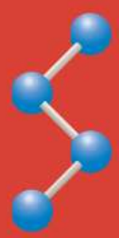
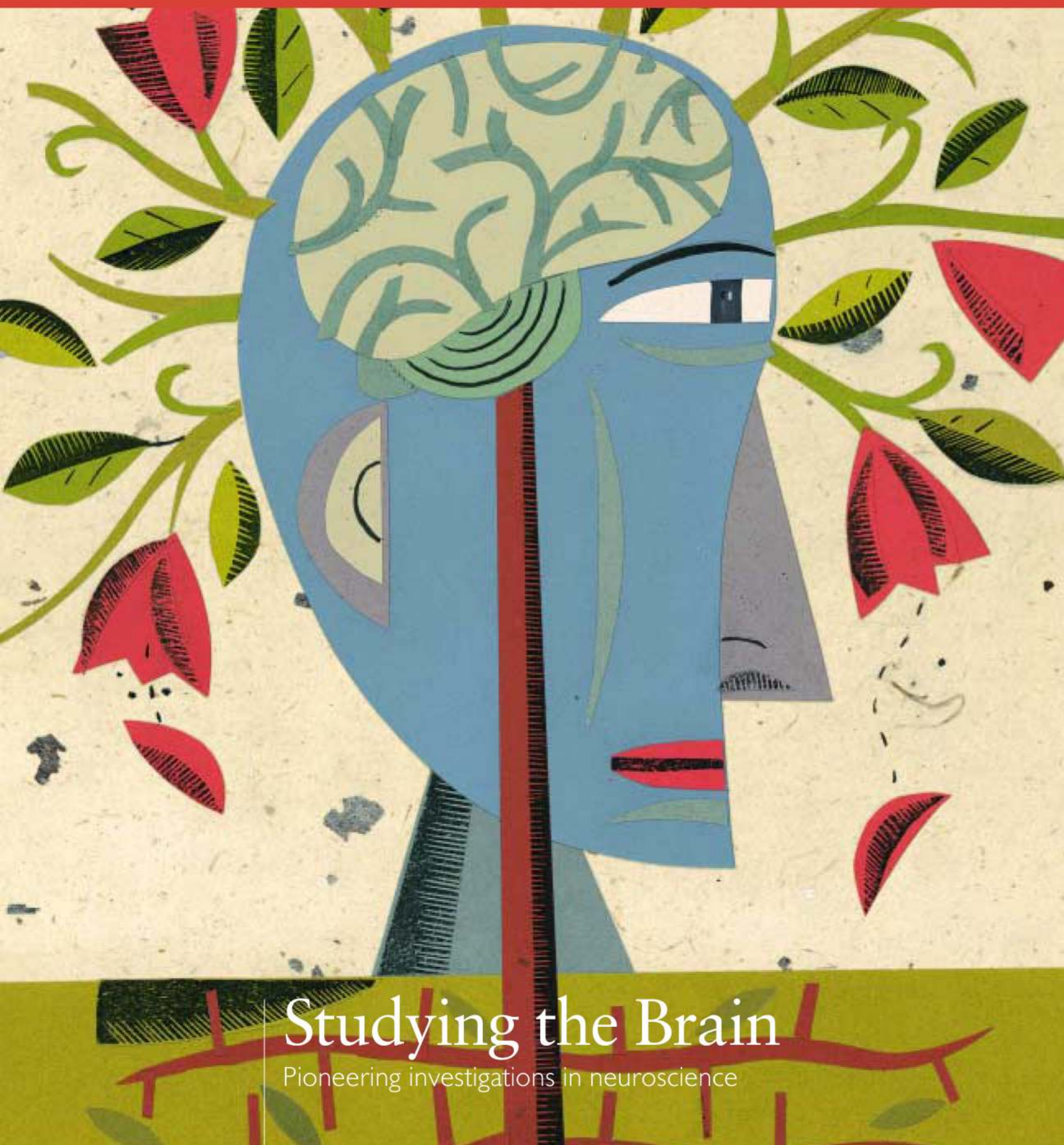
VOLUME SEVEN | NUMBER ONE



THE SCRIPPS RESEARCH INSTITUTE

Endeavor

Spring 2004



Studying the Brain

Pioneering investigations in neuroscience

Endeavor

VOLUME SEVEN | NUMBER ONE

Spring 2004

The effort to understand the mind is among the oldest of human endeavors. Hippocrates named the brain as the seat of intelligence 2,400 years ago. Leonardo da Vinci rendered superb illustrations of the brain's structures in the Renaissance. And Camillo Golgi and Santiago Ramón y Cajal published images of neurons and other brain cells in the Victorian period.

In the modern era, the study of the brain has continued. Careful psychiatric observations have elucidated the functions of many of the brain's distinct areas. PET scans and MRIs have enabled scientists to observe the living brain in action. And the human genome project has delivered a wealth of information on genes key for neurological functioning.

Today, 50 million Americans suffer from various neurological disorders, yet the brain remains something of an undiscovered country, and neuroscience, a frontier. This issue of **Endeavor** features some of The Scripps Research Institute's pioneering investigations on the brain.

featured

also

ENDEAVOR IS A PUBLICATION OF THE SCRIPPS RESEARCH INSTITUTE

Scientists Discover Chemical that Turns Stem Cells into Heart Muscles

A group of researchers from The Skaggs Institute for Chemical Biology at The Scripps Research Institute and from the Genomics Institute of the Novartis Research Foundation has identified a small synthetic molecule that can control the fate of embryonic stem cells.

Led by Professor and Scripps Family Chair Peter G. Schultz, Ph.D., the researchers found that this compound, called cardiogenol C, causes mouse embryonic stem cells to selectively differentiate into heart muscle cells, an important step on the road to developing new therapies for repairing damaged heart tissue.

Reference: J. Am. Chem. Soc., *126(6), 1590-1591 (2004).*

Mixing a Dangerous Cocktail of Alcohol, Brain Peptides, and Neurotransmitters

A team of scientists led by Professor George Siggins, Ph.D., has described the cellular mechanism underlying the brain's response to alcohol, which suggests a possible method for treating alcoholism.

This work ties together the effect of the brain peptide corticotropin releasing factor (CRF) with alcohol. Both appear to influence one particular neurotransmitter called gamma amino butyric acid (GABA). The research suggests that compounds that block CRF receptors might offer a potential new therapeutic for alcoholics.

Reference: Science, *303, 1512-1514 (2004).*

News Flashes
New Researchers
Award Announcements
And More

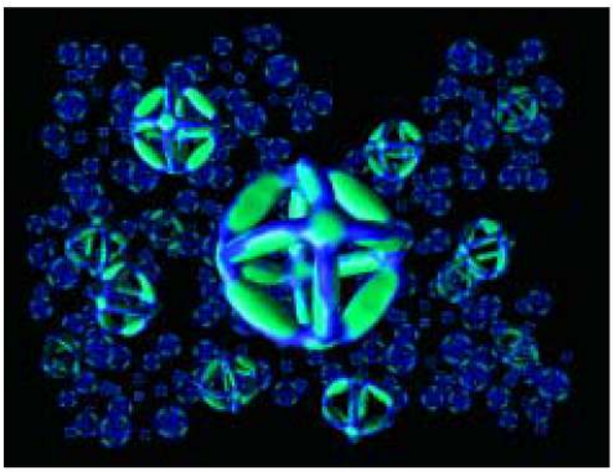
At the Forefront

Nano-Origami: Researchers Create Single, Clonable Strand of DNA That Folds into an Octahedron

Scientists in the laboratory of Professor Gerald Joyce, M.D., Ph.D., have designed, constructed, and imaged a single strand of DNA that spontaneously folds into a highly rigid, nanoscale octahedron that is several million times smaller than the length of a standard ruler and about the size of a small virus.

Making the octahedron from a single strand was a breakthrough. Because of this construction, the structure can be amplified with the standard tools of molecular biology and can easily be cloned, replicated, amplified, evolved, and adapted for various applications. These octahedra are potential building blocks for future projects, from new tools for basic biomedical science to the tiny computers of tomorrow.

Reference: Nature, *427, 618-621 (2004).*



Investigators have created a clonable DNA octahedron, roughly the size of a small virus, which has numerous potential applications in biomedical science.
Image, visualized using cryo-electron microscopy and single-particle reconstruction analysis, courtesy of Mike Pique.

A New Twist on Mad Cow

In a surprising twist on a timely topic, Scripps Research scientists are presenting evidence that mad cow disease prions cannot kill neurons on their own and that normal, healthy cellular prion proteins may be a direct accomplice in unleashing destruction in the brain.

Scripps Research scientists are presenting evidence that normal prion proteins play an essential role in mad cow disease.

Unlike most infectious diseases, the infectious material of mad cow and other prion disease is not a virus, bacteria, or some other pathogen, but a protein. Normally, prion proteins are expressed throughout the body and sit anchored on the surfaces of cells in a wide variety of tissues. The abnormal prion protein, however, has been linked to mad cow disease and related diseases, such as the human variant Creutzfeldt-Jakob disease.

The scientists, led by Professor R. Anthony Williamson, Ph.D., discovered they were able to induce catastrophic neurotoxicity *in vivo* without any abnormal prions at all by adding antibody molecules, which cross-linked the normal prion protein.

Reference: Science, *303, 1514-1516 (2004).*

Investigators Describe a Structure from Deadly 1918 Flu Virus

Professor Ian Wilson, D.Phil., and colleagues have described for the first time a structure of a protein from the deadly 1918 "Spanish Flu" virus—a virus that took more lives than World War I and caused the largest and deadliest influenza outbreak in recorded history.

The team's work suggests why this was so devastating an outbreak. The newly solved structure of a protein called hemagglutinin shows features primarily found in avian viruses, suggesting that the virus jumped directly from birds to humans. This type of transmission is rare and many people's immune systems would have been untrained in successfully recognizing and attacking the unusual pathogen.

Reference: Science Express, *10.1126 (2004).*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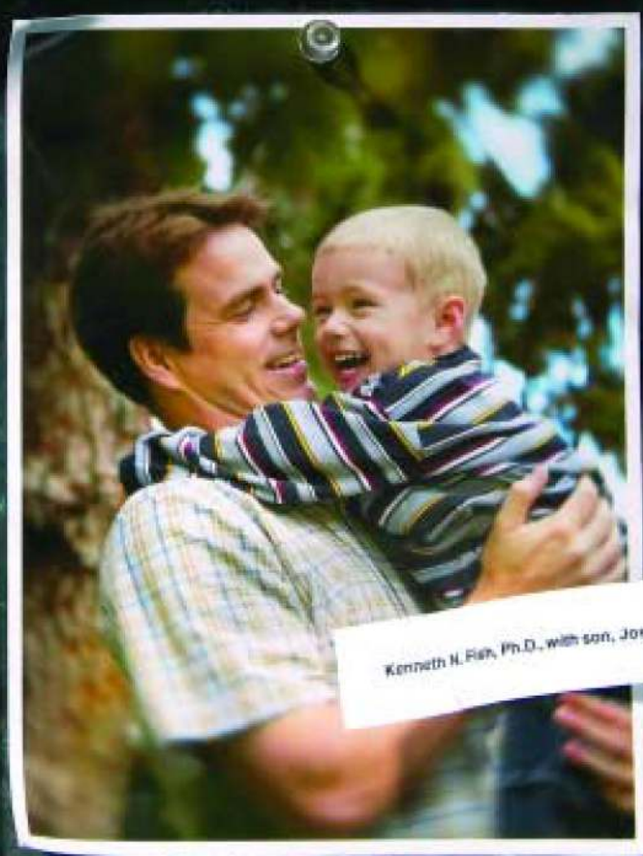

Kenneth N. Fish, Ph.D., with son, Joseph

Focusing on What's Important

KEN FISH TARGETS SCHIZOPHRENIA

When Ken Fish thinks about schizophrenia, something he does every day, a particular image, a kind of cautionary parable, comes into his mind and sticks.

"For someone with a genetic disposition towards schizophrenia, onset can be as simple and as devastating as an act of adolescent rejection," he says. "For instance, a high school senior, one of the most promising students of his class—because schizophrenics are some of the most talented and creative people around—asks a girl to the prom. She laughs in his face. That emotional rejection triggers his first episode of psychosis. One occurrence like that can take all these inherent tendencies and flip the switch."

A severe, chronic disease of the brain, schizophrenia has been diagnosed in all countries and ethnic backgrounds. It can affect anyone at any age, but the majority of cases develop between ages 16 and 30 when people are often at their most vulnerable emotionally. Schizophrenia is best described as an extended psychosis, a type of mental illness that causes severe disturbances of normal thoughts, speech, and behavior. Schizophrenia isn't curable in the true sense of the word, but it can be treated to a degree with antipsychotic drugs.

What Ken Fish is trying to do with his research is to better understand the molecular and cellular alterations that occur in the brains of schizophrenics so that more effective treatments can be developed.

"The multiple behavioral deficits associated with schizophrenia are the result of a group of endophenotypes, measurable components seen only through biochemical tests or microscopic examination of the intersection of disease, genomics, and environmental factors," he says. "Because I started out as a virologist, I know how valuable a cocktail regimen is to treating disease. So, why are we looking for a big hammer that reverses all the behavioral deficits associated with schizophrenia, which is what antipsychotic drugs do today? Why not find individual treatments that specifically target each deficit and then combine these therapies to make a drug cocktail? Why not target individual drugs to their specific site of action? That's the long-term goal of what we're trying to do, and we can't do it alone. It requires a collaborative effort among scientists who are the best in their fields—like the ones at Scripps."

WORKING ON SEVERAL FRONTS

At the moment, Fish is working on several different fronts, a situation not unusual for the 37-year-old assistant professor at the Harold L. Dorris Neurological Research Center. Fish describes himself as a "person who covers a lot of bases because I don't like failure. Attacking a problem from two to three different angles increases our chances of making a significant discovery."

One of his research projects is focused on building mouse models that can mimic individual biologically induced behavioral deficits often associated, at least hypothetically, with schizophrenia. Two of these models lack the ability to produce either the very low density lipoprotein receptor (VLDR) or the apolipoprotein E receptor-2 (ApoER2), which are the receptors for the protein Reelin, a critical component for what is known as neuronal positioning during development. Without these receptors, the Reelin signal is not transmitted to migrating neurons, which results in the abnormal development of different brain regions, one of them the cerebral cortex.

"When I started working on schizophrenia, I was really charged up. But when I held my own child in my arms for the first time, my involvement became something else entirely." –Ken Fish, Ph.D.

The cerebral cortex—the area of the brain responsible for everything from perceptions to simply being able to walk about—is composed of specific layers of cells that develop normally in an inside-out fashion; the youngest neurons are those close to the top of the skull. The correct positioning of neurons in these layers requires the Reelin signal, and is crucial for normal brain function. If the signal is disrupted, the cerebral cortical architecture is completely inverted. The oldest neurons do not pass the youngest, and end up closest to the top of the brain—the wrong spot for them. →

In many cases, these misplaced neurons are unable to perform their normal function. Interestingly, the developmental changes in neuronal positioning in mouse models with a complete disruption in the Reelin signal are reminiscent of the changes that scientists believe occur in schizophrenics, albeit much more severely. Recent findings have shown there is an approximately 50 percent decrease in the expression of Reelin mRNA in the human neocortex, hippocampus, and cerebellum of schizophrenic brains.

Fish is focused on mouse models that lack either the VLDL or ApoE receptors because the brain architectural changes in these mice are much more subtle. These mouse models are proving to be invaluable in his efforts to understand the basic biology behind some of the behavioral abnormalities found in schizophrenics.

"We now have animal models with only small architectural changes in the brain that result in significant behavioral changes," Fish says. "Specifically, we found both similarities and differences while examining the role these receptors play in regulating key reflex responses in mice, specifically sensorimotor gating."

TOWARD BETTER DRUGS

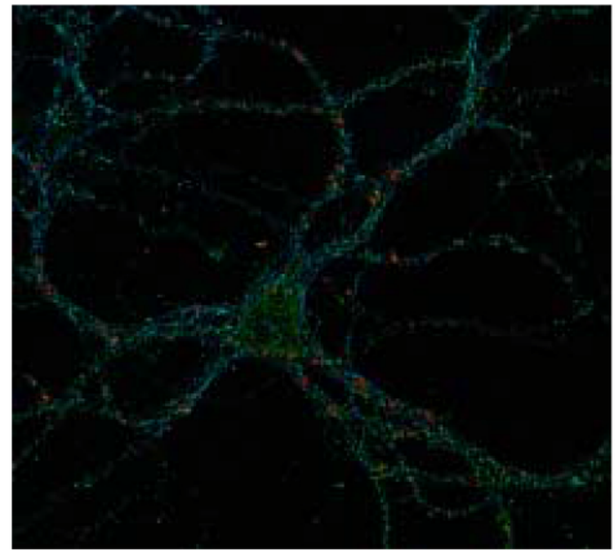
Sensorimotor gating is the ability to filter out certain auditory responses. For example, prepulse inhibition (PPI) is the normal suppression of the startle reflex, something that occurs when a relatively loud tone is preceded by a barely detectable one (the prepulse). Schizophrenics lack the ability to "gate" or reduce their startle response no matter how many times they hear the same set of paired tones, which may help explain certain deficiencies such as disorganized thinking.

"Why are we looking for a big hammer that reverses all the behavioral deficits associated with schizophrenia? Why not target individual drugs to their specific site of action?" –Ken Fish, Ph.D.

What Fish has discovered through anatomical analyses and *in vitro* studies of these mouse models suggests that the differences in PPI response are a direct result of developmental changes in brain architecture.

"Together, these mice create the perfect model system for understanding one of the major behavioral deficits in schizophrenics," he says. "We were very lucky to identify a set perfect for studying the basic biology involved in sensorimotor gating."

These mouse models provide the foundation. Although it will take several years and a strong multidisciplinary approach to understand the molecular and cellular alterations that result in these behavioral changes, Fish is certain the hard work will pay off. From these studies he expects to gain a better understanding of the specific brain regions in sensorimotor gating. Because the better we understand specific areas of the brain, the more accurately we can create new, more efficient drugs.



Scripps Research investigator Ken Fish uses cell cultures of neurons from the primary hippocampus in his research on schizophrenia.

"A great example is one of the drugs used for the treatment of depression," Fish says. "When you first take it, you actually get more depressed. It's amazing to me that the primary function of the drug is reached within 12 hours, but it takes a week or more for the patients to start feeling less depressed. The point here is that we prescribe drugs where the true mode of action is unclear. In a perfect world, we'd know how a drug works before prescribing it. But this is the real world. It can take many years to even scratch the surface of how they function."

Today, Fish explains, there are several antipsychotic therapies to treat schizophrenia. They remain hit or miss, treating some psychotic symptoms, but leaving others unchanged. More to the point, these drugs are ineffective in one quarter of all schizophrenic patients. This is where his research comes in: "We want to build animal models to understand the biology of schizophrenia and how existing antipsychotic medications function. Then we can make better drugs."

A WELL THOUGHT-OUT PASSION

Ken Fish comes at the problems of schizophrenia somewhat obliquely but with a well thought-out passion that is neither a contradiction in terms nor something that is likely to diminish. If anything, it seems to grow apace with his research; the more he knows, the closer he seems to get to the emotional heart of the matter.

When he decided to shift from virology to the study of schizophrenia in 1999, the first thing Fish did was to "sit down and read for nine months straight." After that, he came up with various research projects that received immediate funding from two of the most prominent mental health organizations—the National Alliance for Research on Schizophrenia and Depression (NARSAD) and the National Institute of Mental Health (NIMH).

NARSAD is the largest organization in the world devoted to supporting scientific research on brain and behavior disorders. NARSAD, Fish says, is a fantastic organization to be associated with because of the people involved—many of its supporters have had family members touched by the disease. NIMH is one of components of the federal government's National Institutes of Health (NIH).

Fish's intense and relatively recent focus on schizophrenia is the direct result of, well, no results. Science may have learned a tremendous amount about the brain in recent years, but that knowledge hasn't translated into new drugs to treat schizophrenia. Ken Fish is one of a new wave of researchers determined to find them.

GROWING UP AT SCRIPPS RESEARCH

Something of a poster child for Scripps Research, Fish has spent his entire professional life at the institute and several years before that. While pursuing his bachelor's degree from the University of California, San Diego, he first worked as a student lab technician, then as a research assistant: "I started out as a dish washer, and then got promoted to a media maker. You name it, I've done it. I like to tell people that I worked my way up from the bottom."

Starting in 1994, Fish moved from San Diego to the Oregon Health Sciences University to work on a doctorate in molecular microbiology and immunology with an emphasis on virology. It was during graduate school that Fish began his virology research, receiving several grants and awards to study the pathogenesis of human cytomegalovirus. He received his Ph.D. in 1998 and returned immediately to Scripps Research as a research associate in the laboratory of Professor and Department of Cell Biology Chair Sandra Schmid, Ph.D.

"Even though I was trained as a virologist, I knew one day I wanted to make an impact in the field of neuroscience," he says. "But in order to do that, I also knew that I needed to become a well-rounded scientist. So, to broaden my research base, I went to Sandra Schmid's laboratory because she's a known leader in cell biology. It was clear from the beginning that she was going to be an incredible mentor. I was also able to collaborate with members of Bill Balch's laboratory—Dr. Balch is another leader in cell biology—and that collaboration is still very productive today."

After a while, Fish wanted to return to more disease-oriented research and even considered leaving Scripps Research. He changed his mind when he was offered a fellowship at the Harold L. Dorris Neurological Research Center. In 2001, a year and a half after joining the Scripps Research Department of Neuropharmacology, Fish was named assistant professor. It was after meeting Helen Dorris that Fish first became aware of the full dimensions of the problem.

"It was Helen's passion to accomplish something concrete in schizophrenia that stuck with me," Fish says. "And, for such a widespread and devastating disorder, it was clear little was known about the biology of the disease. So, I thought, what a perfect area for me to focus on."

NOW IT'S PERSONAL

It is the unknown quality of the disease that makes schizophrenia such a frightening and baffling condition, and helps explain the lack of treatment progress. No one knows precisely what causes the disease, so no one knows precisely what a schizophrenic drug should do. Fish considers schizophrenia to be the result of a combination of both hereditary and environmental factors.

"We know that some genetic mutation must be involved, just as we know something in the environment triggers what is essentially a psychotic episode," he says. "With schizophrenics, the psychotic episode doesn't stop. It continues."

The onset of puberty is a well-documented trigger for schizophrenia. The disease also seems to have something to do with the health of the mother during pregnancy. The children of mothers who are either infected with the influenza virus or are

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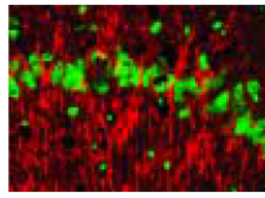
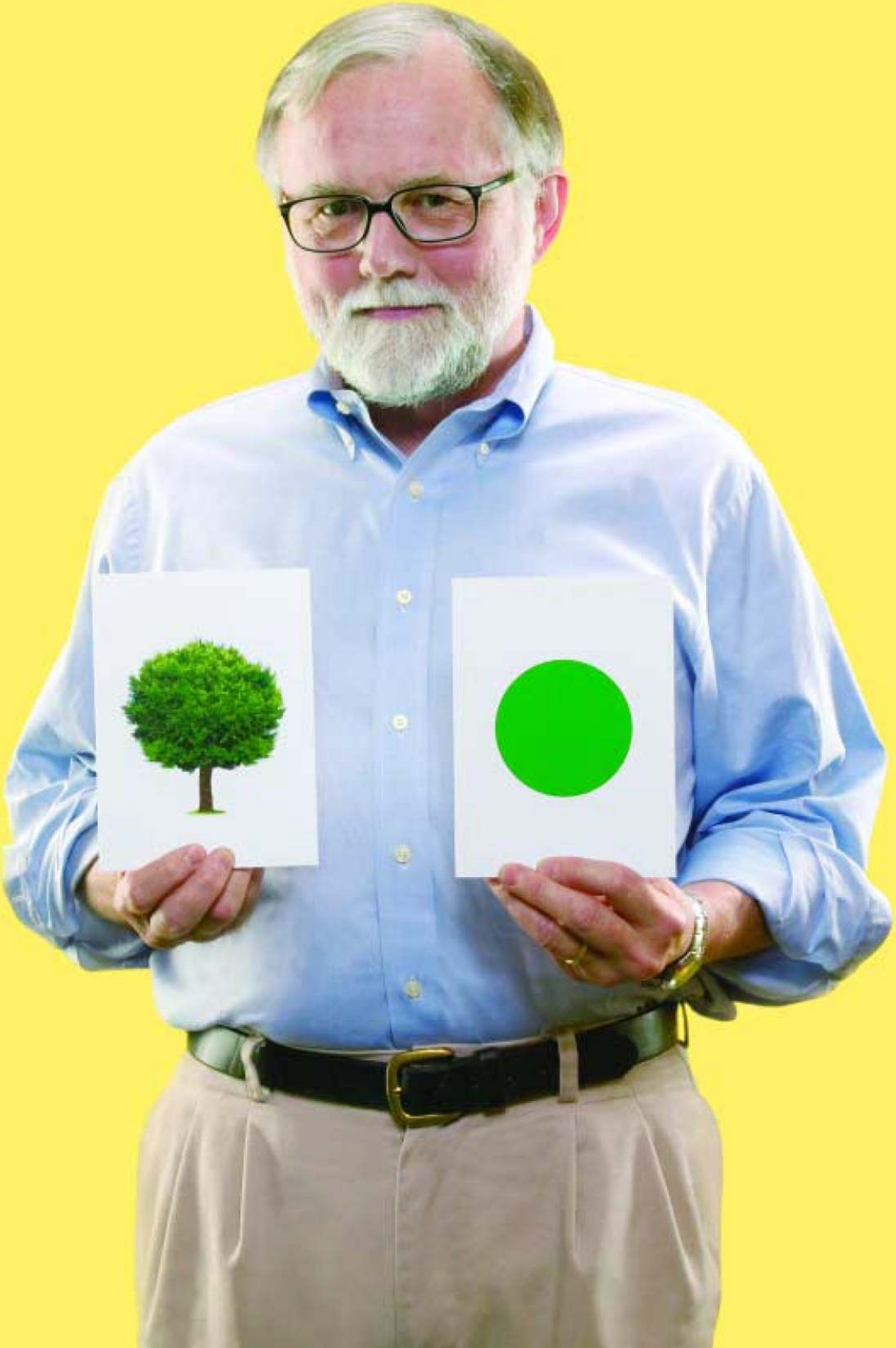


Developmental changes in the brain's hippocampus can alter normal behavior.



Beyond the Brain

JOHN POLICH TACKLES PHILOSOPHY WITH SCIENCE

The basic technique hasn't changed much since 1929 when Hans Berger, a German neurologist, published his paper on electroencephalography (meaning electric brain writing). When electrodes are attached to the scalp and connected to an amplifier, the amplifier reveals variations in voltage over a specific period of time, a measurement called an "electroencephalogram" (EEG). If a human subject is stimulated, say from auditory or visual cues, the brain's response can be measured as specific wave patterns by averaging the EEG responses.

Early researchers labeled these stimulus-induced events "evoked potentials." By the 1960s, evoked potential had become relatively easy to record, and had even begun to provide information about sensory processes. Still, it was a relatively young science.

Within a decade, however, computer and software technology caught up and it became possible to average a variety of different stimuli separately. A human subject could respond to stimuli and accomplish a particular task (pressing a button, for example) with the computer sorting out the averages, quickly and accurately. This produced a new kind of measurable response, an "event-related potential." With an event-related potential it became possible to delineate the electrical brain activity of human subjects while they were engaged in the process of thinking, giving scientists their first real opening into the cognitive process.

In short, they could finally start to measure exactly how the brain became the mind.

All of this extraordinary progress came about just in time to snare John Polich, now an associate professor at The Scripps Research Institute's Department of Neuropharmacology and head of the Cognitive Electrophysiology Laboratory.

PHILOSOPHER, SCIENTIST

Polich had thought seriously about the process of cognition years before arriving in La Jolla. In fact, Polich, who grew up on a farm in rural Iowa, can remember the moment when he first realized that consciousness would be a fascinating topic of study. "I was thirteen and driving a tractor and looking at a cottonwood tree in the distance," he recalls. "I started to wonder why I knew the tree was green. I knew about the retina, that it inverted the image and that the brain somehow made it right. But I wanted to know how the brain produced the perceptual and cognitive experience."

In college at the University of Iowa, Polich first pursued liberal arts, then turned to science, particularly experimental psychology, although he still dabbled in philosophy. For Polich, as for many who grew up in the 1960s, contemplative study was set aside in favor of active involvement. Philosophers read; scientists did experiments in the real world.

Polich eagerly pursued basic neuropsychology. The real action, he decided, was finding out how electrochemical signals generated by the brain somehow turn into what we call "us." It was a convergence of interests, the philosopher reemerging beside the lab man.

After graduating from the University of Iowa and completing a two-year stint in the Army where he was trained as a medic but ended up as a biostatistician, Polich pursued his doctorate at Dartmouth College. All the while, he moved closer and closer toward the cognitive science that was emerging in the 1970s.

> *"I was thirteen and driving a tractor and looking at a cottonwood tree in the distance. I started to wonder why I knew the tree was green."*
> –John Polich, Ph.D.

But when he walked into the laboratory of Emanuel Donchin, a leading figure in the field of cognitive brain wave research at the University of Illinois for an interview for a post-doctoral fellowship, his worldview shifted.

"The computer-based ERP [event-related potential] laboratory Donchin had built was just remarkable for that era," says Polich. "Now, of course, the technology has become so commonplace, you can almost buy brainwave machines in a box. But back then it was spectacular. I realized I was in the best cognitive brain laboratory in the world." →

Despite the fact that it was a major career move for Polich, he quit his tenure-track job as an assistant professor to work with Donchin. He never looked back.

HOW TO MEASURE THOUGHT

After Illinois, Polich moved to the University of California at Irvine. It was there, making rounds in a neurology ward, that Polich first came face-to-face with a patient who couldn't think. The patient suffered from dementia, and Polich began to wonder how to measure a patient's cognitive processes.

Some researchers were measuring diseased cognitive function with event-related potentials—one of the assessment tools available at the time. But even in the early 1980s, this was new and, in some places, hardly recognized as science. At many universities, behaviorism—the accepted wisdom of Pavlov and Skinner that human thoughts and feelings could be conditioned through the manipulation of their environment—reigned supreme: You rang the feeding bell and the dog salivated.

"At that time, people would laugh at the idea of actually studying cognition," Polich says. "Everything was mechanistically assessed. The fundamental questions, though, were fascinating: What constitutes a sentient human being? Is it thought or behavior? And how do you measure it?"

"There will come a day when a multi-technology scan will simultaneously measure many brain activities, and we'll be able to say, 'Yes, you show potential for drug abuse or depression or neurologic dysfunction'…" –John Polich, Ph.D.

The approach to event-related potentials was still pretty basic at that time. Scientists simply put on the electrodes and measured the brain waves that resulted from a series of presentations of auditory or visual stimuli. The event-related potentials produced a series of peaks and valleys—a literal rendering of the subject's electrical brain activity. If there was something neurologically wrong with the brain, as in the case of dementia patients, the timing of the brain response would be delayed, and that delay could be accurately measured.

Increases in computing power and development of new software pushed the science forward, as did the advent of magnetic resonance imaging (MRI) in the late 1980s, and then functional magnetic resonance imaging (fMRI) in the 1990s. In particular, fMRI offered a method of visually determining which parts of the brain were activated by different types of physical sensations, such as sight, sound, or finger movement. The technique began to be taken more seriously, not only as a useful diagnostic tool but as a potential assessment tool and predictor of brain disease.

Brian Lopez and David Berg of the Polich laboratory monitor the frontal lobe brain wave response of a subject while she views a checkerboard pattern.

P300

Polich, who arrived at Scripps Research in 1984, has centered his research around a particular event-related potential known as P300. Presented graphically, the P300 resembles a series of small foothills rising toward a tall peak, then a rapid falloff followed by a slight rise. P300 results when the cognitive system discriminates different stimuli, attention is given to the input, and memory processes are engaged. As Polich himself described it in the *Encyclopedia of Psychology:* "…the P300 component is thought to reflect attentional (cognitive) resource allocation and memory-updating operations…Put simply, the P300 is produced whenever the mental representation of the stimulus environment is changed."

In other words, P300 is what happens in everyone's brain when they're confronted with something out of the ordinary, something that doesn't fit into their knowledge base. It's a universal response that can be readily measured and used to study human cognition.

Polich has taken his understanding of P300 and applied it to young adults to see if, indeed, these universal brain wave readings could help predict which subjects were at high risk for future onset of alcoholism or other drug use. In a paper published last July, Polich reported on his study of a group of 18- to 24-year-old undergraduates who were pre-screened for a family history of alcoholism. High-risk subjects were defined as having a father or other first-degree

relative with a diagnosis of alcoholism; low-risk subjects had none. The subjects were given both auditory and visual tasks and responded with a button press to target stimuli. The simplicity of the testing is part of Polich's strategy to develop useful diagnostic assays using brain waves, something that could be given in a clinic or physician's office.

"Right now, we're trying to understand the underlying neurophysiology of people at risk of drug abuse, so I keep my probes straightforward," he says. "We're trying to make sensitive measures of front and back parts of the brain that appear affected by drug use."

THE QUESTIONS BEGIN

What Polich discovered was less than clear cut. The students determined to be high-risk did produce smaller P300 amplitudes for visual tasks than low-risk students; however, the auditory tasks did not show such strong differences. Does that mean that Polich and his Scripps Research colleagues are on the verge of creating a diagnostic for potential alcoholics? While Polich would be the first to say "No," he would add that he and other researchers have reached a place where that has become a possibility. Polich knows that at this point the questions begin.

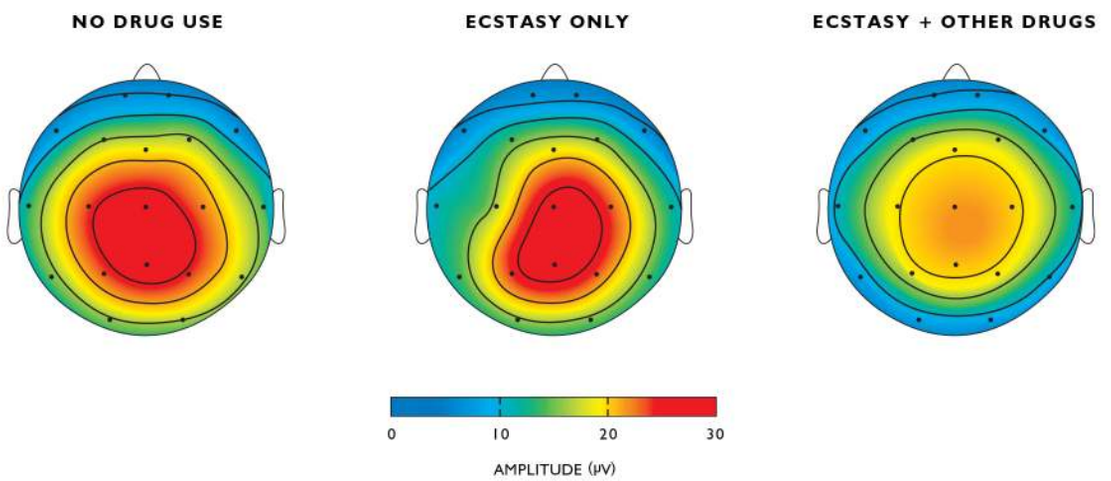
"Let's say that these P300 measures prove ultimately to be true, and that the brains of people at risk for alcoholism are different," he says. "So, if you had a test that said you're at risk for alcoholism and drug abuse, wouldn't you want to know? I don't see this as any different than a blood test for diabetes. You'd want to know. But I also understand that it's not much of a step to ask, well, what about the brains of people with a potential for violent behavior? The question isn't whether you'd want to know, but how society would handle the knowledge. It intrudes on areas that bother a great many people—because we're still talking about potential, something like the idea of pre-crime. Plus we're talking about the larger issues of nature versus nurture."

Polich points out that the human genome is ahead of brain wave research in finding physiological markers for specific traits, and that the biology sometimes drives the psychological research. Someday, he predicts, science will get to the point where event-related potential measurements may be as useful as those genomic markers.

"There will come a day," he says, "when a multi-technology scan will simultaneously measure many brain activities, and we'll be able to say, 'Yes, you show potential for drug abuse or depression or neurologic dysfunction because of the electrical activity pattern in this part of the brain.' But again, in some ways, it's no different than finding out that your liver is abnormal. It all depends on what's done with the information."

While U.S. researchers are generally more interested in mapping basic cognition, many European scientists are investigating possibilities with provocative implications, such as patients' ability to modify their own brain potentials. All of this may seem like giddy science fiction but, as Polich points out, the research has serious moral and philosophical dimensions, not easily dismissed. For Polich, "It's great fun and a privilege to do science in this way, a calling almost."

• Eric Sauter



Brain waves from controls and drug-using young adults.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DEPARTMENT OF NEUROBIOLOGY

1 Gerald M. Edelman, *chairman*
2 Robyn Meech, *gene expression-regulation*
3 Frederick S. Jones, *transcription-regulation and neuronal physiology*
4 Kathryn Crossin, *neural stem cells*
5 Bruce Cunningham, *cell adhesion, RNA granules*
6 Vincent Mauro, *translational regulation of gene expression*
7 Pete Vanderklish, *synaptic plasticity, dendritic translation*

Anatomy of a Brain Department

GERALD EDELMAN AND HIS COLLEAGUES ADDRESS THE FUNDAMENTALS

"Thy gift, thy tables, are within my brain
Full character'd with lasting memory
Which shall above that idle rank remain
Beyond all date; even to eternity…"
-William Shakespeare, Sonnet CXXII, circa 1600.

For a science writer, the prospect of writing a story on the brain can be somewhat intimidating. That three pounds of tissue in our heads has already been the topic of nearly three thousand years of philosophy and research.

When an *Endeavor* reporter sat down for an interview with Department of Neurobiology Chair Gerald Edelman, he got even more than he bargained for. In the room was not one scientist, but five—practically the entire Department of Neurobiology at The Scripps Research Institute. How, he thought, could he possibly write a story about so many different perspectives on this most complex of organs?

But as the hour wore on, the logic of the interview became clear. What emerged was a composite view of the work of the department, which in many ways itself resembles the brain—relatively small, highly interconnected, and very energetic.

SMALL AND INTERACTIVE

The Neurobiology Department is not much larger than it was a dozen years ago when Edelman and his colleagues founded it. Asked about the department's size, Edelman said that it has been kept deliberately small to maximize interactions among the groups.

"It's not the only way of doing things, but it's one that has worked very well for us," says Edelman, who is a member of The Skaggs Institute for Chemical Biology at Scripps Research and winner of the 1972 Nobel Prize in Physiology or Medicine for his discoveries concerning the chemical structure of antibodies. Edelman is also the director of the independent Neurosciences Institute, connected to the Scripps Research campus by a walkway.

The small size of the Neurobiology Department and the proximity to The Neurosciences Institute makes for a highly stimulating environment, says Professor Bruce Cunningham. While each investigator works individually, the investigators have close day-to-day interactions. To an outsider, this is apparent in the way that the department's scientists often nod in agreement when one of them speaks and sometimes finish one another's sentences.

"We work on fundamental problems, and that usually requires spanning a number of disciplines," says Cunningham. The statement is met by nods all around the table.

These fundamental problems revolve around the development of neural function. The overall goal of the department is to understand the fundamental molecular and cellular mechanisms that regulate the differentiation and function of neurons—knowledge that has significant implications for the diagnosis and treatment of a wide range of diseases.

"We work on fundamental problems, and that usually requires spanning a number of disciplines." –Bruce Cunningham, Ph.D.

One important question in the development and morphology of the nervous system is how collectives of interacting cells and cell products give rise to the complex connectivity of the brain.

In the late 1970s, Edelman and his colleagues discovered a class of proteins called cell adhesion molecules that are crucial players in neuronal development. In the brain, neural cell adhesion molecules mediate cell–cell interactions in development and in adult tissues, and their binding induces a variety of intracellular signals, including those leading to changes in gene expression.

Edelman and the other members of his department have long had a program studying the effect of cell adhesion molecules on cells in the central nervous system—establishing the relationship between these molecules and the primary processes of development.

"Like all science," says Edelman, "this has led us into a number of arenas that looked at first to be byways, but really turned out to be fundamental matters." →

KEEPING CELLS TOGETHER

Some of the most basic work on cell adhesion molecules in the department is led by Cunningham. His group looks at the structure and function of these molecules using traditional biochemical and molecular biological technique—looking, for instance, at the structures and activities of domains of cell adhesion molecules. They study primarily the neural cell adhesion molecule N-CAM as an exemplar of those molecules that bind cells homophilically, that is, a molecule on one cell binds to the same kind of molecule on another cell.

Cunningham is specifically investigating the binding mechanism of cell adhesion molecules. These are thought to be critical for cell aggregation, which is known to be critical for the development of the brain.

Edelman notes that, despite the known significance of cell adhesion for neural development, "it's a funny fact the binding mechanism of no homophilic cell adhesion molecule has been fully worked out."

Part of the reason for this is that there is a need for model systems that mimic what happens in nature in a way that can be controlled and observed in the laboratory.

"What you can measure in solution does not necessarily translate directly into what happens on the cells," says Cunningham.

To address this problem, Cunningham and his colleagues use a number of innovative biochemical techniques to study the binding of cell adhesion molecules to other cell adhesion molecules on different neurons, including methods such as attaching the cell adhesion molecules to tiny plastic beads one thousandth of a millimeter in diameter.

He also collaborates with other investigators at Scripps Research and applies the techniques of structural biology—x-ray crystallography, electron microscopy, and nuclear magnetic resonance (NMR). These techniques have allowed his colleague Annette Atkins to solve the structure of one critical domain of the neural cell adhesion molecule—one of seven domains that together constitute the structure of a single cell adhesion molecule.

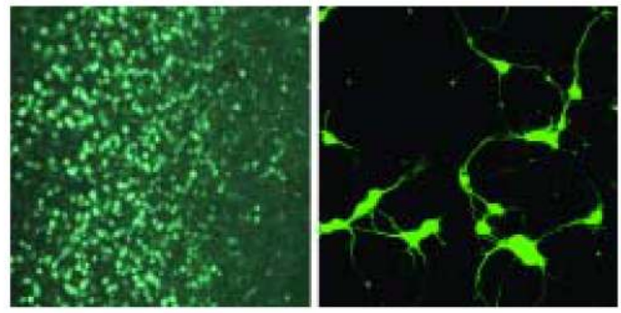
While this group is focused on cell adhesion molecules, they work with a variety of other proteins and usually have one or more active collaborations with the other research groups in the department.

TURNING STEM CELLS INTO NEURONS

Interestingly enough, cell adhesion molecules have turned out to be critical not just because they hold groups of neurons or other cells together, but also for the development of neural cells.

Associate Professor Kathryn Crossin discovered a few years ago that neural cell adhesion molecules have an effect on the emergence of neurons from progenitor cells. Together with other proteins known as growth factors, neural cell adhesion molecules are required during neuronal development to get networks of firing neurons.

By adding neural cell adhesion molecules to neural stem cells in tissue culture, Crossin found, the stem cells developed into neurons in the normal several-week span that development takes in a test tube, and at the end of this period the neurons began to fire, but only when N-CAM plus growth factors were present and not with growth factors alone.



The research group discovered that neurons can be distinguished from progenitor cells based on levels of reactive oxygen species. This finding may open new avenues for addressing disease.

"These results were consistent with earlier studies indicating that cell adhesion was critical for neuronal differentiation, and suggest new approaches for regulating stem cell differentiation for potential clinical use," says Crossin.

Crossin and the other members of the department are attempting to come up with a complete description of this process. They face obstacles, however, because of the materials they are working with. For one thing, isolating and growing batches of neuronal stem cells that are homogeneous is a difficult task. These cells have the ability to differentiate into multiple cell types, and cultures of these cells tend to be heterogeneous mixtures of cells with different fates.

Crossin and the other members of the department persist, however, because results may one day suggest a method to grow more neurons from stem cells and point the way to novel treatments for a number of

neurodegenerative diseases. Cellular therapy, in which neural stem cells are implanted to treat conditions like Parkinson's disease, has shown only limited success because most of the implanted cells don't become neurons. But conditions found by the investigators in the department were able to bias neural stem cells to become neurons—*in vitro*—as well as to increase their survival and enhance their physiological function.

In the course of these studies, the group made a novel observation that neurons can be distinguished from progenitor cells based on their levels of reactive oxygen species. This finding is important for two reasons. First, it allows newly born neurons to be sorted from progenitors, providing a new means of enriching for neuronal cells. Second, high levels of these reactive oxygen compounds usually reflect cellular stress and cell death and appear in brains of patients with neurological disease and during aging. Their appearance in a normal developmental context raises new avenues bridging development and disease.

FINDING THE ELEMENTS THAT CONTROL GENE EXPRESSION

As the conversation continues, several of the faculty members discuss how one of the really interesting questions they have been addressing is how cell adhesion molecules are themselves affected by other genes and proteins in the brain—an area broadly known as gene regulation. Interest in this area, says Edelman, has led to a number of unexpected findings and sent some of their research off in completely unexpected directions.

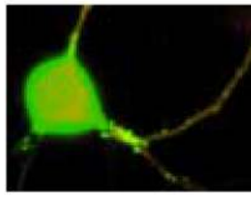
Assistant Professor Robyn Meech navigates one particular area of research in the department that began with work on gene regulation of cell adhesion molecules, but quickly moved on to broad questions of gene regulation in general—through what are known as "*cis*" elements in the DNA.

Cis in Latin literally means "on this side" and *cis* elements are pieces of DNA adjacent to genes that promote, enhance, silence, terminate, or otherwise control the transcription of those genes. Transcription is the first step in the expression of a gene, the process that copies the information from a single DNA gene into a single mRNA message or transcript, which can then be translated into a protein.

Meech and her colleagues are interested in how proteins known as transcription factors interact with these *cis* elements and control the expression of the nearby genes.

Associate Professor Frederick Jones discovered one member of the homeobox family of transcription factors, called BARX2, that bound to *cis* elements in several cell adhesion molecule genes. However, the sequence of the *cis* element that is recognized by BARX2 is very short and many similar sequences appear scattered throughout the genome. This raised the question: How many elements does BARX2 bind to, and how many genes does it control?

"Gene regulation is a multidimensional problem and the *cis* and *trans* components must be studied together in order to really understand what is going on in a given cell type at a given time," says Meech. To do this, Meech and her colleagues utilized a relatively new method called "chromatin immunoprecipitation" that enables them to study transcription in their natural chromatin environment in different cell types. This enabled them to quickly move beyond the cell adhesion molecules and to start investigating other targets of the BARX2 protein.



Several members of the Department of Neurobiology are studying the composition of mRNA granules, shown above, and their regulation by synaptic activity.

One of the cell lines they used in these studies was a breast cancer epithelial cell (epithelial cells are the cells that line the major cavities of the body). Using this cancer cell line and the chromatin immunoprecipitation technique, they discovered a large number of potential DNA *cis* elements to which the BARX2 protein binds, including sequences close to the gene encoding the estrogen receptor.

"Gene regulation is a multidimensional problem." –Robyn Meech, Ph.D.

"We also found an inverse relationship between the expression of BARX2 and the expression of the estrogen receptor in breast cancer cell lines, and by inhibiting BARX2 expression in cells we were able to show that BARX2 could regulate estrogen receptor expression," says Meech. More recently, she adds, they have shown a functional consequence of this regulation in that BARX2 can stimulate the estrogen response and influence the estrogen-dependent growth of breast cancer cells.

CONTROLLING PROTEIN SYNTHESIS WITH RIBOSOMES

Next in the conversation, Associate Professor Vincent Mauro began discussing the control of protein synthesis by ribosomes—the molecular machines that synthesize proteins from messenger RNA (mRNA). Proteins are synthesized in a cell when a ribosome "reads" an mRNA and uses it as a template to synthesize a protein chain. →

But herein lies the interesting twist: the ribosomes themselves may control the translation of some mRNAs.

A few years ago, Mauro says, he and others in the department were looking at gene expression in mammalian cells when they found a large number—perhaps thousands—of sequences in mRNA that are similar or complementary to corresponding nucleotide sequences in the ribosome, much of which is also made out of RNA. He became interested in investigating whether these ribosomal RNA (rRNA)-like sequences, many of which occur in the untranslated or non-protein coding regions of mRNAs, regulate the expression of certain genes by interacting with ribosomes.

"If you asked five years ago if we thought we would be doing these things, [I would have said,] 'I doubt it.'" –Gerald M. Edelman, M.D., Ph.D.

Mauro found that some of the rRNA-like sequences could bind to ribosomes directly and function as internal ribosomal entry sites (IRESes). Apparently, says Mauro, ribosomes and some mRNAs have matching sequences, suggesting that mRNA sequences could be binding directly to the ribosomal RNA through base pairing, similar to the way that two strands of DNA bind to each other.

"This is a startling finding," says Edelman, "the meaning of which we are still exploring."

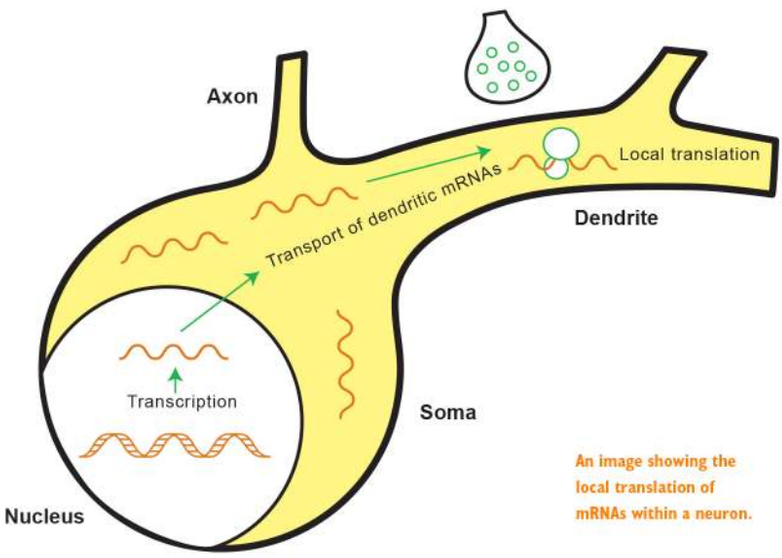
Mauro and his colleagues soon discovered experiments that had been done by scientists at other institutions that supported the idea that short mRNA sequences directly affect translation. For instance, when other scientists had sequentially deleted one end of a piece of mRNA, many of the shorter fragments still had some IRES-activity.

Could these observations be due to the modular composition of IRESes, they wondered, and if so, could the IRES-modules themselves be removed from or added to mRNA? They found that increasing the number of small IRES-modules in an mRNA resulted in a large increase in the rate of protein synthesis and a dramatic increase in the amount of protein generated.

Such amplification of protein output may have potential applications in gene therapy and in biotechnology. This work also suggests a more sophisticated way of understanding the translation of genetic messages, which was elaborated in the ribosome filter hypothesis by Mauro and Edelman. In this model, there are enhancers and inhibitors within the mRNA that influence protein synthesis, in some cases by interacting with binding sites in ribosomes. Different mRNA molecules with different IRES combinations may form a competing population for translation, enabling the cell to preferentially translate one message over another.

The scientists have now developed a model system using yeast cells to manipulate the ribosomal RNA sequences. Manipulating these sequences will enable them to see if the efficiency with which ribosomes translate particular mRNAs changes when the degree of complementarity between the ribosomal RNA and the IRES is changed.

"This is an interesting story," says Edelman, "because it has implications for how you might manipulate protein synthesis." Around the conference table, everybody nods.



An image showing the local translation of mRNAs within a neuron.

10,000 PLASTIC SYNAPSES

Then Mauro turns to his colleague, Assistant Professor Peter Vanderklish, who is also studying the fundamental process of translation of mRNA.

Instead of focusing on the developing brain, Vanderklish is looking at how the control of translation affects the function of a mature brain. In particular, he is looking at the control of translation in dendrites—the spiny parts of neuronal cells that receive input from other neurons. They appear spiny under a microscope because the highly branched dendrites are studded with thousands of small protrusions, each of which forms the postsynaptic element of a synaptic connection between neurons. This architeture allows a single neuron to receive input from thousands of other neurons.

Vanderklish is asking what happens in these dendritic spines when RNA messages enter them and begin to be translated into proteins in response to synaptic activity. Understanding how this translation of mRNA is controlled is of critical importance for understanding how the brain stores information, says Vanderklish, because long-term forms of memory and the synaptic changes that underlie them both require *de novo* protein synthesis shortly after their induction.

A working hypothesis is that when synaptic plasticity is induced, new proteins are synthesized that help change the shape of dendritic spines, and these changes are required for learning and memory.

In addition to studying what new proteins are doing at the synapse, department researchers are now addressing the questions of how different combinations of new proteins may be matched to different forms of plasticity by a process of differential translation, and how the protein synthesis is confined to specific synapses. In addition, since some neurons have as many as 10,000 synapses, they are asking how diverse sets of proteins are getting to a presumably large subset of plastic synapses in a rapid and site-specific manner.

A new and intriguing set of findings on mobile packages of mRNA in dendrites called "granules" offers a glimpse of how neurons may achieve these feats. In collaborative efforts, Vanderklish, Cunningham, and several other members of the department are studying the composition of mRNA granules and their regulation by synaptic activity.

THE EVER-BRANCHING REEF

Studying protein structure and energetics, gene regulation at the DNA level, and gene regulation at the mRNA level seem topics far afield for one department, says Edelman, concluding the hour-long interview, but the investigators go where the science leads them even though they don't know where they will end up.

"If you asked five years ago if we thought we would be doing these things," says Edelman, "[I would have said,] 'I doubt it.'"

Asked what he thinks of this evolution, Edelman shrugs and says whimsically, "I look at science as a giant coral reef consisting of animals slightly more egotistical than [those in the ocean]. You never know where the reef is going to branch."

• Jason Socrates Bardi

Focusing on What's Important

continued from page 5

severely malnourished during the later part of the first trimester and the second trimester are at a higher risk for becoming schizophrenic.

The idea that environmental factors during pregnancy can greatly increase the chance of schizophrenia has particular resonance for Fish. Since he has begun working on schizophrenia, he and his wife have given birth to two children, a boy and a girl. When that happened, his researcher's frame of reference became highly personal.

"When I started working on schizophrenia, I was really charged up. But when I held my own child in my arms for the first time, my involvement became something else entirely. Every day I look at my own children and it becomes a serious motivating factor."

MAKING AN IMPACT

To study something as complex as schizophrenia, there is a clear need to get as many disciplines involved as possible, something less difficult at Scripps Research than almost anywhere else.

"From the standpoint of being able to make the largest impact on the problem, Scripps Research is one of the best places around," Fish says. "The reason why there is so much good collaboration here is because the people at Scripps Research are the best at what they do. I have a lot of those people here to bounce ideas around with. There is an abundance of intellectual resources here."

Fish first hopes to validate his new mouse strains for their efficacy in the creation of new antipsychotic drugs, and then to involve both the pharmaceutical industry and other scientists to use his models to actually develop those drugs.

While he's pointing the way towards better therapeutics, Fish spends as much time as he can learning about schizophrenics and the effect their debilitating disorder has on family members because it keeps him focused on what's important.

"As a society we give them drugs to lessen the burden on society," Fish says, "but not on their families. It's easy to get emotionally involved in your research if you take the time to understand what the families are going through. If we just give them another drug that turns their children into living statues, none of us have done our jobs."

• Eric Sauter

Interview with Charles Weissmann

SCRIPPS FLORIDA, PRION DISEASE, AND THE NATURE OF SCIENTIFIC DISCOVERY

Eminent scientist Charles Weissmann will head the Department of Infectology at the new Scripps Research campus in Palm Beach County, Florida.

Endeavor *spoke with renowned scientist Charles Weissmann, who will head the Department of Infectology at The Scripps Research Institute's new biomedical research operation in Palm Beach County, Florida. Weissmann, who comes most recently from University College London, was a pioneer in molecular biology and has been recognized as one of the most creative investigators in the field. He contributed to the first cloning of alpha-interferon genes, the development of site-specific mutagenesis, and the regulation of red blood cell components. In recent years, Professor Weissmann has turned his attention to prions—the proteins that cause mad cow disease and its human form, new variant Creutzfeldt-Jakob disease.*

ENDEAVOR> What attracted you to Scripps Florida?

WEISSMANN> I liked the idea of building something from scratch and making it successful. I also was attracted by the opportunity to expand my own group and to recruit others in the field. To top it off, my wife and I liked Palm Beach and the people in Florida were enthusiastic, positive, and helpful.

ENDEAVOR> What's your vision of the Scripps Florida Department of Infectology?

"I believe neurobiology is poised for the next major technological breakthrough. If I were a young scientist today, I would go into neurobiology. I'd want to be there, ready to go, when this breakthrough happens." –Charles Weissmann, M.D., Ph.D.

WEISSMANN> My own interest is in prions and the spongiform encephalopathies [such as mad cow disease] they cause. I would like to attract scientists working on problems of interest both for science and public health—diseases such as Hepatitis C, tuberculosis, and malaria. This focus would also help promote the biotech industry in Florida. The specific research topics in the department, however, will also depend on the top scientists available.

ENDEAVOR> What are the main questions in prion research today?

WEISSMANN> The main question in my mind is what the agent looks like. There's one opinion that it is a host protein with an altered conformation, but it may be more complex than that. The main thrust of our research is finding what the agent is really like and understanding how it replicates. These are questions that are only partially understood.

ENDEAVOR> You have shown that mice devoid of the prion gene are resistant to prion disease and noted that cattle or sheep without prions might also be resistant. Are you suggesting that we could raise cattle without prion proteins to control the disease?

WEISSMANN> That's a possibility. But from a practical point of view, this approach would need to be limited to special herds, perhaps those from which we derive pharmaceutical products. To replace entire breeds of cattle is a different question. You can't just take one cow in which the prion gene has been knocked out, breed it, and expect to get a healthy population. To avoid running into a genetic bottleneck, you need to start with dozens or hundreds of individual cows without the protein.

So far, making knock-out cattle is an expensive technology and hasn't worked well. As far as I know, there is currently no such animal.

ENDEAVOR> Is a vaccine for cattle more promising?

WEISSMANN> It would be more practical, but so far there has been little success. Unlike a virus or bacterium, the protein is a natural constituent of the body so there is a lot of tolerance for it. A number of groups are trying different tricks to create a vaccine. It may happen, but it hasn't happened so far.

ENDEAVOR> Is there any approach to treating prion disease in humans that is particularly promising?

WEISSMANN> The answer, unfortunately, is "no."

The use of passive immunization [the administration of antibodies against a pathogen] is one potential approach to the disease. However,

R. Anthony Williamson [professor at Scripps Research in La Jolla] recently published a paper showing that high concentrations of antibodies directed against PrP actually kill neurons, so we are now more cautious in this regard.

Preventive vaccination is not realistic in humans because every vaccine is associated with a certain risk, however low. If the human disease has a very low incidence, we are better off doing nothing.

I think, ultimately, it may well be possible to find a drug that would prevent accumulation of the abnormal protein. That's more likely.

ENDEAVOR> How has science changed over your career? Not long ago, cloning a gene was a major project, certainly a Ph.D. thesis. Now cloning is something that a kid can do with a ready-made kit.

WEISSMANN> When I started in molecular biology, we had to make all our reagents ourselves—everything. In fact, most of our time was spent making the reagents, and the product wasn't always very good. Now, we can buy high-quality purified enzymes, which saves years of research time. The kits we use today were a major development in the field. Unfortunately, some students no longer understand the process of purifying an enzyme. They only understand that if they mix the contents of tube A and tube B they get to the right end point.

ENDEAVOR> How much further is there to go?

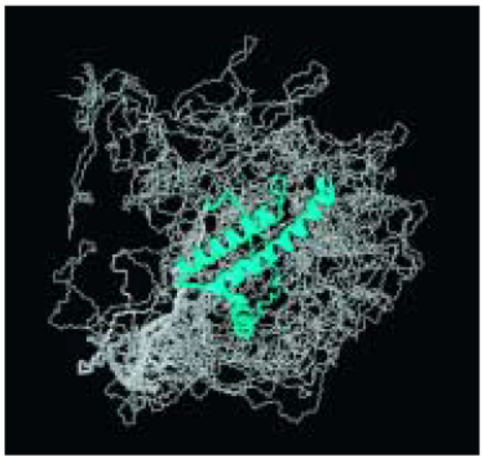
WEISSMANN> A lot further—especially in the neurosciences.

Basically, advances in science are triggered by new methods. When isotopes became available after the Second World War, scientists were suddenly able to do experiments on intermediate metabolism. This research illuminated biochemical pathways, such as how amino acids and fatty acids are synthesized step-by-step.

Then came the realization that DNA codes for proteins. The question became how that information is extracted. A new technology enabled researchers to find out.

After the genetic code was broken, the problem was how to find genetic sequences. It is impossible for people today to imagine how difficult the concept of sequencing nucleic acid was. In the 1960s, it was considered a virtually insoluble problem. But methods were first developed to sequence RNA, then—in a big breakthrough—DNA.

Scientists then wrestled with the issue of extracting specific pieces of DNA from the human genome. Since most of the DNA has more or less the same chemical composition, scientists couldn't fractionate it using classical fractionation techniques. Then came cloning. All of a sudden, like with a magic wand, isolating a human gene and making sense of the human genome became possible.



Charles Weissmann's research currently focuses on prion proteins, which cause mad cow disease and related disorders. *This image shows an NMR structure of the bovine prion protein, bPrP(23-230), solved by another Scripps Research investigator, Kurt Wüthrich, 2002 Nobel laureate.*

I believe neurobiology is poised for the next major technological breakthrough. If I were a young scientist today, I would go into neurobiology. I'd want to be there, ready to go, when this breakthrough happens.

ENDEAVOR> What are the questions that this breakthrough will address?

WEISSMANN> There are many questions. For example, it's well understood that in sight, the perception of color, movement, and shape occurs in different parts of the brain. But how are these different pathways integrated? How does information start in the retina, move to different parts of the brain, then come together to form not only an image but also our perception of the world? Then come more subtle questions. What does thinking mean? How are memories formed? How are they recruited?

We don't really know the answers, but it is my belief that they will come once the technology is there. *• Mika Ono Benedyk and Jason Bardi*

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Jason Socrates Bardi
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