

**W**hen Kathryn Rutland, a UK student working toward a master's degree in public health, walked into Don Gash's office in the fall of 2003, he couldn't have guessed that they would soon be launched on a medical investigation that would for the first time convincingly link a common degreasing agent with Parkinson's disease.

"Some months before, I had suggested that as part of her coursework Kathryn do a detailed clinical history of 10 patients who were part of a study here on Parkinson's disease," says Gash, chairman of UK's anatomy and neurobiology department. "Her focus was on possible genetic factors that caused the onset and progression of the disease. But after she talked with the man known then as Patient Number 10, she rushed to my office and convinced me that, instead, we should focus on a possible environmental factor that might cause Parkinson's."

Rutland explained to Gash, an affable man who has been studying Parkinson's disease for nearly 30 years, that this patient had cleaned grease from metal gauges at a Berea, Kentucky, factory for 25 years, dipping the gauges in a vat of trichloroethylene, or TCE, a chemical solvent. It soaked through his cotton gloves, when he wore them, and into his skin. It splashed onto his clothes, and he breathed in its vapors. The man, whose name is Eddie Abney (he later went public with his story) was sure that his exposure to TCE was the cause of his Parkinson's disease and added

that several of his co-workers had also developed Parkinson's—especially those who had been closest to the vat.

"Kathryn really caught fire on this trail of clues, and it helped that she has a detective's mentality," says Gash. He was familiar with previous research on the possible toxicity of TCE and papers published on other toxic substances that might cause Parkinson's, and he directed Rutland to these sources. TCE had been identified as an environmental contaminant in at least 852 of the 1,430 Superfund priority sites listed by the EPA. Superfund is the federal government's program to clean up the nation's uncontrolled hazardous waste sites. There was also a recent assessment by the National Academy of Sciences on the health risks of chronic exposure to TCE and nervous system toxicity which, the EPA admitted, was not well understood by scientists.

"And there were some relevant scientific studies," Gash says, "including a breakthrough study in 1982 that has been referred to as the Case of the Frozen Addicts." He sets the scene. "Hospital emergency rooms in the San Francisco Bay area were suddenly confronted with mysteriously 'frozen' patients—young men and women who, though conscious, could neither move nor speak." Doctors were baffled until neurologist J. William Langston, recognizing the symptoms of advanced Parkinson's disease, administered L-dopa—the only known effective treatment—and 'unfroze' his patient.

# The Mystery of the Toxic Workplace

by Jeff Worley

“Langston determined that his patient and five others had all used the same tainted batch of synthetic heroin, laced with a toxin that destroyed an area of their brains essential to normal movement. The same area, the substantia nigra, slowly deteriorates in Parkinson’s disease.”

A more recent study in Germany was also revelatory. In 2002, researchers at the University of Würzburg found that in rats TCE was converted to a neurotoxin that led to mitochondrial dysfunctions—mitochondria are the cells’ power sources—in substantia nigra dopamine neurons. The team speculated that this TCE conversion could be the linchpin for an environmental TCE-induced Parkinson’s disease.

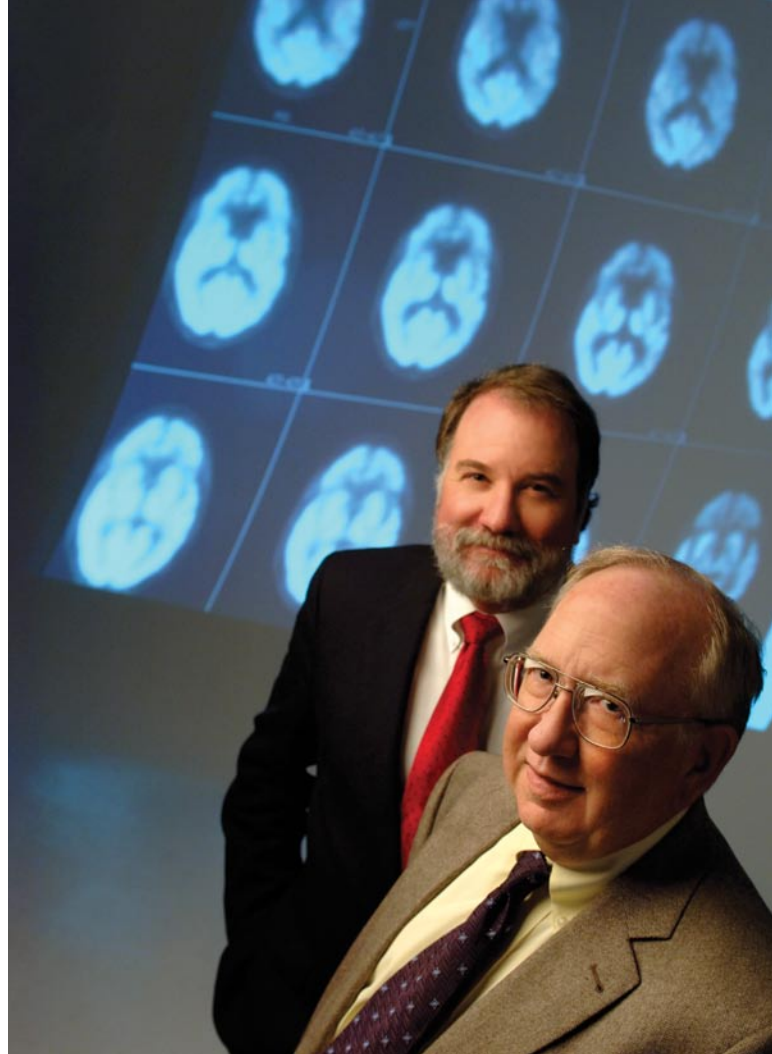
Gash suggested that Rutland talk with former workers at the Berea factory, which is no longer open, to see if there was a TCE-Parkinson’s connection. “We realized that for the first time, researchers could work with a ‘cluster’ of human subjects to see if this connection could be established,” adds Gash.

## Recruiting the Research Team

The first step in this investigation was to secure Institutional Review Board (IRB) approval for the project—any study that involves human subjects or animals must be approved by UK’s IRB. With this approval, Gash, who directed the project, asked his colleague John Slevin, a professor of neurology and molecular and biomedical pharmacology, to join the research team. Slevin, who has worked at UK for 27 years and has headed up several previous Parkinson’s trials at UK, was intrigued by this possible TCE-Parkinson’s connection and eagerly joined in. Scott Prince, an expert in environmental health in UK’s College of Public Health, was brought in, too, to help develop a questionnaire focused on symptoms of Parkinson’s.

“This investigation included a tour de force of UK researchers, working through the Morris K. Udall Parkinson’s Disease Research Center of Excellence,” says Greg Gerhardt, a professor of anatomy and neurobiology, and director of the Udall Center. “The project involved 13 researchers—some of the top people we have here at UK,” he adds. Gerhardt’s role in this study was to help coordinate the project and review critical data.

With IRB approval and the research team in place, Slevin began by examining Eddie Abney, the foreman



Greg Gerhardt (left) and Don Gash in the UK anatomy and neurobiology department have been collaborating for over 14 years to better understand, and fight, Parkinson’s disease. Gash directed the recent project focused on the connection between trichloroethylene (TCE) and Parkinson’s disease.

at the Berea plant, and his two co-workers who were closest to the TCE vat. Slevin found that all three had Parkinson’s disease. The following case studies of these co-workers show the common thread of coming in direct contact with TCE and close proximity to the TCE vat.

Case 1: A 49-year-old man with 25 years’ exposure to TCE, and no family history of the disease. He routinely submerged his arms to the elbows in the TCE vat to clean metal parts. He had had blepharospasm, a twitching of the eyelid, and “tics,” uncontrolled repetitive movement, for 10 years and had probably had Parkinson’s for seven years.

Case 2: A 76-year-old man at the time of his death, with 25 years’ exposure at the plant to the TCE who worked closely with Case 1. He also routinely submerged his arms to the elbows in the vat and suffered tremor, balance and cognitive problems, and



John Slevin, a UK professor of neurology and molecular and biomedical pharmacology, examined 30 former plant employees who had worked with and around TCE. He diagnosed three workers with Parkinson's disease and 24 others with symptoms of the disease. "This is a phenomenally high percentage," he says.

involuntary movement four years before diagnosis of Parkinson's. He had two siblings who also had the disease.

Case 3: A 56-year-old woman with 29 years' exposure to TCE and no family history of Parkinson's. She shared similar work experience with subjects 1 and 2, and sat at a work station adjacent to the TCE vat. She received parts that were often wet with TCE from subjects 1 and 2. She had shown parkinsonian symptoms for five years. Her tongue movements caused "communication difficulties."

The researchers now wanted to gather information about the other co-workers. Prince and Rutland developed a questionnaire which—thanks in large part to a list of names and phone numbers Abney passed along to the researchers—was mailed to 134 former workers. Sixty-five co-workers responded, and of these, 21 reported at least three Parkinson symp-

toms—slowness of movement, stooped posture, trouble with balance, slow walk or dragging feet, rigidity or stiffness, tremor, or decreased facial expression. Twenty-three respondents reported one or two of these signs, and 21 reported no symptoms. Fourteen of the 21 workers who reported three or more signs and 13 co-workers without any symptoms agreed to participate further in the study.

"We were pleased that so many agreed to come in," says Slevin. "Another man, the foreman who worked alongside Abney, would have come in, too, if he had been able. Unfortunately, he was dying—from Parkinson's disease."

Slevin completed physical and neurological exams, and rated the patients according to the Unified Parkinson's Disease Rating Scale, which measures mental ability, physical behaviors and mood. He also evaluated the fine motor speed of each subject.

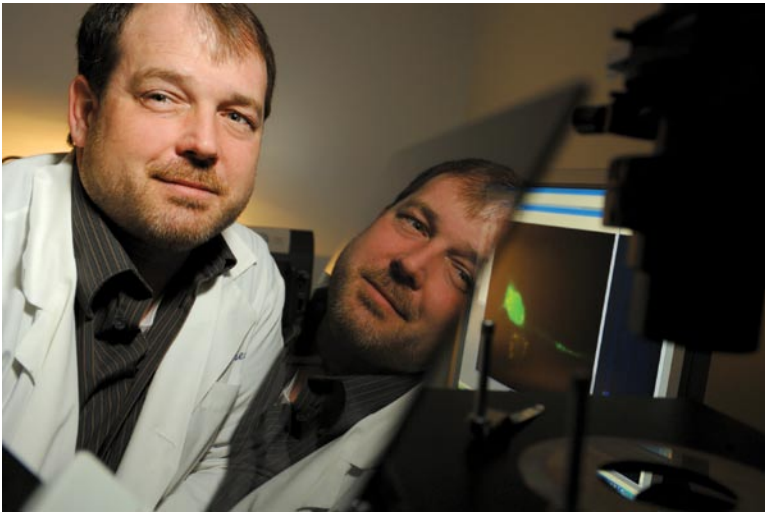
Slevin says that he didn't really expect to find that anyone in this group would have Parkinson's. After all, in the United States only one or two people in a thousand have the disease, with the prevalence increasing after age 60. But Slevin's examinations led to further sad surprises. In addition to the three workers earlier diagnosed with Parkinson's disease, 24 of the other 27 workers had symptoms. The 14 employees who self-reported Parkinson's symptoms performed significantly more slowly than normal controls—age-matched people without signs of the disease—on the test of fine motor speed. Even more startling, the group of 13 who reported themselves asymptomatic also performed significantly more slowly than normal controls, though not as slowly as their 14 co-workers.

"This is a phenomenally high percentage," says Slevin, his voice underlining the word "phenomenally." "Parkinson's disease is certainly more common in older people, but even in the over-65 general population, it affects only one in 100 people."

"Through this 'case-finding study' with this cluster of workers at the Berea plant, we were able to establish for the first time a strong potential link between chronic TCE exposure and parkinsonianism," says Prince.

## The Rat Studies

To complement the human studies and perhaps further establish TCE's effect on the nervous system, the research team used adult male rats. Previous studies had shown that damage to the mitochondria leads to the degeneration of dopamine neurons in Parkinson's disease. How would TCE affect these basic energy producers in the rat brain?



In a study using rats, Patrick Sullivan found that TCE significantly reduces enzyme activity in the substantia nigra, the midbrain lesion site in Parkinson's disease, and that dopamine neurons in this region were also damaged.

This is when Gash brought in another top UK researcher, Patrick Sullivan, who Gash calls the university's "mitochondrial guru."

Sullivan's work is focused on spinal cord and traumatic brain injury, and developing novel therapeutic interventions targeted specifically at mitochondria to treat these injuries.

"Parkinson's is typically thought of as a mitochondrial disease, with what we call Complex I being severely impaired," says Sullivan, an associate professor in anatomy and neurobiology, and associate director of UK's Spinal Cord & Brain Injury Research Center. "Complex I is the first step of the energy chain, the gatekeeper for ATP production. Without a well-functioning Complex I, the cells don't efficiently make ATP, which is what they need to drive the energy train."

The rats were given high doses of TCE five days a week for six weeks. The high doses replicated in weeks what may require years of exposure in humans, Gash explains. The animals were then anesthetized, and Sullivan, with the help of Jignesh Pandya, a research scientist in Sullivan's lab, dissected brain and liver sections to obtain mitochondrial samples.

"The results strongly showed that TCE significantly reduces Complex I enzyme activity in the substantia nigra and that dopamine neurons in this region were damaged," says Sullivan.

"It's important to recognize that this study was not a large-scale epidemiological investigation," Gash adds. "But the results demonstrate a strong potential link between chronic TCE exposure and parkinsonianism."

And now what?

Gash and Gerhardt plan to follow the health of the former plant workers, even those that were asymptomatic, to see if TCE exposure has long-term effects. They also hope that the EPA will take good notice of this study in their periodic review of carcinogens and other toxic substances.

"The most important thing our study did was connect the dots," Gash says. ■

## A UK Center of Excellence

The University of Kentucky Morris K. Udall Parkinson's Disease Center of Excellence, one of 12 Udall Centers in the United States and the only such center in Kentucky, was established in 1999. In 2005 the Udall Center received \$6 million in funding for the next five years from the National Institute on Neurological Disorders and Stroke.

The Parkinson's Disease Research Centers of Excellence program was developed in response to Senate Bill 535, also known as the Udall bill in honor of former Congressman Morris K. Udall, who died in 1998 after a long battle with Parkinson's disease.

"The Udall Center has enabled our research teams to develop a new approach for treating Parkinson's disease, which likely would not have been possible without this support," says center director Greg Gerhardt, who came to UK in 1999. "This new treatment approach is one of the first to try to slow the progression of Parkinson's and possibly restore function to part of the brain affected in the disease."

Working through the Udall Center, nearly two dozen research faculty, scientists and postdocs are looking for ways to repair damage to the brain. Projects include work on a protein called glial cell line-derived neurotrophic factor (GDNF) as a potential therapy for advanced Parkinson's disease, pioneering research on the causal relationship between exposure to TCE and Parkinson's, and illuminating work on Neurturin, a trophic factor that is a close cousin to GDNF, in animal models of Parkinson's.

"Patients and their families in Kentucky have benefited from the increased emphasis on Parkinson's disease research and novel treatments," says Gerhardt.