



# UNCHARTED TERRITORIES

BY ALINE MCKENZIE

Dedicated investigators are making advances against neurodegenerative diseases by collaboratively searching high and low through thousands of compounds.

Like the expeditions of old, scientific research requires several key elements: an unexplored destination, supplies and a skilled crew brimming with curiosity.

The most successful endeavors balance adventure and risk-taking with prudence and careful planning. Without enough food the crew might starve along the way, but too many supplies would bog down progress. And if the adventurers happily terminate their exploration at the very first interesting point, who knows what they might have found had they continued on? 

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WHEN DR. STEVEN MCKNIGHT and his protégé, Dr. Andrew Pieper, set off on their own research expedition at UT Southwestern Medical Center several years ago, they knew they might never arrive at their destination.

They were seeking whether there was a way to enhance the birth or survival of newly formed nerve cells in the adult brain. It was an undertaking that could have profound payoffs. If successful, the research could ultimately lead to new therapeutic strategies for neurodegenerative diseases such as Alzheimer's and Parkinson's.

Dr. McKnight, a member of the National Academy of Sciences, is chairman of biochemistry at UT Southwestern and holds the Distinguished Chair in Basic Biomedical Research and the Sam G. Winstead and F. Andrew Bell Distinguished Chair in Biochemistry. Dr. Pieper is an assistant professor of psychiatry and biochemistry who was part of Dr. McKnight's laboratory team as a postdoctoral researcher before joining the UT Southwestern faculty. Together they focused on an area of the mammalian brain called the hippocampus, which plays an important role in learning and memory. The hippocampus is one of the few areas of the brain that makes new nerve cells throughout life in adult rodents and primates, including humans.

But not all newborn nerve cells survive. In a normal winnowing-out process, many of the new cells die within a month.

"We asked, 'What if we could discover a chemical that could enhance the formation or survival of newborn nerve cells?'" Dr. McKnight said.

#### Old-fashioned methods

Drs. Pieper and McKnight chose an old-fashioned method to search for such a drug. Spurning robotic testing systems and high-throughput technologies, they instead leapfrogged over experiments in isolated cells and went directly to screening 1,000 chemical compounds in living mice – an ordeal that took more than two years and identified a handful of promising compounds.

"This is a retro way of chasing after new drug candidates," Dr. McKnight said. "It's so slow and low-tech that no one does it anymore."

But where should they start their search? No drugs were then known to enhance the birth rate or survival of newborn nerve cells.

Fortuitously, during his time as biochemistry chairman, Dr. McKnight had been laying the groundwork for the department to tackle this sort of problem. The faculty now comprises not only traditional biochemists but also synthetic chemists who specialize in analyzing compounds, finding efficient ways to synthesize them and creating variations that can work more efficiently in biological systems.

"Without access to first-class capabilities in synthetic chemistry, a venture like this would be doomed from the start," Dr. McKnight said.

"It took two and a half years just to complete the initial screen," he said. "More typically, researchers get an idea that takes two, four, five months to get the results."

The pair had two resources vital to any expedition – a team with a wide variety of skills and enough funding to get them where they hoped to go.

In 2004 Dr. McKnight received a Director's Pioneer Award from the National Institutes of Health, becoming one of nine U.S. researchers to receive the award in its first year. The NIH initiative was designed to support exceptionally creative investigators, encouraging them to take on unexplored avenues of research that carry a relatively high potential for failure, but that also possess a greater chance for truly groundbreaking discoveries. The award provided Dr. McKnight with \$500,000 a year for five years.

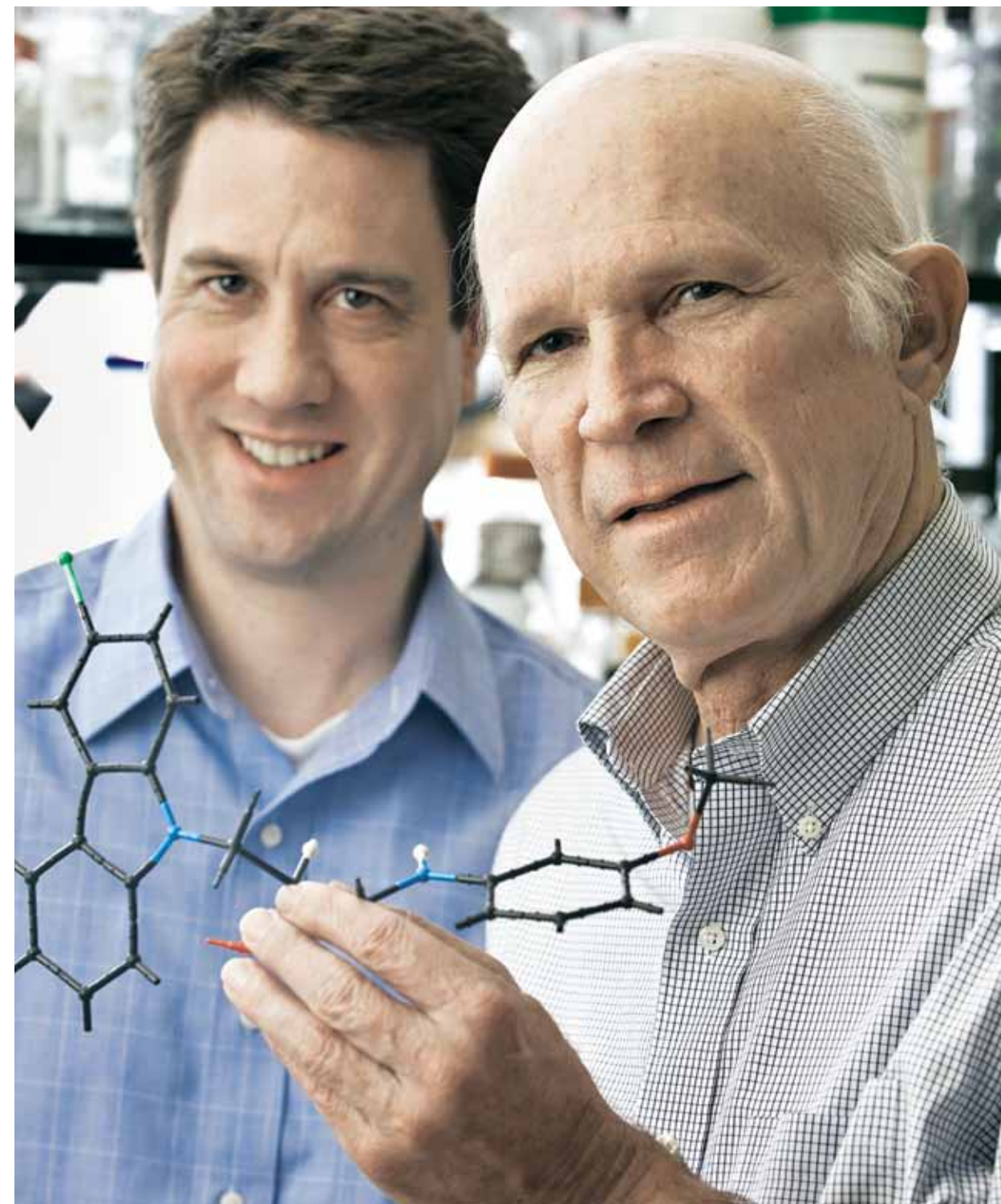
Dr. Pieper has received funding from The Hartwell Foundation, a Memphis-based organization that supports innovative research. And multimillion-dollar gifts from the A.L. Chilton Foundation have supported biochemistry research programs at UT Southwestern for five decades.

#### Teamwork is key

The interdisciplinary collaboration within the biochemistry department was key. "We never could have done this project had we not, over the last 15 years, built a faculty that includes a half-dozen first-rate chemists," Dr. McKnight said. "In fact, this could not have been done in almost any other academic setting at all. It would be like an adventurer who says, 'I want to sail across the ocean,' but who doesn't have a boat."

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Dr. Andrew Pieper (left) and Dr. Steven McKnight hold a model of the P7C3 molecule.



Dr. Joe Ready and colleagues have produced hundreds of versions of the P7C3 molecule to improve its effectiveness.

The vessel that carried forward this project was UT Southwestern's small-molecule library, a chemical ark containing more than 200,000 compounds chosen for their potential drug-like actions. Generally, robotic studies using the library can test hundreds of thousands of chemicals on cultured cells in short order.

To equip its expedition, the research team selected only 1,000 compounds – a balance between what was available and what was doable. They also chose a cache that was chemically diverse and endowed with favorable drug-like qualities.

"The length of time it took to test each compound in living animals is what limited the number of substances we could test," Dr. McKnight said. "It was tedious. We had a library of 200,000 chemicals, but we could only test 1,000."

But this approach had its advantages, Dr. Pieper said. "The thought was that we'd be able to bypass a lot of the pitfalls that you find when screening in high-throughput test-tube assays, such as finding toxicity or side effects when you test compounds later in living animals."

To save time, they pooled the 1,000 substances into batches of 10. If any pooled group kept the newborn nerve cells alive, they then tested each compound individually to see which was the most effective. The mice were given not only the test substances, but also a chemical that would stain newly formed cells.

Dr. Pieper – who did the bulk of the lab work – examined the animals' brain tissue for new cells, which showed up as black dots under a microscope. "We counted every one of those cells," he said.

The first figure in their published study, which appeared in the journal *Cell*, shows a bar graph indicating the rate of cell survival for the 100 batches. A few bars extend higher than the rest, indicating compounds that helped protect the new cells. The most promising compound showed up early in the experiment. They named it P7C3, because it was the third compound in the seventh pool.

In elderly rats, which characteristically show a decline in the birth and formation of hippocampal neurons, the researchers found that P7C3 increased both the birth and survival of new neurons, and improved the memory and learning capabilities of the aged rats.

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They also tested P7C3 in a line of genetically engineered mice that lack a gene that protects new brain cells. In these animals, all newly formed hippocampal cells die. These mice have problems with learning and memory and are often used in research as a model for various forms of neurological disease. Humans who bear similar mutations in this gene also have learning disabilities and suffer from mental illnesses such as bipolar disorder and schizophrenia.

When these mice received P7C3, they formed many more properly shaped brain cells.

Even with this early success under their belts, the researchers soldiered on.

"P7C3 was the 73rd compound we tested," Dr. McKnight said. "It was encouraging that we looked like we had a success. But we wanted to finish screening each and every one of the 1,000 chemicals chosen for study. We found seven other likely ones, although P7C3 remained the best.

"But if we'd stopped at that first success, we'd only have one," he said. "Years from now, it might turn out that one of the seven other chemicals leads us to a much better starting point for drug discovery."

Toward the end of the project, they learned that research teams in Russia and the U.S. were studying an antihistamine called Dimebom as a potential anti-Alzheimer's drug. Remarkably, the chemical structure of Dimebom is similar to that of P7C3, Dr. McKnight said.

But instead of viewing this as a setback, "we were very excited because while Dimebom was in Phase 3 clinical trials, no one knew how it works in the brain," Dr. Pieper said.

Moreover, head-to-head studies of Dimebom and P7C3 at UT Southwestern have shown that P7C3 is far more potent in fostering neuron formation.

"The idea that Dimebom promotes new nerve growth in the brain was totally unanticipated," Dr. McKnight said.

On the heels of their success, the researchers already are peering over the horizon to the next set of challenges. For example, will

P7C3 have any effect on Lou Gehrig's disease, spinocerebellar atrophy or Parkinson's disease?

"All these diseases entail the death of existing cells," Dr. McKnight said. "While we know that P7C3 can block the death of newborn nerve cells, we don't know whether it might be capable of blocking the death of mature brain cells, as happens in so many forms of neurodegenerative disease."

Animal models exist for these neurodegenerative diseases, so the researchers are testing whether P7C3 eases symptoms. They also are looking at whether it can help restore brain function after traumatic brain injury.

Dr. Joe Ready, associate professor of biochemistry, led a team that has made hundreds of different versions of P7C3 to improve its effectiveness. "We want to improve the physical characteristics, such as solubility, and make some changes that might help us figure out how it works in the cell," he said.

### The joy of science

Despite the riskiness and tedium of the work, the scientists say it has been satisfying.

"It's been a wonderful experience," Dr. Pieper said. "At first there was a lot of doubt, because we could have gone through this whole screen and not found anything. Now I couldn't be happier. It's exactly the kind of thing I want to do with my career – something that might be translatable into helping people."

Dr. Ready said the project was, above all, fun. "It was such an unconventional approach to finding a molecule," he said. "It's a detective story."

Since the publication of the study, the skepticism of other researchers during the early stages has been replaced by optimism, Dr. McKnight said.

"It's why I get up in the morning," Dr. McKnight said. "Science is a wonderful occupation. It is – quite literally – the final frontier of adventure. Although it's too early to tell if we've made a fundamental discovery that might really help people, the fun of the hunt is more than enough to overcome our recognition that the road ahead is filled with obstacles." ✦