Neuroscientists Seek Answers To Brain Function and Disease

WASHINGTON, D.C.—Neuroscientists gathered here late last month for their largest annual meeting ever. The presentations covered a wide range of topics, from spinal cord injury and obesity (*Science*, 29 November, p. 1466) to learning and neurodegenerative disease.

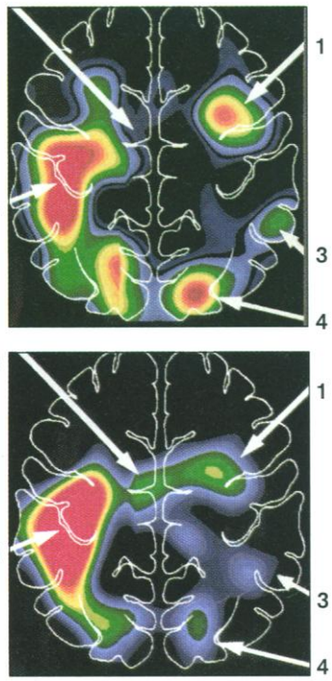
The Anatomy of Learning

Neuroscientists have known for some time that there is no "learning center" in the brain; instead, various parts of the brain work together like cogs in a machine to learn, store, and reproduce even the simplest task. But how does the brain divide the labor? Which brain areas are responsible for which parts of the learning? In an experiment reported at this year's Society for Neuroscience meeting, Steve Petersen and Hanneke van Mier of Washington University in St. Louis have made a first step toward addressing that question for the learning of a simple motor task.

By imaging the brains of human subjects as they learned to trace a maze with a pen, the researchers were able to see activity shifts in different brain regions as different elements of learning the skill came to the fore—such as reducing pauses and errors, then doing the task faster. Neuroscientist Peter Strick of the State University of New York Health Science Center at Syracuse calls the work a "great first attempt" at dissecting the roles of different brain areas in task learning. "How we take individual movements and build meaningful sequences of movements ... is one of the challenges for motor-systems physiology," he says. "This work is beginning to get at that."

Van Mier and Petersen used positron emission tomography to image metabolic activity in the brains of 14 subjects during six 1-minute sessions in which they practiced finding their way through a simple maze with a pen. Over the course of the six trials, the activation of certain brain areas changed. By tracking the subjects' improvement on different aspects of the task, the researchers identified distinct learning curves. Then, by correlating those curves with changes in brain activation, they could link individual brain areas to different elements of the task.

For example, subjects paused often in the maze during the first trial—a phase of learning that was correlated with high activity in the right premotor cortex. The number and duration of those pauses dropped rapidly in the next few trials, however, corresponding to a drop in premotor cortex activity and a rise in activity in the supplementary motor area. The subjects' errors followed a different curve, staying high for the first two trials before they fell. And as errors dropped, so did activity in the left cerebellum and right parietal cortex. In the last few trials, with stops and errors reduced, the subjects' speed in tracing the maze increased, mirrored by stepped-up activity in the primary motor cortex. That rise seemed to be simply a reflection of the speed with which the subject moved the pen, because it occurred even on a control task that involves no learning. But the other correlations suggest, says Petersen, that "different brain regions are responsible for these different phases" of learning.



HANNEKE VAN MIER

Signs of learning. From the first *(top)* to the sixth *(bottom)* maze-learning trial, activity in right premotor cortex (1) and parietal areas (3, 4) drops, while supplementary motor area (2) and primary motor cortex (6) activity increase.

Some of the associations make sense, while others remain a puzzle. The premotor cortex's activity early in maze learning fits with its apparent role in planning movements. And the link between activation of the supplementary motor area and the reduction of pauses, says van Mier, agrees with monkey studies showing that the area is activated when monkeys learn a sequence of movements. "If you stop less, that means you have learned the sequence" of movements needed to trace the maze, says van Mier. "It is possible that the supplementary motor area stores the sequential elements of this task."

Likewise, "we have some notion about the cerebellum being involved in the tuning of behavior, in getting it right," says Strick. The fall in cerebellar activity with the reduction in errors is consistent with that idea, he says. Association of the parietal cortex with error reduction "is a little tougher to explain," says Petersen, although that brain area has been linked to spatial awareness, so it is not surprising that it is somehow involved in maze learning.

But Petersen is reluctant to spin too many stories to explain the correlations at this early stage in the work. Strick agrees that it "may be premature" to jump to specific conclusions about brain-area functions from this experiment alone. The exciting thing about the finding, he says, is that it generates new and testable hypotheses about how brain areas share the job of learning.

–Marcia Barinaga

Faulty Protein Linked to ALS

When scientists found the gene at fault in some cases of the neurodegenerative disease amyotrophic lateral sclerosis a few years ago, the discovery was the first real clue to what might cause ALS. But mutations in the gene, which makes an enzyme called superoxide dismutase (SOD1), account for only about 1% of all cases of ALS—also known as Lou Gehrig's disease—which causes a gradual paralysis and usually results in death. And it is still unclear whether SOD1 defects contribute to unexplained "sporadic" cases, which are not inherited and which constitute 95% of all cases. Now a team led by Johns Hopkins University neurologist Jeffrey Rothstein has uncovered an abnormality that seems to be widespread in patients with sporadic ALS.

In a poster session here, Rothstein and his colleagues presented evidence that a brain protein called EAAT2 is improperly made in almost half of the patients they studied. If confirmed, Rothstein says, the finding would strengthen earlier suspicions that a deficiency of the protein, which deactivates and recycles the neurotransmitter glutamate, is a "potential cause or contributor" to the disease, because such a deficiency could lead to a glutamate buildup. Glutamate is toxic to cells in high concentrations, and some researchers have theorized that the paralysis of ALS might be caused by increased levels of the neurotransmitter and the resulting death of neurons that control the muscles.

Albert Ludolph, a neuroscientist at the University of Ulm in Germany, is cautious about the results, which are not yet published. "It's a promising finding that needs confirmation," he says. "It's another piece in the puzzle

we're trying to solve." Rothstein agrees. "We don't believe there's only one cause" of ALS, he says, but if the defect does turn out to contribute to ALS development, the finding could someday lead to a useful diagnostic test and perhaps to improved treatments.

Rothstein, postdoctoral researcher Glen Lin, and research associate Lynn Bristol discovered the EAAT2 defects while following up on earlier observations that many ALS patients have abnormally low levels of the protein in areas affected by the disease: the motor cortex and spinal cord. To track down the cause of that deficit, the Hopkins team examined the messenger RNAs (mRNAs), which provide the direct instructions for making the protein, in brain and spinal cord tissue obtained at autopsy from ALS patients. They found that those mRNAs were often edited incorrectly in precisely the same tissues where the low protein levels were seen.

Most genes of higher organisms contain non–protein-coding sequences, called introns, that have to be removed from the mRNAs before the proteins themselves are synthesized. But the Hopkins workers found that the machinery that removes the introns from the EAAT2 mRNAs made mistakes in 11 of the 20 sporadic ALS patients they tested. It either failed to remove an unneeded intron, or it deleted an essential protein-coding sequence, called an exon. As a result, the mRNAs make no EAAT2 at all or make a faulty version. Rothstein and his colleagues did not find the abnormalities in brain tissue from 12 normal subjects or in 16 patients who died of other neurodegenerative diseases, including Alzheimer's and Huntington's. This suggests that the EAAT2 defect is specific to ALS and not just a general consequence of nerve cell death.

Many questions remain unanswered. The researchers do not yet know what causes the mistakes in the mRNAs. It may be that a mutation in the EAAT2 gene gives the cell's RNA-processing machinery the wrong directions for removing the introns. Or the fault may lie in the processing machinery itself. Also unexplained, Rothstein says, is how the defect could be restricted to the motor cortex and spinal cord.

Nevertheless, says neurologist Wim Robberecht of the University of Leuven in Belgium, the evidence for a common denominator in at least some sporadic ALS cases is exciting. Even if the primary cause is "something completely unrelated" to glutamate metabolism—for example, the buildup of damaging free radicals from a malfunctioning SOD1 protein—Rothstein's findings "would finally give very conclusive evidence that an abnormality of glutamate metabolism is indeed involved" in ALS, if only as an important secondary mechanism.

–Gretchen Vogel

CELL BIOLOGY

Expanding the Eukaryote's Cast of Chaperones

Textbooks often depict cells as roomy compartments in which proteins go about their business relatively unimpeded. But in reality, the cell interior is more like a crowded marketplace, with proteins hustling from one job to the next, jostling and potentially interfering with one another along the way. In this rough-and-tumble milieu, proteins often need help to fold up into, and then maintain, the three-dimensional structures necessary for them to function normally. With three papers in this issue, it's becoming clear that, at least in higher organisms, a whole retinue of helper proteins is involved, and the task of helping—chaperoning—protein folding is far more complex than researchers had suspected.

In the past, the so-called heat shock proteins, in particular the two called Hsp90 and Hsp70, seemed to play the lead role in protein folding. Among other things, Hsp90 helps to prevent the clumping together of other proteins that have unfolded because of increased temperatures until they can refold into their proper conformation. It also keeps certain key components of the cell's internal signaling pathways, including steroid hormone receptors and kinase enzymes, folded in the right conformation to respond rapidly to their triggering signals. But the new findings bring a whole new set of chaperone proteins into the picture: a large protein family, found in organisms ranging from bacteria to mammals, called the immunophilins.

"There might be a whole slew of chaperones in eukaryotic cells that we just don't know about yet."

—Susan Lindquist

One report (p. 1713) provides the evidence that, in yeast, an immunophilin cooperates with Hsp90 in maintaining the signaling pathways. And results from the other two studies (pp. 1715 and 1718), done in the test tube, suggest that the immunophilins by themselves can keep proteins on the right track for folding. "These proteins themselves have the properties of a molecular chaperone," says F. Ulrich Hartl, a cellular biochemist at Memorial Sloan Kettering Cancer Center in New York City. "They play a role, somehow influencing the conformation [of proteins]. Previously it was rather mystical what these proteins would do."

Besides expanding the cast of characters in protein folding, the new findings add to the intricacy of the plot. They suggest that each of the many chaperones could play a different role in a molecular assembly line that gradually moves a protein toward its final form. And they hint that, with the help of particular chaperones, proteins may sometimes remain poised in an intermediate stage of folding, enabling them to react quickly when they meet up with the right protein partner. The new complexity, say investigators, may be just the beginning. "There might be a whole slew of chaperones in eukaryotic cells that we just don't know about yet," predicts yeast cell biologist Susan Lindquist of the University of Chicago, a co-author of one of the papers, after learning of the other two papers.

The first hints of this complexity came almost a dozen years ago, when cell biologist David Smith, now at the University of Nebraska Medical Center in Omaha and David Toft of the Mayo Clinic in Rochester, Minnesota, found that Hsp90 tends to be accompanied by an entourage of a half-dozen other proteins when it chaperones a steroid receptor. Among them were molecules called immunophilins, primarily cyclophilins, then best known as the targets of immunosuppressive drugs, such as cyclosporin. The appearance of the immunophilins in such a wide range of organisms had already suggested to Smith and others that they have an essential function in the everyday life of the cell. Now, their link to Hsp90 hinted that their crucial role could be protein folding.

To test this possibility, Lindquist, geneticist Richard Gaber of Northwestern University in Evanston, Illinois, and their colleagues first showed that both of the immunophilins in yeast (*Saccharomyces cerevisiae*), which are called Cpr-6 and -7, bind to yeast Hsp90. They also found that Cpr-7, at least, is necessary for normal cell growth. When they knocked out the gene for that protein, the yeast cells grew much more slowly than normal yeast, which led the researchers to wonder whether Cpr-7, like Hsp90, is crucial for proper intracellular signaling. Further experiments suggested that it might be.

When they inserted the gene for the