of synapses falls, just as dramatically, to a level that is surprisingly constant in all cortical areas throughout adulthood.

Activity seems to play an important role where the strength of synaptic connections within neuronal circuits is concerned. These more subtle changes probably occur during learning both in developing and adult brains. For example, during the development of the visual cortex in mammals, an immature synapse may stabilize only if the cell is active. And researchers agree that activity must reach a certain threshold level in order for synaptic connections to strengthen.

Many neuroscientists believe that, when several neurons synapsing on the same cell fire together, they activate the cell to this threshold level. With simultaneous firing among neurons, a competitive situation at synapses exists. Some synapses may strengthen while others weaken. There is also some evidence that new synapses have a competitive edge over old ones.

Deborah Barnes

Mark Konishi (left) and Jean-Pierre Changeux (right) *were the scientific organizers of a recent Dahlem conference on the neural and molecular bases of learning.*

Dahlem participants again pointed to calcium ion influx as a major factor in modifying neuronal circuitry. Wolf Singer, of the Max-Planck-Institute for Brain Research in Frankfurt, also identified modulatory neurotransmitters like acetylcholine and norepinephrine as playing key roles. Both close potassium channels, causing depolarization and increasing the effect of incoming excitatory activity. This could stimulate more calcium entry, trigger second messenger systems, and possibly promote structural changes.

Researchers who study neuronal assemblies and their importance in learning grapple with an inexhaustible supply of theories and a relative dearth of data. They have gleaned much of their information from changes in different areas of primate sensory cortex under experimental conditions, and from clinical studies of individuals who have specific learning and memory deficits.

Neuroscientists generally agree that different forms of learning and memory exist. The clearest example occurs in someone suffering from global anterograde amnesia.

"The changes for learning must be at synapses where circuits are recruited into functional assemblies." Wolf Singer.

This individual retains the ability to learn new skills or habits, but is unable to acquire new cognitive memory. Based largely on this example, researchers segregate procedural learning, or habit formation, from cognitive learning. But they debate about how neuronal assemblies fit into the picture.

For instance, Mark Konishi of the California Institute of Technology in Pasadena thinks there are qualitative differences between learning and memory in lower organisms and in man. He sees a neuronal assembly as a population of neurons that performs a function, such as song production in birds, or coordination of walking or swimming. Konishi thinks simple organisms, like *Aplysia* and *Hermissenda*, have neuronal assemblies, although their form of learning is mostly procedural. But at the same time, he says, "the term assembly is unfortunate because it is theoretical and hypothetical."

In contrast, Mortimer Mishkin of the National Institute of Mental Health associates a cell assembly more with cognitive processes. He thinks that the brains of higher organisms, including man, store copies or representations of activity present when some object in the environment is processed by the brain. He calls the storage of this form of learning representational memory and equates it with cognitive memory. Mishkin views the brain's stored copy of a representation as a neuronal assembly.

Despite their unending struggle to define terms and processes, Dahlem participants agreed that neuronal assemblies are coactivated sets of neurons, capable of changing depending upon the kind of activity they experience. They theorized that a Darwinian-like selection process may operate during the generation of neuronal assemblies. Certain patterns of cooperative activity might reinforce and stabilize connections within the assembly. Thus, one assembly could survive another because it experiences more cooperative activity, from within itself and from external sources.

Understanding the bases for learning from gene regulation to functional neuronal assemblies is an ambitious objective. But the recent Dahlem conference left behind some basic principles and observations.

Coactivation of neurons is fundamental to the learning process. Researchers have evidence from models of gene regulation, long-term potentiation in hippocampus, synapse maturation during development, and neuronal assembly formation that activity of presynaptic and postsynaptic cells is important for long-term modifications. But why?

Neurons are more likely to achieve a threshold level of activity, triggering calcium entry, when they fire together. Calcium itself functions as a second messenger and enhances other second messenger systems, stimulated by neurotransmitters like norepinephrine, serotonin, and acetylcholine.

"How one synapse learns tells you nothing about what can be learned." Pasko Rakic.

Such relatively transient changes may set the stage for longer lasting ones in gene expression, increased neurotransmitter synthesis, and membrane structures. Some changes may be highly localized and restricted to specific synapses. Others may be neuron-wide and affect all the synapses on a cell, which in turn, could induce changes in more neurons. ■ DEBORAH M. BARNES

What Does It Mean to Be Random?

A new theory of randomness multipliers may explain what randomness is and why most phenomena that are thought to be random are not so random after all

PERSI Diaconis has spent much of the past several years thinking about randomness—a subject that is proving to be as slippery as it is important. Diaconis, a statistician from Stanford University who is spending this year at Harvard, says his work is leading him to conclude that, "the more you think about randomness, the less random things become. But sometimes you can take advantage of a lack of randomness."

In particular, Diaconis and his colleagues Bradley Efron and Eduardo Engle are developing a tool that they call randomness multipliers that, Diaconis explains, "take a small amount of uncertainty and deterministically produce highly random outcomes." As a direct consequence of this work, these researchers have, for the first time, a quantitative definition of chaos.

This is not the first time that researchers have tried to analyze randomness, of course. "There have been heroic efforts to understand randomness," Efron remarks. But the concept remains elusive. "Randomness is not an easy concept to define," says Efron. In fact, books on probability do not even attempt to define it. "It's like the concept of a point in geometry books," Efron says. "What we're trying to do is like taking points apart and seeing what's inside."

Randomness is of interest not only to statisticians, Diaconis stresses. It touches all aspects of life and can have political and sociological importance. For example, in 1970, a draft lottery was carried out and, in order to make the drawing appear as fair as possible, the planners decided to have individuals pick birthdays out of an urn. The 365 days were put in capsules and placed in the urn, the urn was shaken for several hours, and then the capsules were chosen, one by one, from the urn. But, says Diaconis, "when you looked at the data, it was very striking that the dates were not random." Birthdays in December tended to be drawn first, those in November next, then those in October, and so on.

The reason the draft lottery drawing was not random is that the capsules were put in the urn according to a definite pattern. The January birthdays were put in the urn first and fell to the bottom, the February capsules