FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

NATIONAL INSTITUTES OF HEALTH ■ OFFICE OF THE DIRECTOR ■ VOLUME 11, ISSUE 5 ■ SEPTEMBER–OCTOBER 2003

NIDA's New Image: Nora Volkow

TRACING THE PATHWAYS OF THE ADDICTED BRAIN

by Fran Pollner

In the 22 years since she got her M.D. degree from the National University of Mexico, in Mexico City, Nora Volkow has taken brain research by storm, almost single-handedly harnessing brain imaging to the service of addiction research.

She is uncovering the neural pathways to addiction—be it to stimulant drugs, alcohol, or food—and charting the potholes left in addiction's wake.

Fran Pollner

Nora Volkow

She has contributed enormously to the scientific literature—in words and pictures—and, this spring, she became a "first" at NIH: She is the first woman to become NIDA director since the institute was founded in 1974.

Volkow officially took office on Thursday, May 1, and on Monday, May 5, she delivered a neuroscience seminar lecture at Lipsett Auditorium on "Mechanisms Underlying Use and Abuse of Stimulant Drugs," an exploration of why the strength of cocaine's addictive grip exceeds methylphenidate's despite the fact that inhibition of the dopamine transporter is the pivot on which both of their actions turn.

In accepting the NIDA position, Volkow exchanged several hats for one. She'd been based at the Brookhaven National Laboratory in Upton, N.Y., since 1987, serving as director of nuclear medicine, director of the NIDA-DOE Regional Neuroimaging Center, associate di-

continued on page 4

On the Road to Crystal Clarity

SOLVING THE MYSTERIES OF MEMBRANE PROTEINS: MULTIPLE CHOICES FOR NIH SCIENTISTS

by Peter Kozel

Question: What's complex, wiggly, mysterious, not very abundant, hard to pin down, risky, essential for life—and something NIH is getting to know better than anyone else in the world?
A. slime molds
B. giant amoebae
C. policy on stem cell research
D. membrane proteins
Answer: D

Although membrane proteins were once thought impossible to crystallize, their structures are now being published routinely and understood—at least a bit—and NIH's intramural research program has something to do with this progress. According to NINDS' Joseph Mindell, NIH now has "[one of] the strongest groups of membrane protein crystallographers [and] membrane protein structural biologists there is."

These investigators are exploring new techniques for the analysis of membrane proteins and are applying new developments to understand how membrane proteins work. Spread throughout NIH's institutes and centers, these scientists are increasingly confident of their abilities. "Our view is, once you have enough of the membrane protein, you can do the structure," says NIDDK's Susan Buchanan.

Membrane Protein Mysteries

Estimated to comprise about one-third of all proteins, membrane proteins act as critical gatekeepers to the cytoplasm. Some convey signals across the phospholipid bilayer in response to conditions outside the cell by changing their shape. Other membrane proteins transport vital small molecules, frequently in a regulated manner.

Essential to the life of the cell, membrane proteins are also increasingly essential to medicine. Between 40 and 60

from published works by NCI's Sriram Subramaniam and colleagues in *Nature Structural Biology*, August 2002, and the *Journal of Bacteriology*, March 2003

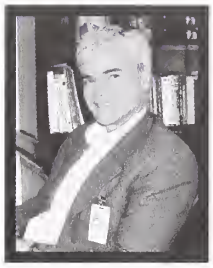
Three-dimensional density map of the oxalate transporter, with the 12 idealized helices grouped into three sets and superimposed manually on the map. One set is nearly perpendicular to the plane of the membrane; another contains bends in the transmembrane region; and the third set contains both a bend and a curve in the transmembrane region [color coding visible in the online Catalyst *—<http://www.nih.gov/catalyst/2003/03.09.01/page1.html>—makes these distinctions clearer].*

percent of pharmaceuticals today target membrane proteins. Astonishingly, how-

continued on page 6

CONTENTS

The Zen of Parking



Michael Gottesman

In response to the threatened loss of 1,700 parking spaces at NIH because of various construction projects, I have had the privilege of chairing a Parking Committee consisting of a cross-section of talented NIH staff. Committee members share the abiding belief that life at NIH is impossible without parking. All of you have been the recipients of my sanguine—yet realistic—all-hands bulletins about the state of parking on our Bethesda campus.

Now, thanks to the hard work of the committee and the Offices of Research Facilities and Research Services, we have been able to delay some loss of parking spaces and replace others on campus. The bottom line is that the shortage of spaces has been reduced from 1,700 to about 400, and we need only embrace alternative transportation plans for the drivers of these 400 cars by December 2003 for a period of about one year.

To solve the remaining problem, we are appealing to a sense of community, to the desire for clean air and healthy exercise, and to a willingness to make some life- and workstyle changes that will be to our advantage in the long run.

Rather than reiterate the content of my recent e-mails to the NIH community, I am taking this opportunity to reveal some of the reasoning that has gone into the current plan.

Finding Middle Ground (and Not Paving It Permanently). As you may surmise, there is a range of opinion at NIH, running along a continuum from the utmost value of ready parking on one end to the utmost value of trees on the other. A few NIHers favor removal of all parking (and some buildings) from the campus in favor of grass and trees; some others maintain that so long as there is a single tree standing at NIH, looking for a parking space would be an intolerable burden. But we found middle ground: Starting in late September and early October, we will be covering some of our grassy spaces with temporary gravel and stacking the parking in all suitable asphalt lots.

During the next 12 months, construction of two new multilevel parking garages will be completed, replacing all the spaces lost to the construction of these garages, Building 33, an underground stormwater management facility, and a commercial vehicle security checkpoint. So by the end of 2004, we will be back to our usual blissful parking state (which, I am proud to say, includes the lowest percentage of single-occupancy vehicles of any major federal or private suburban facility).

Self-interested Selflessness. During a portion of the next year, there will be a shortfall of up to 400 spaces (5 percent of our total) on the NIH campus. The Parking Committee considered a variety of approaches to reduce the demand for parking, including excluding some of our staff from on-campus parking or restricting parking for some individuals for one or more days a week or month.

But, in keeping with the evidence that NIHers will gladly make sacrifices for the common good, we decided to develop a series of transportation alternatives that, for many people, would be more attractive than gridlock on campus when hundreds of drivers look for nonexistent parking spaces.

We now have 400 parking spaces at Mid-Pike Plaza, with an improved shuttle schedule, and 200 new spaces at Twinbrook for those who wish to use TranShare (see <**http://www.nih.gov/od/ors/dps/cpb/etso/transhar.htm**>). With the new security entrances, it will be easier to drop people off and turn around, and carpools, as always, will be encouraged by guaranteed spaces on campus. For those who live nearby, safe bicycling and walking are strongly encouraged.

To put the sacrifice we are requesting in perspective, if all current NIH parkers use an alternative to driving to campus one day in 20 (or one working day in 4 weeks), we will not have a problem.

Who says Life Isn't Fair? Much discussion at the Parking Committee has been devoted to issues of fairness and other matters associated with the change in our parking situation. We have been assured that adequate handicapped parking will be available near all buildings, and the ratio of red parking spaces to general parking spaces will not change (as an aside, it should be noted that the ratio of red permits to red parking spaces is the same as the ratio of general permits to general parking spots).

Some administrative fixes that would change work schedules are under consideration, but much thought needs to go into this approach to ensure that the productivity of our workforce is not compromised.

Going Bananas. There is also considerable concern about the effect of increased pedestrian traffic during rush hours on campus, since most replacement parking will be farther from buildings than current spaces, especially in the northeast corner of the campus near Building 31. Although pedestrians have the right-of-way at properly marked crosswalks, if they cross one-by-one, traffic backs up all over campus. Thus, we are now asking pedestrians to "go bananas"—to cross in bunches of 4 to 6, leaving time between for cars to move. An alternative for some would be to use the new express shuttle to Buildings 31 and 10 from lot 41. More bicycles on campus also mean that drivers must be alert and bicyclists must follow the rules of the road.

The Parking Committee will closely monitor the situation as spaces are lost and alternative parking comes on line. If the situation deteriorates despite voluntary efforts, then we will consider implementing alternative plans that involve scheduled exclusion of some of our staff from parking on campus. We hope this approach will not be necessary and we will all work together as outlined above to solve our parking problems. As always, please send your comments and suggestions. ■

—Michael Gottesman
Deputy Director for Intramural Research

CARTOGRAPHERS DRAFT NIH RESEARCH ROAD MAP

by Peter Kozel

Elias Zerhouni

Before "road map" became the catchword for a Middle East peace plan, NIH Director Elias Zerhouni used the term to describe his nascent plans for redirecting NIH research (see "Roadblocks, Road Maps, and 'The Perfect Storm,'" *The NIH Catalyst*, January-February 2003, page 5). At the June meeting of his Advisory Committee, Zerhouni and some of his chief cartographers presented in greater detail some of the research road map's byways and smaller avenues.

The road map is the product of 16 working groups comprising more than 300 NIH and outside experts and chaired by IC leaders. It emphasizes new tools and building blocks to explore individual molecules and whole systems; multidisciplinary, cross-institute research; and substantial expansion of translational research.

Allen Spiegel

Peter Kozel

Lawrence Tabak

Peter Kozel

Stephen Katz

From Road Blocks To Building Blocks

NIDDK Director Allen Spiegel elaborated the goals of the building-block initiative—to characterize and elucidate the roles of all functional elements in genomic DNA, RNA species, proteins, carbohydrates, lipids, and metabolites in multiple organisms.

He cited NIDDK research showing that glycolipids have metabolic, structural, and signaling properties as an example of uncovering the multiple roles that molecules may have.

Increased access to tens of thousands of small molecules through the creation of new molecular libraries, Spiegel said, will most certainly expand the scope of pharmaceuticals, which currently target fewer than 500 gene products.

The solving of the 3-D structure of the integral membrane protein rhodopsin is a model of what the road map will achieve, Spiegel said. He noted that although membrane proteins are the targets of between 40 and 60 percent of current pharmaceuticals, very little structural information is known about this class of molecules (see story, page 1).

The image we now have of these key proteins, Spiegel said, is akin to a 19th-century daguerreotype, but what we want to produce is a motion picture.

From Solo To Ensemble Performance

NIDCR Director Lawrence Tabak presented the road map's vision of its drivers—that is, multidisciplinary and interdisciplinary research teams. It is NIH's responsibility to break down any cultural or administrative barriers to multidisciplinary research and to identify research problems that transcend the focus of several ICs. The road map calls for the creation of trans-NIH committees to address these issues.

Emphasis is also placed on training investigators to be conversant in several fields and, especially, on the creation of several new granting mechanisms to accelerate the funding of innovative, high-risk, and multidisciplinary and interdisciplinary research.

Translational Research: From Cross-NIH to Cross-Country

NIAMS Director Stephen Katz presented the final aspect of the road map, a series of proposals designed to streamline and expand clinical research.

Translational research would benefit from enhancement of the clinical research infrastructure across the country. This would include the better integration and coordination of clinical research networks and the expansion of their current missions.

Katz also talked about the establishment of 20 to 30 regional Translational Research Centers, each providing core support in biostatistics, pharmacogenetics, novel reagent preparation, animal toxicity testing, and other services.

The behavioral and social sciences would be tapped to develop improved means of assessing clinical outcomes, including quality of life measures.

To bolster clinical research and accelerate the process for performing clinical studies, NIH would work with other federal agencies and with industry to harmonize and simplify many of the current regulatory burdens.

Rules and procedures governing existing national and international genetic and histological repositories would be standardized, and infrastructure would also be built to increase repository availability within the clinical research community.

Finally, the clinical research workforce needs to be boosted by increased training and by expanding current efforts throughout the ICs.

There was a general consensus that these initiatives will require some changes in NIH's approach to basic and clinical research and in granting mechanisms.

Michael Gottesman, deputy director for intramural research, indicated that the flexibility of the intramural program makes it an ideal place for piloting some road map activities and complementing others.

The road map continues to receive congressional blessing: The House of Representatives allocated $45 million in its 2004 budget to the Office of the Director, urging NIH "to maximize the use of the fund to implement the 'roadmap' being developed by the NIH to structure its future research portfolio." ■

rector for life sciences, and chair of the medical department. She had also served concurrently as professor of psychiatry and associate dean of the medical school at the State University of New York, Stony Brook.

The NIH Catalyst interviewed Volkow in mid-August, three and a half months into her NIH job.

Q: What factors informed your decision to accept the offer to become NIDA director?
VOLKOW: It was a very difficult decision. I loved my job (at Brookhaven).

But I recognized what a great opportunity it would be. I have been in the field of drug addiction since medical school and devoted all my professional career to understanding why drugs of abuse promote addiction and the changes in the brain of people who become addicted.

The possibility of going beyond the constraints of my own work and actually having an impact on so many people and on the research of the future was very challenging.

My predecessor, Alan Leshner, had set an example of how you can shape a field. He fought the stigmatization of addiction. He changed the perception that addiction is something an individual chooses out of weakened moral standards. Addiction is a disease, not a choice.

Q: How did this approach dovetail with your own research?
VOLKOW: My own research has concentrated on identifying the changes in the brains of addicted people that lead to loss of control, at how these drugs take away a fundamental aspect of human behavior—free will.

In addiction, the volitional component of human behavior is very much disturbed. You can exert some control in not putting yourself in certain situations, but once you are in those situations, it's almost like a reflex that overtakes the ability to choose.

How have you got to that state? What has happened to the brain?

The other question is, why can some drugs do that? You know, very few compounds can. What are their characteristics?

I have been investigating multiple drugs of abuse in parallel—cocaine, alcohol, heroin, methamphetamine—to determine the common elements, to identify the main skeleton of what each addictive drug has done.

Q: And your methodology?
Volkow: Imaging. Most of my work has concentrated on positron emission tomography [PET], which shows the neurochemical changes in the brain and the pharmacologic properties of drugs in the brain.

Q: What are some of your findings?
Volkow: I was the first to document that cocaine is toxic to the human brain. The belief at the beginning of the '80s was that cocaine was a benign drug. I did studies that showed areas of small cerebral strokes in the brains of cocaine abusers. Nobody believed it, and it took me a long time to get published.

The first study where I documented these brain changes was in 1985; I presented my findings at the Society of Nuclear Medicine, and they didn't believe it. And when I submitted the study, the reviewers said, "There is no evidence that cocaine is toxic to the human brain." I finally got it published in 1988. *(Br J Psychiatry*, **152**:641–648, 1988).

I actually started working with imaging in 1981, when it was not very common. I was one of the first people to use PET technology, first for the investigation of brain tumors, then to study schizophrenia [during a psychiatry residency at New York University from 1981 to 1984, which involved research at Brookhaven] and then with cocaine and alcohol [at the University of Texas Medical School in Houston, as an assistant professor of psychiatry and behavioral science from 1984 to 1987].

I returned to Brookhaven in 1987 specifically to learn what happens in the brain of drug abusers and why drugs of abuse cause addiction.

Q: And you went to Brookhaven because there was a program?
Volkow: No. I went to Brookhaven on condition that I could develop a program on drug addiction. People had not yet recognized how powerful imaging could be for the study of addiction. Brookhaven had the equipment but not yet the program. Now, 90–95 percent of the imaging research at Brookhaven is in drug abuse and addiction.

Q: Do you maintain a connection with Brookhaven?
Volkow: Yes. I keep my laboratory (as part of the NIAAA intramural program), although I handed over many of my ongoing projects to my colleagues there when I became NIDA director. I go there once a month for three or four days—it's hard, but it's very important to me to continue doing research.

We are investigating the long-term effects of drugs and alcohol on the reward circuits of the brain, the neurobiological mechanisms underlying vulnerability to addiction, and the effects of expectation and context of the effects of drugs of abuse. If you can understand this, you can manipulate the outcome.

Another area that fascinates me, and for which there is an ongoing project, is the study of the therapeutic effects of stimulant drugs that can also be abused, such as methylphenidate and amphetamine. Why are they addictive in some contexts and therapeutic in others?

More recently, about seven years ago, I started to work on obesity as a model of an addictive disorder.

Q: Will you be doing any work with NIDDK in the area of compulsive eating and obesity?
Volkow: Yes. I would like very much to work on that initiative and I've met with [NIDDK director] Allen Spiegel.

I am struck by the similarities of loss of control and compulsive behavior in people with compulsive patterns of eating and in people addicted to drugs. The difference, of course, is that you need food to survive, and so the interventions would be different.

But reward mechanisms, conditioned responses, environmental influences (such as stress and drug availability)—these variables that are so important in the prevention and treatment of drug addiction—can be brought to the prevention and treatment of obesity.

Q: Are you contemplating any other collaborations?
Volkow: Yes. We are very much interested in collaborating with other institutes, including those that study the brain, and we are working on a document that will allow us to integrate areas of common research in neuroscience.

I am also very interested in collaborating with NICHD regarding the special vulnerabilty of children and adolescents to addiction.

I want to collaborate with NIAID be-

cause drugs of abuse are extremely important in AIDS transmission, both through drug injection and through inducing mental states that lead to risky behavior.

Collaboration with NCI is also very important because overcoming nicotine addiction is the number-one public health intervention that will have an enormous impact on cancer.

Q: Dr. Zerhouni recently appointed a 10-member steering committee of NIH institute directors. You are among them and the member most newly arrived to NIH. Do you think your voice will be as strong as those who have been here longer?

Volkow: It's actually very good to bring on someone who is new, who brings a fresh perspective. We have a very good combination on this committee of new people and people with knowledge of the system.

Q: What do you think of the suggestion in the recently released Institute of Medicine report that NIDA and NIAAA be integrated into one institute?

Volkow: What's important is not whether there are one or two institutes but that we recognize that these addictive disorders are co-morbid, that they should be studied jointly. For example, there are very few alcoholics who don't smoke, yet the animal models are mostly for alcoholism. They pertain, therefore, only to the 10–15 percent of alcoholics who do not also smoke. That gap has happened not because there are two institutes—NIAAA and NCI—instead of one but because people have not worked together.

T. K. Li [NIAAA director] and I have had several working meetings and are planning some brainstorming sessions to integrate our studies on genetics and drug and alcohol addiction—nicotine, too. And we will prepare an agenda for clinical trials in these areas.

Q: How long do you think you'll be here, and where do you want to take NIDA?

Volkow: I don't think anybody in their right brain would take the position of NIDA director if they are not committed to staying at least five years. I'd like to see NIDA use the tremendous developments in science and technology to fight drug abuse and addiction.

I'm very committed to blending basic and clinical research, to deriving concepts from basic research and applying them clinically, and to taking clinical findings back to the bench.

This is a common goal for all the institutes, and at NIH we can do it. I want to create an environment where scientists of different backgrounds and cultures blend together—physicists, mathematicians, biologists, geneticists, clinicians, chemists.

Q: Is there a dearth of certain disciplines within NIDA? Would you want to hire people?

Volkow: There are areas of expertise that may not be well represented, but we can either hire new people or create collaborations to take advantage of the expertise from other institutes. You don't want to replicate resources. It's not necessary for every single intramural program to have everything.

Q: Are there any research areas calling out for more NIDA support, any where there are huge gaps in knowledge?

Volkow: There are huge gaps in knowledge in the prevention sciences. Why is it that the brain of adolescents is more sensitive to drugs? What are those developmental changes that make it more sensitive? How do we introduce prevention at this stage? It is not so straightforward, not so simple.

Another area of missing information is the whole area of marijuana addiction. It has been, in a way, the forgotten drug, especially in light of the fact that it is the number-one illegal drug of abuse. What is the extent to which early exposure to marijuana increases vulnerability? What *are* the effects of early exposure on learning?

Q: Did your family background have any effect on your thinking when you were growing up?

Volkow: If your family has been persecuted and exterminated for a cause—my father was the only one in his family to survive Stalin, and my mother's family experienced the same thing under Franco—you cannot take your life for granted. It is a privilege to be alive, a privilege to have capabilities and education. I have had all along a sense of responsibility—to give something back, to help. That may have come from my family. ■

Fran Pollner

The great-granddaughter of assassinated Russian revolutionary Leon Trotsky ("Trotsky had two sons and two daughters; my father was the son of one of the daughters"), Nora Volkow grew up in the house-turned-museum in Mexico in which Trotsky was killed. She has three sisters—a writer, an economist, and an infectious diseases researcher—all of whom live in Mexico.

When she graduated from medical school in 1981, she earned not only a degree but a "National Award for the Outstanding Medical Student" as well. She came to the United States for her residency and to pursue her research career because "the possibilities for doing this kind of research in Mexico, the resources, were much more limited."

Though the resources in the United States were excellent, she did occasionally encounter discrimination. In one situation, her being Mexican was cited as a "disadvantage" when she was applying for a particular postgraduate program; in another, her being a woman was cited as a reason she should not be considered for a supervisory position. In the latter instance, more enlightened minds prevailed; in the former, she was not admitted to the program. "But I'm flexible—not having that particular program did not stop me," she recalls. "My task was to understand the brain. I simply did something else to get there."

continued from page 1

ever, the fundamental features of most of these clinically important targets, including their structure and function, are poorly understood.

Low expression level, large size, multimeric composition, and complex arrangement of domains conspire to make membrane proteins notoriously troublesome to study. Unlike cytoplasmic proteins, membrane proteins contain very large hydrophobic regions, making purification difficult. The crystal structures of only a handful of mammalian membrane proteins have been solved.

Peter Kozel

Susan Buchanan

Peter Kozel

Reinhard Grisshammer

In the past, scientists could expect to spend a decade divining the optimum conditions for the production, purification, and crystal formation for a single membrane protein.

But the times are changing. Over the past five years, scientists here and elsewhere have solved the structures of nearly 80 bacterial pumps, channels, and receptors. It now takes just one to three years to form crystals and analyze data for some bacterial proteins—just about right for a postdoc project, Buchanan points out.

A sizeable number of NIH scientists are now riding this wave of discovery. *The NIH Catalyst* interviewed seven scientists to survey the range and depth of structural membrane protein discovery at NIH.

From Expression To Crystal Structure And Protein Function

Before scientists can solve the structure of a membrane protein, they have to be able to express it in quantities sufficient for their method of structural analysis. If that method is crystallography, the quantities required are large and the challenges of producing these quantities of usually scarce molecules are huge. Experts here say one of the keys to NIH's lead in analysis of membrane proteins is its growing savvy in expression techniques.

Advances in the expression of membrane proteins have made possible not only crystallographic analysis of those proteins but also their study by complementary techniques.

Iron Transporters

With more than 15 years of experience solving the crystal structures of membrane proteins, **Susan Buchanan**, an investigator in NIDDK's Laboratory of Molecular Biology, observes that there are still a "huge number" of technical developments needed to optimize the expression and purification of membrane proteins. She and other NIH scientists are hammering away at these needed advances.

Buchanan focuses on iron transporters from several gram-negative bacterial species. Iron is necessary for bacterial proliferation; if iron uptake could be blocked, she observes, an infection could be stopped in its tracks.

"Pathogens such as *Neisseria meningitidis* and *Yersinia pestis* have completely different iron transporters from [*Escherichia*] *coli*, and that's a good thing to study structurally," Buchanan notes, also pointing out that these transporters are highly antigenic and probably good vaccine targets.

Iron transporters have other important qualities. Energy is required for transport, and it appears that many different outer-membrane iron transporters are coupled to a common inner-membrane protein involved in energy transduction. Bacterial iron transporters are picky about their passengers, usually ferrying iron bound to carrier proteins rather than lone iron atoms. In fact, they are highly specific for proteins binding Fe(III).

Buchanan's first crystal structure of an outer-membrane iron transport protein yielded not only a smaller-than-expected 22-stranded transmembrane β-barrel (all predictions at the time suggested 30 or more β-strands), but an additional surprise as well—a globular domain sitting inside the pore.

This globular domain is thought to play roles in ligand-binding specificity and interaction with the inner-membrane protein involved in energy transduction.

"So we view the barrel as a rigid scaffold that allows the rest of the protein to be positioned across the membrane to interact with components in the extracellular space and the periplasm," Buchanan says.

She says such structural details are the key to molecular understanding of transport and maybe even the design of some new vaccines.

G-Protein–Coupled Receptors

Reinhard Grisshammer, a staff scientist in NIDDK's Laboratory of Molecular Biology, studies the enormous family of G-protein–coupled receptors (GPCRs). About 1,000 GPCRs have been identified, 300 to 400 of which are found throughout the body binding endogenous ligands (the remainder are chemosensory GPCRs for odors, pheromones, or tastes).

Recognizing signaling molecules like epinephrine and neuropeptides, GPCRs "are, of course, major drug targets for disease therapy," Grisshammer says. But his interests lie in understanding "the basic mechanisms of how these receptors see their ligand, how they change conformation, and how they transfer the signal across the membrane and lead to activation of a heterotrimeric G-protein."

A crystal structure for only one mammalian GPCR is known—bovine retinal rhodposin. Kryzysztof Palczewski's group at the University of Washington took many years to solve its crystal structure.

For the receptors Grisshammer is interested in studying, which are much less abundant than bovine rhodopsin, "you need to do the tedious expression, purification, and then crystallization. And so we have worked for 15 years on developing methods to express membrane proteins, including GPCRs, in functional form and to establish robust purification schemes at large scale."

In collaboration with Joseph Shiloach at NIDDK's Biotechnology Unit, Grisshammer uses a 200-liter fermentation tank to obtain receptor material of high quality for the regular purification of receptors.

These techniques have been honed to the point that he can produce 5 mg of functional receptor molecules each week. These lots are used to set up crystallization experiments and to develop specific antibodies that aid crystallization.

While scientists believe that the overall structures of GPCR families—for example, the rhodopsin family—are similar, small differences in the ligand-binding regions may determine how tightly those ligands bind. "That's the exciting bit, actually, so having structures from a few representatives would be quite good," Grisshammer observes.

Peter Kozel

Di Xia

P-Glycoprotein

Di Xia, an investigator in NCI's Laboratory of Cell Biology, focuses his crystallographic efforts on proteins responsible for multidrug resistance. "Resistance is everywhere," Di Xia says.

Xia's main focus is on P-glycoprotein (Pgp), the product of the *MDR1* (multidrug resistance) gene.

First cloned by Ira Pastan and Michael Gottesman at NCI in the mid-1980s, Pgp belongs to a family of transporters that includes the gene for cystic fibrosis. Pgp is especially important in cancer management: Once selected in one anticancer drug, cells express the Pgp gene and pump many clinically important drugs, including anticancer agents and HIV protease inhibitors, out of the cell. This ATP-requiring pumping action reduces the intracellular concentration of drugs below their effective dose.

Because cancer cells appear much more sensitive to the function of Pgp than do normal cells, drugs designed to block Pgp could be extremely useful in the clinic, Xia remarks. (According to *CA: A Cancer Journal for Clinicians*, in 2001, there were more than 1.2 million new cancer cases in the United States and more than half a million deaths, most the consequence of chemoresistance.)

How Pgp captures and removes diverse drugs is not known. Xia's lab is pursuing this by seeking to understand how the protein works at an atomic level.

In collaboration with Suresh Ambudkar at NCI, Xia began by expressing and purifying human Pgp. Several common expression systems lack the cellular machinery to produce, modify, and transport human Pgp to membranes and therefore do not generate protein.

Only in insect cells could human Pgp be generated in reasonable amounts, but the yield has been low, and larger cultures have proved less efficient for protein production than small ones. Additionally, purification protocols need to be tweaked. "This is risky business," Xia says.

There are many hypotheses as to why high-level expression of membrane proteins is difficult. One of them is membrane crowding.

To address the problem, Xia is using a novel system that places expression of transporter genes under the control of bacterial photosynthesis promoters to take advantage of the ability of photosynthetically grown bacteria to produce large amounts of fresh membranes.

While developing innovative techniques for expressing human Pgp, Xia is also working on bacterial and yeast Pgp homolog proteins. He hypothesizes that Pgps from all three groups will have similar structures and mechanisms of action.

Bacterial and yeast proteins are easier to study because cranking up expression of native Pgp is easier than modifying the yield of human transgenes. Xia's group can now purify 200 mg of protein using NIDDK's 200-liter fermentation tank—sufficient to begin growing crystals. With crystals in hand, Xia will test his hypothesis by determining the similarity of bacterial and yeast Pgps

Brain Receptors

Mark Mayer, head of NICHD's Section on Neurophysiology and Biophysics, only recently tried his hand as a crystallographer. Trained as a neuroscientist, he has been studying the functional properties of glutamate receptors for more than 20 years, "way before they were cloned."

Found throughout the mammalian central nervous system, glutamate receptors transfer excitatory signals between cells by allowing sodium to enter a cell in response to the binding of a molecule.

Glutamate receptors "do all kinds of wonderful things in the brain," Mayer notes, indicating that his goal is to understand how they work at the molecular level. Mayer says ligand binding is "still quite difficult to model . . . until you've got really detailed side-chain information for a particular subunit."

Mayer currently works on kainate receptors, one of four members of the glutamate receptor family. Biochemical studies indicate that the protein has four domains, and it's believed that four protein molecules—each with 1,000 amino acids—combine to form a functional kainate receptor.

This large size and degree of complexity make it impossible to study the intact, functional channel. "We've resorted to protein engineering to generate soluble constructs of interesting bits of the molecule," Mayer says.

Peter Kozel

Mark Mayer

The bit on which his lab is currently focused is the ligand-binding core. This domain determines subtype selectivity for substrates. "The receptors that have functional roles in the brain are strikingly diverse," Mayer says. "That's due to small numbers of amino acid substitutions having very, very dramatic effects on selectivity."

The ligand-binding core can be thought of as a clamshell, with the ion channel separating the two halves of the shell. Linkers were designed to excise the membrane-spanning region and tie the two halves together. The product is a soluble protein that still binds ligand, implying that the structure has not been perturbed.

This approach works because "these multidomain proteins have quite clear domain boundaries," Mayer says. "One can excise the portion of the molecule that is selective for the ligands."

The engineered eukaryotic protein can be expressed in bacteria. "It's slightly cumbersome juggling 12 liters" of cultures in shaker flasks, but, Mayer notes, "growing bacteria is really not a prob-

lem."

His lab has expressed, purified, and crystallized two members of a low-affinity kainate subfamily that have "striking differences in binding properties." In fact, he remarks, "the drug industry is very interested in these because they are potential analgesic and anticonvulsant targets.".

Chloride Channels

Joseph Mindell, an investigator in the NINDS Membrane Transport Biophysics Unit, applies a range of methods to examine how chloride channels (ClCs) work. Broadly expressed in tissues, ClCs play roles as diverse as salt transport, brain function, muscle activity, and maintenance of intracellular pH.

Peter Kozel

Joseph Mindell

For historical reasons, the ClC family wasn't well studied, but that's changing, Mindell says. He developed a structure of a ClC using electron microscopy and is now "going back to look at function in light of structure."

Peter Kozel

James Hurley

Mindell is taking a two-pronged approach to this goal. Little is known about which—and where—portions of bacterial ClC molecules move during chloride ion transport. Mindell's lab is addressing this by attaching fluorophores to different regions of the protein. The fluoresence characteristics of the fluorophores change depending on the hydrophobicity of their environment.

Armed with this information, Mindell can then create, express, and eventually crystallize and solve X-ray structures for mutants "to get insight into how the whole thing works," he says. "The bacterial system is a great system for that," he notes. "We can make as much [ClC] as we want, and it's easy to manipulate in the large quantities that we need for these sorts of classical biochemical experiments."

Mindell says that while he was focusing on structure, lots of fascinating "biology has emerged," including indications that proteins belonging to at least one of three ClC families are located on the intracellular membranes of lysosomes.

The development of new methods for measuring ion currents from currently inaccessible places, such as lysosomes, is the second prong of Mindell's research program.

"The hope is to broaden the number of channels that we can actually look at and therefore start to look at the conserved properties of the family vs. things that might be specific to [just] one or two. And in the meantime, we may learn something about how the channels in lysosomes work."

New Approaches To Structure

Although the structures of even large membrane proteins can be solved by X-ray crystallographic techniques, these snapshots have key limitations beyond crystallization.

For example, movement is an inherent property of functioning transporters, receptors, and channels. A crystal, however, is a static structure; proteins must be locked into one, and only one, conformation for X-ray crystallographic analysis.

Structural Genomics

One critical class of molecules, amphitrophic membrane proteins, resides in membranes only when they've been activated. **James Hurley**, a senior investigator in NIDDK's Laboratory of Molecular Biology, says "a great deal of signal transduction involves signaling enzymes that translocate from the cytosol to membranes upon activation." Hurley's group studies these proteins, which are involved in trafficking as well as signalling.

Hurley's undergraduate training was in particle physics. He developed his interest in signal transduction during his doctoral training. Upon arriving at NIH in 1992, he began his research program by "opening up Stryer [Lubert Stryer, *Biochemistry*] and looking at what was downstream of receptors."

Hurley's lab went on to crystallize and solve structures of important domains from such classical second messengers as protein kinase C, phospholipase C, and adenyl cyclase.

Hurley's lab has now moved from looking at protein domains to tackling protein complexes using a structural genomics approach. Thus, instead of looking at individual molecules, his lab will now be surveying and comparing domains and structures of whole protein families.

"A lot of trafficking is carried out not by single domains but by complexes that fold together," he observes. He is working in collaboration with Juan Bonifacino's group in NICHD to study sorting into endosomal pathways. Hurley has also begun to look at other protein complexes involved in subcellular targeting and membrane trafficking.

Electron Microscopy

Sriram Subramaniam, chief of the Section on Biophysics at NCI's Laboratory of Biochemistry, is focused on developing and refining an alternative imaging modality: high-resolution electron microscopy.

The immediate product of a high-resolution electron microscopy experiment is an image of a protein or protein crystal that contains three-dimensional data compressed into two dimensions, much like an X-ray image in a computed tomography (CT) scan. Subramaniam's lab uses a suite of techniques to extract the information present in these images to reconstruct the structures of proteins, protein complexes, organelles, and even whole cells.

Electron Crystallography. One of the techniques being used is electron crystallography. Membrane proteins can be crystallized in the plane of lipid bilayer to form two-dimensional crystals.

"The goal of electron crystallography," Subramaniam says, "is no different from that of X-ray crystallography. We want to solve atomic structures." However, because the protein is in a lipid bilayer, it is closer to its native environment than it is in the 3-D crystals used for X-ray crystallography.

This special feature provides an op-

portunity to study physiologically meaningful conformational changes using electron crystallography.

By analyzing and comparing diffraction patterns of 402 different crystals of the proton pump bacteriorhodopsin, each pattern taken at a slightly different angle, Subramaniam was able to do just that and describe the structure of the protein caught in the act of pumping a proton across the membrane.

Peter Kozel

Sriram Subramaniam

"We now have a detailed understanding of how a proton goes across the membrane, how it is pumped. What these structures show is that opening of the cytoplasmic side allows the helices to move and the proton to come in."

A similar approach is also being used in his lab to generate a high-resolution structure of the 12-helix membrane protein that transports oxalate (see graphic, page 1).

Electron Tomography. Another technique Subramaniam's group is using to peer at the organization of membrane proteins in vivo is electron tomography (ET). ET is similar in concept to the CT used in clinical imaging, with one difference—the sample is moved relative to the electron beam in ET, whereas the beam is moved relative to the object in CT. ET is a powerful method for visualizing at molecular resolution the structures of things such as whole cells that cannot be crystallized in 2-D or in 3-D.

One of the projects Subramaniam's lab is using ET to pursue is bacterial chemotaxis.

Bacteria possess an elaborate collection of protein machines that can direct the movement of the bacterium in response to molecules detected at the cell surface. The ultimate goal of using ET is to describe the structure and changes in organization of the molecular apparatus as it swings into action to generate the bacterium's response.

By combining previously published X-ray data with tomographic analyses of fixed cells, Subramaniam's team has observed and analyzed the arrangement of the serine receptor, Tsr, in intracellular membranes. "We discovered that these proteins form these remarkable assemblies inside cells, which we called zippers," Subramaniam recounts. The observation of this novel form has led the lab to propose a new picture of the mechanisms by which bacteria respond to external stimuli.

. . . and More. Another technique that Subramaniam and colleagues are pursuing in collaboration with Jacqueline Milne, also of NCI, involves averaging images from thousands of individual molecules to generate 3-D structures of large protein complexes that cannot be easily crystallized. The team recently reported the molecular structure of pyruvate dehydrogenase, a 10-megadalton protein complex, using these approaches.

Despite these advances, "it's still very early days," Subramaniam says. He is setting ever more ambitious goals—and knows even more powerful techniques will be needed.

For example, his lab is trying to develop new methods of identifying specific proteins in a cell, much like green fluorescent protein is used today—only at "10 to 100 times greater spatial resolution. In the end, we want to be able to take a volume and [get] a three-dimensional localization of all molecules of a particular kind," he says.

To that purpose, the lab is developing new tools for automation of data collection and processing.

Rising to the Challenge

Technical advances notwithstanding, the continuing problems with expression, size, complexity, and purification add up to enormous investments in time and money to achieve structure determination of membrane proteins, NIH's membrane protein masters say. Nevertheless, they are uniformly undaunted. All note that the generous and stable funding of the intramural program increases their productivity. Grisshammer says that one can "really go full speed" at NIH.

One key to their progress is that, although scattered among various institutes, the structural biologists share expertise to address technical difficulties. Buchanan notes that the wide variety of scientists at NIH is one of its strengths. Grisshammer concurs, saying that at most institutions "you have to solve the problems by e-mail or telephone. Here, you can just go and ask for help or advice or materials."

To spread NIH's experience even further, Grisshammer has organized a membrane protein scientific interest group (<**http://www.nih.gov/sigs/mpig**/>) that meets regularly and has grown to more than 60 members.

As complex, wiggly, mysterious, rare, hard to pin down, and risky as they might be, membrane proteins remain well worth the challenge to the scientists the *Catalyst* interviewed. Grisshammer proceeds "without fear or hesitation." Mindell finds it "fun to work on problems where you don't know the answer."

And Xia is sure that he and his colleagues have the right approach: "We know what we are doing, avoid problems, and solve problems one by one. Eventually it should work." ■

Open House/Open Call: RFB&D Needs Scientists

RFB&D, a nonprofit organization that provides recorded textbooks for blind and dyslexic students, has a much greater demand for high-level science texts than it can fulfill. **Its most critical need is for readers who are specialists such as chemists, physicists, doctors, computer scientists, and mathematicians.**

If you have a background in any of these areas or a related field, come to an RFB&D Open House: Wednesday, **October 8**, Building 31, Conference Room 10, 10:00 a.m.–2:00 p.m.

RFB&D has a recording space at NIH, for the convenience of scientists and medical experts who can record college and postgraduate level science texts. All necessary training on recording equipment is provided. A 1-hour per week commitment for a minimum of six months is requested.

For more info, stop by the open house or contact Sarah Scully at (202) 244-8990, or email <**sscully@rfbd.org**>.

STUDENT POSTERS SHINE THROUGH A RAINY SUMMER

by Peter Kozel and Celia Hooper

This year's Summer Poster Day (August 7), the annual exhibition of their research by students in NIH summer research programs, attracted so many participants it had to be divided into morning and afternoon sessions. Following are but eight of the record 484 posters presented throughout the day.

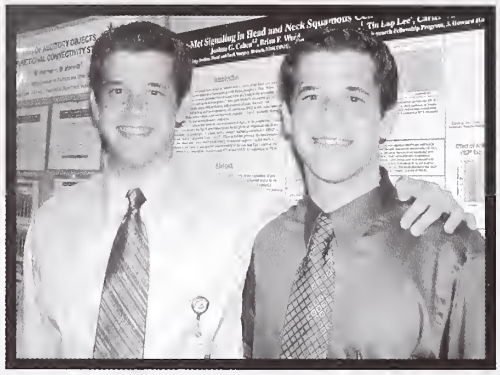
Angiogenesis Inhibition

Many kinds of tumors secrete molecules that promote the formation of new blood vessels, allowing them to continue their rapid growth. NIDCD investigators Zhong Chen and Carter Van Waes had previously observed high levels of hepatocyte growth factor in patients with head and neck squamous cell carcinomas. Brian Worden, an HHMI scholar working in that lab the past year, identified Egr-1 as a potentially important transcription factor in the expression of platelet-derived growth factor and vascular endothelial growth factor.

Continuing Worden's work, **Joshua Cohen** succeeded in elucidating the Egr-1 signaling pathway that turns on these two angiogenic growth factors. He also showed that antisense oligos to Egr-1 inhibited expression of Egr-1 in a squamous cell carcinoma cell line.

This type of head and neck tumor is often detected too late for the usual therapeutic regimen to be effective; it's hoped that blocking Egr-1 will eventually prove clinically useful, Cohen notes.

Cohen, a second-year medical student at Northwestern University Medical School in Chicago, said that working at NIH has been an "amazing" experience. "You study and read about stuff in medical school. Here you get to work with the people doing that research." He thanked by name his preceptors Van Waes and Chen—and everyone else in the lab—for teaching him how to carry out experiments. His fate, he says, is now sealed: "I have to stay in research."—*P.K.*



Celia Hooper

Double Take: *Seth (left) and Joshua Cohen attend the same medical school but worked in different NIH labs this summer*

Stem Cell Platforms

Perhaps it is fitting that **Seth Cohen**, who declares himself the better-looking one of two identical twins working at NIH this summer, also got the sexy research topic—stem cells. Cohen, working with NIAMS mentors Wan-Ju Li and Rocky Tuan, cultured mesenchymal stem cells from adult human bone marrow.

His goal was to compare growth of the cells on the usual flat surfaces to growth on biodegradable, potentially implantable 3-D nanofibers made of poly(e)caprolactone (PCL). The results—equal or slightly superior proliferation on the PCL film—bode well for the possibility of someday implanting the nanofiber disks, loaded with stem cells, as frameworks for regrowing cartilage. The cells on nanofibers also showed greater predilection for calcium mineralization, beginning this differentiation into osteocytes on day 1 vs. day 4 for the monolayer.

Like his twin, Cohen is a second-year medical student at Northwestern University in Chicago. He came to NIH—"The Big House"—for its world-class research and researchers and would like to combine research with medical practice in his career. —*C.H.*

A Model Receptor

Celia Hooper

Michael DiPrima

Michael DiPrima says the most important thing he learned at NIH this summer was "how to learn." A senior this fall in Carnegie Mellon's biophysics program (Pittsburgh, Pa.), DiPrima built upon his biophysical coursework to refine a model for the GluR6 glutamate receptor. Upon binding this excitatory neurotransmitter, the receptor opens, allowing ions to enter a postsynaptic neuron.

Mark Mayer's lab in NICHD, where DePrima did his work, is studying the mechanisms of ligand binding to GluR6 based on X-ray crystallographic diffraction patterns (see p.8). These patterns are translated using computer algorithms into atomic models—but you have to know which algorithm to use.

DiPrima's project was to generate a model using one software package and compare it to a model his preceptor created using a different program. When DiPrima's model proved more accurate, the student became the teacher: He spent his last week here teaching Mayer how to use the better computer program! DiPrima hopes to continue teaching after graduate training in biophysics. —*P.K.*

Bipolar Comorbidity

Studies in adults show that anxiety disorders are prevalent in those with bipolar disorder (BPD). **Anna Binstock**, working with NIMH's Ellen Leibenluft and Daniel Dickstein, sought to learn whether that would be the case in children as well. Binstock examined medical records of young patients with BPD, collected as part of a larger, ongoing study of pediatric BPD.

Celia Hooper

Anna Binstock

Most of the 31 patients in the sample displayed anxiety, a comorbidity rate in line with the 25–90 percent range reported in adults. Moreover, children with both BPD and anxiety were more functionally impaired, with an earlier age of BPD diagnosis and more frequent psychiatric hospitalizations. Though not a focus of the study, Binstock notes that treatment of these patients may be tricky, because standard antianxiety agents may exacerbate BPD symptoms.

A sophomore at Cornell University, in Ithaca, N.Y., Binstock plans to be a pediatric psychiatrist. She enjoyed her experience here shadowing clinicians as they interacted with children and seeing how psychiatric diseases manifest themselves. —*P.K.*

Sights and Smells

Celia Hooper

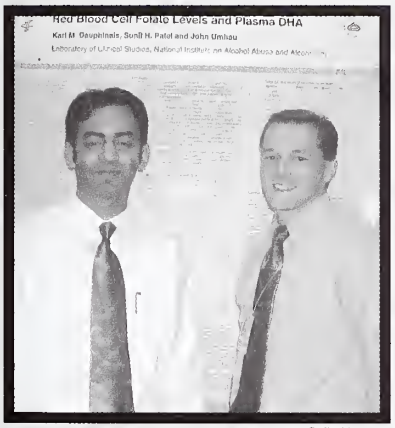
Rhonda Moore

Rhonda Moore is a unique participant in the summer research program: She has a Ph.D. in anthropology. She was pursuing postdoctoral research at the M.D. Anderson Cancer Center in Houston when she caught the neuroscience bug.

Working in Alan Koretsky's lab in NINDS this summer, she used manganese as a contrast agent to enhance the visualization of the rat olfactory system. She anesthetized and intubated rats, applied a manganese chloride solution to their noses, and placed the animals inside an MRI instrument. Manganese reduces T_1 relaxation time, causing positive contrast enhancement in tissues where it accumulates. Future studies will determine which portions of the olfactory bulb respond to specific odorants.

Moore notes that while this particular contrast agent may not be as useful in human brain studies, it is an invaluable means of studying olfaction in knockout animals that are models for human disorders. Now committed to mastering various imaging modalities, she says a key factor in her selection of a graduate biology program will be the availability of an MRI system. —*P.K.*

Fishing for Folate Effects



Celia Hooper

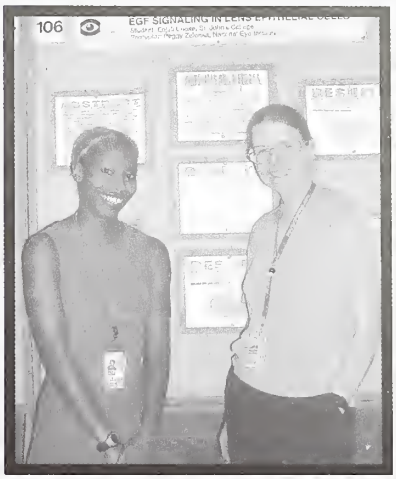
Sunil Patel (left) and Karl Dauphinais

Eating ω-3 fatty acids from fish can ameliorate risk factors for heart disease, but levels of consumption of fish are low in the United States. Working with NIAAA investigator John Umhau, medical students **Sunil Patel** and **Karl Dauphinais** set out to explore whether folate supplementation might increase the absorption of dietary ω-3 fatty acids, especially of the long-chain DHA form —a possibility suggested by a correlation between red blood cell folate levels and plasma levels of DHA in rats.

Using human serum samples collected for another study in the lab, they observed a similar correlation in humans. Interestingly, high levels of folate correlated with high levels of DHA, but not of other ω-3 or ω-6 fatty acids. They are proposing future studies to determine whether plasma DHA levels rise in people who take folate supplements.

Patel, a second-year medical student at Midwestern University, Chicago College of Osteopathic Medicine, at Downer's Grove, Ill., wants to become a cardiologist and tackle the problem of sudden cardiac death. Being able to transform research findings into novel ways to treat patients, he says, "is the best way to practice medicine." Dauphinais, a fourth-year medical student at the University of Miami, will be starting an internal medicine residency and hopes to do clinical research as an adjunct to a practice that emphasizes preventive care and patient education. —*P.K.*

Signaling Surprises



Peter Kozel

Enjoli Cooke (left) and mentor Peggy Zelenka

Enjoli Cooke points proudly to her picture-perfect Western blots. The senior from St. John's College in Annapolis, Md., says the most important thing she learned this summer in Peggy Zelenka's NEI lab was the patience it takes to get clear data—even when they point to negative results. "It took two and a half months to get there," she laughs.

Cooke was following up on studies suggesting that epidermal growth factor (EGF) has different effects on cells at different concentrations. Using her lab's immortalized rabbit lens epithelial cells, she exposed the cells to a range of concentrations of EGF, then looked at the response of the three key initial pathways set off by EGF signaling to see whether they responded differently from one another.

The beautiful theory—that the varying response of the cells to low vs. high EGF levels was due to different responses of the MAPK, PI3K, and PLC-γ signaling pathways—is now threatened by an ugly fact. In Cooke's experiment, all three pathways responded similarly, switching on and staying on from low concentrations to high. Cooke says more experiments are needed to put the theory to rest—and that her enthusiasm for molecular biology remains intact. She wants to pursue postbac studies in proteomics. —*C.H.*

Stem Cell Markers

Celia Hooper

Zishuo Hu

Zishuo Hu, working with Tobi Limke and Mahendra Rao at NIA in Baltimore, has been in pursuit of useful markers that would distinguish neuronal stem cells in the subventricular zone (SVZ) of the embryonic mouse brain from cells in the ventricular zone (VZ) of the developing cortex. Other scientists have found transcription factors that distinguish VZ cells from more mature cells in the developing spinal cord.

Hu wanted to see whether these markers could be used in developing cortex but also sought extracellular markers that could be used to sort cells. Working with tissue from 16-day mouse embryos, Hu was foiled in his goal of distinguishing SVZ from VZ cells, but did come up with some nice markers that could be used to distinguish SVZ from VZ stem cells jointly from more differentiated surrounding cells. This preliminary work pointed to CD24 and PDGFRα (platelet-derived growth factor receptor–α) as non-VZ and SVZ cell markers, and Sox1 as a potential VZ and SVZ marker.

Hu says he greatly values this lab experience and learning that "science can be difficult and exciting at the same time." A sophomore at Brown University in Providence, R.I., he says he is strongly leaning toward a career in research. —*C.H.*

People

Recently Tenured

Yavin Shaham *received his M.A. in 1988 from the Hebrew University, Jerusalem, and his Ph.D. in 1992 from the Uniformed Services University of the Health Sciences, Bethesda. His postdoctoral training from 1992 to 1995 was at Concordia University, Montreal, in the laboratory of Jane Stewart. Subsequently, he was an affiliated scientist at the Addiction Research Center, Toronto, and an assistant professor in the Psychology Department at the University of Toronto. He joined NIDA in 1998 as a tenure-track investigator, and he is currently the chief of the Neurobiology of Relapse Section of the Behavioral Neuroscience Branch.*

Yavin Shaham

In the Neurobiology of Relapse Section,* we use a rat model to study neuronal mechanisms that may underlie relapse to heroin, cocaine, and methamphetamine seeking induced by stressors and drug-associated cues. In the rat relapse model, we measure resumption of lever-pressing behavior induced by drug or nondrug stimuli, following training for intravenous drug self-administration and subsequent extinction of the drug-taking behavior. Our main projects are described below.

■ **Stress-induced relapse.** Human studies report that stress increases relapse to drug use, but until recently there has been no animal model to study the mechanisms mediating this effect. During my postdoctoral training, Jane Stewart and I developed a rat model to study the effect of stress on relapse in rats. We found that exposure to mild intermittent footshock, a common stressor in animal studies, reliably reinstates heroin seeking after prolonged withdrawal periods.[1]

Subsequently, we and others found that this effect extends to other drugs of abuse (cocaine, nicotine, alcohol) and to certain other stressors.[2] We also identified two brain neurotransmitters—corticotropin-releasing factor (CRF) and noradrenaline—and two brain sites—the central nucleus of the amygdala and the bed nucleus of stria terminalis—that play major roles in stress-induced relapse.[3,4]

Over the last several years, our research has been extended to successful human studies on the effect of stress on drug craving. Also, two drug classes found effective in our rat studies are either in clinical trials for relapse prevention (α-2 adrenoceptor agonists) or in planning phases for such trials (CRF_1 receptor antagonists).

■ **Incubation of cue-induced cocaine seeking.** Cocaine addiction is characterized by high relapse rates after prolonged withdrawal periods. Gawin and Kleber hypothesized that human addicts become progressively more sensitive, over the first months of withdrawal from cocaine, to drug-associated cues. Experimental evidence for this hypothesis, however, was not available.

We developed a rat model to study the effect of the cocaine withdrawal period on cue-controlled drug seeking. We found a progressive enhancement, over two months of withdrawal from cocaine, of lever-pressing behavior after exposure to cocaine cues.[5] These data suggest that the individual is most vulnerable to relapse provoked by drug cues at time points that are well beyond the acute withdrawal phase.

In a collaborative study with Tsung-Ping Su and Teruo Hayashi from our institute, we found that the time-dependent changes in cue-induced drug seeking over the first 90 days of withdrawal are associated with alterations in brain-derived neurotrophic factor within components of the mesolimbic dopamine reward system.[6]

In another collaborative study with Bruce Hope from our branch, we also found that in the brains of cocaine-experienced rats, glutamate receptors in regions of the mesolimbic dopamine systems are upregulated for up to 90 days after drug withdrawal.[7]

We and several other laboratories are exploring further the neuronal mechanisms involved in the new phenomenon of incubation of cue-controlled cocaine seeking after withdrawal.

■ **The drug environment and relapse to heroin and cocaine.** In humans, environmental stimuli associated with drug intake play an important role in drug relapse. These stimuli can be divided broadly into two categories: 1) discrete drug cues (such as a needle or white powder) that are temporally associated with the acute rewarding effects of the drug and 2) contextual drug cues (such as a specific bar) that can become predictors of drug availability, but are not temporally associated with the acute rewarding effects of the drug.

Many laboratories currently explore the neuronal events underlying the effect of discrete cues on relapse. In contrast, little is known about the mechanisms mediating drug context-induced relapse.

We recently adapted a rat model (originally developed by Bouton and Bolles in fear-conditioning studies) to study the effect of the drug context on relapse. We found that in rats trained to self-administer speedball (a mixture of heroin and cocaine), re-exposure to the drug-taking context reinstates drug seeking after extinction of the self-administration behavior in a different context.[8]

We are currently exploring neurotransmitters and brain sites involved in context-induced reinstatement of drug-seeking.

References

1. Y. Shaham, J. Stewart, "Stress reinstates heroin self-administration behavior in drug-free animals: an effect mimicking heroin, not withdrawal," *Psychopharmacology* **119**: 334–341, 1995.

2. Y. Shaham, U. Shalev, L. Lu, H. de Wit, J. Stewart, "The reinstatement model of drug relapse: history, methodology and major findings," *Psychopharmacology* **168**:3–20, 2003.

3. Y. Shaham, S. Erb, J. Stewart, "Stress-induced relapse to heroin and cocaine seeking in rats: a review," *Brain Res. Rev.* **33**:13–33, 2000.

4. U. Shalev, J.W. Grimm, Y. Shaham, "Neurobiology of relapse to heroin and cocaine: a review," *Pharmacol. Rev.* **54**:1–42, 2002.

5. J.W. Grimm, B.T. Hope, R.A. Wise, Y. Shaham, "Incubation of cocaine craving after withdrawal," *Nature* **412**:141–142, 2001.

6. J.W. Grimm, L. Lu, T. Hayashi, B. T. Hope, T.P. Su, Y. Shaham, "Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving," *J. Neurosci.* **23**:742–747, 2003.

7. L. Lu, J.W. Grimm, Y. Shaham, B.T. Hope, "Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats," *J. Neurochem.* **85**:1604-1613, 2003.

8. H.S. Crombag, Y. Shaham, "Renewal of drug seeking by contextual cues after prolonged extinction in rats," *Behav. Neurosci.* **116**:169–173, 2002.

* *The section currently comprises two visiting fellows, Lin Lu and Jennifer Bossert, and two students, Jack Dempsey and Shirley Liu, whose hard work and dedication are gratefully acknowledged.*

DOE Expands Half-Life Service

Is Your Research Taking Some New Isotopic Directions?

Radioisotopes can be used for much more than just Southern blots—they are also powerful tools for the diagnosis and treatment of disease. But more exotic biomedical research applications may call for more exotic radionuclides. NIH scientists can now request special—and more mundane—isotopes for their research from the U.S. Department of Energy (DOE).

Some of the clinically important isotopes, such as those used for positron emission tomography scanners, are so short-lived that they must be produced on the NIH campus. The DOE produces and distributes diagnostic and therapeutic isotopes with half-lives longer than about three days.

The DOE distributes four classes of isotopes. Stable isotopes, such as helium-3, and "essentially stable," long-lived radioactive isotopes, such as aluminum-26, are sold from inventory. The final two categories, research and commercial isotopes, have much shorter half-lives—2.6 days to years—and are produced as needed.

Actinium-225 is an interesting example. This isotope, with a half-life of 10 days, is produced from fissile, Cold-War legacy uranium-233. An α-emitter, Ac-225 shows promise as a cancer treatment.

Although the DOE builds and maintains unique facilities to produce isotopes such as Ac-225 that simply aren't available anywhere else, it does not have the funds to produce isotopes that do not have buyers. The agency has thus established a peer-review process, called the Nuclear Energy Protocol for Research Isotopes (NEPRI), to determine which research isotopes will be produced in a given fiscal year.

The NEPRI process begins in February with the distribution of pre-applications to research, medical, and commercial customers. These forms are due in late spring, when they are peer-reviewed. The final list of isotopes to be produced in a subsequent fiscal year is made public by late summer, and orders are accepted until the end of September for the next fiscal year. (Scientists have until September 30 to place their orders for fiscal year 2004.) Payment must be made 30 days before the start of production to cover production and isolation costs.

A fact sheet with contact information and lists of isotopes available in FY2004 can be downloaded from **<http://www.nuclear.gov/infosheets/snm_nepri.pdf>**. This document also contains information on how to request isotopes for FY2005. —*P.K.*

For a glimpse of this year's pre-application form, go to ***<http://www.ornl.gov/isotopes/nepri_04.pdf>***.

A New Kind of NAPster

Suggestions for NIH Organizational Changes, Compliments of the National Academies Press

Anyone interested in the full text of the latest outside effort to evaluate the structure of NIH—entitled *Enhancing the Vitality of the National Institutes of Health: Organizational Change to Meet New Challenges*—may now "read it free online" at

<http://www.nap.edu/catalog/10779.html>.

First released July 29, the report was prepared by a blue-ribbon panel convened by the National Research Council and the Institute of Medicine in response to a congressional mandate.

After a year-long study that focused on whether the proliferation of institutes at NIH is or may become an impediment to NIH's ability to respond efficiently to the country's research needs, the panel declined to recommend the "widespread consolidation" of institutes in NIH.

Instead, the panel suggested establishment of a formal process to review and act on any proposals for IC restructuring—and urged that two particular mergers be first in line for consideration: NIDA with NIAAA and NIGMS with NHGRI.

The panel had suggestions for the enhancement of clinical research, high-risk research, and trans-institute research initiatives consistent with the "road map" plans already under development at NIH (see "Cartographers Draft NIH Research Road Map," page 3).

Other recommendations include taking special care not to outsource administrative functions that are deeply tied to scientific functions, reconsidering NCI's special legislative status, and establishing "term limits" for institute directors and the NIH director. ■

Don't Get Stuck Without Your PIN

Beginning **October 1, 2003**, NIH employees will use Employee Express, an automated system that provides access 24/7, for management of the following transactions:

- Tax withholding (federal & state exemptions/amount)
- Direct deposit/financial allotment changes
- Home address change
- Federal employee health benefit plan/enrollment changes (during open season)
- TSP percentage of salary deductions (during open season)

Changes can be made to benefits information anywhere and any time.

To find out how you can get your PIN and to learn more about Employee Express, visit **<http://www3.od.nih.gov/ohrm/ee/niheeinfo.htm>**.

The Good News: People Want To Keep Their NIH Jobs

Fran Pollner

Jacque Ballard (foreground, right), president of the NIH chapter of Blacks in Government, addresses a noontime rally July 17 protesting A-76. A sea of signs proclaim such sentiments as "A-76 A Weapon of Mass Distraction" and "A-76 Bush, Not Federal Workers."

Displays at NIH are usually in the form of scientific poster presentations, and protests at NIH (rare) are usually conducted by outside groups. But on July 17, the demonstrators were NIH employees, and the poster boards on display decried the A-76 competitive outsourcing process.

A month after a Town Hall meeting that had been dominated by employee concerns about A-76 (see "Bread-and-Butter Dominates Director's Town Hall Meeting," *The NIH Catalyst*, July-August 2003, p. 3), the demonstration revealed continuing dismay over the speed with which competitive outsourcing is being carried out and the potential loss of jobs.

Organized by the local branch of the American Federation of Government Employees and the NIH chapter of Blacks in Government (BIG), the event was billed as an informational rally.

Jacque Ballard, NCI, president of the BIG chapter, noted that for 2002, 466 vacant positions had simply been contracted out, affecting no current NIH employees. The 2003 complement of jobs on the line, however, affects 1,000 NIH employees in grants technical support and real estate property management—"mostly blue-collar workers, minorities, and women," Ballard said in a speech. More of the same is slated for 2004 and 2005, she said.

As the *Catalyst* went to press, the House had passed an amendment to an appropriations bill, submitted by Chris Van Hollen (D-Md.), that would thwart the administration's A-76 plans. The Senate had not addressed the issue. ■

The NIH Catalyst

The online version of *The NIH Catalyst* can be found at **<http://www.nih.gov/catalyst>** and is accessible to all computers within the NIH network.

To be notified when each new issue hits cyberspace and of its approximate contents, subscribe to the Catalyst-L listserve. Send an e-mail message to **<listserv@list.nih.gov>**.

Your message should read:
Subscribe catalyst-L Your Name. ■

Finnish Immunologist: New Fogarty Scholar

NIH's newest Fogarty Scholar, Helgi Valdimarsson, will be on campus for three months each fall from 2003 to 2007.

Professor and chair of immunology at the University of Iceland, Reykjavik, Valdimarrson's early research led to the first report—published in *Nature* in 1975—of what is now known as natural killer cell activity. He went on to explore the immunological underpinnings of such conditions as rheumatoid arthritis and psoriasis, elucidating the mechanisms of phagocytosis, T-cell recognition of antigens, the MHC system, and other immune system pathways.

Helgi Valdimarrson

His hypotheses, first, that psoriasis is a T-cell–mediated disease and, second, that it is triggered by streptococcal superantigens, changed the way the scientific community views psoriasis. His studies have extended to identifying candidate gene loci for susceptibility to psoriasis and the development of effective immunosuppressive therapies.

In continuing work, Valdimarsson intends to focus on identifying alleles predisposing to psoriasis and autoepitopes recognized by psoriasis-causing T cells. He will also explore the use of psoriasis as a model for autoimmune diseases in general.

While at NIH, he will be hosted by Snorri Thorgeirsson, NCI, and Warren Strober, NIAID, and interact especially with the Immunology and Cytokine Interest Groups.

Strober, chief of the mucosal immunity section, anticipates talking with Valdimarsson about the immunological similarities between psoriasis and Crohn's disease—especially regarding the proximity on chromosome 16 of loci associated with the two conditions and the potential role of the gene product NOD2. Bacterial involvement in pathogenesis is another commonality and suggests several collaborative laboratory-based studies focused on the possible roles of interferon-γ, CD25+ regulatory cells, and TGF-β, Strober says. ■

Principles and Practice Of Clinical Research

The deadline for registering for the 2003–2004 course on "Introduction to the Principles and Practice of Clinical Research" is **October 3**. The course runs from **October 20, 2003, to February 24, 2004**, and is held on the NIH campus Monday and Tuesday evenings from 5:00 p.m. to approximately 6:30 p.m. The course is free of charge, but textbook purchase is required. A certificate will be awarded upon successful completion of the course, including a final exam.

For additional information or to register, go to **<http://www.cc.nih.gov/introclinres>** or call the Office of Clinical Research Training and Medical Education at 301-496-9425. An e-mail confirmation will be sent to those accepted into the program.

For reasonable accommodations, call (301) 496-9425, 8:30 a.m.–5:00 p.m., at least seven business days before the event.

The course objectives are:

- To understand the basic epidemiologic methods involved in clinical research.
- To be grounded in the principles of clinical research ethics and the legal issues and regulations involved in human subjects research, including the role of IRBs in clinical research.
- To become familiar with the principles and issues involved in monitoring patient-oriented research.
- To understand the infrastructure required in the conduct of clinical research and the steps involved in developing and funding research studies.

The course is aimed at physicians and other health professionals training for a career in clinical research. Interested persons are strongly encouraged to take a course in biostatistics such as STAT 200 or STAT 500, currently offered at the FAES (see "It's Not Too Late!" p.15). NIH/FAES is accredited by the Accreditation Council for Continuing Medical Education. ■

NIH Research Festival Showcases Clinical Research At the CC 50th Anniversary Scientific Symposium

This year's Research Festival will kick off Tuesday **October 14** with a symposium extravagantly—and correctly—titled "The Past, Present, and Future of Clinical Research" (8:30–5:30, Masur Auditorium).

The people who will trace the progress in the major clinical realms are the people who effected that progress in the last century and continue to carry the work into this one.

All former or current NIH investigators, the speakers are (in order of appearance) Vincent DeVita, Tom Waldmann, Steve Rosenberg, Eugene Braunwald, Elizabeth Nabel, Steve Paul, Henry McFarland, French Anderson, Allen Spiegel, Elizabeth Neufeld, Francis Collins, Harvey Alter, Anthony Fauci, and John Gallin.

The subject matter spans cancer therapeutics, cardiovascular disease, neuroscience, the molecular basis of disease, and infectious diseases.

The Research Festival runs from the 14th through the 17th. Music and food will refresh festival goers as they immerse themselves in the scientific offerings of 12 minisymposia and hundreds of posters. There will also be exhibits of intramural resources and commercial suppliers—and, of course, the Job Fair for NIH postdocs and clinical fellows.

The minisymposia sessions, 10:30 a.m.–12:00 p.m. and 2:00 p.m.–3:30 p.m., will be held Wednesday, **October 15**, at Natcher.

The six simultaneous morning symposia are on host response to infectious diseases, the "new omics" in the molecular epidemiology of chronic diseases, protein-protein interactions, virus entry-virus receptor interactions, programmed cell death, and interconnection of hormones, bone, and brain.

The afternoon symposia address genome instability, bioinformatics from bench to bedside, negative regulation of immune responses, bringing genetics to the public, macromolecular complexes and assemblies, and interfacing the physical and biological sciences.

This year's festival is co-chaired by Joseph Fraumeni, director of the Division of Cancer Epidemiology and Genetics, NCI, and Robert Desimone, NIMH scientific director.

The full festival schedule is at **<http://festival03.nih.gov>**.

It's Not Too Late!

With a $5 late fee, late registration for the FAES (Foundation for Advanced Education in the Sciences) Graduate School at NIH is being accepted through **October 9**. $10 will enable late registration through **October 24**—but that is the last possible day.

The FAES 2003–04 course catalog is available on line as a PDF file at **<http://www.faes.org>**. The hard copy can be picked up at the FAES Bookstore (CC/Building 10, B1 level) and at the FAES Graduate School (One Cloister Court/Building 60, Suite 230). Required texts are also available at the bookstore.

For more info, call 301-496-7976.

FAES could use more classroom space; suggestions are welcomed. ■

Sense and Civility

If the answer to any of the following questions is "yes," CIVIL, the NIH team of experts that promotes civil behavior in the NIH workplace, is available to help sort through the issues and determine the next steps to take.

- Are you or someone you know having difficulty managing anger at the worksite?
- Are you concerned about how to respond to behavior at work that is less than civil—and possibly even intimidating, harassing, or verbally or physically threatening?

- Are family or other personal disputes affecting your ability to think clearly and be productive at work, or are you worried that family members or others with hostile attitudes or behavior may make unwanted visits to the worksite to see you?
- Do you believe that you or any of your colleagues are experiencing overwhelming feelings of depression or thoughts of suicide?
- Have you seen other behavior changes (or behaviors) in yourself or others at work that are cause for worry?

CIVIL may be reached at this phone number: **C-I-V-I-L**, or 2-4845; TTY at 301-402-9499. ANYONE can call CIVIL. For more info, either call or go to **<CIVIL.NIH.GOV>**.

If you think you or others are in IMMEDIATE danger, always call 911 first, if on campus, and 9-911, if off-campus. ■

CATALYTIC REACTIONS?

If you have a photo or other graphic that reflects an aspect of life at NIH (including laboratory life) or a quotation that scientists might appreciate that would be fit to print in the space to the right, why not **send it to us via e-mail: catalyst@nih.gov>; fax:402-4303; or mail: Building 2, Room 2W23.**

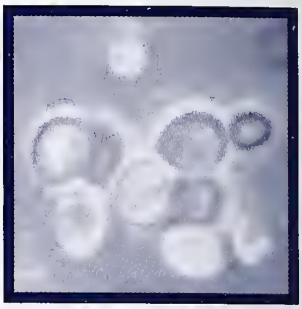
Also, we welcome "letters to the editor" for publication and your reactions to anything on the *Catalyst* pages.

In Future Issues...

- IRP Research Roundup
- Research Festival And CC's 50th
- Phoenix Tales

Kids' Catalyst

BUBBLING BILLIONS: A YEAST EXPERIMENT



Alan J. Cann, Department of Microbiology & Immunology, University of Leicester, U.K.

Saccharomyces cerevisiae

For anything to grow, it needs the right conditions. In this experiment, we're going to find out how to wake up something that looks like sand—dry yeast.

Just looking at this stuff, you'd never think it could be used to make bread, or wine, or pizza. But you'll soon see that you can make this dull, dry sandpile just bubble over with enthusiasm!

First, it might be a good idea to get a grown-up to help you gather the ingredients and equipment you'll need (and don't be surprised if the grown-up gets curious and wants to stick around to see what happens).

You will need:

- A package of dry active yeast
- Four small glasses (cordial size would be good, but test tubes would be even better)
- A packet of sugar
- Warm and cold tap water

Your sense of what is warm and cold is just fine. (You might also write down your observations, glance at the clock every now and then, and use some wooden chopsticks to stir.)

Now let's get started.

So that you remember what to do and what is in each glass container, label the four glasses (with a Post-It) with the following: 1) **Yeast-warm-sugar**. 2) **Yeast-cold-sugar**. 3) **Yeast-warm**. 4)**Yeast-cold**.

Step 1: Open the package of yeast and pour it on a plate. There's not much there at all—and we're going to divide that little bit into quarters, too! (Not much? We'll see.) Shake the dry yeast so it covers the plate, then divide it into quarters (an index card is a good tool for this). Put each quarter in its own glass.

Step 2: Into the two glasses that are marked to have sugar, sprinkle enough sugar to cover the surface of the yeast—a healthy "pinch."

Step 3: Now for warm and cold. Fill a separate container with warm tap water that feels like bathwater and another separate container with cold tap water that feels like a cold soda. Then pour the warm water into the two yeast glasses that are marked for warm, and pour the cold water into the two yeast glasses that are marked for cold (look at the label to make sure, and pour the water almost to the top).

Step 4: Stir. Use separate stirrers for the glasses with and without sugar.

Step 5: Wait and wonder—but you won't have to wait long at all to see something happening.

So, what is it that makes dry, dull yeast wake up and bubble over? When you decide what that is, see if you can get all four yeast samples to behave like that.

—Jennifer White

For more information about yeast (and action videos), see **<http://www-micro.msb.le.ac.uk/Video/Scerevisiae.html>**.

The NIH Catalyst is published bi-monthly for and by the intramural scientists at NIH. Address correspondence to Building 2, Room 2W23, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; fax: (301) 402-4303; e-mail: <catalyst@nih.gov>

PUBLISHER
Michael Gottesman
Deputy Director
for Intramural Research, OD

EDITORS
John I. Gallin
Director, Warren Grant Magnuson Clinical Center, and Associate Director for Clinical Research

Lance Liotta
Chief, Laboratory of Pathology, NCI

SCIENTIFIC EDITOR
Celia Hooper

MANAGING EDITOR
Fran Pollner

COPY EDITOR
Shauna Roberts

CATALYST INTERN
Peter Kozel

CONTRIBUTING WRITER
Jennifer White

EDITORIAL ADVISORY BOARD
Jorge Carrasquillo, CC
David Davies, NIDDK
Dale Graham, CIT
Hynda Kleinman, NIDCR
Elise Kohn, NCI
Susan Leitman, CC
Bernard Moss, NIAID
Michael Rogawski, NINDS
Joan Schwartz, NINDS
Gisela Storz, NICHD

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
Building 2, Room 2W23
MSC 0235
Bethesda Maryland 20892

FIRST-CLASS MAIL
POSTAGE & FEES PAID
DHHS/NIH
Permit No. G-802

Official Business
Penalty for Private Use $300

Printed on 50% recycled content paper and can be recycled as office white paper.