THE DANA FOUNDATION'S

BRAINWORK

The Neuroscience Newsletter

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News FROM THE FRONTIER

••• **New Compound for Chronic Pain.** Chronic pain from inflammation or nerve damage, like the kind associated with rheumatoid arthritis, can be debilitating. Previous research has suggested that two ion channels—known as $P2X_3$ and $P2X_{2/3}$— located on sensory nerves, play a role in the sensation of pain. In the *Proceedings of the National Academy of Sciences* (week of December 2-8), Researchers from Abbott Laboratories present evidence that a compound with the technical name A-317491 blocks receptors for $P2X_3$ and $P2X_{2/3}$, reducing pain due to nerve injury and chronic inflammation in rat models.

Michael Jarvis, from the department of anesthesiology, University of Iowa, and colleagues tested the compound on rats with chronic constriction injuries (CCI). They found that A-317491acted as an agonist for both $P2X_3$ and $P2X_{2/3}$, reducing pain sensation caused by nerve constriction and thermal hyperalgesia (increased pain sensation due to inflammation). Furthermore, the compound showed no major side effects, and

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SOCIETY FOR NEUROSCIENCE ANNUAL MEETING

Editor's note: BrainWork devotes this issue to reports from the 32nd annual meeting of the Society for Neuroscience, held November 2-7 in Orlando, Florida. Covering the meeting were Rabiya S. Tuma, a New York, NY-based science writer, Brenda Patoine, a science and medical writer based in LaGrangeville, NY, and Andrew Cocke, editor at The Dana Press. This year's conference catch phrase was "It's About the Science," and there was certainly much for the 26,000 attendees to absorb.

Immune System Molecule Plays Essential Role in Brain Development

BY RABIYA S. TUMA

In the opening public lecture at the 32nd annual meeting of the Society for Neuroscience in Orlando, Florida, Carla Shatz presented her new data illustrating how neural connections are established during brain development. Shatz revealed that, in an unexpected twist, a molecule previously thought to be involved only in the immune system plays a critical role in the process of brain wiring.

Unlike the hard-wired connections that are soldered into place on computer chips, the brain's neural connections, the synapses, are plastic. That is, they are made as well as broken during development, and later, in the mature brain, rewiring is a necessary part of learning and memory. But just how the brain knows which synapses to preserve or cleave has remained largely a mystery. Now, Shatz, a professor of neurobiology at Harvard Medical School in Boston, Massachusetts, finds that a major histocompatibility class I protein (MHC I), which scientists know is critical for one cell to recognize another in the immune system, is required for such rewiring to occur.

During development of the mammalian eye, neurons stretch from the immature retina to the lateral geniculate nucleus (LGN) and from there to the visual cortex of the brain, the area that organizes images from visual information arriving through this pathway. Early in development the connections

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Carla Shatz reveals a surprising new role for an immune system molecule.

(IMMUNE SYSTEM, continued from page 1)

in the LGN and the cortex are a tangled mess, with inputs from one eye mingled with inputs from the other one. However, in the adult brain the connections from the two eyes are no longer intermixed but rather form a pattern of alternating layers in the LGN and the visual cortex such that the inputs from one eye form stripes with the inputs from the other eye.

Shatz's team found that signals from the young retinal neurons drive this rewiring. Before the eye is open or the retina has any sensory input at all, groups of neighboring retinal neurons fire electrical impulses, called autoimpulses, to the LGN. As waves of these autoimpulses flow over the retina, the groups of neighboring connections in the LGN and cortex are stimulated and the synapses strengthened. By contrast, those connections that do not receive impulses gradually weaken and erode altogether, leaving the striped pattern of the mature LGN and visual cortex.

Without the autoimpulses, which occur approximately once a minute for several weeks during development, the immature, intermixed pattern of wiring remains into adulthood. Shatz describes this as a "use it or lose it" strategy for brain wiring.

To find out what molecules are responsible for this remarkable pattern formation, Shatz and her colleagues designed the following experiment. In some animals, the researchers chemically blocked the autoimpulses, while allowing them to continue in control mice. Then they looked to see if there were any differences in what genes were turned on in the LGN neurons from the blocked animals versus those from the controls. To the researchers' surprise they found that one of the predominant differences was the activity of a gene called MHC-I. This gene was active only in the normal control LGN, not in the signal-blocked LGN. Scientists know that the protein is involved in cell-to-cell recognition in the immune system, but nobody expected to find it in use elsewhere in the body, let alone in the brain, which is isolated from much of the immune system by a barrier between the blood system and the brain that blocks movement of molecules from one system to the other.

To counter any nagging doubt about the unexpected result, Shatz used genetics to remove the MHC-I gene from mice and found that their LGN and visual cortices looked just like those in the animals that had their retinal autoimpulses blocked. That proves, rather concretely, that MHC-I is required in the brain for the normal rewiring that occurs during development.

Now that the scientists know what they are looking for, they find that MHC-I is expressed in numerous regions in the developing and adult brain. The protein appears to be positioned at the synapse, which is where you would expect a molecule involved in such connections to be. Shatz thinks it is an "anti-glue" that helps to weaken the unwanted connections, while allowing the other connections to remain in place.

The protein is involved in cell-to-cell recognition in the immune system, but nobody expected to find it in use elsewhere in the body, let alone in the brain.

Knowing that the protein is expressed more widely and more permanently in the brain than just in the developing LGN, Shatz and her colleagues looked to see if other brain regions have normal cellular behavior in the mice lacking the MHCI gene or if, like in the LGN, rewiring is disrupted. They found that the normal plasticity of the neural connections in the hippocampus, which is vital for learning and memory, is diminished in these mice. Although the researchers have not yet had the opportunity to evaluate the animals in memory tests, Shatz speculates that they will not have as high an acuity as normal animals, simply because they cannot rewire the connections in their brains—something researchers know is required for long-term memory formation.

Thinking Outside the Box

So how surprising is it really to find an immune system molecule playing such a critical role in the brain? The answer to that question, like much of science, depends on whom you ask. Colin Blakemore, Professor of Physiology at the University of Oxford, who

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Jane Nevins, Editor in Chief
Walter Donway, Director

Editorial office:
The Dana Center
900 15th Street, NW,
Washington, D.C. 20005
E-mail: danainfo@dana.org

THE DANA FOUNDATION
745 Fifth Avenue Suite 900
New York, New York 10151

For free subscription or change of address:
rtalley@dana.org, or fax to 202-408-5599

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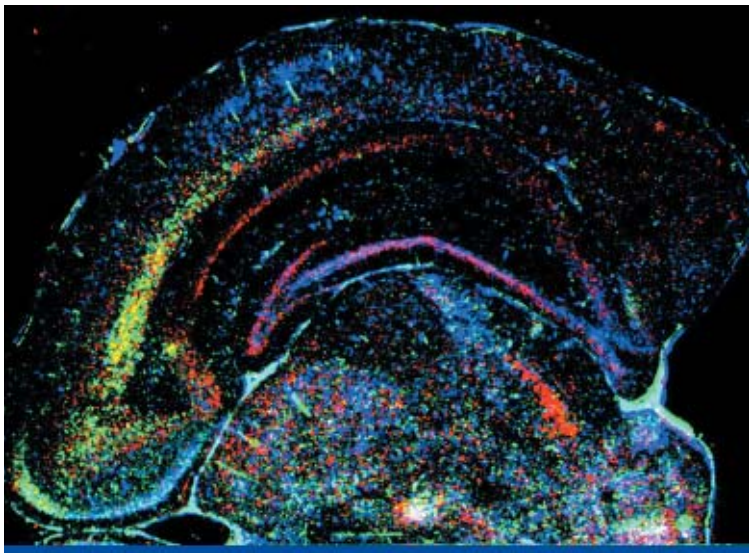

A lab tissue culture shows the mRNA expression patterns for three different class I MHC genes (red, green, blue) in a section from an adult mouse brain.

heard Shatz's presentation and was impressed, told *BrainWork*, "For decades, biologists have contrasted the immune system and the nervous system. Both have the task of representing huge amounts of information—about foreign proteins or about activity passing through the network of nerves. Shatz's new work suggests that the same family of genes may be involved in processes of recognition in both systems."

However, David Colman, director of the Montreal Neurological Institute in Canada and someone who has written quite a bit about the similarities and differences between immunological and neuronal synapses, is more cautious. "It may be the case that the MHC molecules are important in defining synaptic relationships," said Colman, "but there are so many molecules implicated at this moment in synaptic specificity or adhesion, (that is in which synapses are made and maintained during development) that it is hard to make a definitive conclusion."

For her part Shatz thinks the finding is exciting and likely the start of a whole wave of similar work. Already in two other presentations at SFN, researchers described finding similar molecules in the nervous system. "I think it is time for people to think outside of the box," said Shatz. "All bets are off when it comes to how these immune molecules are functioning in the brain."

Possible Roles in Disease

But, she says, knowing about their presence could be critical for understanding several neurological disorders. For example, in Parkinson's disease, in which neurons critical to movement die, some investigators have found evidence that there may be an autoimmune component to the disorder, such that the patient's own immune cells may be killing the neurons. Maybe, she says, the MHC-I protein—or another as yet undescribed immune system protein—is present in the membrane of those dying neurons and, when the immune system somehow becomes dysregulated, those neurons are the unlucky targets of immune cell attacks and are killed.

Additionally, she notes that in humans, HLA genes, which are homologues of the mouse MHC genes, are located on a segment of human chromosome 6 that has been associated with a number of neurological disorders, including dyslexia. And now she knows from her own work in mice that knocking out the MHC gene causes subtle visual defects. Obviously, she says, she cannot test the mice for dyslexia, but the plot has certainly thickened with her new discovery.

Piecing Together the Puzzles of Brain Aging

BY BRENDA PATOINE

Why do some people retain superb mental capabilities well into old age, while others struggle with a failing memory or succumb to the ravages of dementia? It is a question an aging population is anxious to have answered, and one on which neuroscience researchers are working hard.

A growing body of research is helping to identify the cell-signaling patterns that underlie cognition, and these findings are driving a shift in scientific views about age-related cognitive changes, Molly V. Wagster, director of the neuropsychology of aging program at the National Institute on Aging, said in her opening remarks to a symposium at the Society for Neuroscience meeting. By embracing new understandings about neural plasticity—the brain's inherent ability to change and to some extent regenerate, even into old age—Wagster said, a "more promising view of aging has emerged that will hopefully open doors to novel interventions."

"Bifocals for the Mind"

The average American can now expect to live to nearly 80, but while aging per se is a certainty, said Michela Gallagher, a behavioral neuroscientist at John Hopkins University, "the condition of aging *well* is less certain." Cognitive decline is quite common among the elderly, she said, which may or may not herald the beginning of a progressive decline into dementia. Either way, she said, "It's clear that many people would benefit from something like bifocals for the mind."

The prospects for potential treatments, however, will depend upon a better understanding of normal aging versus disease. Right now, it is often

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the case that by the time a diagnosis of dementia is made, the damage to the brain may be too far along for effective intervention. Treatment would likely be most effective if it were started during the "prodromal" phase before symptoms of dementia are overt. Yet, differentiating between early dementia and cognitive changes not associated with dementia is difficult. Likewise, she said, "There is no clear boundary between normal aging and age-related cognitive decline."

Gallagher and her colleagues have been trying to solve this puzzle by comparing the behavior of young rats with two groups of aged rats: those deemed to be "cognitively impaired" and those not impaired. Cognitive impairment is determined by water maze tests, a commonly used technique in which an animal must remember where a submerged platform is in a pool of water. The animals' brains are then examined to try to correlate brain differences with how well they performed in the tests.

The studies have already helped debunk a long-standing myth about aging, which is that cognitive impairment results as the aging brain loses nerve cells, especially in critical areas like the hippocampus, a brain structure important to short-term memory. Gallagher's team found no indication of a loss of neurons in the hippocampus, even in impaired aged rats. Rather, they found evidence for a loss of "connectional integrity" at synapses in an important class of cells (layer II CA-3 pyramidal neurons) that relay nerve signals between the hippocampus and a nearby brain structure, the entorhinal cortex. Pre-synaptic (or signal-sending) activity was markedly lower in these cells, suggesting that, as animals age, something interferes with the normal transmission of nerve signals in this particular region of the brain. Other evidence has suggested that the earliest symptoms of Alzheimer's disease are also linked to changes in the entorhinal cortex, making this brain area a hot spot for further investigation.

Using the same rat model of aging, Gallagher's group has also investigated whether age-related decreases in the production of new nerve cells (neurogenesis) in memory-related brain areas may help explain cognitive decline. They found that, although neurogenesis does decrease somewhat with age, these decreases in cell proliferation can occur without having an impact on learning. Based on these preliminary findings, she said, "Restoring neurogenesis in old brains might not do the trick" of stopping or reversing cognitive decline.

Aging Neurons Lose Excitability

Like Gallagher, cellular neuroscientist John Disterhoft of Northwestern University Medical School in Chicago has also been focusing on pyramidal neurons, but in an adjacent region of hippocampal tissue (CA-1). CA-1 pyramidal cells receive signals from CA-3 and have a distinctive pattern of nerve fibers that project upward into the "higher-thinking" cortical areas of the brain. Disterhoft called CA-1 pyramidal neurons the "output cells of the hippocampus" and noted that their activity—or excitability—increases early in the process of learning new behaviors.

Disterhoft's team found that this activity is altered in aging animals, which may help explain age-associated learning deficits. Drugs such as metrifonate that increase the rate of learning in aged animals also increase the excitability of these neurons, further evidence that the activity of these cells is linked to learning and may mediate the decline in learning that often accompanies aging.

Calcium: Not Just for Bones

Philip Landfield, a neuroscientist at the University of Kentucky, has been investigating the role of calcium dysregulation in brain aging, and has uncovered intriguing information about age-related genes and gene products that may contribute to cognitive impairment in complex ways.

Landfield said there is growing evidence for the "calcium hypothesis of aging," that is, that subtle alterations in how calcium is regulated in the brain occur early in the aging process and that these alterations seem to impair plasticity in the brain, which may lead to neurodegeneration and cell death. It has become clear, he said, that a specific type of channel that funnels calcium in and out of nerve cells (L-type calcium channels) is important to calcium dys-

regulation during aging, but the question of how has not been answered. One clue lies in the observation that the density of calcium channels in neuronal membranes increases with aging in the CA-1 pyramidal neurons of the hippocampus, and this "may account for altered calcium-dependent processes and impaired cognition," he said.

Landfield's team has undertaken an ambitious investigation of aged rats to try to determine if changes in the activity of certain genes underlie these alterations. They used sophisticated gene microarray analyses, which permit investigators to measure the expression of as many as 15,000 genes at once, to identify which genes change with age and which are correlated with results of behavioral tests such as the water-maze test previously described.

The researchers discovered five categories of genes that were downregulated—that is, their activity decreased—with aging and five categories that were upregulated, or more active. Of the genes whose activity was downregulated, about 75 percent began to show decreasing activity in middle age, and on average, these downregulated genes played roles in nerve cell processes. In contrast, the genes that were upregulated primarily affected processes specific to glia, the specialized cells that provide support to nerve cells.

Landfield said that, while preliminary, these findings suggest that "there is a widespread, orchestrated change in genomic regulation that begins early in life" and that the changes affect "a vast number of genes—much more than previously believed." Altered calcium signaling, he proposed, "may be the initiator of a complex cascade of neural changes, including altered gene expression, that eventually leads to cognitive impairment."

Researchers Explore the Boundaries of Regeneration

BY ANDREW COCKE

Regeneration refers to the process of damaged neurons regrowing after injury, whether stroke or other trauma to the brain and spinal cord. Research is currently focusing on understanding the mechanisms of regeneration, and ways to enhance the brain's ability to facilitate recovery. At this year's Society for Neuroscience annual meeting, researchers reported findings offering more detailed understanding of how regeneration works—or does not work—with the hope of possible therapies in the future.

Inhibiting the Inhibitor

After an injury in the brain or spinal cord, neurons do not spontaneously repair themselves. Why? One of the culprits is believed to be the growth-inhibiting protein dubbed "Nogo" by Martin Schwab, researcher at the University of Zurich and a pioneer in the field of regeneration. Nogo is present in the brain white matter and binds to receptors on axons, stopping axonal growth that is needed if recovery is to occur. Stephen Strittmatter and colleagues in the department of neurology at Yale University School of Medicine have been toiling to uncover the mechanisms of Nogo action. In 2001, his team reported in *Nature* the discovery of a receptor for the Nogo protein. The researchers then hypothesized that blocking this receptor would stop the inhibiting action of Nogo and allow regrowth of neurons after injury.

In a continuation of this line of research, Strittmatter reported at this year's conference that his team had discovered a peptide—called NEP1-40—that blocks the action of Nogo. In the study, one group of rats with spinal cord injuries was treated with the peptide, while the control group went untreated. On a twenty-one point functional recovery scale, treated rats scored an average of three points higher than control models, recovering more walking ability. NEP1-40-treated rats also had more robust nerve fiber growth, with 5 to 10 percent of nerves growing 1.5 cm below the injury site.

Strittmatter commented that while this particular peptide blocks the effects of Nogo as a "receptor antagonist treatment," other inhibitors are also at work after nerve injury—including MAG and OMgp—and compounds that efficiently and selectively block the action of all three regrowth inhibitors in animals have yet to be discovered. Recent work in Strittmatter's laboratory and in Zhigang He's laboratory at Harvard revealed that all three inhibitors act via the same protein, raising the possibility that one compound may be able to kill three birds with one stone. Though many steps remain before reaching human clinical testing, Strittmatter says that Nogo receptor blockers "might have significant therapeutic potential."

Using Human Cells to Treat Rats

Two areas of the brain have the distinction of containing nerves able to regenerate throughout life: the dentate gyrus of the hippocampus and the olfactory bulbs located in the upper surface of the nasal cavity. Twin bundles of nerves, the olfactory bulbs contain olfactory ensheathing cells (OECs) and facilitate the sense of smell. Some of the nerves of the olfactory bulbs grow into the nasal epithelia, where the OECs can be extracted from a patient in a simple five-minute procedure.

Studying the properties of OECs, Hans Keirstead and colleagues at University of California, Irvine extracted human olfactory ensheathing cells, cultivated them in the lab, and transplanted them into spinally-injured rats. (One of Dr. Keirstead's

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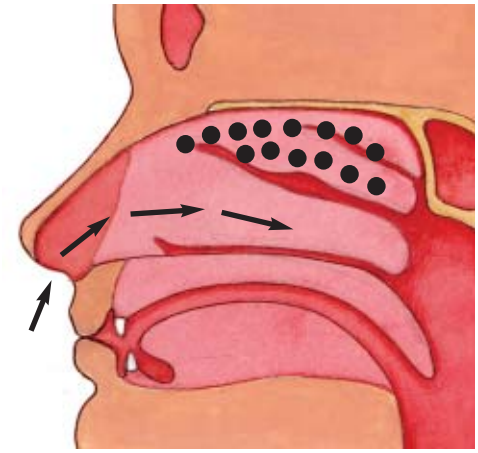
colleagues in the study actually donated his own cells for the process. In an unexpected side effect, he experienced a period of what Dr. Keirstead described as "olfactory hallucinations," smelling things that were not there.)



Cells in the nerve bundles that enable our sense of smell may become useful in regeneration efforts. The black dots indicate areas from which researchers took cells to transplant in spinal-cord injured rats.

Adult rats with contusion injuries to the spinal cord received transplants of human OECs seven days after injury. The rats were monitored, and by eight weeks after transplantation, all treated rats could walk and had regained coordinated locomotion. While Keirstead admits that the researchers do not know exactly why recovery occurred, he offers three possibilities: Perhaps the human cells encourage long-distance growth of axons (an unlikely scenario, says Keirstead, since axon growth occurred but was not robust enough to explain the results); the increased myelination (the white fatty substance that covers health nerves) may aid in nerve repair; or maybe the transplanted cells decrease the tissue loss—also known as secondary degeneration—that occurs after injury.

Keirstead says that though the study is in the preclinical stage now, the results are promising. The study

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New Drugs May Add Up to Good News in Schizophrenia

BY RABIYA S. TUMA

Scientists expect that new discoveries about drug and nerve-cell interaction will result in improved treatment for patients with schizophrenia in the not too distant future, according to research presented at the Society for Neuroscience annual meeting.

Currently many patients benefit from the typical and atypical antipsychotic drugs available to them. But the benefits come at a significant cost. While patients on the drugs have fewer hallucinations and more organized thoughts, they often have to put up with debilitating side-effects, such as feelings of sedation or the shakiness called dyskenesia that resembles the symptoms of Parkinson's disease.

These side-effects occur because the drugs affect large regions of the brain, rather than just the circuits that are out of balance in schizophrenia. Specifically the typical antipsychotics, like haloperidol, exert their affects by blocking a receptor called "D2," a major receptor for the neurotransmitter dopamine. This may exert a positive effect when there are excessive amounts of dopamine around, as is thought to be the case during psychotic episodes. However, between such episodes, the dopamine levels in most patients are relatively normal, so the drug-induced blockade actually causes a harmful block of the neurotransmitter's action, leading to the dyskenesia and other problems. For their part, the atypical antipsychotics, such as clozapine, act on both minor dopamine receptors and some serotonin receptors. These drugs more effectively control psychosis in many patients than the typical antipsychotics but still have unpleasant side effects such as significant sedation and weight gain.

Now, researchers think that as they develop a more precise understanding of neurotransmitter and receptor responses to medication in the brains of schizophrenics, they will be able to develop more targeted therapies that will correct the problem, with little or no effect on the surrounding brain circuitry.

The Drugs

Already one new drug, aripiprazole, has been approved by the FDA for use in the treatment of schizophrenia. It is the first drug in a class of compounds called partial dopamine agonists, or helpers, stimulating both dopamine and serotonin receptors, but only under certain conditions. This drug is thought to adjust the amount of dopamine so that the neurotransmitter stays within a normal range and is the first agonist compound approved for use—both the typical and atypical antipsychotics are dopamine antagonists, or blockers. Thus far, the patients who participated in clinical trials found it to be as effective as the older drugs and reported fewer side effects.

But no one drug is going to work for all patients, so the search continues for new ways of controlling schizophrenia. At the neuroscience conference, Arvid Carlsson, who won the 2001 Nobel Prize for his discovery of dopamine and its relevance to schizophrenia, presented his new results on a partial dopamine antagonist, ACR16, which is under development by the Carlsson Research Company in Sweden. Unlike the partial agonist aripiprazole, which assists or mimics dopamine binding at the receptor, the partial antagonist ACR16 inhibits dopamine binding to some degree.

In contrast to the typical antipsychotics that block the D2 dopamine receptor, which sits squarely in the middle of the synapse, ACR16 has been designed to bind to a different dopamine receptor, called the autoreceptor, located off to the side of the main synapse.

Significantly, these autoreceptors lie in the membrane of dopamine-

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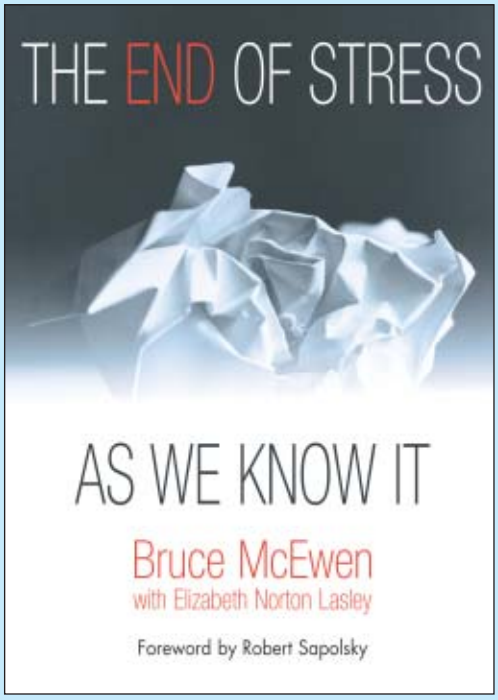
New From THE DANA PRESS



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—from the Foreword by Robert Sapolsky, author of *Why Zebras Don't Get Ulcers* and Professor of Biology and Neurology, Stanford University

"Central to this new understanding [of disease vulnerability] is a novel conception of stress, developed by Dr. McEwen, who has been studying the subject for more than three decades. According to his model, it is not stress per se that is harmful."

—From "The Heavy Cost of Chronic Stress," *The New York Times*, December 17, 2003

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M. Flint Beal, M.D.,
Neurologist in Chief of the New York and Presbyterian Hospital, and Chairman of the Department of Neurology and Neuroscience at the Weill Medical College of Cornell University.
David J. Kupfer, M.D.,
Thomas Detre Professor and Chairman of Psychiatry at the University of Pittsburgh School of Medicine.

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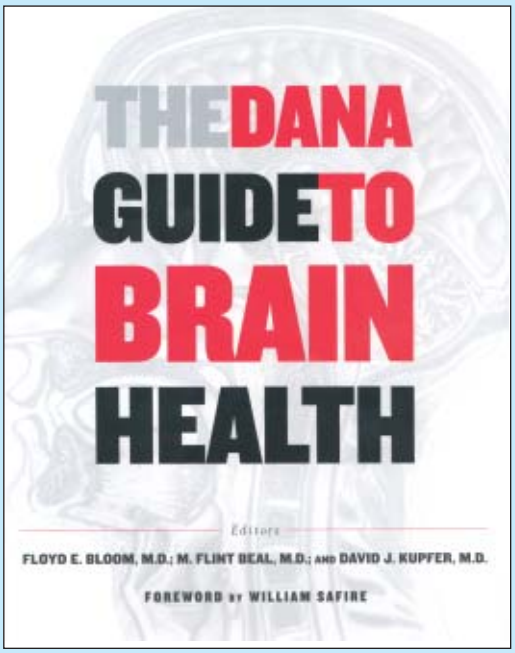

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(SCHIZOPHRENIA, continued from page 6)
secreting neurons on the presynaptic, or sending, side of the signal, as opposed to the D2 receptors that are in the membrane of post-synaptic neurons, which receive the signal. This localization to the presynaptic neuron is important because Carlsson and others have shown that the autoreceptors help control the amount of dopamine released during nerve cell firing. If excess dopamine is released, it binds to the autoreceptor, which then tells the cell to shut off dopamine release; if the receptor is unoccupied, the presynaptic cell knows to keep releasing the neurotransmitter.

To visualize how a compound that inhibits the autoreceptor might work, Carlsson described an experiment he performed in mice. When he gave normal mice ACR16, they become hyperactive—a behavior that results from excess dopamine and is seen in many schizophrenic patients. However, if he first treated the mice with methamphetamine, which induces hyperactivity and excess dopamine release, and then gives them ACR16, the animals calm down, behaving very much like the untreated control mice. These data, he concludes, mean that ACR16 can both upregulate and downregulate the amount of dopamine depending on the baseline level of the neurotransmitter in the brain when the drug is given.

Putting all this biology together, one can imagine that ACR16 might be a very effective drug. Carlsson said, "ACR16 may help quell the symptoms of schizophrenia without causing any serious side effects," because it acts at the regulatory level rather than by an all-out blockade of the major dopamine receptor.

The Role of Serotonin

Herbert Meltzer, from Vanderbilt University in Nashville, Tennessee, emphasizes that, though hallucinations are the most familiar symptom of schizophrenia, other facets of the disease can be seriously debilitating. For example, he says the patient population has a high rate of suicide due in part to negative symptoms, such as withdrawal and depression. Additionally, at the onset of the illness many individuals suffer a significant drop in their cognitive ability, causing them to fall to within the lower 25 percent of the general population in terms of mental ability. These symptoms, perhaps as much as the hallucinations, cause social problems for patients, said the experts at the meeting.

But like Carlsson, Meltzer thinks the horizon looks brighter for people with schizophrenia because of new developments in types of therapeutics and their uses that are becoming available.

The FDA advisory board—which recommends to the agency whether or not to approve a drug or an indication for a drug—recommended that clozapine be used in preference to other atypical antipsychotics for the prevention of suicide. This decision was in large part due to a study Meltzer did in which he showed that clozapine is 25 percent more effective in eliminating suicide attempts or suicides in patients with schizophrenia, relative to other drugs in the class. He thinks this increased efficacy is due in part to clozapine's preferential binding to serotonin receptors in the brain cortex to a greater extent than the other atypicals.

If the FDA approves its advisory board's recommendation for clozapine, Meltzer predicts it will have a rapid and dramatic impact on the development of drug therapies for the treatment of schizophrenia. "It is very important to emphasize this study and the committee recommendation," said Meltzer, "because it represents a huge change in the direction of developing drugs for schizophrenia. The FDA—if they approve the indication—recognizes that psychosis is only one component of schizophrenia." Other factors—cognition, mood, negative symptoms and suicide—can each now be targeted for drug development, he said. This change in regulatory approval will likely increase the number of pharmaceutical companies willing to spend time and resources on developing therapies for schizophrenia, because the task becomes much more feasible if they need only develop a therapeutic agent that treats a single symptom, rather than the whole complex cohort of the disorder.

Recently, Meltzer has followed up on his clozapine work and has shown that when schizophrenic patients took either tandospirone or buspirone, they had improved cognitive ability. Unfortunately these drugs had unacceptable side effects, so they cannot be used in the general patient population. Meltzer, however, remains excited these data show which receptors need to be targeted for new therapies: These two drugs act on the same serotonin receptor as clozapine, but unlike the atypical, they do not affect other receptor systems in the brain. Therefore, researchers now know exactly which serotonin receptors need to be targeted to improve cognition in schizophrenia patients. "Serotonin has now emerged as a new strategy for treatment and for rational drug design," said Meltzer.

A Dose of Caution

Although all the developments that Meltzer and Carlsson describe will likely benefit some patients, Daniel Weinberger from the National Institute of Mental Health, who is trained as a neurologist and a psychiatrist, cautions that scientists are a long way from a cure for schizophrenia. "These drugs are clearly much better tolerated than the old drugs," said Weinberger. "As such there is a penumbra of benefit that accrues to a much better tolerated substance, but it would be misleading to leave you with the impression that these drugs are dramatically more effective in wiping out these diseases. These drugs do not cure these diseases. And most patients who are on these drugs...are still much more ill than most clinicians would like them to be."

Neurobiology of Addiction:

Toward Therapeutic Interventions That Help the Brain 'Just Say No'

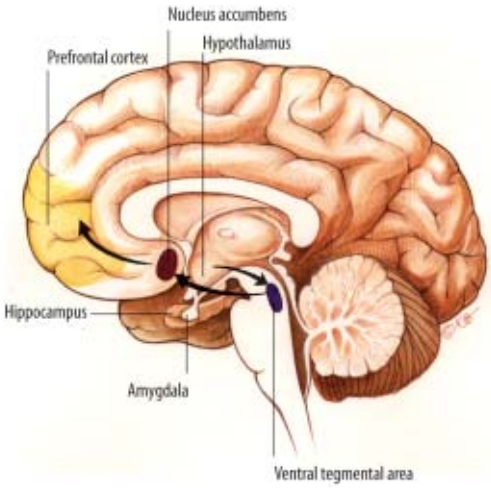
BY BRENDA PATOINE

Addiction and relapse were recurring themes at the Society for Neuroscience meeting—the subjects of various symposia, a special lecture, and a press conference highlighting new findings in the field. Common threads among all these events were the recognition of drug addiction as a biological disorder and how new understandings of the associated brain changes are pointing the way to novel therapeutic targets.

"Addiction is a chronic, relapsing disorder," said Glen Hanson, acting director of the National Institute on Drug Abuse (NIDA), in his opening address for a NIDA-sponsored symposium, the Neurobiology of Relapse. "It has features similar to other kinds of chronic diseases, like diabetes or cardiovascular disease." As such, he said, addiction will require continuing or even lifetime treatment. In the absence of a cure, treatments must target the chronic symptoms of addiction, he said, the most complicated of which is relapse.

Anthony Basile, director of biological research at Alkermes, Inc. in Cambridge, Mass., echoed this sentiment, noting that there is a growing realization that "drug addiction is a disease, not a personality disorder." Basile chaired a separate symposium on the mechanisms underlying drug abuse and spoke at a press conference on the subject. He reviewed new results from research by Alkermes and the National Institutes of Health on type M5 muscarinic receptors, a class of brain proteins that have previously been shown to be located exclusively on nerve cells in the brain's "reward" region, the areas activated by drugs of abuse.

Basile and colleagues developed mice lacking the gene that encodes for the M5 muscarinic receptor (M5 knockout mice), and used them to investigate the role of these receptors in addiction to morphine, an opiate drug used clinically to suppress pain but which can become addictive. They found that, unlike normal mice, the knockout mice showed no compulsion to continuing taking morphine after they had been repeatedly administered the drug; the rewarding effects of morphine were almost completely absent.



The "reward pathway" helps condition the brain to crave drugs.

Moreover, when morphine was discontinued, the severity of withdrawal symptoms in the M5 mice was substantially reduced. The results suggest that the M5 receptor "represents a novel target for future development of anti-drug-addiction medications," Basile said. The tests also showed that removing the M5 receptor had no effect on the beneficial, painkilling properties of morphine.

Tracking Reward Pathways

Barry Everitt, a university professor in behavioral neuroscience at the University of Cambridge, England, has been taking a "neurosystems approach" to drug addiction, with investigations aimed at understanding how neural pathways involved in drug use change as the duration of exposure to a drug of abuse increases. Initially, he said, drug use is a "voluntary, goal-directed" behavior with the characteristics of classic instrumental conditioning: taking the drug results in a reward (i.e., the euphoria of a drug high), which conditions the user to take drug again. In addition, stimuli in the drug-taker's environment become associated with the drug's effects through Pavlovian conditioning, and these "drug cues" can support drug-seeking, induce craving, and precipitate relapse.

This latter conditioning response is mediated by a specific brain pathway involving a portion of the amygdala that connects to the prefrontal cortex (PFC), a frontal part of the brain involved in the control of compulsive behaviors. Both the amygdala and PFC interact with a region of the nucleus accumbens, a brain structure located in the striatum and recognized as a critical element of the brain's "reward circuit."

As drug use becomes chronic, drug-seeking behavior may change from voluntary to involuntary and compulsive. Drug self-administration becomes an automatic response, a habit over which the user loses control. Underlying these behavioral changes, Everitt said, is a "metaphorical switch in the brain" that acts like a train-track switch, shifting neural processing to a different pathway. A distinct part of the striatum, the dorsal striatum, seems to take over, becoming progressively more engaged the longer the drug is used.

When compulsive drug-seeking continues, Everitt said the prefrontal cortex becomes less active, as if its role as control center of the brain has been hijacked. Everitt said this "chilling out of the prefrontal cortex" is critical to the compulsive behaviors associated with drug addiction. Moreover, studies show, it does not recover well, which may help explain the propensity for drug relapse. Even after three or four months of abstinence, he said, "the prefrontal cortex is extremely quiet" in people who are addicted.

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Retaking Control of Relapse

Everitt's research team has shown that drugs that block specific receptors in these pathways can decrease drug-seeking behavior in animals. At least two classes of drugs—AMPA kainite antagonists, which act on glutamate receptors, and D3 antagonists, which block a subset of dopamine receptors—show particular promise in preliminary studies. The idea behind such treatments, he said, would be to interrupt the brain switch that underlies involuntary drug-seeking behavior by pre-treating individuals who are trying to abstain from taking drugs. The goal is "a treatment that may pharmacologically help your brain to resist the compulsion to abuse drugs," Everitt said.

In the NIDA symposium, Peter Kalivas from the Medical University of South Carolina reviewed evidence showing that blocking the synaptic projections that run from the prefrontal cortex to the core of the nucleus accumbens stops animals' drug-seeking behavior immediately, making this "a real target for intervention," he said. In this case, glutamate—not dopamine—is the primary neurotransmitter involved.

Changes in gene expression that may be triggered by the release of various neurotransmitters also represent targets for intervention. Kalivas reviewed evidence that two proteins—cysteine and AGS-3—are associated with drug-seeking behavior, and when these proteins are manipulated, drug relapse is prevented in animals.

Altogether, these new understandings about the neural systems and molecular changes underlying addiction illustrate "the multiplicity of potential therapeutic targets," said NIDA's Hanson. "If we can appreciate these neural underpinnings, we'll know where to target therapies." On that endeavor, he said, "We're making a lot of progress, but we've got a long way to go."

Getting to Genetic Dysfunction in Epilepsy

BY RABIYA S. TUMA

Despite all the talk about the genome project and the identification of genes associated with various disorders, such as epilepsy, there is another aspect of genetics that has received much less attention: How do the mutated genes actually cause problems?

At a symposium on epilepsy at the annual meeting of the Society for Neuroscience, one researcher addressed just that question. Like the mutations made in transgenic mice that cause epilepsy-like syndromes, the mutations identified in human epilepsy patients cause a change in the behavior of sodium channels in neurons.

Voltage-gated sodium channels are transmembrane proteins that lie in the membrane of neurons and are responsible for transmitting signals throughout the nervous system. The proteins are exquisitely sensitive to changes in electrical potential across the membrane—that is, the difference between the number of positive ions on the outside versus inside of the membrane. When that difference, or potential, decreases the sodium channel opens and then closes again very quickly. During the brief time the channel is open, sodium ions flood into the cell. The influx of positive sodium ions causes a further drop in the membrane potential and forces the neighboring channels to open. Thus the sodium channels pass a signal down the axon, eventually causing the neuron to send a signal through its synapse to the adjoining neuron.

What Miriam Meisler, from the University of Michigan, found is that even small changes in the sequence of these channels can cause epileptic seizures. Working first in a mouse model, called Q54, her team found that 100 percent of these mice, which carry a mutant copy of a sodium channel gene in addition to all of their normal wild-type genes, experience regular, brief seizures.

When she attaches intracranial electrodes to neurons in the hypothalamus of these mice, she sees that about 2 percent of the sodium channels stay open for a fraction of a second longer than they should. In wild-type animals she finds that such extended openings occur 1 percent of the time. That small difference is enough to cause the transgenic mice to have approximately 20 seizures per hour, each lasting 10 seconds.

But that is an artificial experimental situation in mice; what everyone really wants to know is what happens in human patients with epilepsy. Already, Meisler and other geneticists have found numerous mutations in sodium channel genes in epilepsy patients. To find out how these mutations generate seizures, neuroscientists have made copies of the mutant human genes and caused the proteins encoded by the genes to be expressed in cultured human cells. As unlikely as this experimental design may sound, this protocol allows the scientists to measure the electrical currents generated by the individual sodium channels, something they certainly cannot do in human patients.

That small difference is enough to cause the transgenic mice to have approximately 20 seizures per hour.

Similarities in Human Epilepsy

To Meisler's surprise the mutations associated with human epilepsy act very much like the mutant channel from the Q54 mouse: The human mutations cause a slight delay in the closure of the sodium channel, allowing excess sodium ions to flow into the cell. These

small changes—in the range of millisecond differences between normal and abnormal—are somehow the difference between no epilepsy and epilepsy. "Very subtle kinetic changes can have severe effects," said Meisler. "But these are episodic effects and most of the time the channels are functioning fine in the brain."

Ruth Ottman, a professor of epidemiology at Columbia University in New York and a specialist in epilepsy genetics, said that although this isn't the first work on voltage-gated ion channels in epilepsy—other projects have looked at potassium channels, for example—"What impresses me the most is the exquisite sensitivity of the channels to such small changes in activation kinetics."

Ottman and Meisler also stress that one big question is why not everyone who carries one of these sodium channel mutations suffers from epilepsy. Even within a single family, one person with the mutation will have epilepsy and another will be fine. It isn't clear yet why people who have the same mutation are so variable, but Meisler might be on the way to illuminating at least one reason. She has already found that a second gene in her Q54 mouse can strengthen or weaken the effect of the sodium channel gene. In the strain of mice she originally used to make Q54, all of the animals have seizures, but in a second breed of mice only 10 percent of the animals do. When she finds the mitigating genetic factor in the mice, she may find a clue as to why some family members have seizures and others do not, say the researchers.

So how do these small changes actually cause episodic seizures? Meisler speculates that the mutations make the neurons hyperexcited. Under conditions where they would normally remain closed, the channels open and remain open too long. And at some sporadic frequency, enough of the channels respond in this way to cause the synchronous nerve firings that are characteristic of seizures.

News
FROM THE FRONTIER

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treated rats showed no loss of motor function or change in heart rate or blood pressure.

The possible advantage of this compound is its high degree of selectivity. A-317491 appears to block $P2X_3$ and $P2X_{2/3}$, almost exclusively, and "shows only very weak or no affinity for a large selection of other cell surface receptors, ion channels, and enzymes," the study authors write. Finally, the researchers note that the compound "shows reduced potential to produce tolerance compared with morphine," after repeated doses. The major problem with using morphine to treat chronic pain is that patients acquire a tolerance to the drug, requiring higher and higher doses to be effective.

••• Mind What You Say Around the Baby. When does the human brain develop the capacity to understand language? Does it come through experience, or is it hardwired into our anatomy at birth?

Researchers in France, conducting functional magnetic resonance imaging (fMRI) studies on infants, have taken steps to answer this question. They discovered that when hearing speech, the infant brain activates in many of the same areas as the adult brain, indicating that language processing centers in the brain already begin to develop as early as in the first two months of life.

In the study, which appeared in the December 6 issue of *Science*, the research team tested 20 healthy infants, all between two and three months old. Each infant listened to 20 seconds of speech, 20 seconds of silence, followed by 20 seconds of their native language (French) played backwards. While hearing regular speech, the left temporal lobe of the infant brain activated (the same pattern of activation seen in the adult brain). Interestingly, the pattern of activation in the left temporal lobe did not change when the infant heard the backward speech. The study authors note that "this suggests that this area is undergoing changes during infancy and has not yet acquired its full competence for the native language by three months."

The team concludes that although development of the language centers of the brain are by no means complete in infants, their results support the theory that development is well underway at birth, rather than the "blank slate" theory that all brain development results from outside experience.

••• Sunlight and Serotonin. The neurotransmitter serotonin plays a role in depression, and drug treatments called specific-serotonin-reuptake inhibitors are effective because they raise serotonin levels in the brains of depressed patients. Now researchers are investigating the connection between serotonin levels and sunlight amounts in seasonal affective disorders (the so-called "winter blues"). The research appears in the December 7 issue of *The Lancet*.

Gavin Lambert and colleagues at the Human Neurotransmitter Laboratory, Baker Research Institute, in Australia measured serotonin levels in 101 healthy men by extracting blood from the jugular vein. Researchers could thus test blood as it directly left the brain, allowing for accurate serotonin level measurements. They discovered that the turnover of serotonin by the brain was lowest during winter months (July and August in Australia).

The research team obtained data on the number of sunlight hours each day from the Australian Commonwealth Bureau of Meteorology and correlated the data to serotonin readings taken on each day. The data showed that the rate of production of serotonin was directly related to amounts of bright sunlight, "with val-

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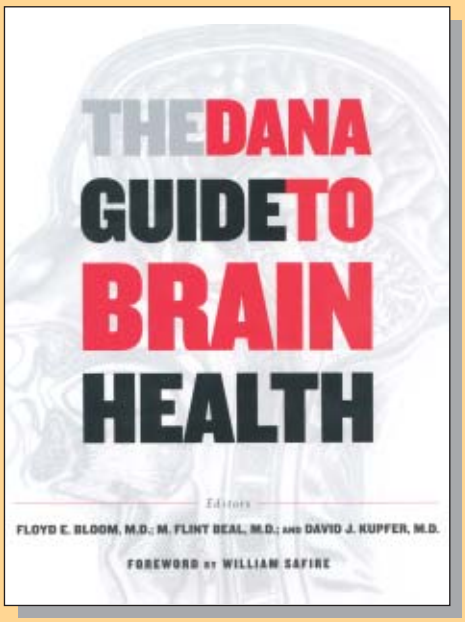

Now Available!

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has been repeated several times, with up to 30 animals in each trial. The next step is to evaluate toxicity studies and have an independent group repeat the results. The team is applying for a National Institutes of Health grant for a preclinical report. If progress continues, the OECs could become a viable treatment for injured brains and spinal cords. Harvesting the cells from the patient's own body eliminates the risk of transplant rejection, and the availability of the cells allows quick harvesting and purification. "Because our study used human cells," says Keirstead, "it has direct significance for clinical use."

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ues being higher on bright days than on dull days," the authors write. Furthermore, since the researchers tested all subjects in the morning—and found no correlation between serotonin levels and amount of sunlight on the day before testing—they say the evidence "suggests that brain serotonin turnover adjusted promptly to sunlight intensity on the morning of the study."

The authors admit that the study shows only how healthy brains adjust serotonin levels to sunlight, and they "do not know whether patients predisposed to affective disorders are affected by environmental factors in the same way."

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