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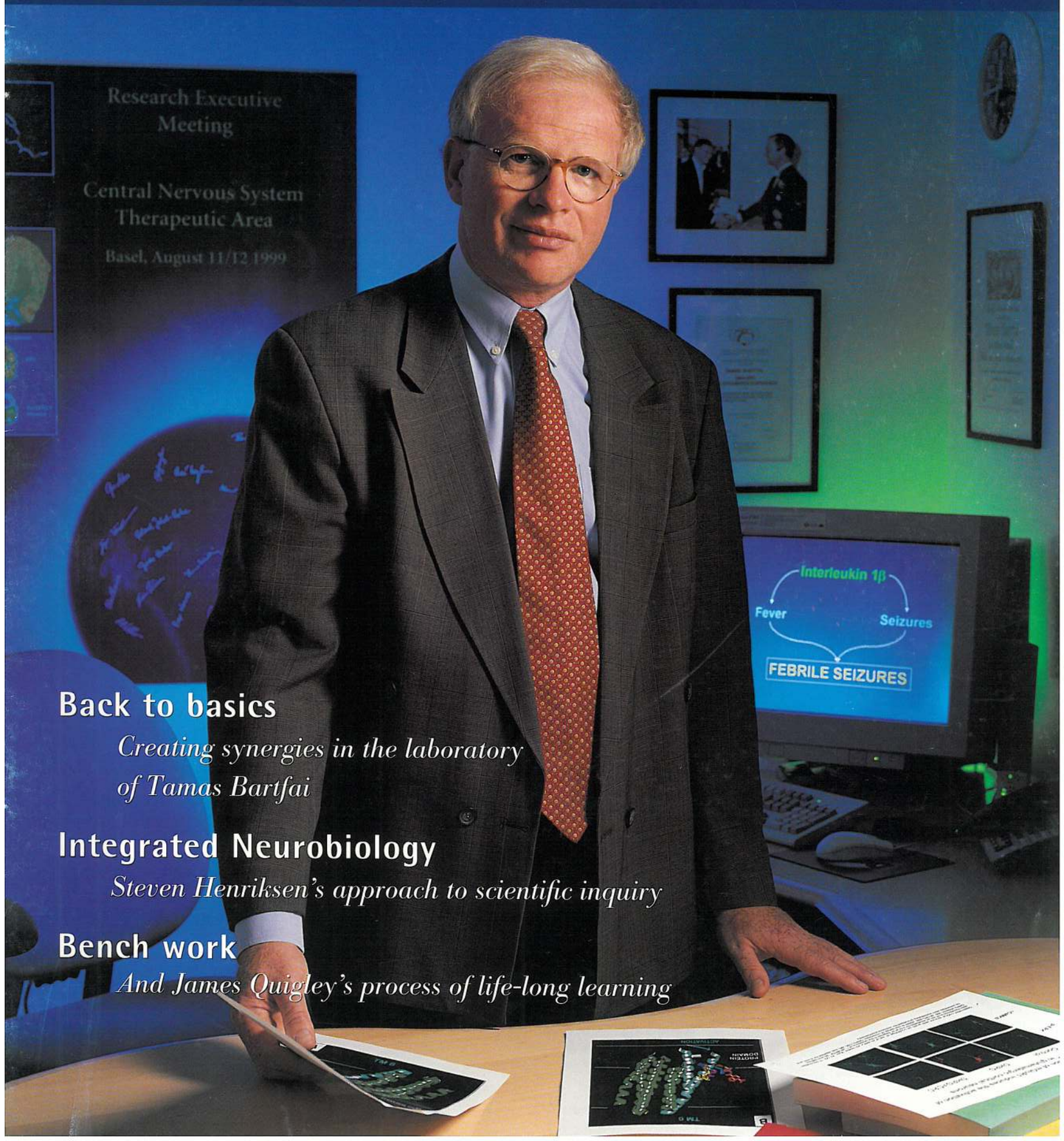
THE SCRIPPS RESEARCH INSTITUTE

Endeavor



Back to basics

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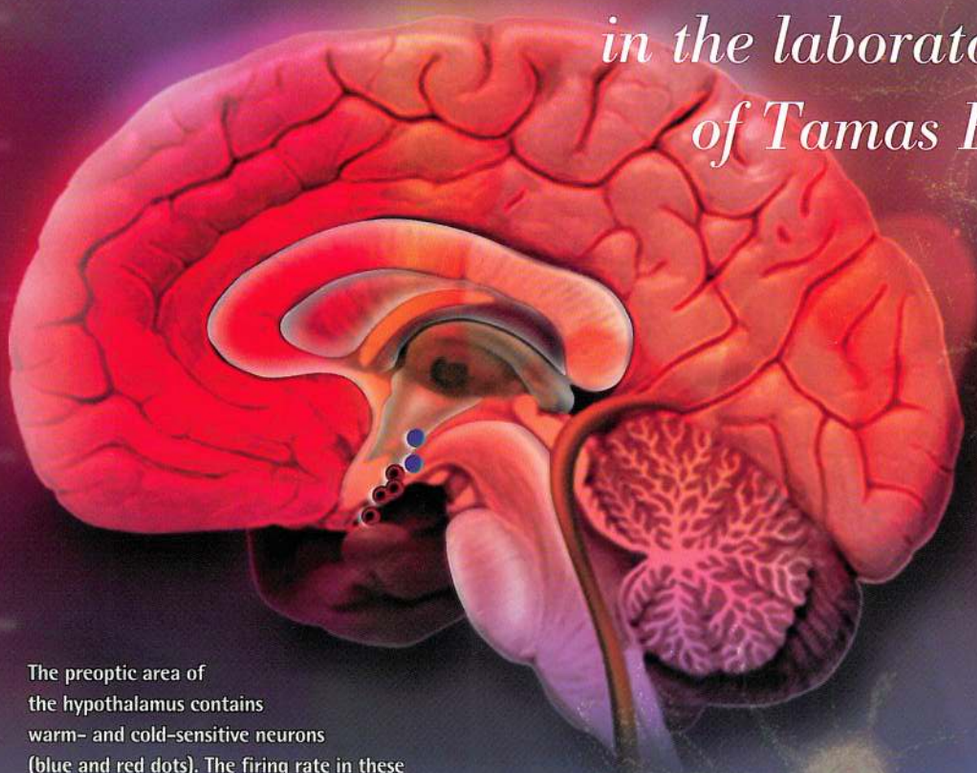
back to basics

Creating synergies in the laboratory of Tamas Bartfai



The preoptic area of the hypothalamus contains warm- and cold-sensitive neurons (blue and red dots). The firing rate in these neurons is different depending on the state – awake, fever, or sleep (blue, yellow and green bars). EEG recordings of febrile seizures in wild type mice and IL-1 receptor antagonist transgenic mice give different results (oscillations shown at the bottom part of the picture).

Understanding fever and making a cognitive enhancer are two of Tamas Bartfai's ultimate goals.

When Tamas Bartfai, Ph.D., decided to leave his job as a senior vice president in charge of central nervous system research at Hoffman-LaRoche to join the faculty of The Scripps Research Institute, the company did what it could to get him to stay. He did not look back. Bartfai, a native Hungarian who speaks in a delightful Eastern European burr, wanted to return to full-time research in biochemistry, the field in which he received his doctorate from Sweden's Stockholm University in 1973. TSRI was his primary choice.

"At this stage of my life, my professional work is most important," he says. "So Scripps was the absolute right choice for several reasons. One, it does outstanding chemistry, fantastic chemistry; in fact, several of the top chemists in the world are here — and biology can't be done without chemistry. The other reason is that I like the entrepreneurial atmosphere of Southern California, even though I don't want to start a biotechnology company. Finally, there are very bright people here in every field. With its location and reputation, it's easy for Scripps to recruit bright people."

Bartfai joined the Institute in 1999 as Director of the newly established Harold L. Dorris Neurological Research Center. The center was formed with a $10 million commitment from Helen L. Dorris of San Diego — the largest TSRI had ever received for research in the neurosciences. Bartfai also holds the Harold L. Dorris Chair in Neuroscience and is a Professor in the Department of Neuropharmacology.

The Dorris Center conducts research into a variety of neurological disorders, including depression, schizophrenia and Alzheimer's disease, as well as studies into the effects of aging on the brain. TSRI has recruited five new senior scientists to the Center to uncover some of the basic mechanisms of the brain, a large-scale collaborative effort that involves ongoing research in the departments of neurobiology, neuropharmacology, chemistry and molecular biology.

For Bartfai, who was born in Budapest and spent nearly all of his professional life in Europe, with spells at Yale, Rockefeller and UCLA, Southern California is the best place to be at the moment. In the life sciences, he claims, nothing else compares.

> *"I like to work on problems that everyone thinks have already been solved."*

"It's a unique combination of scientific excellence and tremendous ambition," he says. "In biotechnology and the life sciences, San Diego is ahead of Boston and Chicago, better than New York City, and second only to the Bay area. This is mainly because TSRI, The Salk Institute and the University of California, San Diego are all successful organizations — and we're all within walking distance of one another. The hiring strategy for places like Scripps and Salk is that they go for absolute excellence, they attract the best of the best."

THE AMBITION COMES FROM WHAT BARTFAI WITNESSES EVERY DAY

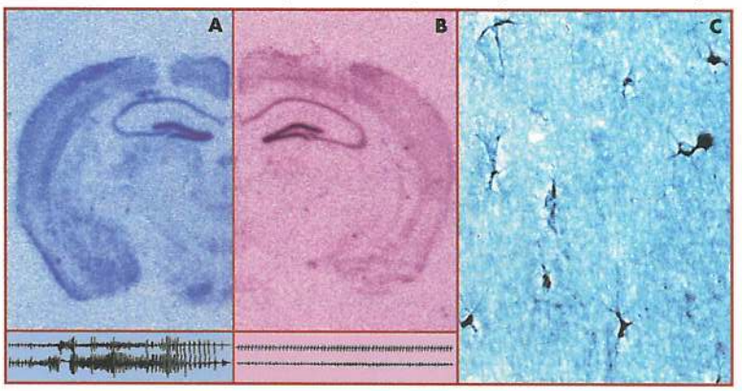
"There are about 100 biotechnology companies on the same street with TSRI," he says.

"But unlike Boston or San Francisco where the top schools run independent shows, something unique goes on here — the industry and the three research centers all work together. Because all of us are concentrated in such a small place, you really do feel the ambition, the entrepreneurial drive of the business. I find it tremendously interesting, this biotech community, vital, very forward-

On The Cover:
Tamas Bartfai Ph.D.,
Director,
Harold L. Dorris
Neurological Research
Center, and
Professor,
Department of
Neuropharmacology.

looking, and very interested in science and technology. They're struggling to grasp what the human genome project means for them."

Professionally, Bartfai's goal for the Dorris Center is to establish a research link between chemistry, neurobiology and neuropharmacology, expanding the synergy between chemists and biologists to build a novel research program. He has his own hero to help him, metaphorically at least.



Interleukin-1 expression is induced in the hippocampus by (A) seizure activity and (B) no seizures. Figure C shows Interleukin-1 expression in neurons under seizures. Bottom Figure: EEG tracings.

"I like to work on problems that everybody thinks are no longer problems, that everyone thinks have already been solved," he says. "The best example of this is Kapitza, the man who discovered how and why water boiled. For me, the heroes of the world are the Kapitzas, not the ones who do huge projects with linear accelerators — even if I started out studying particle physics myself."

For the record, Peter Kapitza was a Russian physicist, educated in the former Soviet Union and in Cambridge (UK), who died in 1984. He was perhaps best known for his work in low temperature physics, and as an outspoken advocate of more openness in science in the USSR. Bartfai studies temperature, but not of water. He has a longstanding interest in the properties of mammalian fever. Like Kapitza, Bartfai's fascination comes from the fact that fever is a common phenomenon that no one fully understands.

"We all have fevers, and we think we understand them," Bartfai says, "so I was shocked when I found out that no one knows how fever is regulated or whether it's good for us. In terms of treatment, nobody knows why acetaminophen breaks a fever, either." Bartfai's own work with the subject began ten years ago, and he soon came to realize what an enormous problem fever was for the medical profession — a sizable number of patients who fill up emergency rooms are there to find the reason for their fevers. As a practical matter, Bartfai wants to know too, and he has his own ideas.

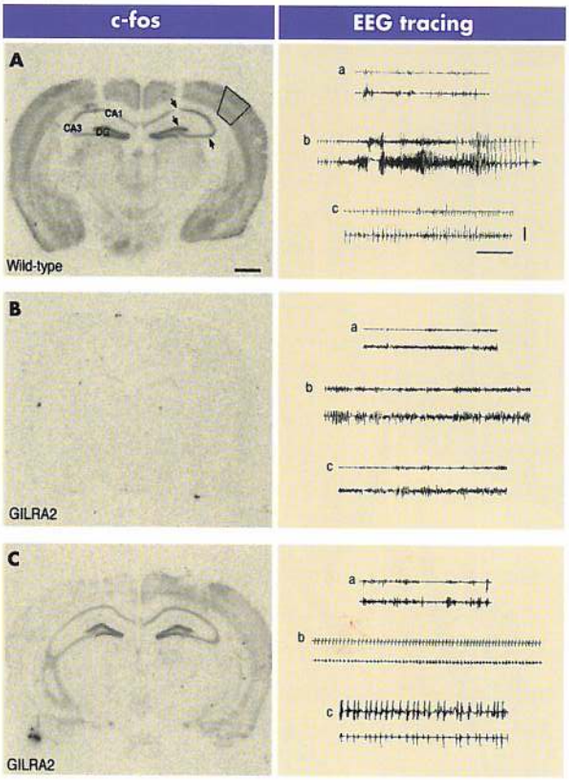
"First, fever is a regulated stress response that is mediated by the brain. You have a sore on your foot and you get a fever. How? No one knows. The best way to study fever is to study temperature set point in mammals. This is one of the key problems of mammalian biology today, how do mammals regulate their own temperatures? There is something truly magical about how temperature is set at a constant point. Why is it the same for the mouse and the elephant? A woman's fertility cycle is set by temperature — women maintain a regulated fever for perhaps one third of their lives. Why is that?"

Tamas Bartfai studies the phenomenon of fever in the hope he can catch a glimpse of the mechanism at work — and then go to work on it. "I want to look at the system, maybe bang it against the wall once or twice to see just how it works," he says. "We understand vision, hearing, taste yet we don't understand heat sensation," he claims. "But if you are burned, you feel it. Is the basis of thermal sensation molecular or cellular? How is the set point kept, or what moves the set point up when you have a fever or down when you go to sleep?"

Scientists who study the brain quickly end up knocking on sleep's door. Our ability to regulate temperature has the room right down the hall, so sleep and temperature regulation live in tandem. Both are common occurrences that scientists know little about yet both have an enormous impact on day-to-day life.

"It's the constancy of our need for sleep that fascinates me," he says. "The temperature set point works together with sleep because your set point has to drop when you sleep." Sleep is also linked to one of Bartfai's longstanding goals, the development of a quick-acting compound for the treatment of depression. Although none exists at the moment, the two known methods of bringing on a rapid anti-depression response are electroshock and sleep deprivation. While effective, neither method is long lived. The antidepressant effect of sleep deprivation, for example — a period of 12-18 hours of enforced wakefulness after a full day's activities — lasts about 48 hours.

Bartfai's work with antidepressants goes back twenty years to his study of the molecular and biochemical basis of both fever and cognition. The study of cognitive impairment includes a wide range of neurological disorders beyond depression, including Alzheimer's disease, schizophrenia and sleep disorders. He was involved in the development of Zimelidine, the first selective serotonin reuptake inhibitor (SSRI) and two antipsychotic agents used in the treatment of schizophrenia. He also has done major work on the peptide galanin, a major regulator of neuronal excitability in the hippocampus, a part of the brain that influences and regulates how you imprint or retain information, the basic process of cognition.



C-*fos* mRNA expression after intrahippocampal injection of bicuculline methiodide in B6/CBA wild-type and transgenic mice overexpressing IL-1Ra in astrocytes (GILRA2). Left column: C-*fos* was widely and massively expressed in the central nervous system after injection of bicuculline methiodide in wild-type B6/CBA mice (n = 5) (A). In contrast, mice overexpressing IL-1Ra (GILRA2, n = 7) showed no induction (B, n = 4) or considerably milder induction (C, n = 3) in most forebrain areas, particularly in the neocortex and hippocampus. Right column: Representative EEG tracings of the hippocampus in each row depict epileptic activity after injection of bicuculline methiodide in the corresponding wild-type (A) and GILRA2 mice (B and C).

GALANIN RECEPTORS AS DRUG TARGETS

Recent studies have shown that the regulation of galanin plays an important role not only in cognition, but also in seizures and an individual's threshold for pain. What they also show is that neurons inundated with galanin are the only ones that survive in the brains of Alzheimer's patients. Bartfai's work has led to three galanin receptors becoming the target of more than 20 projects in the pharmaceutical industry.

Right now, Bartfai and his colleagues in the Dorris Center are attempting to establish combinatorial libraries that he hopes will eventually lead to the creation of more effective galanin receptor antagonists for antidepressants and cognitive-enhancing therapies. Human cognition is determined by numerous attentional and emotional abilities — if you are emotionally disturbed or a victim of Alzheimer's disease, for example, you may not be able to learn certain tasks as quickly or as thoroughly, or remember what you have learned. There are no drugs that can enhance human cognitive ability or that can restore damaged human cognition. The development of the drug that restores cognitive ability remains one of Bartfai's top priorities.

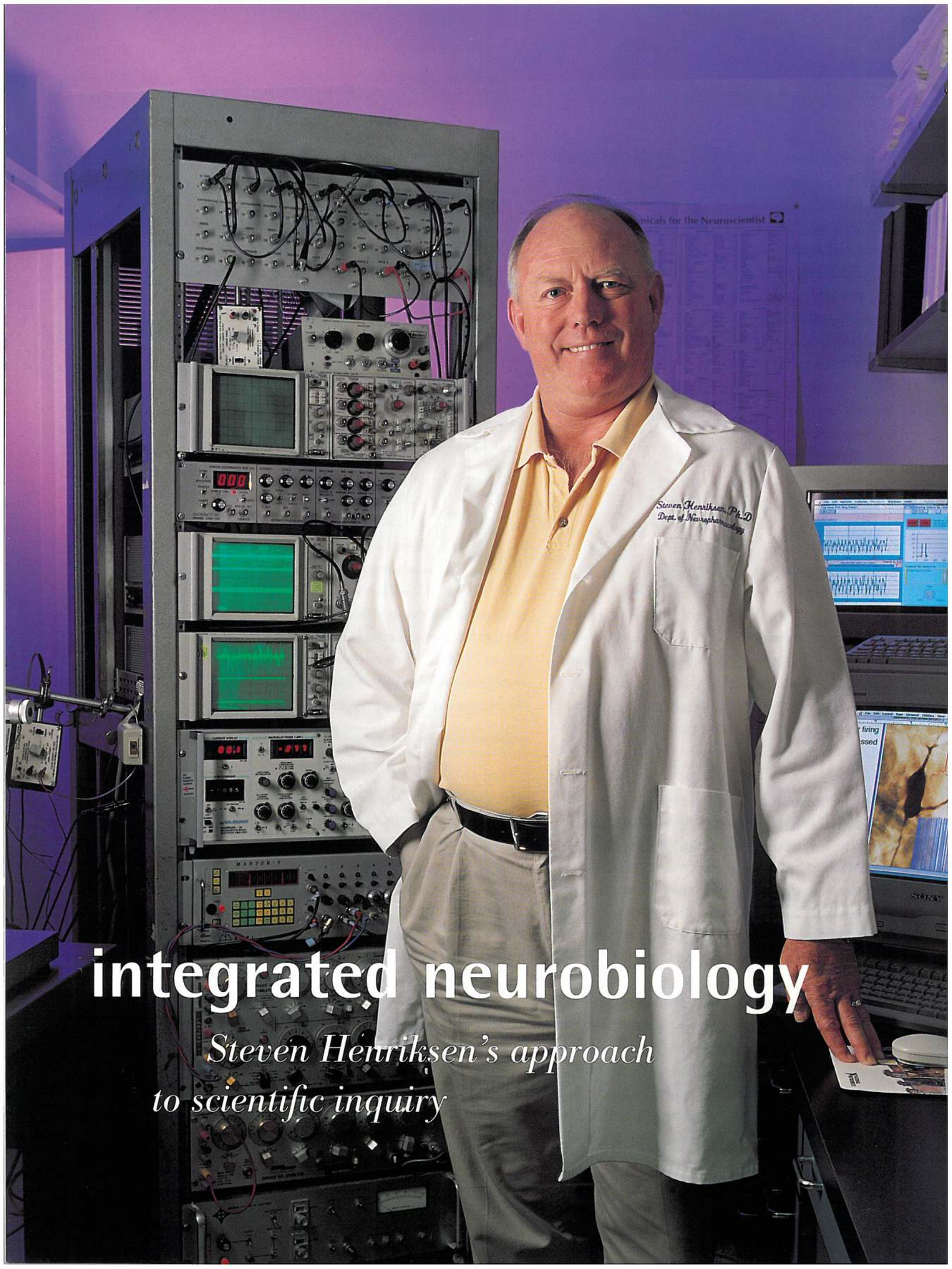
While he readily talks about his scientific work, when asked to describe his work outside the realm of research, Bartfai does so almost reluctantly. Given his background, it's not surprising that he is something of an internationalist and a humanitarian. Working with the International Red Cross, for example, he donates his scientific knowledge to help eliminate biological and chemical weapons.

He was involved in the invention of a biosensor-based device to detect land mines, participated in negotiating an agreement between animal right activists and Swedish researchers, and led a project to develop chlorine-free paper bleaching methods to protect the environment.

Given his background, it's not surprising that he is something of an internationalist and a humanitarian.

He participates in risk assessment and vaccination programs, and serves on ethical committees and boards of scientific institutions around the globe. He admits to these with a reticence that seems striking because it is so rare in public life. His refreshing lack of self-reverence shows up most clearly in his comments on teaching, a job he still enjoys immensely.

"It is time well spent, because the chances of many of your students being brighter than you are very high," he says, sounding very much like a man who knows he's made the right choice. ■



integrated neurobiology

Steven Henriksen's approach to scientific inquiry

Steve Henriksen is the sort of man who sees the forest, not only the trees. He has another name for it.

"The best way of looking at what I do," he says, "is to see it as systems biology or integrated neurobiology. It isn't a traditional discipline but it has become much more current with the realization that most of the brain-related pathologies neurobiologists want to understand involve the entire structure. Abnormalities do not occur simply as a result of loss of a single neuron or a single structural protein, but ultimately because of changes in specific brain circuitry. And those abnormalities can come from a variety of sources — viruses, genetics, the environment or drugs."

"...This could have tremendous consequences for how we treat arousal disorders, insomnia and the serious sleep disorders."

Associate Professor in the Department of Neuropharmacology at TSRI, Steven Henriksen, Ph.D., was born in Santa Barbara, and completed graduate work in neuroscience at the Stanford University Medical School. It was there he found a quiet revolution underway in neurological science. The emphasis was shifting from a single system focus to a fully integrated vision of the brain and its functional circuitry. Henriksen liked the new way of thinking. "I found myself very comfortable in looking at systems that involve both normal behavior and pathological behavior as part of a whole," he says. "I was fortunate to be part of this scientific evolution, which continued full-bore during postdoctoral work at the NIH with Dr. Floyd Bloom."

Henriksen's laboratory studies the neural circuitry of mammals, looking for the mechanisms behind complex cognitive functions such as memory, learning, sleep, even motivational factors leading to addiction. By studying the interactions of the various neural transmitters in these circuits, Henriksen hopes to gain new insights into treating myriad human diseases. By monitoring the electrical activity of the brain circuitry of various experimental mammals, they can determine just how that neural activity underlies normal or abnormal behaviors.

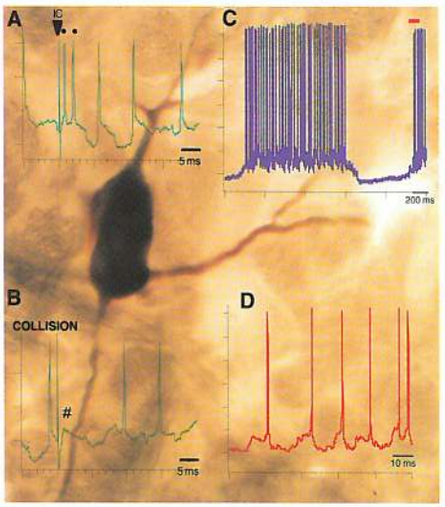
For example, diseases like drug abuse appear to involve interconnected neurochemical systems in the brain, complicated even further by genetic and environmental factors. It's becoming increasingly clear that drugs such as cocaine, heroin or even alcohol, somehow usurp a common neural system to produce the complex need to take more. By recording the electrical activity of specific circuits in the brain of experimental animals, Henriksen and his colleagues can even monitor this urge. Maladies such as Parkinson's disease, or Alzheimer's disease, Henriksen believes, are caused by other kinds of brain insults that are translated into the circuit abnormalities characteristic of the individual disease.

INTEGRATED BIOLOGICAL SYSTEMS

"To study the whole system, we need whole animal studies, whole tissues," he says. "I can't find the answers to my particular questions, for example, by relying on the anatomic structure of the brain alone. Just like I can't find out how a television works by tinkering with just the green wires. I need to study the hierarchy of closely connected circuits, and I need to view them while they're working." Henriksen believes that the next major advance in biology will come not from deciphering the mammalian genome, but from understanding how genes and their proteins operate within the context of integrated biological systems, in his case, brain circuitry.

It's an idea that's appreciated at TSRI, where

Steve Henriksen, Ph.D., Associate Professor, Department of Neuropharmacology.



A neurobiotin-labeled neuron located in the dorsal tier of the ventral tegmental nucleus of Gudden. These GABA containing neurons are the critical component of an ascending circuit related to neocortical activation. A, B, C, and D represent different neurophysiological characteristics of this cell type determined by both intra- and extracellular recording techniques.

collaboration and data sharing between diverse disciplines is the norm. Henriksen has regular collaborations with immunologists, chemists and cell biologists, constantly exchanging ideas and insights with fellow scientists about structural physiology and systems biology.

"We don't have jobs," he says, laughing. "Really, this is not a job. We have a privileged opportunity to ask questions of nature and search for answers. This is our big secret, every scientist at every level of research knows that it's true."

He first came to the Institute in 1985, and became an associate professor in 1996. As head of the Laboratory of In Vivo Neurophysiology, Henriksen has about a dozen or so postdoctoral fellows and technicians who work for him. "It's so important that you have that feeling of opportunity, of being able to explore. We have been able to attract some exceptional people to our lab, some very bright people, and I'm basically responsible for their performance. It's been uniformly terrific."

Henriksen's projects carry forward his efforts in deciphering the workings of the brain as a holistic system, and that cut across some of the nation's most up-to-the-minute problems: drug abuse, HIV infection, neurological diseases and sleep disorders.

"People who are exposed to HIV or who are long-term methamphetamine addicts are inclined to exhibit some similar cognitive abnormalities," he says. "The consequences of HIV infection and chronic drug usage both appear, in part, to affect people in a specific part of the brain, the neostriatum, the middle part of the brain that deals with complicated motor and motivational actions. I'm directing a large project at TSRI, involving six or seven other laboratories, searching for the potential synergistic pathological actions of these two diseases. Our hope is to find a final common path leading to the cognitive decline in these types of brain disorders."

AROUSAL AND SELECTIVE HUMAN ATTENTION

Interestingly, Henriksen believes that many of these disorders take place in the context of specific brain mechanisms that underlie arousal and selective human attention. He has identified a type of brain circuit that he believes is clearly involved in the human arousal system but with another interesting twist. These neurons don't produce feelings of excitement in the rest of brain; rather, they are long-projecting inhibitory neurons that release the brain from inhibition.

Henriksen became interested in attention and arousal while working as a graduate student at Stanford University in the early 1970s. In returning to some of these studies at TSRI, he and another researcher, Dr. Scott Steffensen, were studying the effect of alcohol on the hippocampus, the part of the brain involved in memory and learning. Originally, they thought that alcohol worked directly on the structure. But they soon found something different, that the most sensitive effects of alcohol were on other structures that projected into the hippocampus. Those cells happen to be GABAergic neurons — neurons that release GABA, a powerful inhibitory neurotransmitter. Henriksen is now investigating how these long-projecting neurons translate their inhibitory signal into behavioral alertness and consciousness.

"My bet is that these neurons directly inhibit local circuit GABA neurons in the forebrain, releasing an excitatory barrage to the rest of the brain," he says. "We're just working out the critical circuits right now, but this could have tremendous consequences for how we treat arousal disorders, insomnia or other serious sleep disorders."

"Sensory events come into the brain and get processed so we can respond to our environment in a meaningful way," he continues. "That event can't be linked to a direct motor response because that would put us in harm's way almost every minute of the day. Our response has to be conditional on the confluence of a number of things — memory, experience, and other sensory input. The inhibitory circuitry is most developed

within that part of the brain where decisions are made. Our response is held in check by this circuit until this confluence of factors releases the brain from inhibition and the appropriate motor activity can take place."

This same process applies to other external or internal stimuli involving motivated behaviors. Responses to environmental challenges don't happen instantaneously or even automatically. They have to be learned. Even such complicated instinctual mammalian behaviors like maternal or nurturing behavior have components that must be learned. But illicit drugs can usurp the delicate processes involved in motivated behaviors. These drugs unnaturally alter basic circuits involved in motivational circuits, and essentially overwhelm them.

UNDERSTANDING SLEEP AND WAKEFULNESS

Henriksen also uses specific abused drugs to probe the "reward" system circuitry. As he sees it, this circuitry is critical for understanding the brain and deciphering neural pathologies and disease. And all this relates to a key system from Henriksen's past, one that he claims has come back to haunt him. His original predoctoral work at Stanford was done under the direction of Dr. William Dement, the father of the modern study of sleep. As Henriksen has come to realize, the point is that these motivational behaviors are all built on the scaffolding of sleep and wakefulness, which is why he got into the study of sleep in the first place.

But it's how the brain processes the information from our environment and that we keep stored in our memories that arouses Henriksen's imagination, especially dreams.

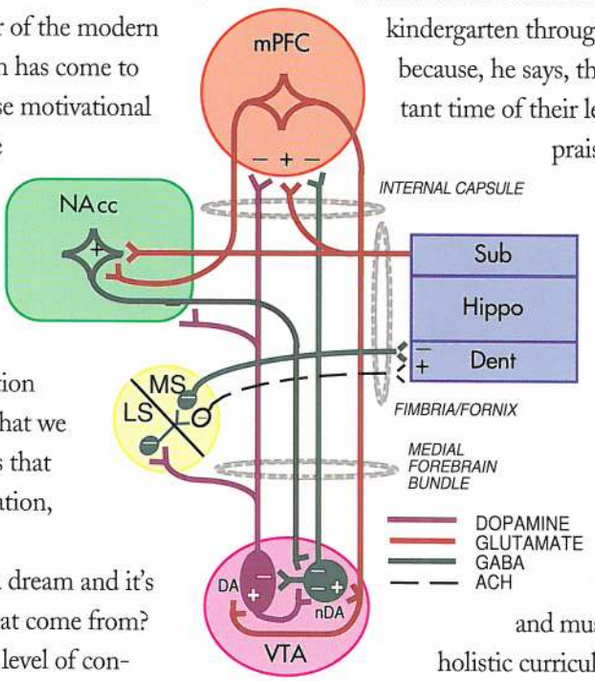
"Every night you have a dream and it's like reality — where does that come from? It is an internally-generated level of consciousness that is as real as everyday reality to some people. At Stanford, we often theorized that about schizophrenics — that their thought disorder might in fact be an abnormal dream emerging into wakefulness. Indeed, some major sleep disorders can be seen as sort of state-boundary problems. The circuitry loses some of its inhibitory capability."

In a very real sense, Henriksen misses the observational power of science, something he calls a lost art. We often forget that there are many examples from nature, creatures living in extreme environments, that can show us a great deal about how our different physiological systems including our brains work.

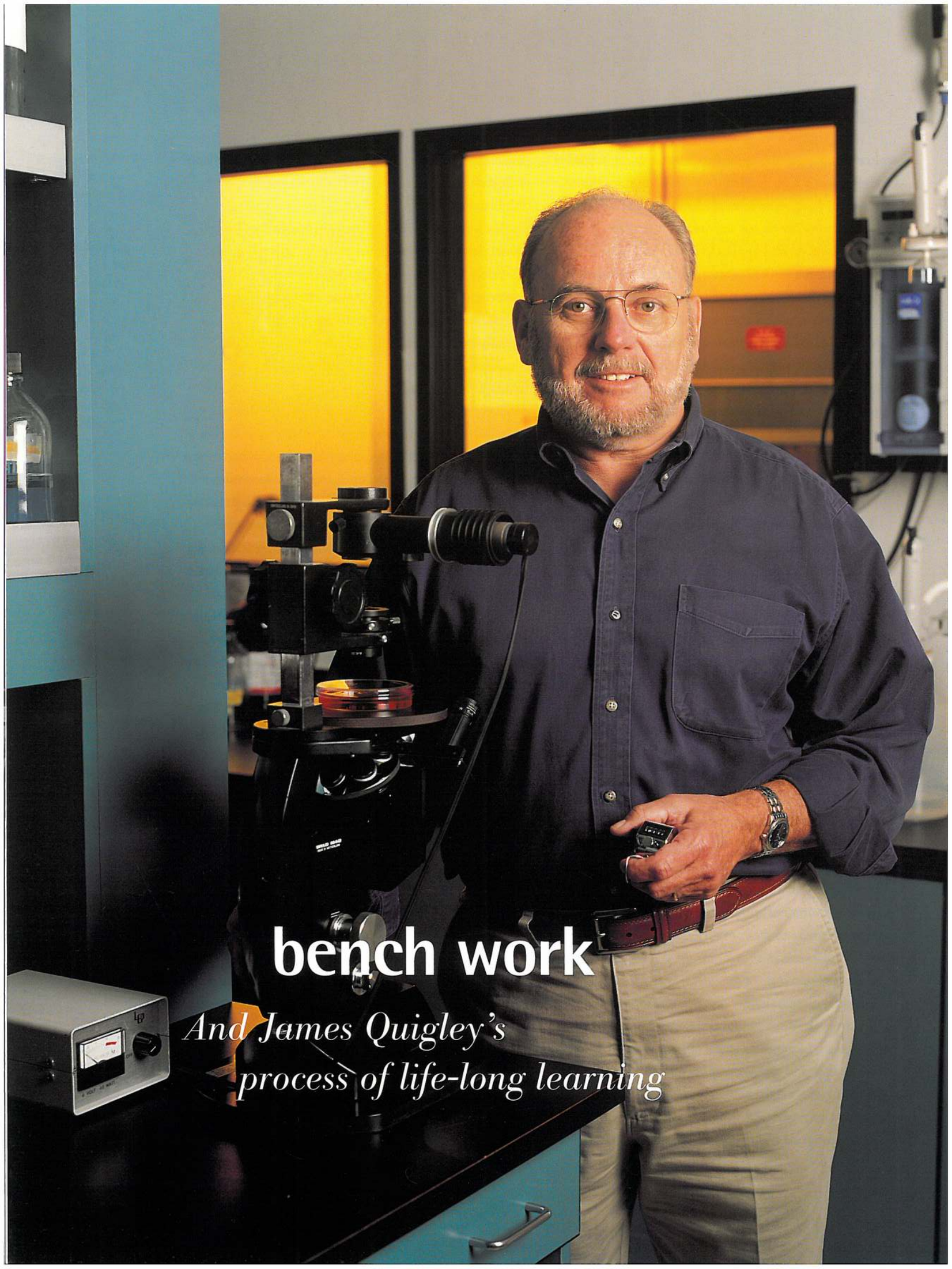
"Biologically, there are some fascinating examples," he says. "Mammals that are poikilothermic — they no longer possess the ability to regulate their own body temperature. Maintaining body temperature is one of the most important things mammals must do, but these animals have lost the need for that in their environment. This makes them the perfect animal to study in terms of temperature regulation and sleep/wake phenomena — because our body temperatures fall during sleep, and during wakefulness cognitive acuity is directly related to body temperature. So again, we're looking at the whole organism."

Henriksen brings the same holistic approach to education. He has served as a trustee of the Solana Beach School Board since 1990. It's a small district, kindergarten through sixth grade. Henriksen likes it because, he says, they have them at the most important time of their learning experience. He gives high praise to teachers. "To be able to recognize special skills in kids or reach special-needs children is the most important job parents and educators do," he says. "My daughter has a minor learning disability, and I feel it is important to recognize and applaud how educators strive to help each child. We believe we must teach the whole child, so we've fought very hard to integrate science and music and physical education into a holistic curriculum."

"You'd be surprised at how many scientists are musicians, or have other artistic drives," he says. "In a way, we're just like one of those little kids, trying to learn in the very same way." ■



Schematic representation of relevant attentional circuits. This diagram illustrates the brain circuitry believed to be involved in both natural and drug-induced craving. It may also be important in alerting the brain and in coordinating the activity of wide-spread brain areas to respond to specific sensory events that predict "reward."



bench work

And James Quigley's process of life-long learning

At 26, James Quigley, Ph.D., became a student of cancer, a subject that has held his interest for more than thirty years.

It sustained him through the Nixon administration's War on Cancer campaign, a time, he remembers, of great promise and unwarranted optimism, when many people thought cancer might be virally induced and could be conquered with a vaccine.

After receiving his doctorate in biochemistry from Johns Hopkins University, James Quigley, a professor in the Department of Vascular Biology, accepted a Leukemia Society fellowship at New York's Rockefeller University. He took the fellowship because it didn't restrict him to study leukemia, but allowed him to investigate whatever aspect of cancer he chose.

"I wanted to work on a basic research problem that had some meaning, something that would justify my existence as a biologist," he says. "Cancer was a prominent disease, and it was the start of an exciting time in cancer research."

Four years later, he became an assistant professor at the Downstate Medical Center in Brooklyn, New York, (SUNY) at the age of 31. In the meantime, he continued doing what he has done all his life — studying. He turned out to be good at it.

"What I learned at Rockefeller is that you can create cellular models for cancer in the lab," he says. "You didn't need to study animals with tumors. In those days, we used tumor viruses to turn normal cells malignant. One, the Rous Sarcoma Virus, caused cancer in embryonic and adult chickens. This was startling, that you could study the process of cancer in a culture dish simply by injecting a virus into cells."

Quigley first worked on defining the structure of the virus, then studied how the virus went about transforming normal cells. As it turned out, the Rous Sarcoma Virus carried an oncogene, bits of genetic material with the potential to cause cancer that gets passed down from one generation to the next. The presence of the oncogene in the genetic material of the Rous virus was a pure accident. Somewhere, the virus had picked up the oncogene from a cell it had once infected, and simply carried it forward from that point on.

"I wanted to work on a basic research problem that had some meaning, something that would justify my existence as a biologist."

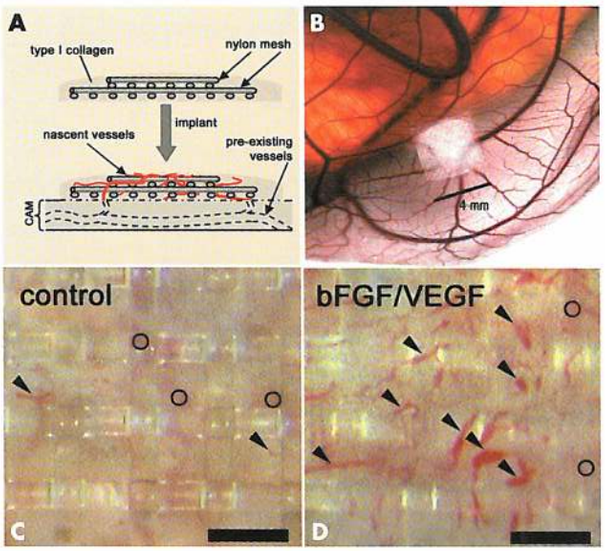
Quigley carried that knowledge with him to the Downstate Medical Center, starting as an assistant professor and leaving eleven years later as a full professor. Teaching oncology and virology complemented his molecular research into malignancy. When he looked at the properties of these newly infected transformed cells, he soon discovered one of their most distinguishing characteristics: they had become very invasive and you could measure their invasive ability in culture.

CELL GROWTH OUT OF CONTROL

"Everyone knows that cancer migrates and invades other cells," he says. "Our cell culture was a recapitulation of what was going on in the body. We also know that cancer cells have basically learned how to circumvent the controls that keep normal cells from staying in their proper place, controls that exist in various stages of biological development. Cancer cells either circumvent suppressor molecules or express positive initiators of the malignant process, either by mutation or genetic expression. Either way, the brakes are off and cell growth accelerates out of control."

The theories that surround this acceleration process argue in favor of a combination of random genetic insults

James Quigley, Ph.D., Professor, Department of Vascular Biology.



Basis for quantifying new blood vessel growth (angiogenesis) involves implanting a gridded nylon mesh surrounded by collagen onto the chorioallantoic membrane of a chick embryo (A and B). When the collagen contains specific growth factors, bFGF and VEGF, enhanced appearance of new blood vessels occur in the upper grid of the nylon mesh (C vs. D), which can be easily quantified.

that alter cells in such a way that certain genes are turned off or certain gene expressions initiated. It's a multi-stage process, advanced over time and involving more than a single gene, Quigley suggests, even in cases like exposure to radiation or a carcinogen. Genetics may also play a strong role in whether or not the mutations occur and how quickly they might be adopted, if at all.

The theory, Quigley argues, has had a dramatic impact on the way cancer is viewed today by the public and by researchers.

"The war on cancer was overly optimistic in thinking a cure was just around the corner," he says. "We rarely talk about cures anymore, but early diagnosis and prevention. Depending on its tissue origin, it's a series of different diseases, and each one may need a different therapeutic approach."

The one possible exception to Quigley's view is angiogenesis, the creation of new blood vessels. Cutting off the supply of new blood vessels that feed tumor growth is for him, other researchers, and more than a few biotechnology investment firms, a reasonable pan-cancer hypothesis, something he'd been looking at for quite a while.

ANGIOGENESIS AND METASTASIS

When Quigley left Brooklyn and moved to Stony Brook on the eastern end of Long Island, his research began to shift toward the vascular biology of tumors. Even though he had never published any research on the subject, he developed a collaboration with two vascular biology labs at TSRI. In 1996, he came out for what he calls a micro sabbatical. In 1999, he moved permanently to TSRI to work in what he sees as the interrelated areas of angiogenesis and metastasis.

"If you wanted to describe my work in a sentence, it would be that I'm trying to determine the identity and the function of extracellular molecules, and cell surface molecules involved in two processes — metastasis and angiogenesis," he says.

Although Quigley believes firmly in the potential of inhibiting angiogenesis, he is realistic about it ever becoming a workable therapy. "I think the concept is pure and simple enough to work," he says. "You go after what the cancer needs to expand, the endothelial cells in the blood vessels, and you stop it. But if blood vessel growth around a tumor is no different from that which occurs in normal inflammation or following a wound, then it won't work. Some researchers were able to inhibit the blood vessels that infiltrated the tumor, while not affecting those in the surrounding area. That's a good sign but the fact is, nobody knows for sure."

One possibility is that the molecules tumor cells produce to attract new blood vessel growth are a modification of the normal pathways of blood vessels. Quigley confesses the whole idea makes him somewhat pessimistic, but he still believes that eventually researchers will find a way to control tumor angiogenesis and maybe something more. By controlling tumor angiogenesis, Quigley theorizes, they may cut off the conduit that carries the metastatic cells into the rest of the body.

The process of learning, working at his knowledge, is important to who he is as a scientist and as a person.

To study the mechanism by which human tumor cells migrate to secondary organs, Quigley and members of his lab use an in vivo model of a developing chick embryo, the same basic model he used at Rockefeller. So far, some of the angiogenic tumor cells they have tested formed highly invasive large tumors, but have yet to produce secondary tumors in other organs of the embryo. The fact that these tumors can attract new

blood vessels, but are unable to use them as conduits doesn't surprise or discourage him.

"The answer is that tumor cells have to have the right environment to spread to a secondary organ and grow," he says. "Metastasis is not an easy process. Tumor cells must escape from the primary tumor, survive in the circulation, and come to rest in a secondary organ. Then they have to grow. Sometimes the reason they don't is that the right growth factors aren't around to make them grow."

In fact, the laboratory's first major paper in the field, recently published in *Blood*, was about growth factor induced angiogenesis. After implanting native collagen onto a chick embryo, they injected angiogenesis growth factors into the collagen, and then studied the effect of new vessel growth on the surrounding area to uncover the active molecules that contributed to the remodeling of the new tissue. Some of the active molecules turned out to be enzymes that break down proteins, the same proteolytic enzymes that Quigley had been studying for years as part of his research into the tumor invasion process. Now his research had turned up the fact that the same enzymes were also involved in the formation of new blood vessels, a gratifying breakthrough.

Cover of a recent issue of *Blood* that illustrates data from Quigley's article. The cover shows that new blood vessels (red stain) vigorously enter tissue composed of normal collagen (WT), but blood vessel density is reduced in tissue composed of mutant (r/r) collagen. The mutant collagen is resistant to certain types of proteolytic enzymes.

In other studies he has tried to trace the passage of human tumor cells from the primary tumor on the surface of the chick embryo to a secondary organ, in this case, the lungs. The migration turned out to be incredibly fast. In less than a week, 4-7 days, he was able to detect tumor cells in the embryo lungs. He used a molecular trick to find these early metastatic tumor cells in the lungs of the embryo.

Human DNA contains unique repeat regions known as alu sequences that can be amplified by the technique of polyerase chain reaction (PCR); chick embryo DNA contains no such alu repeats. By isolating the total DNA from chick embryo lungs and carrying out the alu repeat PCR, Quigley's group is able to detect the presence of small amounts of human DNA. The method is so sensitive that the presence of as few as 50 human tumor cells in the embryo lung will yield a signal, allowing the earliest arriving metastatic tumor cells to be detected and monitored.

GOOD THERAPEUTIC TARGETS

"What I hope to accomplish as a biologist," he says, "is by identifying these very early events, we may stumble upon a good therapeutic target. We normally do not generate organs by sending cells through the blood stream to take up residence somewhere else. It really only happens with cancer cells, and it's one of the most interesting unsolved cancer problems around."

Studying interesting phenomenon is something that James Quigley likes to do. The process of learning, working at his knowledge, is important to who he is as a scientist and as a person. Starting in 1983 and continuing until 1998, Quigley spent every summer at the Marine Biological Laboratory in Woods Hole, Massachusetts studying the defense mechanisms of two primitive invertebrates, the horseshoe crab and the sea urchin. Working with a University of California professor of zoology he had met at Oxford, the two would spend the summer months doing bench experiments, the sort of work he had no time for during the academic year, and something that he missed.

Although he stopped spending his summers in the Woods Hole lab, they have kept a house there, and he and his wife Joan go back for several weeks each year. His friend and colleague still spends summers at the lab, and Quigley is considered an unofficial member of the team. In the early mornings before work, they go fishing to catch that evening's meal. The late afternoons are often spent kayaking the waters around Woods Hole.

"I never found any invertebrate models for tumor angiogenesis, so I could never really justify the summer research on those grounds," he says. "I was in academia, so when the summer came around, I went to Woods Hole to do what I really want — to study things that were new to me in a beautiful location."

And, of course, get back to his bench work. ■

Scientists at TSRI Receive Prestigious Honors and Awards

Gerald F. Joyce, M.D., Ph.D., *Professor in The Skaggs Institute as well as the Departments of Chemistry and Molecular Biology,* has been elected a member of the National Academy of Sciences. He is one of 72 new members and 15 foreign associates named "in recognition of distinguished and continuing achievements to original research. Election to membership in the Academy is considered one of the highest honors that can be conferred on a U.S. scientist. Joyce is the only researcher from San Diego to be elected to membership this year. His research involves the test-tube evolution of nucleic acids and the application of these methods to the development of RNA and DNA molecules with novel functional properties.

Richard A. Lerner, M.D., *TSRI President,* has been named a recipient of an honorary degree from the Technion — Israel Institute of Technology in Haifa, Israel. The conferral ceremony was held in June, 2001, to coincide with the Technion's annual international Board of Governors meeting. Lerner is being recognized for "outstanding pioneering contributions to chemistry and immunology, and their impact on biocatalysis, and, in particular, the groundbreaking demonstration that antibodies can be converted into enzymes, that catalyze chemical reactions, considered impossible to achieve by classical procedures." Additionally, he was honored for "scientific and public leadership and contributions to the cooperation between the United States and the Technion." In collaboration with TSRI Professor Peter Schultz, Lerner conceived of and demonstrated the idea that antibodies could selectively catalyze chemical reactions. While it took enzymes acting on natural biological systems millions of years of evolution to reach their present level of efficiency, antibodies can be produced overnight, for obtaining an almost limitless variety of products with an efficiency that may exceed that of natural enzymes.

K. Barry Sharpless, Ph.D., *The Skaggs Institute for Chemical Biology and W.M. Keck Professor of Chemistry, Department of Chemistry,* is the recipient of two prestigious honors, the 2001 Wolf Prize in Chemistry, and the 2001 Benjamin Franklin Medal from the Franklin Institute in Philadelphia. Sharpless has provided innovative contributions to the development of broadly useful and commercially viable catalytic oxidation chemistry for the selective production of bioactive molecules with the proper right or left "handedness" which allows for the manufacture of safer and more effective drugs and agricultural chemicals. Chirality, or handedness, is the structural characteristic of a molecule that makes it impossible to superimpose it on its mirror image. Proteins, DNA and carbohydrates are all chiral molecules; without the correct handedness, they will not function as the basic molecules of life. Many drugs must also be of correct chirality; in some cases, the wrong handedness can be toxic.

Jeffrey Kelly, Ph.D., *Professor, The Skaggs Institute and Department of Chemistry, Vice President of Academic Affairs and Dean of Graduate Studies,* has won the 2001 American Chemical Society Arthur C. Cope Scholar Award. This award recognizes excellence in the field of organic chemistry. Kelly's research seeks to elucidate the chemistry and biology of peptides and proteins and develop new ways of manipulating these properties through small molecule design. Of particular interest to Kelly are the biophysical causes, neurological effects and methods of prevention of amyloid fiber formation in diseases like Alzheimer's.

Peter G. Schultz, Ph.D., *Professor, The Skaggs Institute and Department of Chemistry,* has received the 2001 Alfred Bader Award in Bioinorganic or Bioorganic Chemistry from the American Chemical Society. He conducts research that aims to combine the tools and principles of chemistry with the molecules and processes of living cells to create molecules with new properties and functions. By studying the structure and function of the resulting molecules, Schultz gains insight into the molecular mechanisms of complex biological and chemical systems. His research, which spans the interface of biology, chemistry, and materials science, includes techniques to study the structures of macromolecular complexes involved in regulation of DNA transcription and fatty acid synthesis.

K.C. Nicolaou, Ph.D., *Chairman of the Department of Chemistry and L.S. Skaggs Professor of Chemical Biology,* was recently honored by three separate international bodies. The Council of the British Royal Society of Chemistry has invited him to the United Kingdom to give a lecture tour of research centers in the upcoming academic year. Nicolaou also has won the 2001 Ernst Schering Prize, sponsored by the German nonprofit research foundation from which its name derives. He will be awarded the prize later this year at the University of Madrid, and will deliver a lecture there and at the University of Berlin in conjunction with the award. And later this year he will travel to Nagoya University in Japan, where he will conduct a one-day seminar and accept The Nagoya Medal of Organic Chemistry. The three organizations recognized Nicolaou's outstanding contributions to chemical synthesis and chemical biology, particularly his work in the total synthesis of complex natural products.

Phillip Dawson, Ph.D., *Assistant Professor, Departments of Cell Biology and Chemistry,* has been selected to receive the Alfred P. Sloan Research Fellowship and its unrestricted two-year grant. His work utilizes the total synthesis of proteins to study hydrogen bonding, enzymatic catalysis and complex protein — protein interactions.

Engineering Bacterial Cells to Encode 'Unnatural' Proteins

Scientists at The Skaggs Institute for Chemical Biology have published two separate papers in a recent issue of the journal *Science* in which they describe two different ways of engineering bacterial cells to encode "unnatural" proteins.

These proteins differ from those produced by other living organisms because they incorporate novel amino acids, the subunit molecules of which proteins are composed.

Both of these methods could provide powerful new mechanisms for studying protein and cellular functions, because they are proof-of-principal that bacterial strains can be made to incorporate novel amino acids into proteins. In addition, they could enable scientists to envision the possibility of engineering completely new proteins.

According to TSRI President Richard A. Lerner, M.D., "One of the holy grails of modern genetics is the extension of the genetic code to increase the ability of proteins to perform new chemical tasks. We at The Skaggs Institute of TSRI are extremely proud to have accomplished this by two separate routes."

"...They could enable scientists to envision the possibility of engineering completely new proteins."

Principal Investigators Peter Schultz, Ph.D., Scripps Family Chair, The Skaggs Institute and Department of Chemistry; and Paul Schimmel, Ph.D., Ernest and Jean Hahn Professor and Chair, The Skaggs Institute, and Departments of Molecular Biology and Chemistry, led the two separate efforts.

The research article, "Enlarging the Amino Acid Set of Escherichia coli by Infiltrating the Valine Coding Pathway," is authored by Volker Döring, Henning D. Mootz, Leslie A. Nangle, Tamara L. Hendrickson, Valérie de Crécy-Lagard, Paul Schimmel, and Philippe Marlière.

The research article, "Expanding the Genetic Code of Escherichia coli," is authored by Lei Wang, Ansgar Brock, Brad Herberich, and Peter G. Schultz.

Encoding proteins from DNA is one of the most fundamental requirements for life, since proteins do much of the microscopic work of the cell and make up a large part of the physical structure of cells and tissue.

When a protein is expressed, an enzyme reads the DNA bases of a gene (A,G,C, and T), and transcribes them into RNA (A, G, C, and U). This so-called "message RNA" is translated by another enzyme, called the ribosome, into a protein, which is a chain of amino acids. For every codon on the mRNA-every three bases-the ribosome attaches another amino acid to the chain.

But even though there are 4x4x4 = 64 different codons (UAG, ACG, UTC, etc.) there are only 20 amino acids that all organisms use to produce proteins. One of the great unanswered questions of evolutionary biology is why there are only 20.

Some of the 64 codons are redundant, with several coding for the same amino acid, and a few of them are nonsense codons — they don't code for anything at all.

Schultz and his colleagues have developed a general method to make the bacterium Escherichia coli incorporate novel amino acids site-specifically. Their approach starts with generating an orthogonal transfer RNA/synthetase pair, which does not interact with other pairs existing in E. coli. Then the orthogonal synthetase was engineered so that it charges the orthogonal tRNA with an unnatural amino acid but not any natural amino acids.

The orthogonal tRNA delivers the attached novel amino acid into proteins in response to a UAG codon inserted at any position of interest.

Using this method, they have incorporated O-methyl-L-tyrosine into proteins with purity higher than 99 percent, which is close to the translation fidelity of natural amino acids.

Schimmel and his colleagues used a more general approach, which broadly incorporates the novel amino acid aminobutyrate into proteins where the amino acid valine should go. To do this, they modified the valine tRNA synthetase enzyme.

Using mutagenesis and screening, they were able to find a valine tRNA synthetase with no proofreading mechanism. In this mechanism, another part of the enzyme checks to see if it accidentally attached an aminobutyrate to a tRNA where it should have attached a valine.

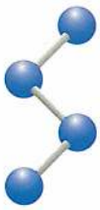
Though aminobutyrate and valine are almost identical, the proofreading mechanism is highly specific, allowing fewer that one mistake in 100,000 tries.

But the proofreading mutants are so good at making mistakes that in their paper, Schimmel and his colleagues report that 24 percent of all the valines are randomly replaced with aminobutyrates.

These strange new proteins can then be purified and studied in isolation, or left in vivo and used as a probe to study cellular functions.

Furthermore, proteins with novel amino acids may prove to have enhanced or emergent properties. Having a bacterial expression system will make them trivial to produce on a massive scale.

The research was funded by The Skaggs Institute for Research, National Institutes of Health, National Foundation for Cancer Research, and the Office of Navy Research. ■



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