

How does cocaine give a rush? Susan Amara pretty much answered this question at the molecular level. Her lab's work has implications for drugs of abuse, neurodegenerative diseases, and the molecular basics of how we think and feel. Amara, a Howard Hughes investigator, joins Pitt's Department of Neurobiology as chair this academic year.

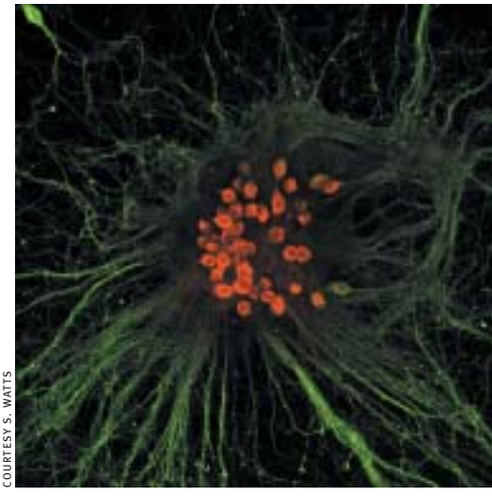
COCAINE HIJACKERS AND OTHER ADVENTURES IN NEUROSCIENCE | BY ROBIN MEJIA

ADDICTIVE RESEARCH

When journalist Jane Stevens visited the Bolivian Andes, she expected to fight the light-headedness and headaches that visitors get at such high altitudes. She didn't expect what the hotel staff had left in her room. Next to her bed, she found the traditional remedy for altitude sickness, coca tea.

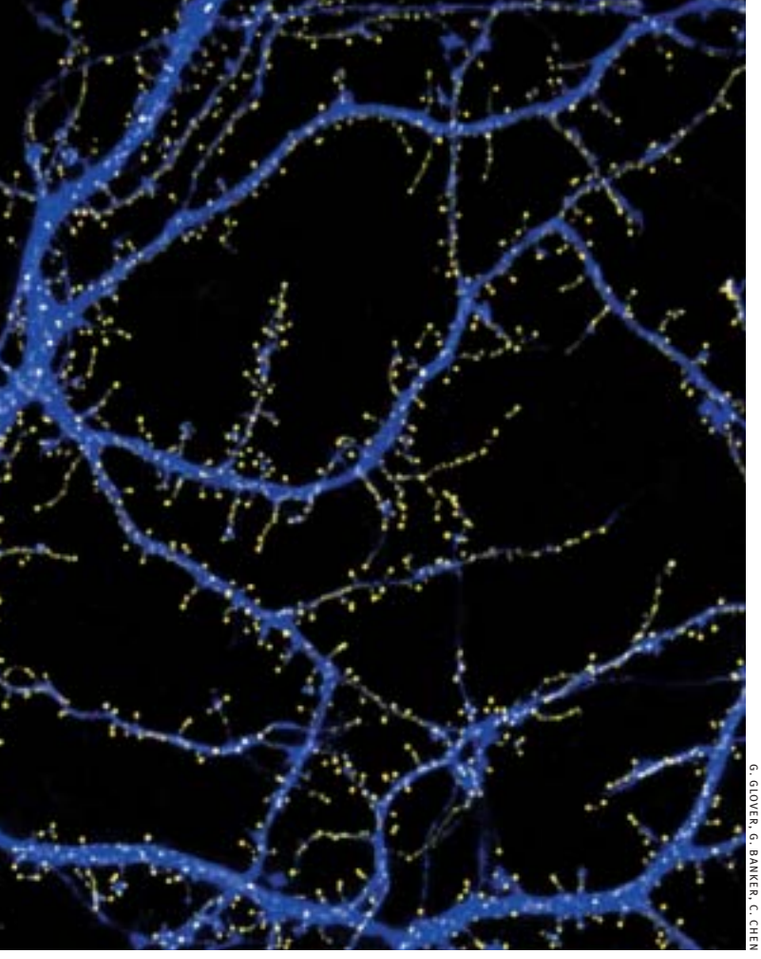
A common drink in Bolivia, the tea is made from dried leaves of the traditional coca plant. Cocaine is purified from a more potent strain of coca, one bred to increase its drug content. Coca tea helps visitors cope with thin air. The small amount of the drug in the tea helps the body get more oxygen. Native laborers chew coca leaves while they work. The tea worked so well for Stevens that when she ran into an American man who was clearly battling altitude sickness, she suggested he try it. He told her he couldn't—he was a DEA agent.

PORTRAIT | LÁSZLÓ BENCZE



COURTESY S. WATTS

Dopamine transporters (red) do more than anyone thought. They recently surprised Amara.



G. GLOVER, G. BANKER, C. CHEN

Glutamate transporters, which glow here on stained neuron dendrites, are key to a healthy brain.

The man told her that employees of the Drug Enforcement Administration are prohibited from drinking the mild traditional tea. Yet, cocaine itself was initially welcomed as a cure-all when it arrived in the United States. The stimulant was believed to improve brain function and cure impotence. Like opium, cocaine was a key ingredient in many over-the-counter medicines until the early 1900s. Sigmund Freud is one of the more famous early proponents of the drug. Thomas Edison also believed in cocaine's miraculous effects. France exported Vin Mariani, or coca wine, to these shores, and in 1886, John Pemberton introduced Coca Cola, named after the drug that gave the original formula its kick.

As cocaine use climbed in the late 1800s and early 1900s, the more negative effects of the drug started to mar its reputation. Doctors noticed the loss of appetite and inability to sleep that came with prolonged use, what we now recognize as symptoms of addiction. In 1914, cocaine sales were banned in the United States under the Harrison Act.

Still, no one knew exactly what cocaine did in the body. And it remained that way through much of the last century. Advances in pharmacological research helped scientists figure out what parts of the brain were excited when rats were dosed with the drug, but through the 1980s, no one knew exactly what it was that the cocaine molecules did to cause those effects.

Susan Amara, a Howard Hughes Medical Institute researcher, was intrigued by the question of how cocaine molecules created the myriad of effects we associate with the drug. Amara joins the University of Pittsburgh this academic year to chair the School of Medicine's Department of Neurobiology. She has built her career by applying molecular techniques to neurons so as to understand how nerve cells work and how they handle chemical signals.

Amara's research focuses on molecules called neurotransmitter transporters, critically important proteins that regulate chemical activity in the brain. These large molecules straddle a neuron's cell membrane, for the most part, scavenging compounds from the extracellular spaces back into the cell. They play crucial roles in maintaining a healthy environment for the brain and nervous system by regulating chemical levels in the fluid between cells.

Neurotransmitters, the brain's chemical messengers, allow nerve cells to communicate with one another. To send a message, a cell releases a batch of neurotransmitters. These molecules travel to the next cell, where they stimulate sensitive receptors. Some neurotransmitters are familiar to us, like serotonin and dopamine, which help modulate our moods. Antidepressants like Prozac are called "serotonin-specific re-uptake inhibitors" because they affect serotonin levels in the brain. Dopamine helps us experience pleasure—it makes necessary activities rewarding. Eating, for example, stimulates the dopamine system.

When they're working correctly, transporters ensure communications between neurons go smoothly by taking up any extra neurotransmitter molecules left floating around between cells. This way, neurotransmitters aren't wasted, and cells have more chemicals in their stash when they need to send another message. Likewise, extra neurotransmitters aren't left wandering around in the brain, accidentally hitting receptors and sending messages that weren't intended.

Some therapeutic drugs target transporters. Prozac slows a transporter's ability to take up serotonin, leaving more serotonin to hit the nerve cell's receptors. Many of what scientists call drugs of abuse, including cocaine, act on the dopamine system in the brain, essentially hijacking our natural reward system.

Thanks to Amara, scientists are learning more about what cocaine does in the brain. The cocaine molecule finds its way to the dopamine transporter and latches on. Then, instead of getting carried into the cell, like dopamine does, it sits on the outside, essentially plugging the system. Dopamine then builds up in the brain fluid and continues to stimulate neuron receptors, creating a rush on the brain's pleasure system.

Recently, Amara cloned a newly discovered cocaine-sensitive transporter in fruit flies, which she thinks may be an evolutionary ancestor of the dopamine and norepinephrine transporters in humans. (Norepinephrine is a neurotransmitter that physically arouses the body; when we face stress, it's released with adrenaline.) This discovery gives researchers a new animal model for behavioral studies of addiction. Scientists have already shown that flies react to cocaine in much the same way humans and rats do—they get hyper. But the fruit fly genome is better understood than that of most other animals, so it should be easier for scientists to alter the flies, for example, creating mutants that are more or less sensitive to the drug.

The National Institute on Drug Abuse gave Amara a MERIT Award for her work studying cocaine action. And in some ways, the cocaine research exemplifies the reasons Amara got into pharmacology. Though she does basic research—she describes herself as "very reductionist"—she says she tries to work on "questions that really have a lot of benefits." Perhaps that's why her research has resulted in 20 patents.

That practical aspect of pharmacology appealed to her from the very first time she was exposed to the field, on a high school field trip to a southern California pharmaceutical company. As a teenager, Amara was already proficient at concocting projects in her family's garden, where she dissected bugs and made plant extracts. (She'd asked if she could pull up the carpet in her bedroom so she could work inside, but her mother nixed the idea.) The field trip showed her that adults could make a living doing experiments full-time. But even more important, she saw that those experiments could actually help solve important problems. The scientists she met were involved in cancer research.

In 1987, Randy Blakely, a neuroscientist at Vanderbilt University, did his postdoctoral fellowship under Amara at Yale, where the two shared a 250-square-foot lab with four graduate students and a technician. They were on the all-too-common junior faculty/postdoc 8 a.m.-to-9 p.m. schedule. The arrangement could have been a disaster if tempers had flared, but Blakely says the interactions he had with his mentor were inspiring; her "creative spirit" was contagious.

Blakely had come to the lab to study a poorly understood receptor Amara had identified during her thesis work. After he'd been there a few months, another group showed that the frog eggs they were using in their project could be used to express a sugar transporter normally found in the gut. At that point, no one had yet cloned a neurotransmitter transporter from the brain. Blakely injected brain RNA into the frog eggs, to see what would happen. It looked like the frog eggs were synthesizing the transporters for all the major central nervous system neurotransmitters.

"We both realized it was a phenomenal opportunity," Blakely says. Amara agreed to put the existing project on hold to see if they could clone the norepinephrine transporter.

The diversion amounted to "a scientific flier experiment," says Blakely. They took a chance by heading off in a completely new direction. It was the kind of problem that appealed to someone like Amara, says Blakely, "a liberal thinker and a mentor who is willing to take chances on a young postdoc."

"She has total scientific taste," says Michael Geoff Rosenfeld, who was Amara's graduate adviser at the University of California, San Diego.

At the time Amara was in his lab, Rosenfeld and his wife were going to have a daughter. One morning, he came into the lab to find Amara looking cheerful. "Geoff, come over," he remembers her saying. She'd named all the frogs they were using for her thesis research. Rosenfeld walked over to the cage: there were all the name plaques, exactly the names he'd been thinking about for his girl. He quickly realized she'd stolen the list he'd been doodling on at his desk the day before.

When Rosenfeld lists the qualities that have helped Amara rise so quickly in the field, her intellect tops the list, but he also thinks her wit and personal style have been an asset. "She's so refreshingly unjaded," he notes. Throughout her career Amara has been known for bringing a fresh perspective to scientific questions.

By using molecular biology techniques to study brain function, Amara has been at the forefront of a transformation in the study of brain transporters. Through the 1980s, neuroscientists typically administered drugs and then measured their visible effects on the molecules. Amara's approach has been to isolate individual transporter systems and study their workings in minute detail.

"Her work on transporters has given us a series of clues [about] the way the brain is regulated moment to moment, minute to minute, and day to day," says Henry Lester, the Bren Professor of Biology at the California Institute of Technology in Pasadena. Lester explains that Amara's research has helped scientists study not only the structure of transporters, but how they work and how the success or failure of their functioning affects how we think and feel.

"We have to have ways of funding research that aren't tied to already knowing the outcome of the experiment and just taking it to the next step."

Amara discovered her interest in transporters soon after she finished her PhD. Her early successes in Rosenfeld's lab had already made her stand out, and within a year of graduation, she got her first faculty appointment, as a researcher at Yale. The school gave her three years to find projects in the area she wanted to pursue—linking microbiology to neuroscience.

Around the same time, the Howard Hughes Medical Institute knighted her as one of its assistant investigators, which gave her incredibly flexible research funding. Most funding agencies require very specific research proposals; HHMI is unique in that the organization picks people and lets them choose their projects. Yale and HHMI's faith in Amara paid off—she, Blakely, and graduate student Tad Pacholczyk promptly cloned the norepinephrine transporter, a first in the field.

"It was the sort of thing NIH would have laughed at" if she'd asked them to fund the endeavor, she says. But they succeeded, in part because of the fresh perspective they brought to the research, she believes.

"We've benefited greatly by looking at things in a different way. … When you're not biased by preconceived notions, you notice things," she says. "We have to have ways of funding research that aren't tied to already knowing the outcome of the experiment and just taking it to the next step."

At the same time she and Blakely were working on the norepinephrine transporter, a different team cloned another transporter. Both groups published papers within six months of each other.

"It suggested that because those two proteins resembled each other, they probably were from the same family," says Marc Caron, the James B. Duke Professor of Cell Biology at Duke University. A flurry of activity ensued, and within a few years, scientists had described almost two dozen brain transporters.

In the 1780s, Luigi Galvani of Bologna made dead frog legs twitch with static electricity. His followers called this phenomenon "animal electricity."

Since Galvani's day, a lot has changed in our understanding of electrical currents in animals. Amara's work has underscored that we've more to learn.

Her breakthrough came by studying glutamate, the most common neurotransmitter in the body. Glutamate is essential for survival, yet at high levels, it's a potent neurotoxin. It can excite most neurons in the brain and central nervous system, making it both critically important and potentially dangerous. Malfunctions in the glutamate system have been linked to degenerative diseases. (In fact, an Amara collaboration with Brazilian scientist Andreia Fontana has revealed that the venom of a spider, *Parawixia bistriata,* could be a key ingredient to cooking up a drug to reduce glutamate levels in the brain. They believe such a drug has promise for preventing brain damage, for example, after a stroke.)

Perhaps because glutamate is so important and ubiquitous, nerve cells have developed a large family of transporters—many of which Amara's lab cloned—to modulate its levels.

A few years ago, Amara showed that glutamate transporters do something no one realized: They act as gated ion channels, transmitting electrical currents under certain conditions. In fact, some transporters transmit electricity better than they transport glutamate. Last fall she demonstrated that dopamine transporters do the same thing. She found that low levels of dopamine make nerve cells fire more quickly.

"The importance of this is not yet fully realized," says Duke's Caron.

Blakely, who now has an endowed chair at Vanderbilt, is not surprised that Amara remains in the vanguard of neurobiology.

"She's like a rabbit's foot," he says. "You just want to stay close to her." ■