FOSTERING COMMUNICATION AND COLLABORATION

The NIH CATALYST

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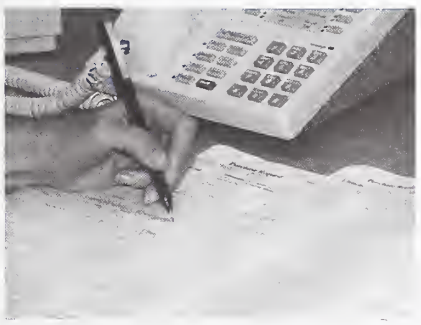
UNTANGLING RED TAPE: RESHAPING BUREAUCRACY TO MEET SCIENTISTS' NEEDS

by BPRC Staff

Have you ever felt like tearing your hair out after trying to make your way through the bureaucratic maze involved in ordering research supplies and equipment? How about filling out a time card or trying to subscribe to your favorite scientific journal? If so, you are not alone, and your cries are no longer falling upon deaf ears.



Lorna Heartley

Inside...

"NIH is a terrific place to work, interacting with some very knowledgeable people," says Michael Cashel, head of the Section on Molecular Regulation in NICHD's Laboratory of Molecular Genetics, but the research milieu would be even better if administrators could eliminate some of the little "day-to-day things that unnecessarily raise the frustration level" for scientists. NIDCD Scientific Director Jim Battey concurs: "Our major product is research;

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BRIDGES TO BALTIMORE: NIDA'S ADDICTION RESEARCH CENTER

by Rebecca Kolberg

Melanie Holden

Gazing out a window at the blue of the Chesapeake Bay framed by the steely glint of shipping cranes and the arch of a distant bridge, NIDA's intramural research center in Baltimore seems a world apart from NIH's main campus in leafy, landlocked Bethesda.

And in some ways — its unique historical tradition, its self-contained camaraderie, and even its plentiful parking — NIDA's intramural research program does stand alone. But when it comes to its scientific endeavors, there may be far less distance between NIDA and other intramural research programs than many scientists realize.

"The general progress of science has provided lots of new ways to explore drug addiction. But, to flip it around, the field of addiction research is also able to contribute to the kind of science of interest to broad parts of the neuroscience community and the NIH scientific community," says NIDA's Acting Scientific Director George Uhl.

Noting that epidemiological evidence indicates that "an incredibly large chunk" of U.S. morbidity and mortality, perhaps as much as 50 percent, may be related to behaviors driven by much the same kind of motivation-reward circuits that drive drug abuse, Uhl says NIDA scientists may be able to help others in the NIH intramural community understand some of these behaviors.

NIDA researchers may also be able to provide some valuable clues in the effort to solve a major neuroscience riddle: how memory works. "By my definition, addiction is a fairly specific form of memory ... a behavior that at some time depends on recalling your past experience with that drug," Uhl says. "Because we now know quite a bit about how drugs work on the brain acutely, at both the pharmacologic and the molecular level, we are actually in a good position to help

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THE CLINICAL CENTER REVIEW: WHAT'S REALLY HAPPENING

John Gallin

I am sure that most of you have read or heard about the recent news articles in *Science, Nature*, and *Nature Medicine* about stress at the Clinical Center stemming from the HHS proposal to contract out all, or a portion, of the Clinical Center's operations as part of phase two of Vice President Al Gore's Reinventing Government initiative, or REGO II. I am also sure that many of you who saw the May 12 article in the *Washington Post*, which was headlined "Administration Unveils Proposal to Cut 2,400 HHS Jobs in 5 Years," were disturbed to read that "HHS sources said ... 1,000 or so jobs could turn out to be positions at NIH's Clinical Center ... ." In my conversations with Health Care Financing Administration Deputy Administrator Helen Smits, who is charged with overseeing the review of the Clinical Center for HHS Secretary Donna Shalala, it is clear that no decision has been made to contract out the entire Clinical Center. The purpose of this column is to bring you up to date on my understanding of what is happening.

Smits, who has an office near mine on the second floor of the Clinical Center, is here every Wednesday. She is anxious to learn about the Clinical Center and soon expects to be joining patient rounds and visiting operating rooms. She has assembled an "Options Team" charged with identifying obstacles to conducting clinical research and evaluating options to ensure that the Clinical Center runs as efficiently as possible. The members of the Options Team, which is chaired by Smits, are Alan Brier, NIMH; Greg Curt, NCI; Michael Goldrich, NIAID; Christine Grady, NINR; David Henderson, CC; Steven Holland, NIAID; Walter Jones, CC; Ruth Kirschstein, OD (ex officio); Harvey Klein, CC; Francine Little, OD; Kathy Montgomery, CC; Griffin Rodgers, NIDDK; Judith Vaitukaitis, NCRR; and myself. Additionally, the following external advisers will provide periodic consultation to the Options Team: John Finan Jr. of Barnes Hospital in St. Louis; William Kerr of the Medical Center at the University of California at San Francisco; Gloria Opirhory of John Dempsey Hospital, University of Connecticut at Storrs; John Rowe of Mount Sinai Medical Center in New York; Steve Shimpf of the University of Maryland Medical Center in Baltimore; Ralph Snyderman of Duke University in Durham, N.C., and Samuel Thier of Massachusetts General Hospital in Boston.

The goal of the Options Team is to identify mechanisms for making the Clinical Center more efficient and a better place to conduct clinical research. The goal is not to save a certain number of full-time employees (FTEs) through costly and inefficient contract mechanisms. Specifically, the Options Team will be divided into groups to look at the Clinical Center's governance, information and reporting, budgeting, benchmarking, and options as a federal entity and at the possibility that the center could become a reinvention laboratory. As a result of the retreats held by the Medical Board and the Clinical Center department heads early this fiscal year, several groups have already started working independently on related issues and these groups will merge their work with the Options Team's efforts. The Options Team is addressing specific questions in these areas:

- **Governance.** How can the governance system of the Clinical Center best ensure its successful function? Is the Clinical Center capable of responding quickly to change and dealing effectively with difficult decisions? Does the current governance structure serve the interests of intramural clinical investigators?
- **Information needs and reporting mechanisms.** What kinds of information, training, and administrative systems are needed to ensure cost-effective performances?
- **Budgeting mechanisms.** Are there other medical research institutions similar enough to use for comparisons of efficiency? If so, in what functional areas?
- **Flexibility.** What are the most serious problems posed by federal rules dealing with personnel? Purchasing? Contracting?

THE GOAL IS NOT TO SAVE A CERTAIN NUMBER OF FULL-TIME EMPLOYEES (FTEs) THROUGH COSTLY AND INEFFICIENT CONTRACT MECHANISMS.

Smits has asked the Options Team to describe a full range of options that could be used to enable the Clinical Center to operate with maximum effectiveness. She has also asked the team to weigh the relative merits, including the cost-effectiveness, of each option.

The Options Team will spend May through August gathering data and visiting other hospitals. In September or October, the team will hold a retreat to develop recommendations, and those recommendations will be submitted to an outside consultant for cost-benefit analyses. Throughout November and December, the team will review draft reports and present its conclusions to the NIH leadership, including the External Advisory Committee to the NIH Director. The final report will be delivered to Assistant Secretary for Health Philip Lee by Jan. 1.

From my vantage point, the Options Team's objectives appear compatible with NIH's long-standing objectives of efficiency in clinical research, training, and patient care. I am confident that the review process will result in an improved Clinical Center, and I urge clinical researchers to make their views and priorities known during this time of transition. Smits welcomes your comments and can be reached by e-mail (smits@qmgate.cc.nih.gov).

John I. Gallin, M.D.
Director
Warren Grant Magnuson Clinical Center
Associate Director for Clinical Research

The Interest Group Gazette

Research Festival

Intramural interest groups are uniting on a new project: coordinating many of the scientific presentations at the 1995 NIH Research Festival. Scheduled for Sept. 18-22, this year's event will include workshops and poster sessions organized according to the broad research interests of NIH's interinstitute interest groups. For example, the **Structural Biology Interest Group** is sponsoring two workshops — one devoted to signal transduction and the other to DNA-protein interactions from the structural-biology perspective — and the **Hard Tissue Disorders Interest Group** and the **Clinical Research Interest Group** are co-sponsoring a workshop focusing on clinical and basic research on skeletal disorders. The festival will be held in the Natcher Building with displays and information booths located in Parking Lot 10-D between the Clinical Center and Building 37.

First NIH-Wide Meeting

NIH Director Harold Varmus enthusiastically welcomed representatives of the 40 interinstitute interest groups to the first meeting bringing them all together on May 5. Among the items discussed at the gathering were the general organization of the groups, scheduling of speakers for the Wednesday Afternoon Lectures series, and ways of improving communications among the various interest groups, such as establishing electronic poster pages, creating "home" pages on the World Wide Web, and setting up e-mail list servers for exchanging ideas and updates between and among the various groups. In particular, a demonstration of the **NIH Campus Yeast Interest Group's** home page (http://www.nih.gov/sigs/yeast/index.html) dazzled everyone.

Always on Tuesday

Meanwhile, interest groups across campus continue to lay out their sumptuous smorgasbord of monthly meetings and research symposia. Consider just one weekday's offerings — Tuesday, for example. NIH's **Drosophila Group** meets on the third Tuesday of every month from 1:15 p.m. to 2:30 p.m. in Building 6B, Room 4B429, to discuss recent research on *Drosophila melanogaster*. Researchers interested in being placed on the *Drosophila* mailing list should send their name and e-mail address or fax number to Susan Haynes (phone: 496-7879; fax: 496-0243; e-mail: sh4i@nih.gov).

The recently formed **Bioinstrumentation Interest Group** emphasizes mutual education for scientists interested in modifying, designing, and building their own instruments for biomedical research. Members convene in Building 13, Room 3W54 at 2 p.m. on the first Tuesday of each month from September through June. The group's meetings offer informal technology tutorials, talks on specific projects, and surveys of various areas of bioinstrumentation, as well as brainstorming sessions on key problems with the technology and possible solutions for improvement. For more information, contact Stephen Leighton (phone: 496-4426; fax: 496-6608; e-mail: leighton@helix.nih.gov).

Another meeting always held on the first Tuesday of the month attracts researchers from NIH, the University of Maryland, George Washington University, and Johns Hopkins University to the **RNA Club**. The focus of this special interest group is post-transcriptional regulation and RNA-protein interactions. Members specialize in research on RNA processing, stability, transport, and translation; RNA binding proteins; ribozymes; and small RNAs. The meetings, which start at 4 p.m. in Building 41, Room C509, usually feature two 30-minute lectures from group members on their evolving research. To join the RNA Club, forward your mailing address, affiliation, and voice or fax numbers to either Carl Baker (phone: 496-2078; fax: 402-0055; e-mail: ccb@helix.nih.gov) or Susan Haynes (phone: 496-7879; fax: 496-0243; e-mail: sh4i@nih.gov).

If that's not enough to whet your scientific appetite, *The NIH Catalyst* plans to publish in a future issue a summary sheet listing the meeting times and contacts for all 40 NIH interinstitute interest groups. ■

—Katie O'Brien

Catalytic Reactions

Below is a comment we received for a topic raised in the January-February issue.

On intramural research couples

The article was interesting, except that most of the couples interviewed were senior scientists who were happily settled with steady jobs at the NIH. Most of the married couples on the NIH campus are postdocs or untenured scientists, and their concerns are quite different. And what's with those "Wedded Words of Wisdom"? What is this, marriage counseling? Most married scientists are primarily concerned with getting jobs in the same city, not with how to communicate with each other! ■

— Susan Chacko, NIDDK

NIH Scientific Poster Conference Page

Are you up to the challenge? As announced in the Hot Methods Clinic in the March-April issue of *The NIH Catalyst*, Editor Lance Liotta is calling on all intramural scientists to help create the first NIH Scientific Poster Conference Page on the World Wide Web. This page will serve as a continuous, electronic poster session, providing references to a wide array of Web pages that feature posters describing cutting-edge research performed at NIH. Liotta and his cyber colleague at NCI's Laboratory of Pathology, Alex Lash (e-mail: alash@helix.nih.gov), welcome your suggestions about how the poster page should be set up, what sorts of research it should feature, and where to locate NIH research that is already posted or soon to appear on the Web. ■

END OF THE ICE AGE: WHAT THE HIRING THAW MEANS FOR SCIENTISTS

by Rebecca Kolberg

After weathering 15 months of hiring and promotion freezes that sent a chill throughout the intramural research community, NIH is back in the business of hiring full-time employees and handing out GS-14-and-above promotions for nonsupervisory and supervisory positions.

"We are out of the freeze fully, although still limited by ceilings," says Deputy Director for Intramural Research Michael Gottesman. "In most institutes and centers, this should translate into some judicious hiring and processing of promotions."

Coming on the heels of January's long-awaited thaw in the full-time-employee (FTE) hiring freeze, the latest warming trend in NIH hiring and promotion affects the number of GS-14-and-above positions. Last year, the Clinton administration, in an effort to curb the swelling ranks of federal midlevel managers, fixed the ratio of higher-level to lower-level positions in the government work force. The mandate required that 10 percent of federal downsizing come in positions that are GS-14 and above. However, that mandate assumed that all federal positions that are GS-14 and above are held by supervisors or managers — an incorrect assumption for NIH, where many highly trained scientists who receive GS-14-and-above salaries do not perform any managerial or supervisory tasks. In April, the Office of Management and Budget took note of the hardship that the GS-14-and-above freeze was causing among scientists and gave NIH permission to hire and promote freely at the GS-14-and-above level if the person is not in a supervisory role. Each institute, center, or division will also have a few GS-14-and-above supervisory positions open, but NIH administrators currently do not know how many of these slots will be available.

"This is good news. It means that anyone hired now into a GS-13 nonsupervisory position will have the opportunity to be promoted in the future and that we can hire senior scientific staff in nonsupervisory and some supervisory positions," Gottesman says. The action is also welcome news for those nonsupervisory, as well as some supervisory, scientists whose promotions have been delayed because of the freeze.

Meanwhile, the recent lifting of the moratorium on general FTE hiring continues to lift the morale of the NIH scientific community.

"The average scientist in an institute that is below its FTE ceiling [which is assigned by the Office of the Director] would be able to bring on the secretary or the technician for which they have recruitment authority," Gottesman says. "We can hire tenure-track investigators, an occasional expert, visiting scientists, and some staff fellows. We can recruit senior-level people now and offer them a few positions to staff their labs."

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Currently, NIH as a whole is 800 to 1,000 FTEs below the ceiling, but the situation at individual institutes varies widely. The institutes, centers, and divisions (ICDs) that are currently most under the ceiling on a percentage basis are NIMH, NCHGR, NINR, and FIC. If an ICD has no FTE positions available, it may be able to convince another ICD that is under its FTE ceiling to lend it an FTE slot for the short term. "But this requires much trust and probably a written understanding since FTEs are now so precious," Gottesman says.

Furthermore, Gottesman notes that NIH's overall FTE ceiling for fiscal 1996 is tentatively about 65 positions below that of fiscal 1995. "Hence, if we fill up to the '95 ceiling, then we will need to lose some people by '96. So, it is likely that we will not hire up to the ceiling, at least not in long-term FTEs," he says. ■

Balancing the Scales: Pay Inequities Corrected

Averting what could have turned into a bitter and divisive lawsuit, NIH has reached an amicable resolution on the issue of pay inequities among tenured, intramural researchers. The NIH plan, approved by PHS this spring, will provide a one-time pay adjustment for approximately 50 women and minority tenured scientists whose salaries were found not to be comparable to their male and nonminority peers with equivalent experience and performance.

"We are very pleased — and particularly pleased that it [the agreement] was a reflection of merit as well as equal pay for equal work," says Acting NIMH Scientific Director Susan Swedo, chair of the Women Scientists Advisors, the group that, with the help of the scientific directors, conducted a detailed, institute-by-institute analysis of pay inequities among tenured scientists. "We feel particularly proud that this issue could be settled without taking action against our academic home, the NIH."

Swedo praised NIH Director Harold Varmus and Deputy Director for Intramural Research Michael Gottesman for their assistance in developing a plan to correct the pay discrepancies. The inequalities were determined using a formula to plot the regression of pay against year-since-degree for given job categories. Only a few tenured scientists per institute are expected to receive pay adjustments. Any intramural researcher with questions about the pay-equalization plan should contact the scientific director or a representative of the Women Scientists Advisors at his or her institute, center, or division, Swedo says. ■

TEACHERS' WORKSHOP FANS INTEREST IN EMERGING INFECTIONS

by Celia Hooper

Some people just have a knack for news. Months ago, when Deputy Director for Intramural Research Michael Gottesman was trying to come up with a topic for the Foundation for the Advancement of Education in the Sciences' (FAES) seventh annual biology teachers' workshop, "The Hot Zone" — Richard Preston's thriller about Ebola virus contamination of a building in Reston, Va. — was just arriving in the bookstores.

By May 3, when the workshop was staged at the Cloisters, the topic Gottesman selected, "Emerging Infectious Diseases: After AIDS, the Risk of Other Plagues," was as hot as hot could be.

"This is fantastic, really exciting," said Leslie Keiler, a biology teacher attending the program from Robert E. Lee High School in Springfield, Va. "This is a topic that is very interesting to my students. It will be interesting and easy to take this information back to the classroom."

Keiler said that recent movies, such as "Outbreak," starring Dustin Hoffman; television shows, such as "Robin Cook's Virus"; nonfiction books, such as Laurie Garrett's "The Coming Plague"; and newspaper articles on the recent Ebola virus outbreak in Zaire have made the topic of emerging diseases very compelling to her students. At the workshop, NIH scientist-lecturers talked about the research problems they have encountered in studying emerging diseases and how they came up with solutions. "Showing students the puzzles is a good way to get them interested," says Keiler, who particularly appreciated the fact that several of the speakers emphasized that "not all the problems are solved yet. They showed us where they think the research needs to go in the future."

Although it was Keiler's first year attending the "Frontiers in Biology" program, the workshops have been held since 1989, when Henry Metzger, then president of FAES, suggested that NIH could reach the most students by talking to teachers. On the basis of that suggestion, Gottesman launched the series as a way of sharing the wealth of NIH expertise with science colleagues at local high schools. FAES does the mailings and provides lunch for the teachers. Previous programs have featured such subjects as molecular medicine, immunology, neurobiology, and the Human Genome Project. This year, after Gottesman came up with the hot topic, he turned responsibility for organizing and running the program over to Richard M. Krause, former director of NIAID and now senior scientific adviser at the Fogarty International Center. Krause says he would recommend the experience of leading and participating in the workshop to other intramural scientists.

Celia Hooper

Science teachers listen to a presentation at an NIH workshop on emerging infectious diseases. Leslie Keiler of Robert E. Lee High School in Springfield, Va., is in the foreground.

"I enjoyed the experience very much. They [the teachers] were very attentive and asked lots of questions," Krause says. "Training the next generation of scientists is what it's all about. We need to work with our colleagues in primary and secondary education — not just at the universities."

By late summer or fall, Gottesman will be on the prowl again for NIH researchers working at the frontiers of science to teach next year's workshop. "We have not decided yet what next year's subject will be. We depend on the advice of the teachers and try to choose a scientific area that is in the news," Gottesman says. "Working with the teachers is a rewarding experience. If you see me coming, don't hide." ■

First NIH Fellows Symposium

This is turning out to be a year of firsts for the NIH Fellows Committee — the first competition for the NIH Fellows Award for Research Excellence, the first sponsorship of speakers at NIH's Wednesday Afternoon Lectures, and now the first opportunity to organize an all-day symposium for the entire NIH community. Scheduled for Oct. 12 at Natcher Auditorium, the inaugural NIH Postdoctoral and Clinical Fellows Symposium will emphasize the latest developments in molecular biology, especially those advances that contribute to an understanding of the etiology of major diseases. Nationally recognized scientists from a diverse range of biological disciplines have been invited to speak at the sessions, which will be chaired by fellows. The complete list of speakers and the titles of their talks is still being finalized. The NIH Fellows Committee, which represents the 2,000 fellows at NIH, was formed to foster communication among fellows, promote fellows' education and career development, and serve as a liaison for fellows to the NIH administration. ■

Cures for Some Common Computer-Related Problems

by Dale Graham, Ph.D., DCRT
(degraham@helix.nih.gov)

Scientists are used to encountering one frustration after another as they pursue their research goals. However, computer-related problems have to rank high on the list of experiences that modern biomedical researchers find the most aggravating. We expect computers to solve our research headaches, not compound them. To help reduce "computer stress" at NIH, we offer solutions to some of the most common computing difficulties reported by intramural researchers.

Problem: *An analysis has been performed using a Genetics Computing Group (GCG) program on the Helix computer, and after the files are transferred to the researcher's personal computer, the data, which looked fine on the screen, are so jumbled that they are practically uninterpretable.*

Solution: The problem is that the analysis file from the mainframe is provided as a text file. When this file is opened with a word processor, it comes up in the "default font," which is usually a proportional font. This means that each letter will take up more or less space, depending on its size. However, for sequence analysis, you *must* use a monoproportional (or nonproportional) font such as Courier or Monaco for sequences to be shown properly. There also may be problems relating to document size and font size.

Here's what you should do:

- Select the entire file.
- Change the font to Courier or Monaco (or some other nonproportional font).
- Change the font size to 10 point.
- If there is still inappropriate "wrap around" of some lines, then either change the document size by increasing the margins or use "page setup" to change to landscape, or wide, orientation. Now, the data should look like you originally expected.

Problem: *Some of the analyses on the GCG, such as "squiggles" for RNA structure or "plot structure" for peptides, won't display properly on the screen.*

Solution 1: The data for these kinds of analyses are presented in "plotter" format. The only way you will see such data properly represented on your screen is if you have a communications program that can "emulate" a Tektronix terminal. This is true whether you are using a modem or a network connection. The best solution, if you do a lot of these analyses, is to buy a communications program that can perform terminal emulation.

Lorna Heartley

Dale Graham

DCRT recommends VersaTerm Pro (*not* just VersaTerm — it must be the *Pro* version) for Macintosh users. Contact DCRT (phone: 594-DCRT) for the latest recommendations for PC users.

Solution 2: The second-best solution is to use a command that will "turn off" the plotter function and to save the file in a text format that can be manipulated with another program to produce output. To do this, you must add some information when you request the analysis from the GCG. That is, you need to type "-fig" on the command line after the analysis name. In addition, you need to provide a name for the output text file, such as "filename.out." Your command line should be patterned after the following example: "helix% squiggles -fig=filename.figure." After the analysis, the "figure" program can be used with this file to print the figure.

Problem: *It can be very difficult to use the GCG suite of programs for restriction-enzyme analyses, simple peptide analyses, and other relatively simple analyses.*

Solution: Personal computer users are far better off doing "simple" analyses on their computers, and not using mainframe solutions. Certainly, there are times when you must use a mainframe, but for simple stuff, using a mainframe is like using a sledge hammer to pound in a tack. Many programs are available for relatively simple analyses. Some of these are even free or cost very little. DCRT also offers a booklet on program options for sequence analysis for the Macintosh (see box, page 7).

Problem: *What is the easiest way to get sequences from databases and perform homology searches? Do I have to use either the GCG or GenInfo computers?*

Solution: If your personal computer is on the network and you are already using something like PC-Gopher or TurboGopher (Mac), you should be able to install and run a World Wide Web (WWW) "browser" such as Mosaic, MacWeb, or Netscape. These programs make it very easy indeed to retrieve sequences from databases on the NIH campus or all over the world. In fact, you can also use browsers to perform several different kinds of sequence analyses. A couple of useful addresses (or URLs, as they are called) are "http://www.nih.gov/," which will take you to the NIH Home Page and a treasure trove of information from scientific data to telephone numbers, and"http://mantis.dcrt.nih.gov:8000/Publications/Internet_Talk/Tools.html," which contains a list of links, arranged by analysis type, for sequence analysis via the Internet.

Of course, this is far from a complete list of the wide range of computer problems encountered by the intramural research community. For help in solving other difficulties, from personal computing to mainframes, contact DCRT. We'll do as much as we can to help, even if it is just to suggest another avenue to explore. DCRT is also providing a lot of computer-related resources on its WWW servers and indicating links to other helpful sites so you will be able to have information and assistance at your fingertips if you are using a WWW browser. ■

Computing Guides

DCRT has a dozen publications that explain how to perform scientific computing tasks and select appropriate types of software. To order these and other pamphlets, contact DCRT (phone: 594-DCRT; fax: 402-0537; e-mail: sdl@cu.nih.gov).

Title	Number
"Comparing Alignment of Two Sequences Using the GCG"	DCRT132
"Creating Internet Documents"	DCRT124
"Getting Sequences and Homologies (From Mainframes)"	DCRT103
"Guide to Cross-Platform Computing"	DCRT111
"Guide to Preparing Posters Using the Macintosh"	DCRT113
"Macintosh Options for Multiple Sequence Alignment"	DCRT112
"Performing Multiple Sequence Alignments With GCG programs"	DCRT105
"Preparing Figures for Publication on the Macintosh"	DCRT106
"Searching for Patterns in Sequences: Regulatory Elements and Motifs"	DCRT107
"Searching for Transcription Factors and Motifs Using Mac Software"	DCRT108
"Sequence Analysis Programs for Macintosh: A Comparison of Features"	DCRT110
"Using Macintoshes and the Internet for Sequence Analysis"	DCRT112

WWW Browsers

NIH intramural researchers can get programs to "browse" the World Wide Web through a variety of avenues. Here are some suggestions from DCRT:

Macintosh computers — You can get the current version of Mosaic or MacWeb from PUBnet by going into the Browser Software folder inside the WWW Browsers folder under Mac Software. These browsers are free. Netscape is a commercial product that can be purchased through Haven and Co. on the Business Purchasing Agreement list for $39 a copy without manuals and $60 a copy with manuals. All three browsers are supported by DCRT.

PCs running Windows — You can get some browser software free to be used without support (that is, at your own risk) on PUBnet. Window users who want to use a supported product should purchase Netscape from Haven and Co. for $60 a copy (with manuals). Support will be provided through Netscape. ■

Clinical Research Core Curriculum

The new core curriculum in clinical research is off to a resounding start — with more than 90 people applying for the first course that ran from April 17 through June 7. The 25 people who took part in the initial offering of this new training program were selected because they will be leaving NIH this summer. A second course will be conducted from late summer to early fall to help meet the remaining demand.

The 44-hour, accredited Continuing Medical Education course, which was organized by a committee of NIH faculty led by John Gallin and administered by the NIH Office of Education, was taught by 30 staff members. The curriculum is divided into four modules, and it uses both didactic lectures and practical experience, such as participating in "meetings" of mock Institutional Review Panels. The first module, which deals with methods and epidemiology, provides instruction on study design and development, measurements and biostatistics, and use of meta-analysis. The second module, which is devoted to ethical and regulatory issues, reviews legal matters, the role of Institutional Review Boards, gender and race diversity in study populations, and scientific conduct. The third module, which centers on the oversight of patient-oriented research, discusses quality assurance, monitoring of clinical trials, relations with the FDA, information systems and data management, and dissemination of information to the research community and the public. The last module, which focuses on preparing and funding a clinical research study, covers the infrastructure and resources for clinical research, writing clinical research and grant proposals, and technology-transfer issues.

For more information on the core curriculum in clinical research, contact Cindy Parker at the Office of Education (phone: 496-3887; e-mail: cfp@helix.nih.gov). ■

The Cyber Catalyst

First, it was just text. Now, it has pictures. Who knows what's next? With each passing issue, the electronic version of *The NIH Catalyst* is improving in both appearance and usefullness. To find current and back issues of the publication, look under the Intramural Research News section of the NIH Campus Information menu on Gopher or the World Wide Web. ■

HIV-1 Induces a New, G2-Phase Form of Cell Death in T-Cells

Abstract

Human immunodeficiency virus — type 1 (HIV-1) infection in humans leads to depletion of CD4+ T-cells and the development of AIDS. Despite intense investigation over the past decade, the mechanisms underlying CD4+ cell death in HIV disease are poorly understood. Genetic-mapping studies of HIV have provided some insight into this process by showing that the most important determinants of the virus' ability to kill T-cells lie within the viral envelope glycoproteins (gp120 and gp41). In the past several years, it became increasingly clear that programmed cell death, or signaling-dependent cell death, plays a major role in many forms of physiologic and pathologic cell death. We and others asked whether the form of programmed cell killing accounts for HIV's cytopathicity. Our experiments suggest that the virus invokes a distinct form of programmed cell death that kills T-cells in the G2 phase of the cell cycle.

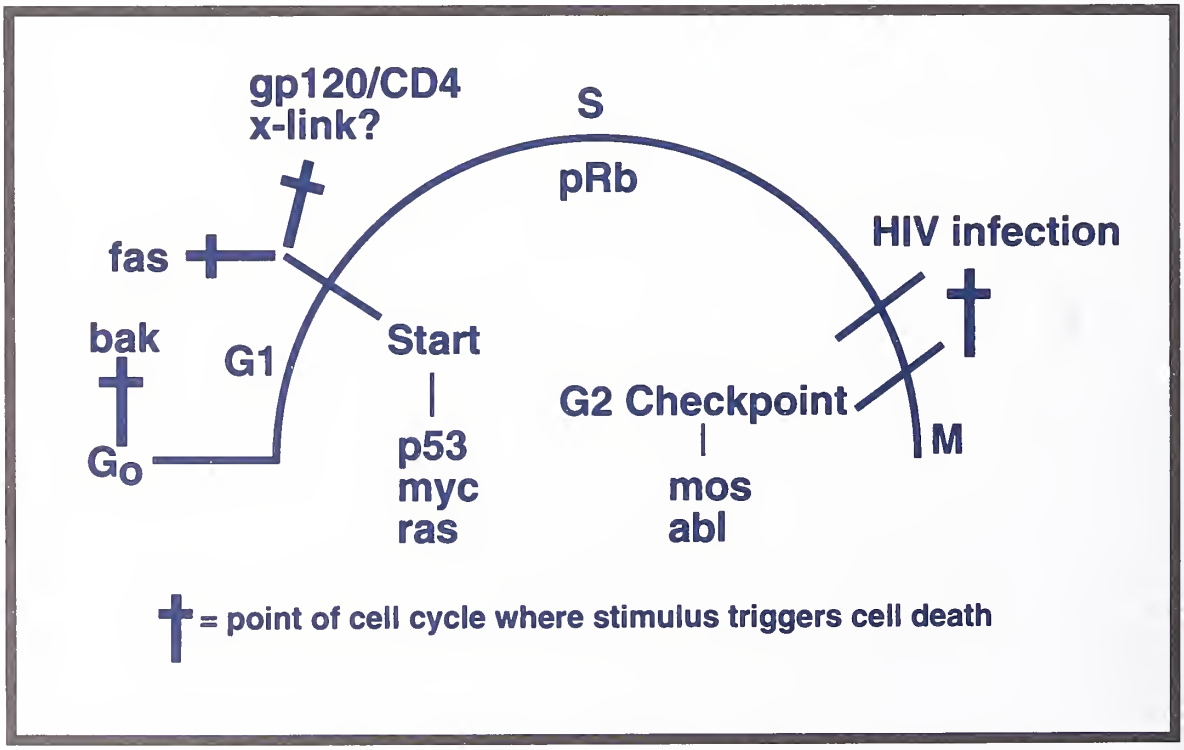
Questions

Q: *What was the starting point for this work?*

A: We initially investigated whether any of the proteins encoded by HIV-1 were capable of initiating intracellular signals that directly program CD4+ cells to die. These studies led us to conclude that processed HIV envelope glycoproteins (gp120 and gp41) expressed on the surface of one T-cell can interact with the CD4 receptor of another T-cell, triggering signaling mediated by protein tyrosine kinases (PTKs) and cell death. Other HIV proteins, including Tat, Rev, Nef, and the matrix polypeptides, are not capable of directly initiating CD4+ cell death. Using the PTK inhibitor herbimycin A, we also showed that interfering with protein tyrosine phosphorylation during HIV infection dramatically reduces viral cytopathicity in vitro.

We followed up this initial observation by attempting to identify the viral and cellular substrates that undergo tyrosine phosphorylation during the course of HIV infection. Our observations with antiphosphotyrosine antibodies suggest that a 34-kilodalton cellular substrate becomes profoundly tyrosine-hyperphosphorylated and that this event has the same kinetics as HIV-induced cell death. We also found that an HIV matrix protein (p17 gag) may also become tyrosine-phosphorylated during the course of HIV infection.

To define these phosphorylated substrates more completely, we performed phosphoamino acid analysis, which showed that the cellular substrate had the unusual property of being phosphorylated at threonine and serine residues as well as at a tyrosine residue. This suggested to



A new, pathologic type of programmed cell death, dubbed "phouskomatosis" — coined from phouskoma, *the Greek word for bloated or inflated, is triggered by HIV infection and kills T-cells in the G2 phase of the cell cycle. HIV acts at the G2 checkpoint via regulatory proteins such as mos and abl. In contrast, classic apoptotic cell death occurs when T-cells are in the G1 phase and operates through regulatory proteins such as p53, Myc, and Ras.*

by David Cohen , M.D., of the Laboratory of Immunoregulation, NIAID. Cohen presented this lecture on March 29, 1995, as part of the Immunology Interest Group's regular seminar series.

us that the pp34 substrate might be a cyclin-dependent kinase (cdk), which was verified when we established that the pp34 substrate is cdk1, the cdk regulator of G2/M transition.

Q: *Which findings have been most surprising to you or to other scientists?*

A: The identification of large quantities of tyrosine-phosphorylated cdk1 in cells that were dying during HIV infection strongly suggested that these cells were trapped in the G2 phase of the cell cycle, where tyrosine-phosphorylated cdk1 accumulates. This observation was surprising because all previously described forms of apoptotic cell death in normal T-cells had been shown to occur when the cells were in G1 or early S phase. We confirmed that observation through additional biochemical experiments, including analysis of the mitotic cyclin, cyclin B. These studies also showed that clinical isolates of HIV-1 that have the greatest cell-killing capacity also most strongly direct the G2 form of cell death. This correlation makes this killing pathway a focal point for understanding depletion of CD4+ T-cells in HIV-1 infection.

THIS OBSERVATION WAS SURPRISING BECAUSE ALL PREVIOUSLY DESCRIBED FORMS OF APOPTOTIC CELL DEATH IN NORMAL T-CELLS HAD BEEN SHOWN TO OCCUR WHEN THE CELLS WERE IN G1 OR EARLY S PHASE.

Q: *What were the greatest stumbling blocks, and what new observations, techniques, reagents or insights helped you to get past them?*

A: Because HIV-1 infection or processed envelope glycoproteins (gp120 and gp41) might also be capable of triggering G1/S forms of apoptosis or might initiate cell death by additional mechanisms such as syncytium formation, it was important to distinguish the pathway that we had identified from these other processes. To overcome this difficulty and to define the G2-cell-death pathway, we eluted dying cells and fractionated a purified population of "balloon" cells. These dying "balloon" cells have a single, open nucleus, are free of syncytia, and show no signs of classical apoptosis, which is characterized by pronounced nuclear condensation.

Transmission electron microscopy studies of purified balloon cells tagged with immunogold demonstrated that the balloon cells have active centrosomes, containing both cyclin B and cdk1 proteins, which again confirmed that these cells are trapped in G2 — the part of the cell cycle in which centrosomes become activated.

Q: *Do you see any potential areas where this research might provide insights for clinical scientists, and how are you following up on your discoveries?*

A: These findings have general significance for understanding pathological forms of programmed cell death and may also provide therapeutic targets in the cell for inhibiting HIV-1 directed T-cell killing. Our studies suggest that HIV-1 infection initiates a pathological form of cell signaling leading to prominent death of cells at G2 (see figure, page 8). In contrast, physiologic forms of T-cell death, and quite possibly other pathways of cell killing triggered by HIV-1 (see figure, page 8), occur at G1, where induction and subsequent cross-linking of the Fas apoptotic receptor is an important mediator of T-cell death. We expect that genes involved in S and G2 cell-cycle progression are likely also to be responsible for initiating and executing the HIV/G2 cell death programs in the balloon cells (see figure, page 8). Such genes include *pRb, c-mos,* and *c-abl* rather than other cell-cycle regulatory genes, such as *p53, myc,* and *ras,* which appear to mediate apoptosis in G1. Because the G2 program is rarely employed during normal T-cell regulation, therapeutic intervention in the G2 pathway might interrupt CD4+ T-cell depletion during HIV infection without interfering with normal T-cell function, and we are currently investigating this interesting possibility. ■

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COMMENTARY

IDENTIFYING SUBSTRATES IN THE BRAIN THAT UNDERLIE COCAINE CRAVING

The epidemic of cocaine abuse in the United States has underscored the need for knowledge about the mechanisms by which cocaine alters behavior. Such information is critical in the development of medications to curb cocaine addiction. As part of this effort, we have applied the [^{18}F]fluorodeoxyglucose method to assess changes in regional cerebral glucose metabolism, an index of local brain activity, by positron emission tomography (PET) in cocaine abusers exposed to cocaine-related cues. Our preliminary findings indicate that the presentation of a videotape with cocaine-specific cues, exposure to drug paraphernalia, and anticipation of cocaine self-administration produce self-reports of cocaine craving, electroencephalographic (EEG) arousal, and a characteristic metabolic pattern in the brain. Exposure to cocaine cues is associated with stimulation of areas of the occipital cortex, presumably because the cues are visual. Also activated are areas of the prefrontal, temporal, parietal, and limbic cortices. This work begins to define anatomical circuits that are important in the response to environmental stimuli. These circuits may link episodic memories of cocaine use to emotions and to planning future drug taking.

Although the role of the mesolimbic dopaminergic pathways in supporting reward and reinforcement from psychoactive drugs has dominated contemporary drug-abuse research (1), it has become increasingly evident that the pharmacological response to the drug per se is only one factor that maintains compulsive drug use. Drug-induced reward is a critical factor in the initiation or acquisition of self-administration behavior, but hedonic responses to the drug may not be critical to subsequent stages in addiction (2,3). In fact, Tiffany (4) has suggested that continued drug self-administration results in automatic cognitive and motoric habit patterns and that relapse results from re-exposure to cues that elicit these automatic patterns.

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It is well-established that individuals who are drug-free can relapse to abuse long after detoxification, and it is, therefore, probable that chronic drug abuse produces persistent changes in the nervous system that outlast the immediate effects on brain reinforcement pathways. The presence of long-term changes suggests that learning and memory are critical to the addictive process. In this regard, almost 50 years ago, anecdotal reports of addicts who left the Addiction Research Center of NIDA in Lexington, Ky., and then relapsed after returning to their old neighborhoods first suggested that learning or conditioning factors may contribute to recidivism (5).

There are two broad and conflicting theories regarding the responses to drug-related environmental stimuli. Both propose that environmental cues promote drug-seeking behavior via classical conditioning mechanisms, but they differ with respect to the proposed relationship between acute drug effects and the responses elicited by the cues. The early proposals held that drug-related cues elicit an aversive motivational state, which the addict attempts to escape by further drug intake. The two major theories focusing on the aversive effects of cues posited 1) that conditioned withdrawal phenomena produced by drug-associated stimuli contribute to relapse (6), and 2) that exposure to drug cues activates an opposing process that contributes to tolerance (7). Either way, responses elicited by drug-related cues would be in the opposite direction of responses produced by the drug itself. For example, because cocaine increases arousal and heart rate and produces euphoria, cocaine-related cues would be predicted to decrease arousal and heart rate and to produce dysphoria.

More recently, researchers have proposed that responses to cues mimic aspects of the hedonic effects of drugs and, therefore, that drug-seeking behavior is motivated by approach rather than by avoidance (2,8,9). According to this view, cues activate reinforcement systems in the brain and the addict seeks to maintain this activation by engaging in drug-seeking behavior. These "approach" theories propose that responses to drug-related cues contribute to sensitization to drug effects because the conditioned response is in the same direction as the acute effect of the drug (2,10,11). In contrast to the "avoidance" predictions, the recent hypotheses propose that cues related to cocaine use would increase arousal and heart rate and would generate euphoria. Because there is empirical evidence of both drug-like and drug-opposite responses to drug-related cues (2,7,12,13), attempts have been made to reconcile these conflicting theories. One suggestion is that the nature of cue-elicited responses depends on the class of the drug taken. For example, cues associated with opioid and sedative drugs have been proposed to elicit drug-opposite and withdrawal-like responses, whereas cues associated with stimulants, such as cocaine, elicit drug-like responses (14). A second suggestion is that the direction of the cue-elicited effect depends on the specific response measured. Drug effects on afferent, or sensory, processes lead to drug-like responses to cues, whereas drug effects on efferent, or motor, processes produce drug-opposite cue-elicited responses (15).

Although conditioned responses to drug-related environmental stimuli were first described by Pavlov (16), the brain systems involved in drug conditioning have only recently been studied. Because direct measurement of many brain systems requires invasive techniques, these studies have been performed primarily in animals. Using the induction of the immediate early gene c-fos as a marker for neuronal activation, Fibiger and colleagues mapped the brain regions in rats that respond to environmental cues associated with cocaine administration (17). They found that drug-related cues increased c-fos expression throughout the limbic system, including cingulate cortex, amygdala, septum, and habenula. This distribution was similar to that of the direct effect of cocaine on c-fos expression, except that cocaine also increased c-fos expression in the caudate and accumbens nuclei. The absence of increased c-fos expression in any portion of the striatum is problematic for both "approach" and "avoidance" models of drug conditioning because they have emphasized the contribution of striatal areas to the responses elicited by drug-related cues (9,18). Furthermore, because dopaminergic input to these regions contributes to the reinforcing and hedonic effects of drugs of abuse, the incentive motivational theories propose that drug-related cues are reinforcing because they also increase

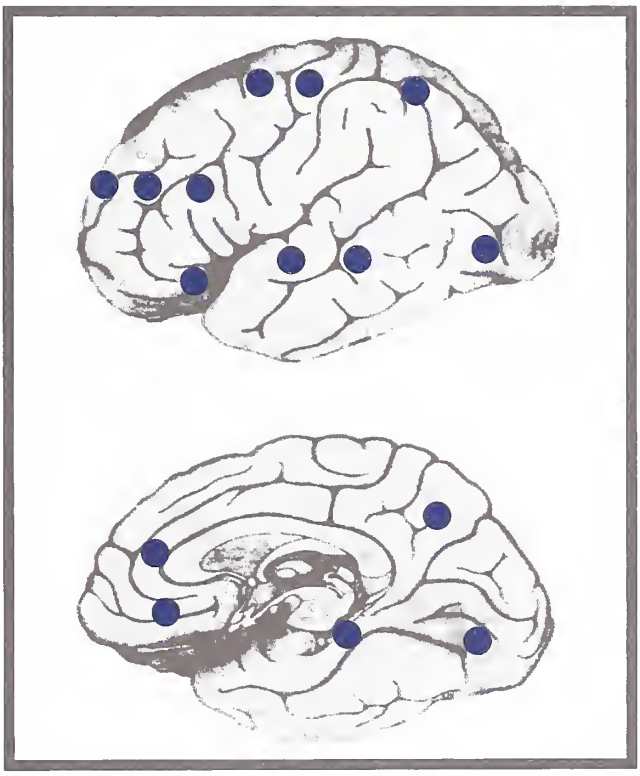
by Edythe D. London, Ph.D., Steven Grant, Ph.D., and David Newlin, Ph.D., NIDA

dopaminergic tone. It is known that cues associated with delivery of biological reinforcers — such as food — increase the firing of dopaminergic neurons in the brainstem and increase dopamine release in the basal ganglia (2,19), but the evidence for dopamine release in response to drug-related cues is contradictory (2). Robinson and Berridge (2) recently suggested that although the reinforcing effects of drugs are initially dependent on alterations in dopaminergic tone, over time, other brain systems become dominant, especially those associated with drug-seeking behavior elicited by environmental stimuli.

Researchers have tested theories of drug conditioning in human volunteers by presenting them with drug cues in the absence of drug administration. Typically, the subjects view videotapes of drug-taking behavior or conduct a self-administration ritual and inject themselves with a placebo (14,20); this way, the conditioned response to drug-associated stimuli is not obscured by the drug. In studies of cocaine addicts, such experiments have shown that drug-related cues lead subjects to report cocaine craving, accompanied by reliable decreases in skin temperature and skin resistance, increases in heart rate, and EEG arousal (21,22).

Although drug abusers may attribute their addiction to craving, the extent to which craving drives drug-taking behavior may be limited (13). For example, research volunteers with histories of cocaine abuse reported less craving when treated with desipramine, a proposed therapy for cocaine abuse; however, the volunteers did not reduce self-administration of cocaine in the laboratory (23). In another study of the efficacy of desipramine in the treatment of cocaine abuse, decreases in self-reports of craving occurred weeks after a decrement in cocaine use was observed (24). Nonetheless, it is generally agreed that craving is "a subjective state in humans that is associated with drug dependence" (25). A current goal of our research on mechanisms of drug dependence is to elucidate the biological determinants of craving for abused drugs and the relationship, if any, between drug craving and drug dependence.

To study the effects of cocaine-related cues on cerebral metabolism, we paired PET measurements with psychophysiological assays and self-reported subjective assessments in cocaine abusers during two sessions. In one session, during the period when the radiotracer ([^{18}F]fluorodeoxyglucose) was taken up after its intravenous injection, subjects viewed a neutral videotape on arts and crafts. In the second session, they were exposed to a cocaine-related stimulus complex — a videotape of cocaine-related activity and paraphernalia, the presence of actual paraphernalia, and a small amount of cocaine. Analysis of data from the first nine subjects revealed increases in self-reports of craving and overall arousal (decrease in EEG power) during presentation of the cocaine-related stimuli compared with the neutral session. Cerebral metabolic activation in response to cocaine-related stimuli differed from activation during the neutral session. The response also differed from the decrease in brain activity observed previously in response to acute administration of cocaine (26). The changes in response to cocaine-related stimuli were "drug-opposite": the stimuli caused selective increases in regional cerebral glucose metabolism, whereas acute cocaine administration reduced glucose metabolism globally. Increases due to cocaine-related cues, as distinct from neutral cues, were observed throughout the prefrontal cortex, occipital cortex and parahippocampal gyrus, with more restricted activations in the parietal and temporal lobes and the pre- and postcentral gyri (see figure). The metabolic changes produced by the cocaine-related cues are consistent with the view that the response to drug-related environmental stimuli may reflect activation of a distributed neuroanatomical network, involving areas that mediate retrieval of memories with affective components as well as those that may participate in the planning of future drug self-administration.



Schematic representation of the lateral (above) and medial (below) aspects of the brain. *Blue dots indicate brain regions in which cocaine-related cues produced increases in glucose metabolism compared with the effects of neutral cues. Affected regions were located in prefrontal (superior, medial, and inferior frontal gyri; pregenual cingulate; and medial and posterior orbitofrontal gyri), temporal (superior and inferior temporal gyri), central (pre- and postcentral gyri), parietal (angular gyrus), limbic (parahippocampal gyrus), and occipital cortices (pre-cuneus, inferior fusiform gyrus, and middle occipital gyrus).*

These and other recent findings concerning the activation of brain systems by exposure to cocaine cues raise interesting questions for further study. The study of neural systems involved in highly motivated behavior, such as addiction to drugs, may have important implications for other forms of both adaptive and maladaptive behaviors. In this regard, several medical disorders and risk factors may entail acquired motivation to engage in other self-destructive behaviors. Understanding cocaine craving may help us understand craving for tobacco, alcohol, and opiates and the brain substrates of normal and abnormal cravings for food in eating disorders. Related questions concern paraphilic sexual motivation and preoccupation with gambling. Moreover, it

continued on page 22.

BRINGING TOGETHER MORPHOLOGISTS AND MOLECULAR BIOLOGISTS: IN SITU PCR AND RT-PCR

The possibility of performing DNA or mRNA amplification in tissue sections has been proposed since the beginning of the polymerase chain reaction (PCR) era. Unfortunately, however, the technique has proved to be more elusive than a quick glance of varions protocols (1,2) suggests. Nevertheless, the potential reward promised to those able to master the technique is high: unlimited sensitivity in the detection of specific nucleic acids expressed in subpopulations of cells with as little as a single "available" molecule inducing a detectable signal. This promise has attracted a great number of investigators who work with molecules that are expressed at low levels (e.g., growth factors, receptors, and developmental signals), with new or rare genes (e.g., viral infections, point mutations, transpositions, and deletions), or who are trying to follow vectors after gene therapy.

The advent of thermocyclers designed specifically for tissue sections solved many of the technical problems associated with detecting these low-level signals in preserved tissue, and our laboratory recently developed a direct protocol that uses one such instrument to detect DNA and mRNA in archival material (3).

The Method and How It Works

In the past, one of the most serious problems with in situ PCR was the lack of reproducible results due to 1) difficulties in performing synchronized "hot start" applications, 2) limitations in the number of slides that can be processed at the same time, and 3) heterogeneous heating of the slides (they were usually placed on top of a regular thermocycler block, with holes for the tubes, and even if a small aluminum foil boat is used, there can be large temperature variations in different areas of a tissue section). We overcame the first obstacle by using a monoclonal antibody that blocks Taq polymerase until the temperature reaches 70°C. At that temperature, the denatured antibody liberates an active enzyme to the PCR solution (4). The other two difficulties were resolved by performing the reaction in a thermocycler specifically designed to accommodate microscope slides (Hybaid's OmniSlide System, National Labnet Company, Woodbridge, N.J.).

In addition to these problems, fixation of tissue is critical for obtaining good results in in-situ PCR. In samples treated with alcohol- or acetone-based fixatives, the PCR products ended up in the supernatant. Conversely, cross-linking fixatives, such as paraformaldehyde or formalin, retain the labeled products in the tissue, possibly by entrapping them in the lattice they created among proteins (5).

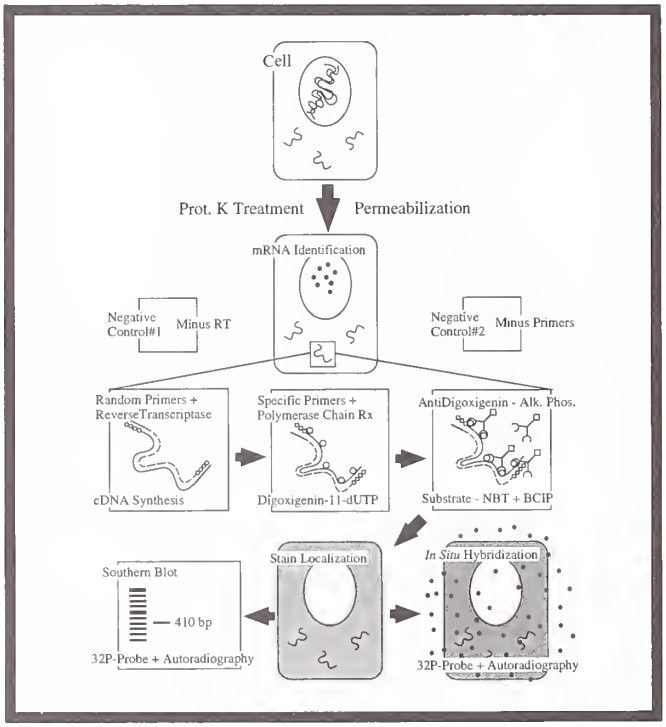


Figure 1. *Schematic drawing of the steps involved in the amplification of mRNA in tissue sections. The basic controls have been also included.*

Conceptually, the protocol is simple (see figure 1). If we want to detect DNA, after dewaxing and rehydrating the sections, three steps must be performed: 1) protein digestion to facilitate reagent penetration, 2) PCR reaction with simultaneous labeling of the PCR products, and 3) visualization of the labeled products by immunocytochemical methods. The detection of mRNA incorporates a reverse-transcription step, to generate cDNA, before amplification.

In situ amplification combines all the advantages of histological and PCR technologies but, unfortunately, it also compounds the possible artifacts of both. For this reason, successful interpretation of results requires attention to appropriate controls (see figure 2). These are some of the controls we use: 1) positive control — a section of a tissue or cell line known to have a high expression of the target nucleic acid as determined by other techniques (such as Northern blot, regular PCR, or in situ hybridization); 2) negative control — substitution of primers by water in the PCR mixture to reveal non-specific endogenous priming (from necrosis, apoptosis, or repair processes, for example); 3) negative control for mRNA — use RNase pretreatment or omit reverse transcriptase; 4) co-localization of the signals for the mRNA — via in situ RT-PCR — and, for its translated protein, via immunocytochemistry; 5) extraction of the DNA from the tissue section after amplification and analysis by electrophoresis and Southern blot; and 6) in-situ hybridization with a labeled nested probe after amplification. The last procedure is routinely used in the indirect method of in situ PCR.

Protocol

In situ amplification can be done on cytospin preparations or on sections from paraffin-embedded and frozen tissues. Here we present a protocol for the detection of mRNA in paraffin sections that is important because of its application to archival material. To detect DNA, step 4 would be omitted. Mention of specific products does not constitute an endorsement.

In Situ RT-PCR

1. Take the usual precautions for working with RNA, even during cutting and handling of sections: wear gloves, bake the glassware, and use diethyl pyrocarbonate (DEPC)-treated water.

2. Deparaffinize the sections by immersion in xylene (20 min.) then rehydrate

by Alfredo Martínez, Ph.D., NCI, and Frank Cuttitta, Ph.D., NCI

in decreasing concentrations of ethanol in DEPC-treated water. Always use new solutions for in situ RT-PCR. You can subsequently reuse these solutions for regular histological procedures.

3. Permeabilize the tissue by incubation with proteinase K. A concentration of 10 µg proteinase K/mL at 37°C for 15 min. is appropriate for most archival material, but varying the concentration or exposure time is recommended to optimize results for each particular application.

4. For reverse transcription, prime the reverse transcriptase. For this step, you may use either specific primers designed to target the proper message or an oligo (dT) that binds to the polyA tail of the mRNAs. We use the SuperScript Preamplification System (Gibco BRL, Gaithersburg, Md.): first, a drop (60 µL) containing the primers is placed on top of the section and covered with a coverslip of parafilm, then the sections are incubated for 10 min. at 70°C in the thermocycler. After removing the coverslips, another solution containing the reverse transcriptase (100 U/section) is added and covered with a new piece of parafilm. The slides are then maintained at room temperature for 10 min., at 45°C for 45 minutes, and at 70°C for 10 minutes.

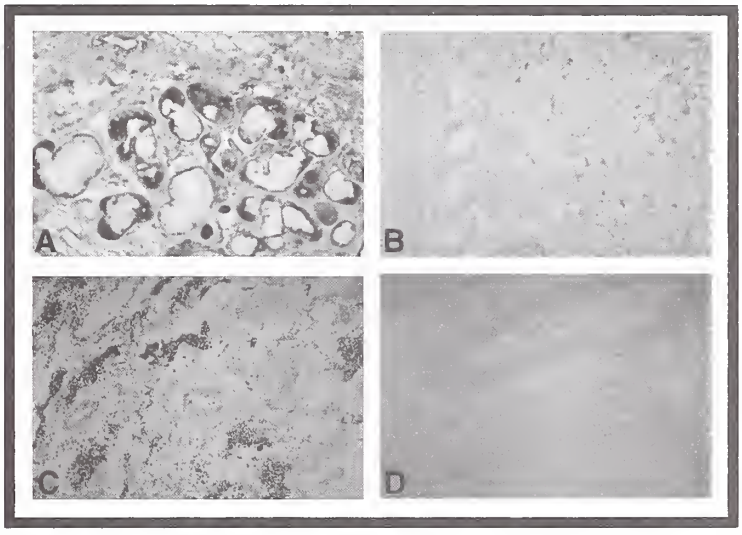


Figure 2. *Detection of mRNA in archival material by in situ RT-PCR. a) Localization of adrenomedullin mRNA in human bronchial glands. This regulatory peptide has been recently described in normal lung and in pulmonary tumors (6). b) Serial section of the same gland used as a negative control, primers were substituted by water in the PCR mixture. c) Large cell pulmonary tumor expressing transferrin mRNA (7). d) Negative control obtained by omission of the reverse transcription.*

5. For PCR, optimize all the parameters (such as pH, $MgCl_2$ concentration, and annealing temperature) for each specific set of primers used in the PCR reaction by regular PCR before attempting in situ amplification. a) Mix in a sterile microcentrifuge tube 0.5 µL of Taq polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) and 0.5 µL of TaqStart antibody (Clontech, Palo Alto, Calif.) per slide and incubate 5 min. at room temperature to block the enzyme. b) Add the rest of the components of the PCR mixture to obtain the following composition: 2.5 mmol $MgCl_2$/L, 200 mmol dNTPs/L, 100 µmol digoxigenin-11-dUTP/L (Boerhinger Mannheim, Indianapolis), 1 ng primers/µL, 50 mmol KCl/L, 10 mmol Tris-HCl/L. c) Apply 80 µL of solution to each slide, then cover the section with a glass coverslip and seal with rubber cement to prevent evaporation. Place the slides in the thermocycler. d) Optimize the number of cycles and the annealing temperature for each tissue and target nucleic acid. A standard run could be like this: begin with 2 min. at 72°C; 15 cycles: 94°C for 15 s., 55°C for 15 s., 72°C for 60 s.; finish with 5 min. at 72°C. e) Remove coverslips and wash the sections twice — for 20 min. each time — in 0.1X SSC at 45°C.

6. For detecting digoxigennin-tagged DNA, we use the Digoxigenin Detection kit (Boehringer Mannheim). To produce a dark blue precipitate, it requires a 2-h. incubation with an antidigoxigenin antibody bound to alkaline phosphatase at a dilution 1:500, thorough washes, and incubation with the proper substrates (nitroblue tetrazolium and a complex phosphate).

7. Check slides under the microscope until the proper color intensity is reached. Stop the reaction before the background in the negative controls begins to increase. Mount the slides in a water-soluble mounting medium (such as Crystal Mount, Biomeda, Foster City, Calif.) because the blue precipitate is soluble in alcohols.

8. Compare the test slides with the controls.

Troubleshooting Tips

1. How to choose a thermocycler? Several companies offer slide thermocyclers for in situ PCR with different designs. Before buying one of them, look for the following characteristics: a) number of slides that can be processed at the same time — remember, the name of the game is *controls* — and the more sections you can accommodate per run, the better; b) ease of operation; try to avoid complex manipulation; c) price, which is unquestionably a critical point and a variety of instruments are available (e.g., Perkin Elmer, Coy, MJ Research, and Hybaid), costing between $3,000 and $10,000; and d) maintenance service support following sale (e.g., is there a regional distributor in the area?).

2. DNase or not DNase? In some protocols for in situ RT-PCR, the authors recommend a digestion with RNase-free DNase before the reverse transcription step. This treatment is intended to remove nuclear and mitochondrial DNA to avoid genomic amplification during PCR. We repeatedly observed, using a variety of DNases, that this digestion step resulted in nonspecific nuclear staining (3). This problem seems to be due to the behavior of the DNase enzyme, which cuts the DNA into oligonucleotides but does not reduce it to mononucleotides. The remaining oligonucleotides are used as primers by the Taq polymerase, lead-

continued on page 22.

THE LAB BEHIND THE LEADER: NEW NIDCD SCIENTIFIC DIRECTOR

As is often the case at NIH, one institute's loss is another's gain. When James Battey recently became scientific director of NIDCD, NCI lost a top-notch researcher, and NIDCD gained a distinguished leader. Battey came to NCI in 1983, after a postdoc at Harvard Medical School in Boston. In 1988, he moved to NINDS as the chief of the Laboratory of Neurochemistry's Molecular Neuroscience Section. In 1992, Battey returned to NCI to head the Laboratory of Biological Chemistry's Molecular Structure Section. Battey offers this description of his research.

Our laboratory focuses on the structure, function, and regulation of mammalian receptors for bombesin-like peptides. Bombesin is a 14-amino acid peptide originally purified from frog skin by the physiologist Vittorio Erspamer and his colleagues at the University of Rome in 1971. Two homologous mammalian peptides have been purified and sequenced. Gastrin-releasing peptide (GRP) was named for its ability to stimulate the release of gastrin from the G cells in the antral mucosa. Neuromedin B (NMB) was isolated from the spinal cord as an effective agent in inducing smooth muscle contraction in a bioassay. Both peptides show remarkable sequence identity to bombesin over their carboxy-terminal ends — the region essential for high-affinity binding to receptors and all biologic responses attributed to mammalian bombesin-like peptides.

Mammalian bombesin-like peptides mediate a variety of biologic responses, including smooth muscle contraction, secretion, and modulation of neuronal firing rate. In addition, they regulate the growth of cultured fibroblasts expressing bombesin receptors and have been implicated as autocrine growth factors that modulate the growth of some human carcinomas. GRP is transiently expressed in the developing lung, raising the intriguing possibility that such peptides and their receptors are important for establishing patterns of growth and differentiation during fetal development. This wide spectrum of biologic activity raises interesting questions about the receptors for bombesin-like peptides: How many receptors are there? Do all the receptors access the same signal-transduction pathway? Are there specialized receptors for different functions?

To learn more about the molecular mechanisms governing the responses elicited by mammalian bombesin-like peptides, our lab has cloned and characterized cDNAs and then genes that encode three structurally similar, but pharmacologically distinct, receptors for such peptides. All three belong to the growing family of receptors that includes cystic fibrosis transmembrane-conductance regulator and the multidrug-resistance protein. These receptors all possess seven transmembrane-spanning domains, and their responses are coupled through heterotrimeric GTP-binding proteins. The first bombesin receptor, GRP-R, binds GRP with highest affinity; the second, NMB-R, binds NMB with highest affinity; and the third, called bombesin-receptor subtype 3 (BRS-3), binds neither GRP nor NMB with high affinity. The existence of bombesin receptor with no known naturally occurring, high-affinity ligand raises the possibility that there are additional mammalian bombesin-like peptides yet to be discovered. NMB-R and GRP-R are expressed in overlapping but distinct regions of the central and peripheral nervous systems and gastrointestinal tract in adult mammals, whereas BRS-3 shows a different pattern of expression limited to secondary spermatocytes in the testis and the uterus during pregnancy. GRP-R also has a widespread pattern of expression late in embryonic life.

Ernie Branson

James Battey

All three receptors appear to activate a similar signal transduction pathway, which involves activating phosphoinositide-specific phospholipase C, elaborating inositol 1,4,5-tris phosphate (IP_3), elevating intracellular calcium, and activation of protein kinase C.

With Robert Jensen's lab at NIDDK, we are investigating which segments of bombesin receptors are critical for high affinity binding of agonists and antagonists. Our preliminary studies indicate that multiple regions of the receptor are necessary, that there are residues which determine preferential binding for either NMB or GRP peptides, and that agonist and antagonist binding sites are overlapping but clearly distinct. We have created mutant receptors, which can no longer couple to G-proteins, to help determine if coupling is essential for receptor internalization and other events, such as phosphorylation, that occur when bombesin receptors are activated. To perform these studies, we have generated a number of stably transfected cell lines that express wild-type or mutant receptors at varying levels, as well as specific polyclonal antisera suitable for both immunoblotting and immunoprecipitation.

Our studies using bombesin receptors ectopically expressed in *Xenopus laevis* oocytes suggest that GRP-R and NMB-R may preferentially initiate signal transduction with different heterotrimeric G-proteins. With John Northup at NIMH, we will address the question of receptor-G-protein interactions and establish a biochemical system for studying receptor coupling in vitro, using purified receptors, heterotrimeric G-proteins, and candidate receptor kinases. Using the yeast two-hybrid system with Richard Kahn's lab at NCI, we plan to search for molecules that interact with the bombesin-receptor region that undergoes ligand-activated phosphorylation. This approach has the important advantage of making no initial assumptions about the identity of potential regulatory molecules.

Finally, working with Heinz Arnheiter at NINDS, we are using gene targeting techniques in embryonic stem cells to generate mice that lack a functional allele for GRP-R, NMB-R, or BRS-3. We hope that these knock-outs will reveal the function of the bombesin receptors in the whole animal. ■

NIDA's Addiction Center
continued from page 1.

contribute to questions about long-term information storage in the brain, which really is at the core of 'memory' processes."

Sixty Years of Science

Although NIDA has only been part of NIH since 1992, its intramural research program traces its roots to the Research Division of the U.S. Narcotics Farm in Lexington, Ky. The 1,200-bed facility, often referred to as "Narco," was established by the U.S. Public Health Service in 1935 for the treatment of opiate addicts. In 1948, Narco's Research Division was renamed the Addiction Research Center (ARC) and placed under the jurisdiction of NIMH's intramural program. In 1973, NIMH's parent agency, the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA) established NIDA. ARC was physically moved to Baltimore in 1979 after a nationwide ban on the use of federal prisoners as research subjects made it difficult to find patients for clinical studies in Kentucky. When ADAMHA's research programs became part of NIH three years ago, ARC — which is now synonymous with NIDA's intramural research program — came along as part of the package.

"When we [NIDA] were within ADAMHA ... there were competing voices. There was a focus on scientific rigor, but also a focus on the provision of service — something that was split off when the Substance Abuse and Mental Health Administration was created," Uhl says. "Moving just the research components of the institute into NIH has really focused the energy on research."

NIDA Director Alan Leshner notes that even though the intramural research program represents only 5% to 6% of NIDA's total budget, it still represents the largest scientific research center dedicated to drug addiction. "Although by NIH standards it is small, by world standards on drug-abuse research, it is a central focal point," says Leshner, observing that very few academic institutions have the resources or the continuity of personnel for the types of long-term drug-abuse studies that ARC has been able to pursue.

Approximately 85 scientists currently work at ARC, which is located atop a hill at Johns Hopkins University's Bayview Campus in southeastern Baltimore, less than an hour's drive from the Bethesda campus. All six branches of NIDA's intramural research program — molecular neurobiology, neuroscience, preclinical pharmacology, clinical pharmacology, etiology, and treatment — are contained in ARC's 60,000-square-foot building and two nearby buildings. In addition to basic research labs, the facilities house 30 inpatient research beds in a closed unit, an outpatient research unit, and an 80-slot outpatient methadone clinic.

The forerunner of NIDA's intramural research program, the old "Narcotic Farm" in Lexington, Ky.

Research Outlook

Recent achievements by NIDA intramural investigators reflect how NIH affiliation has helped to reinvigorate its research outlook. Following up on pharmacologic leads uncovered by former ARC Director William Martin in the 1970s, Uhl, Jia Bei Wang, and their colleagues in 1993 succeeded in cloning the gene that encodes one of the major subtypes of opiate receptors, the μ morphine-preferring opiate receptor, and in subsequent studies, went on to explore the protein's structure and function. Similarly, fueled by the ground-breaking pharmacologic research of NIDA's Michael Kuhar and Steven Goldberg, Uhl and Shioshi Shimada, with help from Kuhar, cloned the gene for the cocaine-sensitive dopamine transporter — a so-called receptor that, when exposed to cocaine, blocks dopamine reuptake in the mesolimbic pathways, initiating a series of events thought to cause the euphoric and rewarding effects associated with cocaine use.

On the preclinical and clinical front, intriguing current research at ARC includes Edythe London's positron emission tomography (PET) studies on the physiological basis of cocaine craving [see Commentary, page 10], Xiao Bing Wang's research using the differential-display method to examine gene-expression patterns in the brains of animals exposed to drugs, Goldberg's attempts to create transgenic mouse and nonhuman primate models that mimic the long-term changes observed in the brains of human drug abusers, Jack Henningfield's work on the psychological basis of drug craving and its utility in treatment, and David Gorelick's studies on the relationship between the rate of cocaine administration and euphoria. Early findings suggest that a slow-release, cocaine-like compound could possibly be used as a methadone-like treatment for cocaine addiction.

Tracking the history of ARC's research, Uhl says the emphasis has

moved from finding out what happens to addicts when they take drugs to using animal models for self-administration to predict which drugs will be abused in humans, to working on defining which molecular sites were involved in this drug reward, and then on to finding the genes that encode such sites. "That's a fairly impressive progression compared with what's known about the biology of many disorders that involve behavior. ... Clearly, our challenge now is to try to have the same impact upon our understanding of the addiction, the use of drugs over the long term."

Life in Baltimore

Working with Uhl to explore the long-term effects of drug abuse on molecular events in the brain is David Vandenbergh, a senior staff fellow in the Molecular Neurobiology Research Branch. Like many of his colleagues, Vandenbergh says he makes it to only the most interesting seminars in Bethesda because of time demands. Although he blames NIDA's remoteness for difficulties in renewing his NIH library card and getting supplies delivered from the NIH stock room, Vandenbergh says he doesn't consider being located in Baltimore a handicap. "Doing science is fairly similar no matter where you are," he says.

Among the NIDA researchers who have spent time in Bethesda is another senior staff fellow, Amy Hauck Newman, a medicinal chemist who designs and synthesizes novel compounds to discover mechanisms underlying drug abuse. Before moving to Baltimore in 1990, Newman spent three years as a postdoc at NIDDK, where she maintains collaborations with her mentor, Kenner Rice, and Anthony Basile on projects centering on dopamine transporters and opiate receptors. She is also working with NINDS' Michael Rogawski and Swami Subramaniam on studies involving *N*-methyl-D-aspartate (NMDA) receptors and potential anticonvulsant agents.

"The biggest advantage of being in Baltimore is space — and no problems with parking!" Newman says. "I also find it very interesting to work in one building where a whole spectrum of scientific backgrounds are focused toward just one mission." However, Newman does miss the convenient access to the NIH library, interactions with other chemists, and ease of attending seminars in Bethesda. "I feel somewhat isolated now."

Peter Johnson, who came to ARC in 1993 as a Pharmacy Research and Training (PRAT) fellow and is now a staff fellow, says he would have wanted to work at NIDA no matter where it was located. "Drug-abuse research was the area where I wanted to go in my career ... and this is one of the world's premier institutions in that field," says Johnson, who is now studying opiate receptors and signal transduction within the cell.

Melanie Holden

Amy Hauck Newman

Staying in the Loop

To keep intramural researchers at outlying campuses "in the loop," Newman advocates increased teleconferencing or videotaping of major lectures; continued improvements in e-mail service, which just became available to many ARC scientists last August; and occasionally moving some events from Bethesda to other campuses. "Not being forgotten is what's important," she says.

NIDA Director Leshner agrees: "Many people on the [Bethesda] campus are not familiar with the absolutely incredible resources available in Baltimore. ... There are lots of opportunities for collaboration that are going untapped." NIDA particularly welcomes collaborative partnerships involving PET, Leshner says, noting that ARC is one of the few research institutions in the world to have its own dedicated PET scanner. In fact, there is an interesting story behind the funding of that PET scanner. Leshner says the millions of dollars of equipment necessary for PET studies came from the federal government's sale of cars, boats, real estate, and other ill-gotten gains confiscated from convicted drug dealers.

NINDS' Rogawski says that more than distance may underlie the reluctance of some intramural researchers to collaborate with NIDA. "One of the problems is that some people may have an outdated perception that drug abuse research is soft science, not realizing that the field is now at the cutting edge of molecular, cellular, and behavioral neuroscience," he says, adding that Newman's scientific expertise in medicinal chemistry — a relatively rare expertise at NIH — has been a "valuable asset" to his studies.

Stephen Heishman, a NIDA research

psychologist, says that currently, his only interinstitute collaboration is with a scientist who's already very familiar with NIDA's resources, Herb Weingartner of NIAAA — an institute that was part of NIDA when it was originally founded. In their clinical studies, Heishman and Weingartner are examining cognitive functioning in polydrug users. Their preliminary findings show that compared with non-abusers, substance abusers not under the influence of any drug effect give two to three times more wrong answers when asked to recall a list of 12 nouns presented to them 20 to 30 minutes earlier. It remains to be seen whether this deficit in reflective processing — the inability to "inhibit" wrong answers — stems from drug abuse or is a predisposing factor for drug abuse, Heishman says.

Echoing the views of others at NIDA, Heishman says he finds that he's more inclined, given ARC's history and location, to set up collaborations with scientists at Baltimore medical schools — Johns Hopkins and the University of Maryland — than with NIH scientists in Bethesda. And like a substantial number of his colleagues, Heishman also enjoys the opportunity to teach at Johns Hopkins, where he is an assistant professor in the Department of Psychiatry and Behavioral Science.

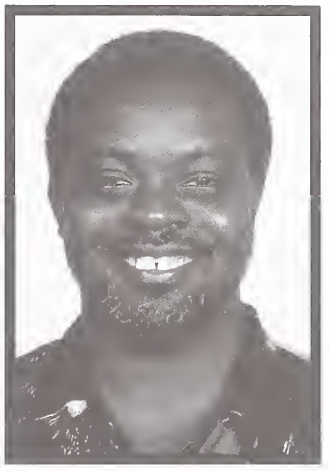
Melanie Holden

George Uhl

Best of Both Worlds?

Although NIDA's Baltimore campus, with its proximity to poor, inner city neighborhoods, is well-situated for recruiting drug-using volunteers for inpatient studies, its urban location and long-standing reputation as an addiction-research center inhibit the recruitment of middle- and upper-class drug users for outpatient studies, says Jean Lud Cadet, head of the Neuroscience Branch's Molecular Neuropsychiatry Section. Cadet says he is exploring the possibility of establishing a NIDA outpost at the Clinical Center in Bethesda for such studies.

Cadet, who spent time at the Clinical Center as a medical student and at NIMH as a medical staff fellow, says he also would like to see more exchange of scientific ideas among what he considers to be NIH's four neuroscience institutes: NIDA, NIAAA, NIMH, and NINDS. For example, no one from NIDA is currently part of the Neurosciences Working Group. "Sometimes it feels like that old saying 'Out of sight, out of mind.' A lot of the clinicians and basic scientists in Bethesda don't know a lot of the people here — and we have some really superb scientists," says Cadet.



Jean Lud Cadet

For his part, Cadet works hard at maintaining his ties to the Bethesda campus. Drawing on what he's learned in copper-zinc superoxide dismutase (CuZnSOD) transgenic mice, which have shown resistance to the lethal effects of some methamphetamine drugs that affect the mono-aminergic systems of rodents, Cadet is collaborating with NIMH's Dennis Murphy and Anne Andrews on a preclinical study assessing the toxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analogs in the CuZnSOD mice. The hypothesis is that SOD will counteract the neurodegenerative effect of the MPTP analogs. "I don't mind driving 45 minutes if there's somebody I really need to talk to," he says.

Contact with colleagues in Bethesda has given Cadet a greater appreciation of Baltimore's relatively spacious labs and its lower labor costs for support staff. However, Cadet says his visits also underscore what he misses about the main campus: the ability to exchange ideas with scientists from a wide range of disciplines by simply walking down the hall or slipping into a seminar. "It's exciting to be in Bethesda. I get so stimulated when I go there that I end up thinking, 'I wish I were here,'" Cadet says. "But after an hour or so, I come to my senses, and realize that, over the long-term, for what I want to achieve, it's better for me to be in Baltimore." ■

NIDA Intramural Research at a Glance

Contact: Acting Scientific Director George Uhl
Phone: 410 550-1538
Location: Addiction Research Center building, Johns Hopkins Bayview Campus, 4940 Eastern Ave., Baltimore, MD 21224.
Resources: Dedicated PET facility available for collaborations with the entire NIH intramural community. Special expertise in systems for assessing reward-behavior phenotypes in transgenic mice and other animals. Secure inpatient unit for studies involving patients who abuse drugs. ■

RESHAPING BUREAUCRACY
continued from page 1.

scientists want as much free time as possible to perform scientific research and spend a minimum amount of time with cumbersome processes. I realize that since we are funded with tax dollars, there are some legal limitations placed on us, that we must live with. If we could just remove some of the additional layers of oversight and bureaucracy placed upon us, we could better accomplish our research tasks."

A key part of the monumental job of turning such visions of a less bureaucratic, more scientist-friendly NIH into a workable reality lies with the recently formed NIH Business Process Reengineering Center (BPRC).

Last fall, a three-year, $2 million Business Process Reengineering (BPR) contract for overhauling the administrative processes that support all of NIH was awarded to Lockheed Martin Corp. in Bethesda, Md., which has done similar work in the past for the Department of Defense. A well-established concept in both private industry and management circles, BPR involves

- forming a team of knowledgeable workers,
- establishing start and end points for a given administrative process,
- making flow charts that show how that administrative process actually works today,
- identifying potential areas for improvement or elimination,
- collecting data and brainstorming,
- making flow charts to outline the new, reengineered, or "to be" process,
- implementing the new, reengineered, or "to be" process, and
- measuring the new process to determine whether the desired streamlining has actually occurred.

At NIH, the administrative areas of payment, purchasing, and human resources are currently taking part in the BPR project, to improve the timeliness of responses to the needs of scientists and their support staffs,

Accounts Payable

The government's often Byzantine payment, or "accounts payable," process is a sore point among many scientists. For example, Cashel says, "Recently, some subscriptions to key journals were canceled because, in the supplier's opinion, the subscription bills were not promptly paid. It was extremely difficult to obtain these journals by the informal network. As a result, some of our smaller libraries have unfillable holes."

NIH Associate Director for Administration Leamon Lee attributes the subscription problems to the fact that the company providing NIH's subscription services, Faxon Co. of Westwood, Mass., was recently bought by another firm that requires advance payment from NIH before journals are delivered. NIH administrators have been told that the missing journal issues will arrive eventually, albeit late. As for recent complaints about a shortage of supplies at NIH self-service stores, Lee blames the consolidation of warehouses and changes in ordering systems for tying up the delivery of stock to significantly those stores. Lee adds that both problems appear solvable, and journal subscriptions and delivery to self-service stores should soon be sharply improved.

To avert a repeat of such problems and to further speed the delivery of research supplies to scientists, Office of Financial Management (OFM) Director Francine Little decided to sponsor the first BPR team in Accounts Payable. "While there are some regulations that control our activities (i.e., the Prompt Pay Act), the team is looking at ways to ensure we pay our invoices within a 25 to 30 day window," Little says. "This means more money will be available for NIH research activities because prompt

Bill Branson

Accounts Payable BPR team. *From left, Bob Schaller, Tony Sambataro, Mary Saab, Anita Bowrin, Kathleen Hall, Joyce Lee, Deon Johnson, Francine Little, Jeanne DeAngelis, Marguerite Kendall, Harold Varmus, Laura McNay, George Dobenecker, Lynda Eckard, and Bob Davidson. Absent were the team's leader, Penny Strong, who has retired, and Sandra Logan, who was traveling.*

payment ensures receipt of discounts from certain key vendors. Also, vendors who get paid promptly are more than eager to supply free journals, catalogs, etc., to our scientific community. This ensures that the scientists receive the most current information on the most current technology available. Ideally, we would like to supply one-stop shopping to all our customers so that any problems can be resolved in a timely fashion."

Currently, 30 technicians at OFM must process and pay more than 600,000 invoices a year. "Being able to redirect the efforts of those men and women will result in a more positive, highly motivated work group, ensuring prompt, courteous service," Little says.

For their progress and readiness to tackle tough issues, members of the Accounts Payable BPR team were recognized at a special OFM all-hands meeting on April 12. Before presenting team members with their awards, NIH Director Harold Varmus stated, "I am extremely proud of OFM for taking the lead in the BPR effort. Accounts Payable was the prototype and the model for all future BPR activities. I really appreciate the effort expended."

Small Purchases

When it comes to small purchases, Battey says he thinks authority for procurement needs to be brought to the lowest level allowed by law, he hopes as low as a technician or research scientist. Cashel also supports the streamlining of small purchases, calling for some type of credit card system "that would allow direct purchases by scientists of any item costing less than $300, with minimal restrictions."

Some short-term fixes are already being implemented, according to Lee, who sponsored both the Small Purchases and Property Management BPR teams. "I have already vowed to cut time for acquiring items costing $2,500 or less from a period of 10 to 30 days to two days by removing unnecessary steps in the clearance process. This has been done by having the ordering of an item and the written confirmation of clearance determination run in parallel," Lee says. "Another short-term fix now allows requests for next year's small purchases to be input in September instead of the old July requirement."

Lee also notes that the credit card pilot [see page 22, January-February issue of *The NIH Catalyst*] has already begun at NCI and NCHGR. In addition to these initial steps toward simplifying the purchase of scientific reagents and equipment, Lee says the Property Man-

The Scientist's Advocate

Of the various teams struggling to untangle NIH's heap of red tape, the Intramural Reinvention Working Group (IRWG) has the well-earned reputation of being the strongest voice for the scientific community.

Formed nearly two years ago to identify the administrative roadblocks to intramural research, IRWG counts eight scientists among its 15 members. In September 1994, NIH Director Harold Varmus approved the IRWG report detailing the bureaucratic obstacles faced by NIH scientists and recommending ways to remove those barriers — a report that has contributed to NIH's Business Process Reengineering (BPR) activities as well as to the reinvention efforts spearheaded by the Office of Financial Management (OFM), the Office of Administration (OA), and the Office of Human Resources Management (OHRM).

"Our group is considered the scientist's advocate. The changes we promote are changes that we think would be positive for NIH and would improve the quality of life for the intramural scientist," says MaryAnn Guerra, co-chair of IRWG and executive officer at NHLBI.

Among the IRWG recommendations that have been placed high on the priority list of OA's reinvention activities is streamlined credit card purchasing. Meanwhile, OHRM is following through on IRWG's call for a simplified pass-fail performance-review process, and the revision of time-keeping procedures has been initiated as a BPR project.

Although most of IRWG's suggested changes have been channeled to the appropriate functional areas within NIH for implementation, the working group itself is not shying away from the monumental task of turning its ideas into reality. IRWG has initiated a new, streamlined approach to internal management controls: the self-assessment of the most sensitive management areas performed annually by scientific directors. A test of the streamlined approach at NHLBI found that the new self-assessment form took fewer than 4 hours to complete. In contrast, the previous, lengthy assessment process required a multidisciplinary audit team, Guerra says.

IRWG is also putting together a Cooperative Research and Development Agreement (CRADA) proposal in hopes of finding an industrial partner to create a fully automated system for facilitating the ordering of intramural supplies and services. The proposed state-of-the-art, computer-based system would feature electronic catalog access, generation of order forms, budgeting, and oversight.

Despite those strides, at least one IRWG member thinks it's far too early for the group to sit back and rest upon its laurels. "We may be moving in the right direction, but so far, to me the motion has been almost imperceptible," says David Ledbetter of NCHGR. "We still have a long ways to go."

For more information on IRWG or to voice your ideas about removing administrative barriers to science at NIH, contact MaryAnn Guerra (phone: 496-2411; fax: 402-3686; e-mail: princess@nih.gov). ■

— *Rebecca Kolberg*

agement and Small Purchases BPR teams are now trying to work out long-term fixes for even greater improvement.

Time and Attendance

"When someone comes into the laboratory from outside [such as a new postdoc], they find many of our rules unbelievably complicated," Cashel says. Although the NICHD molecular biologist was not specifically referring to NIH's methods of keeping time and attendance, no doubt that process — which determines how and what people get paid — is a part of that confusion.

"Resources currently used to operate within this time-keeping process could be better used to support research, which is what we are all about, anyway," says Richard Drury, director of the Division of Human Resource Systems and head of the Time and Attendance BPR team. Characterizing NIH's current methods of keeping time and attendance as "a 1930 system," Drury says, his team is "willing to take some risks" to streamline the process so it better meets the needs of 21st century science.

Getting Involved

For his part, Battey would like to see a couple other things come to pass as part of NIH's business reengineering effort: a simplified personnel system that would make it easier to get postdocs in the lab and quicker travel-approval procedures, especially for international trips. Battey adds that a "retrospective review" of travel might save some time over what he calls the current "prospective review process."

What can other scientists do to make sure that their voices are heard and they receive maximum benefit from the BPR teams? The best approach is to participate is as a team member. Although teams are limited to eight to 10 people, at least one scientist has been invited to participate on each panel. As the primary "customer" in NIH's administrative framework, scientists provide vital feedback to other team members concerning what's urgent, important, and needs fixing from the viewpoint of the research community. The time spent in team activities can be significant — two to four hours per week for approximately six months — but the lasting gains made in the overall quality of scientific life at NIH should be well worth the individual investment. If a scientist who is not a team member would like to offer insights or suggestions on improving NIH administrative processes, he or she can contact the BPR Center (594-4923) for information on whom to contact. Researchers can also make their views known to their administrative officers, who receive periodic updates on the efforts of BPR teams.

"This is a time of excitement if we can streamline many of the frustrating procedures and policies," Cashel says. "Things are progressing, and the efforts of many individuals are heartening." ■

BPRC at a Glance

Contacts:	Bob Schaller and Bob Davidson
Phone:	594-4923
Location:	Building 15, Room F-1
Resources:	Information on becoming a member of a Business Process Reengineering (BPR) team and on how to contact the scientific members of BPR teams. ■

IMPLEMENTING IDEAS:

Putting good ideas into practice is not necessarily easy, especially when the good ideas involve changing the government's time-honored, or some might say hidebound, methods of doing business. Take the example of NIH's pilot project to give intramural scientists charge cards.

Even before any researchers got their hands on the cards, the project was receiving quite different receptions at the two institutes singled out as guinea pigs: cautious optimism at NCHGR and a wait-and-see attitude at NCI.

Although the charge cards — expected to arrive May 1 — had yet to materialize as of mid-May, scientists' various reactions to the charge card training sessions and instruction manuals indicate that how a "reinvention" is presented may be nearly as important as what changes are actually occurring.

Under the pilot program, NCI and NCHGR are each granted 15 charge cards. The VISA cards, which function along the payment lines of American Express cards and are issued by the Rocky Mountain BankCard System, allow scientists who've volunteered or been recruited and who've received a special half-day training course and delegation of authority to buy many types of supplies and equipment. There is a price cap of $2,500 per item, as well as a limit of $2,500 per order, unless a scientist undergoes an additional 80 hours of procurement training. There are no limits on the number of orders that can be placed per month, and the monetary ceilings on monthly purchases are determined by the individual institute.

As long as the basic guidelines are adhered to, NCI and NCHGR have considerable leeway to implement the project in the manner that best

The Charge Card Example

serves the needs of researchers at their own institutes. At NCI, scientists who volunteered or were asked to take part in the pilot were required to attend the half-day training session and received a 20-page general manual, along with a thicker, supplemental guide prepared by NCI administrators to refer to when using the card. At NCHGR, where all participants in the pilot are volunteers, scientists also had to undergo the same half-day training session and pick up a general manual, but they did not receive an institute-specific reference guide.

NCHGR officials said they felt that NCI's supplemental guide was very useful and will be used by NCHGR's administrative office. However, because the actual procurement of items was enough to make cardholders nervous, they chose to wait until later in the process to distribute the thicker guide to interested NCHGR cardholders.

"I didn't want to scare them off," explains Linda Adams, NCHGR's senior administrative officer. "I wanted to get scientists to start using the cards, rather than putting up barriers to their use. ... I wanted them to see what they can do with a charge card rather than what they can't do with it." In addition, Adams, who is a certified procurement officer, has a card that she can use to help NCHGR scientists buy items that cost more than $2,500.

Although there is a requirement that scientists check with mandatory sources before buying something with their charge cards, David Ledbetter, chief of NCHGR's Diagnostic Development Branch and a participant in the pilot program, says it really shouldn't take much checking if a researcher is using his or her card to buy routine supplies from a familiar source. "All it really takes is good judgment on the part of the scientist," says Ledbetter, noting that, in practice, charge card orders will probably be placed by the same procurement-savvy people who place paper orders — high-level technicians, lab secretaries, or administrative officers working in conjunction with the scientist.

"I think the real benefit of the card is for emergency purchases — to buy supplies or equipment that you need right away. I don't think the number of purchases made by charge card will be huge," says Ledbetter, who is one of the Intramural Reinvention Working Group's (IRWG's) representatives on the NIH-wide team that developed the charge card concept.

In contrast to NCHGR, where half of the principal investigators are taking part in the charge card pilot and where there is a waiting list for cards, NCI actually had two participants drop out of the pilot before it even began because of the procurement requirements placed on the cardholder for proper use of the card.

The charge card "is not the answer for all our procurement problems, particularly in view of the monthly reconciliation process," says Janice Romanoff, program administrative officer at NCI. According to Romanoff, NCI has requested relief from some of the more cumbersome requirements, such as advance clearance for certain purchases, and also asked for the use of "record of call" numbers so scientists and other cardholders can directly place orders of less than $2,500 through Blanket Purchase Agreement (BPA) vendors. However, it should be noted that IRWG was successful in working with the Office of Procurement Management to modify the draft charge card guidelines to allow the cards to be used to buy supplies from BPA vendors.

Although she and many other NCI scientists never received official notification of their institute's charge card pilot before the training course began, Claude Klee, chief of NCI's Laboratory of Biochemistry, says that out of curiosity, she thumbed through a colleague's inch-thick instruction manual. "It's not the sort of book you'd like to look at," she says. "Procurement officers might spend an afternoon reading this book — that's their job, but a scientist shouldn't have to."

Nevertheless, Klee says that she thinks that streamlining the NIH procurement process is essential. "A charge card system should be able to help do that if it is implemented correctly. It has tremendous potential," she says, adding that input from scientists is a crucial element in the successful implementation of any administrative change.

Ledbetter says he thinks that part of the difference between NCIs and NCHGR's approaches to the charge card pilot may be the differing attitudes of the two institutes' scientific staffs. "Many NCHGR scientists were academic scientists who've only recently been transplanted to NIH. We don't like the government procurement process, and charge cards are something that we were used to in the outside world," he says.

On the other hand, Ledbetter says NCI has more veteran government researchers who've learned to efficiently navigate the existing procurement process and who would rather not be burdened by the added personal responsibility that comes with a charge card, such as reconciling a monthly charge card statement with supplies and equipment received.

However, NCI's Klee disagrees. "It's not the scientists who are different between the two institutes, it's the administrators who are different," Klee says, adding that she and her colleagues would gladly take responsibility for a charge card if that would make it quicker and easier to get supplies and equipment for her lab. ■

—Rebecca Kolberg

HOT METHODS

continued from page 13.

ing to nonspecific staining. For this reason, we strongly recommend omission of this step. A careful choice of primers and a reduced number of cycles (15 to 20) helps to avoid nonspecific nuclear staining.

3. Designing primers. When choosing primers, consider the following: a) A good size range for the PCR product is 100 to 500 base pairs (bp). If the product is too small, it could leak out of the fixative-induced lattice and be washed out of the tissue. On the other hand, excessively long products could be hard to amplify in tissue sections, and, especially in archival material, the probability of finding nicks in the nucleic acid template that prevent amplification increases with size. b) If primers bridge an intron, it helps to eliminate the possibility of genomic amplification. c) Take precautions to avoid palindromic sequences and hairpin formations in the primers. These formations can block amplification.
4. Double labeling. It is possible to combine in situ amplification with immunocytochemistry or in situ hybridization. For immunocytochemistry, we recommend performing immunological detection first because thermal cycling could destroy antigens in tissue. For mRNA localization, remember to use RNase-free reagents during the whole process by using DEPC-treated water in all solutions and adding RNase inhibitors to antisera. ■

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References

1. M. Corcoran, M. Levin, S. Jacobson, and L. Liotta. "From hot starts and false starts to smart starts: in situ PCR." *The NIH Catalyst* **2**, 12 - 14 (1994).
2. G.J. Nuovo. "PCR in situ hybridization: protocols and applications." New York: Raven Press (1992).
3. A. Martínez, M.J. Miller, K. Quinn, E.J. Unsworth, M. Ebina, and F. Cuttitta. "Non-radioactive localization of nucleic acids by direct in situ PCR and in situ RT-PCR in paraffin-embedded sections." *J. Histochem. Cytochem.* (in press).
4. D.E. Kellogg, I. Ryballkin, S. Chen, N. Mukhamedova, T. Vlasik, P.D. Siebert, et. al. "TaqStart antibody; "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase." *BioTechniques* **6**, 1134 - 37 (1994).
5. J.J. O'Leary, G. Browne, R.J. Landers, M. Crowley, I. Bailey Healy, J.T. Street, et. al. "The importance of fixation procedures on DNA template and its suitability for solution-phase polymerase chain reaction and PCR in situ hybridization." *Histochem. J.* **26**, 337 - 46 (1994).
6. A. Martínez, M.J. Miller, E.J. Unsworth, J.M. Siegfried, and F. Cuttitta. "Expression of adrenomedullin in normal human lung and in pulmonary tumors." *Endocrinology* (in press).
7. M.J. Miller, K. Quinn, M.D. Vos, A. Treston, E.J. Unsworth, J.L. Mulshine, et. al. "Expression of transferrin mRNA in human tumor cell lines." *Proc. AACR* **35**, 552 (1994).

COCAINE CRAVING

continued from page 11.

may be possible to determine whether brain systems underlying acquired motivation for stimuli that are not biologically relevant are similar to those of "normal" motivation for biologically relevant stimuli such as food, water, and sex.

Although our findings suggest that a specific anatomical network is activated by cocaine-related cues and that this activation is associated with cocaine craving, many questions remain unanswered. To date, our studies in this area have focused on identifying the brain regions important in responding to drug-related cues, but the specific neurotransmitters mediating cocaine craving are not known. The availability of radioligands for specific neurotransmitter receptors and the development of tracers and mathematical models for assessing neurotransmitter synthesis will facilitate further work with PET scanning.

In clinical applications, it will be important to determine whether the conditioned response to cocaine cues can be blocked pharmacologically, either by agonists such as bromocriptine (27) or by antagonists such as naltrexone (recently approved by the FDA for the treatment of alcoholism). Key questions will be whether blocking craving interferes with biologically necessary motivational systems and whether suppressing craving will also prevent the actual taking of abused drugs on the street.

Acknowledgment

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References

1. E.D. London, S.J. Grant, M.J. Morgan, and S.R. Zukin. "Neurobiology of drug abuse." In: *Neuropsychiatry: A Comprehensive Textbook.* B.S. Fogel, ed. (in press).
2. T.E. Robinson and K.C. Berridge. "The neural basis of drug craving: an incentive-sensitization theory of addiction." *Brain Res. Rev.* **18**, 247 - 91 (1993).
3. J.S. Altman, B.J. Everitt, M. Fischman, S. Glautier, A. Markou, D. Nutt, et. al. "The biological, social and clinical bases of drug addiction." *Psychopharmacology (Berl)* (in press).
4. S.T. Tiffany. "A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes." *Psychol. Rev.* **97**, 147 - 68 (1990).
5. A. Wikler. "Conditioning factors in opiate addiction and relapse." In: *Narcotics.* D.M. Wilner and G.G. Kassebaum, eds. New York: McGraw Hill, pp. 85 - 100 (1948).
6. A. Wikler. "Dynamics of drug dependence: implications of a conditioning theory for research and treatment." *Arch. Gen. Psychiatry* **28**, 611 - 16 (1973).
7. S. Siegel. "Classical conditioning, drug tolerance, and drug dependence." In: *Research Advances in Alcohol and Drug Problems,* Volume 7. R.G. Smart, F.B. Glaser, Y. Israel, H. Kalant, R.E. Popham, and W. Schmidt, eds. New York: Plenum, pp. 207 - 44 (1983).
8. J. Stewart, H. de Wit, and R. Eikelboom. "Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants." *Psychol. Rev.* **91**, 251 - 68 (1984).
9. J.P. Vincent, B. Kartalovski, P. Geneste, J.M. Kamenka, and M. Lazdunski. "Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes." *Proc. Natl. Acad. Sci. USA* **76**, 4678 - 82 (1979).
10. P.W. Kalivas, B.A. Sorg, and M.S. Hooks. "The pharmacology and neural circuitry of sensitization to psychostimulants." *Behav. Pharmacol.* **4**, 315 - 34 (1993).
11. R.M. Post and S.R.B. Weiss. "Sensitization and kindling: implications for the evolution of psychiatric symptomatology." In: *Sensitization in the Nervous System.* P.W. Kalivas and C.D. Barnes, eds. Caldwell, NJ.: Telford, pp. 257 - 93 (1993).
12. R.S. Niaura, D.J. Rohsenow, J.A. Binkoff, P.M. Monti, M. Pedraza, and D.B. Abrams. "Relevance of cue reactivity to understanding alcohol and smoking relapse." *J. Abnorm. Psychol.* **97**, 133 - 52 (1988).
13. L.O. Bauer. "Psychobiology of craving." In: *Substance Abuse. A Comprehensive Textbook.* J.H. Lowinson, P. Ruiz, R.B. Millman, and J.G. Langrod, eds. Baltimore: Williams & Wilkins, pp. 51 - 5 (1992).
14. A.R., Childress, R. Ehrman, D.J. Rohsenow, S.J. Robbins, and C.P. O'Brien. "Classically conditioned factors in drug dependence." In: *Substance Abuse. A Comprehensive Textbook..* J.H. Lowinson, P. Ruiz, R.B. Millman, and J.G. Langrod, eds. Baltimore: Williams & Wilkins, pp. 56 - 69 (1992).
15. R. Eikelboom and J. Stewart. "Conditioning of drug-induced physiological responses." *Psychol. Rev.* **89**, 507 - 28 (1982).
16. I.P. Pavlov. In: *Conditioned Reflexes; An Investigation of the Physiological Activity of the Cerebral Cortex.* London: Oxford University Press, (1927)
17. E.E. Brown, G.S. Robertson, and H.C. Fibiger. "Evidence for conditioned neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures." *J. Neurosci.* **12,** 4112 - 21 (1992).
18. G.F. Koob, L. Stinus, M. Le Moal, and F.E. Bloom. "Opponent process theory of motivation: neurobio-

NIAID Clinical Associate Wins Henry Christian Award

The American Federation for Clinical Research Foundation recently honored Sharon Jackson, clinical associate for the Laboratory of Host Defenses at NIAID, with its Henry Christian Award for Excellence in Research. Jackson received the recognition for her abstract "Mouse Model of Chronic Granulomatous Disease (CGD): the *p47*^{phox} Knock-out" — one of only 15 abstracts chosen for the prestigious award out of 1,200 submitted for the Clinical Research meeting on May 6 in San Diego.

Jackson came to NIH in 1991 after finishing her residency at Mount Sinai Medical Center in New York. She received her M.D. from the State University of New York at Buffalo School of Medicine, in 1988.

CGD is a genetic disorder of the bactericidal function of the white blood cells that is characterized by widespread granulomatous lesions of the skin, lungs, and lymph nodes, as well as life-threatening bacterial and fungal infections. Patients with CGD are unable to produce superoxide, hydrogen peroxide, and other reactive oxygen metabolites due to a deficiency in any of the four proteins that make up nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.

Lorna Heartley

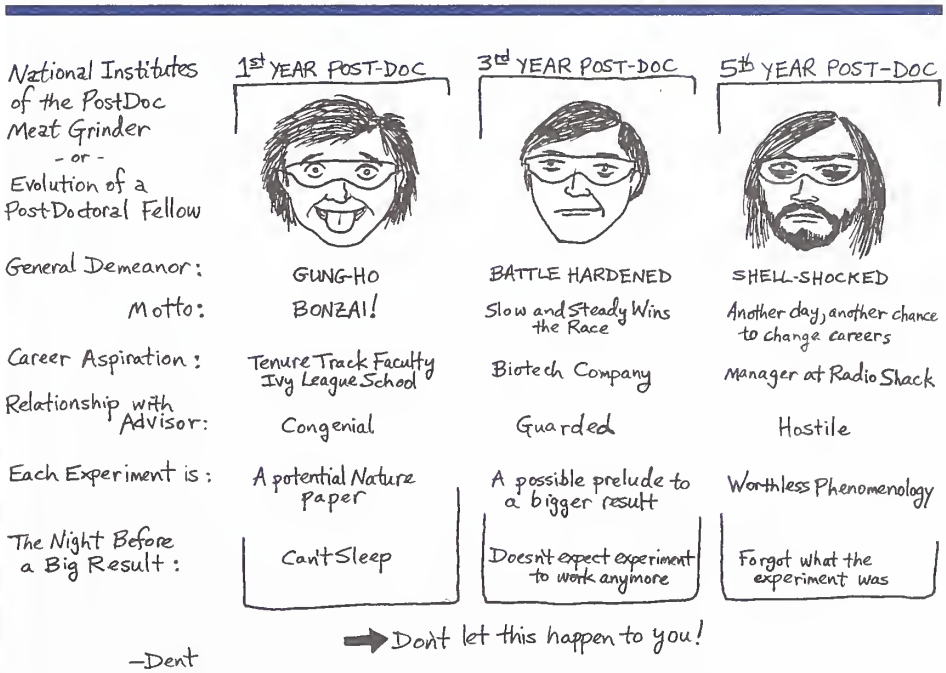
Sharon Jackson

Under the guidance of the Laboratory of Host Defenses' Steve Holland and John Gallin, Jackson created a mouse model of the major autosomal-recessive (*p47*^{phox}) form of CGD by using homologous recombination. A neomycin-resistance gene was inserted into the mouse *p47*^{phox} gene and transfected into embryonic stem cells. Neomycin-resistant colonies were cloned and transferred into blastocysts that developed into fertile chimeras, which were then bred to produce *p47*^{phox}-deficient "knock-out" mice. The knock-out mice could not be physically distinguished from their wild-type and heterozygous littermates. However, 50% of the knockout mice, by 14 weeks of age, developed spontaneous infections, while their wild-type and heterozygous littermates did not. Most of the knock-out mice have developed systemic infections with *Staphylococcus xylosus*. Based on these findings, the *p47*^{phox} knock-out mouse appears to provide an accurate model of human CGD. Such a model will be useful for studying CGD gene therapy, and the role of hydrogen peroxide production by phagocytes in malignant transformation, cataract formation, arthritis, atherosclerosis, and other disorders. ■

— Lorna Heartley

logical evidence from studies of opiate dependence." *Neurosci. Biobehav. Res.* **13**, 135 - 40 (1989).

19. W. Schultz. "Activity of dopamine neurons in the behaving primate." *Semin. Neurosci.* **4**, 129 - 38 (1992).
20. C.P. O'Brien, R.N. Ehrman, and J.W. Ternes. "Classical conditioning in human opioid dependence." In: *Behavioral Analysis of Drug Dependence.* S. Goldberg and I.P. Stolerman, eds. Orlando, FL: Academic Press, pp. 329 - 56 (1986).
21. R.N. Ehrman, S.J. Robbins, A.R. Childress, and C.P. O'Brien. "Conditioned responses to cocaine-related stimuli in cocaine abuse patients." *Psychopharmacology (Berl)* **107**, 523 - 9 (1992).
22. L.O. Bauer and H.R. Kranzler. "Electroencephalographic activity and mood in cocaine-dependent outpatients: effects of cocaine cue exposure." *Biol. Psychiatry* **36**, 189 - 97 (1994).
23. M.W. Fischman, R.W. Foltin, G. Nestadt, and G.D. Pearlson. "Effects of desipramine maintenance on cocaine self-administration by humans." *J. Pharmacol. Exp. Ther.* **253**, 760 - 70 (1990).
24. F.H. Gawin, H.D. Kleber, R. Byck, B.J. Rounsaville, T.R. Kosten, P.I. Jatlow, et al. "Desipramine facilitation of initial cocaine abstinence." *Arch. Gen. Psychiatry* **46**, 117 - 21 (1989).
25. R.W. Pickens and C.E. Johanson. "Craving: consensus of status and agenda for future research." *Drug Alcohol Depend.* **30**, 127 - 31 (1992).
26. E.D. London, N.G. Cascella, D.F. Wong, R.L. Phillips, R.F. Dannals, J.M. Links, et. al. "Cocaine-induced reduction of glucose utilization in human brain. A study using positron emission tomography and [fluorine 18]fluorodeoxyglucose." *Arch. Gen. Psychiatry* **4**, 567 - 74 (1990).
27. H.R. Kranzler and L.O. Bauer. "Bromocriptine and cocaine cue reactivity in cocaine-dependent patients." *Br. J. Addict.* **87**, 1537 - 48 (1992).



CATALYTIC REACTIONS

In this issue, we are asking for your reactions in four areas: the Clinical Center review, efforts to cut bureaucratic red tape, tips for our Hot Methods Clinic, and postdoc life. **Send your responses on these topics or comments on other intramural research concerns to us** via e-mail: kolbergr@nih.gov; fax: 402-4303; or mail: Building 1, Room 334.

In Future Issues. . .

- The Shape of Things to Come, Part II
- Tissue Culturing Prepares to Enter The Space Age
- Commissioned Corps At the Crossroads
- The Postdoc Plight: How Can NIH Lessen the Pain?

1) What suggestions do you have for creating a more responsive, efficient Clinical Center? How do you think one option under consideration — the contracting out of certain services — would affect clinical research?

2) What administrative obstacles at NIH aggravate you the most? What suggestions do you have for removing those obstacles?

3) Do you have any suggestions or comments about in situ PCR and RT-PCR in this issue's Hot Methods Clinic? What updates can you provide on previous Hot Methods? What techniques would you like to see covered in future issues?

4) We are planning a group of articles on the difficulties faced by postdocs at NIH. What issues should be addressed in such articles? What suggestions do you have for improving the training and mentoring of postdocs at NIH?

The NIH Catalyst is published bimonthly for and by the intramural scientists at NIH. Address correspondence to Building 1, Room 334, NIH, Bethesda, MD 20892. Ph: (301) 402-1449; e-mail: KOLBERGR or HOOPERC@od1em1.od.nih.gov

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