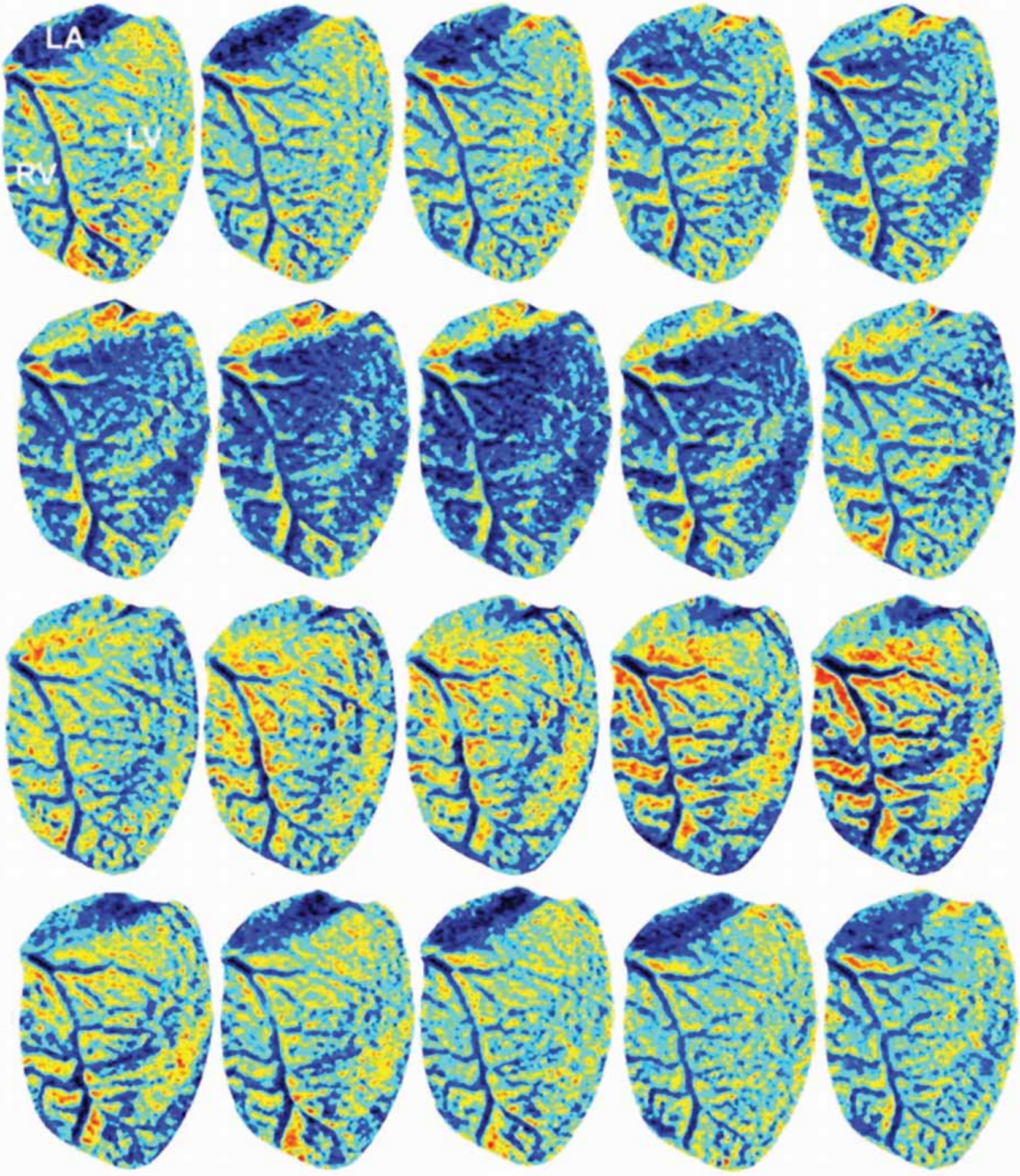

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A PEEK AT HOW AN ARRHYTHMIA IS WIRED

BY CHUCK STARESINIC

TO LIVE AND DYE

It's easy to picture the human heart as a pump. Children readily grasp this concept when introduced to it in school. Most will never forget this basic fact of anatomy and physiology, though they might never learn what the liver does.

But the purpose and mechanics of this pump were not always so obvious. Galen theorized in the second century that venous blood originated in the liver and arterial blood in the heart. He suggested that blood flowed from those organs to all of the body's parts, where it was consumed. This view prevailed until the early 1600s, around the time when an English physician named William Harvey met a man who, as a child, fell from his horse and wounded his chest. Though he recovered, the wound never closed properly. Harvey inquired about the old injury, and the man promptly removed a plate affixed to his chest, revealing what Harvey described as "a vast hole." The physician reached in. He later wrote that he "perceived a certain fleshy part sticking out, which was driven in and out by a reciprocal motion, whereupon I gently handled it in my hand."

Harvey had been told it was the young man's lung that could be observed moving within his chest. The doctor was skeptical, however, so he placed one hand on the man's wrist to measure his pulse as he

Few scientists really understand the electrical system that keeps our hearts ticking reliably. But Guy Salama probes why fatal arrhythmias happen by mapping voltage changes in the heart. LEFT: This series shows a single cardiac cycle of a beating mouse heart. A genetically coded protein reveals changing concentrations of positively charged calcium ions. (Red shows the highest concentrations; dark blue the lowest.) Mapping calcium ions is a way of tracking the changing polarity of heart cells that occurs with each beat.

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reached inside his chest with the other. He noted the synchrony. He carefully watched the man inhale and exhale and measured his breaths against the pulsing of the flesh he held in his hand. He concluded that he was holding the man's heart through a thin layer of skin that had grown within the wound.

Harvey is often remembered as the discoverer of blood circulation. (The Syrian physician Ibn Al Nafis described the circulatory system 400 years earlier, but his text was not rediscovered until the 20th century.) Humanity has now had several hundred years to get used to the rather mechanistic but accurate notion of the heart as a hollow, blood-filled pump in a circular plumbing system.

The electrophysiology of the heart is a different story. The notion of the heart as an electrical organ is newer and less intuitive. It also suggests that your heart has some affinity with your toaster, and that's not really the case.

In 1856, two German researchers dissecting a frog accidentally let the sciatic nerve fall across the frog's heart, which was beating. The result was similar to what you might get if your electrician inadvertently crossed two live wires—say, for the porch light and the doorbell. Ring the doorbell and the porch light turns on. In this case, the frog's leg muscles contracted with each beat of the heart, demonstrating that a pulsing signal controlled the beating of the heart and that nerves conducted such signals.

We've learned since that power surges and other disruptions of the electrical signals in the heart can mean trouble. Yet few understand this vital electrical system in real detail.

Guy Salama, who was born in Egypt and moved to France when he was 12, came to the field of biophysics with a chip on his shoulder. This was in the 1970s at the University of Pennsylvania, where he was working on a PhD in physics. "I was a solid-state physicist working on superconductivity," he says, as a way of explaining why he was so cocksure that physics was real science and anything involving living organisms was a bit, well, fluffy.

"It's not even science," Salama says, jokingly, three decades into a productive career as a biophysicist, the past 15 years in the University of Pittsburgh's Department of Cell Biology and Physiology, where he is a professor. "Needless to say, I can't even figure out the other science now."

Salama's onetime mentor in Penn's physics department, Alan Heeger, would go on to win the Nobel Prize in Chemistry. Salama, however, switched mentors and switched fields as a student, bringing a physicist's mindset to the study of the beating heart. Back then, studying the electrophysiology of the human heart was mostly limited to tools like the electrocardiogram or echocardiogram, which record the voltage changes of large regions of heart tissue as they occur with each cycle of muscle contraction and recovery. What if it were possible to record voltage at the cellular level? Could we learn how electrical signals travel through the entire heart—billions of cells that contract in concert billions of times without fail?

And why would it matter? Revealing this basic electrophysiology of the heart will inform scientists about deadly forms of arrhythmia—when that steady heartbeat inexplicably loses its way and never recovers.

Scientists like Salama's second PhD mentor, Britton Chance, were interested in measuring voltage changes in outrageously small spaces, such as across the mitochondrial membrane within a living cell. It was possible in the '70s, says Salama, to take an electrode with a half-micron tip and impale a single neuronal cell. But that was a struggle, and how would one monitor thousands of cells to learn about the electrical activity of the entire heart? Biophysicists began to dream of other means.

"Could one imagine having a molecule that would report voltage without impaling the cell?" Salama asks rhetorically. "That was essentially the gold ring."

Making a molecule that reports voltage—now that's science, and it's enough to make even a physicist's heart beat faster.

Salama started with dyes from the photographic industry that changed colors in different environments. In the lab, Salama photographed their changing fluorescence in the beating heart. In 1976, while Salama was still a graduate student, *Science* published his report on the most promising of these dyes—merocyanine 540. The gold ring.

The electrical signal that causes your heart to beat begins in the upper right chamber with a cluster of pacemaker cells. They produce electricity by rapidly changing their electrical charge from negative to positive and back again. The signal spreads like a wave through the cells of both upper chambers, causing them to contract and relax. The signal reaches the lower chambers after a slight delay, which causes them to contract immediately after the upper chambers do. This is your normal heartbeat.

Your heart cells change their charge, or reverse their polarity, by alternately taking in and expelling charged atoms called ions. Positively charged ions are missing electrons, and negatively charged ions have extra electrons. Heart cells are swimming in potassium ions, sodium ions, calcium ions, and other ions. These atoms surge in and out of your cells with each beat. They enter and exit through pores called ion channels—large proteins that straddle cell membranes like countless little automated security checkpoints on the perimeter of a stadium. Each type of channel permits the passage of only one type of ion. Some channels allow ions to flow across the cell membrane at rates of 10 million per second with error rates of only 1 in 1,000.

This is the cellular traffic that Salama began to spy on with voltage-sensitive dyes in the mid-1970s; it has kept him occupied ever since.

What he has uncovered about the wiring and cell-to-cell conduction system of the heart can fill books. Yet, he says, "Every time I try to elucidate something new and come to an epiphany that I understand how things work, I discover new things that make me realize that I understand so little." So he continues to make better dyes and to devise better ways to use them.

Salama's lab is a small, windowless room in Pitt's Thomas E. Starzl Biomedical Science Tower. During an experiment, researchers switch off the overhead lights. The only illumination comes from a bright green bulb and a nearby computer screen. The intense green glow—from a 100-watt bulb—shines on a transparent plastic box containing clear fluid and a mass of tissue excised from a rabbit. A sort of lid, also transparent, sits askew across the middle of the box, leaving an opening at either end for tubes, catheters, and electrodes. Catheters deliver nutrient-rich fluid to keep the tissue oxygenated and moist. The box holds the rabbit's central nervous system and heart, with the latter pressed firmly against the underside of the lid and beating steadily. It has been infused with the latest version of Salama's voltage-sensitive dye, and it will continue beating for several hours this way.

Two cameras sit permanently mounted above the beating heart, and they are focused on this plane of visible heart tissue pressed against the underside of the transparent lid. The scientists take high-speed photos of the heart.

The changing fluorescence is not visible to the naked eye. However, the optical imaging system designed by Salama, expertly built and improved upon by Pitt's electricians and machinists, cap-

Such a map would be an enormous step forward in learning exactly what goes wrong to create arrhythmias.

tures the images every few milliseconds.

Few places in the world and few people can carry out this sort of work. Most have some connection to Salama.

Pitt has shared the specialized technology and expertise coming out of Salama's lab with other researchers in the field.

"Our machine shop made five or six units," says Salama, and they are in labs throughout the country.

When switched on, the overhead lights reveal electrical components that resemble stereo receivers and amplifiers. These transmit electrical signals through tiny wires clamped to individual nerve bundles that lead to the heart. To start an experiment, a researcher turns the dial and stimulates a nerve. The rabbit's heart rate increases accordingly, just as yours does as you walk up a steep hill or when you became frightened and experience a spike of adrenaline. When the researcher cuts the electrical stimulation, the heart rate slowly returns to baseline. Salama's cameras capture the entire event in the changing concentration of calcium ions—a strong indicator of voltage—and in the overall voltage changes in very precise local environments. The voltage maps and stop-action movies he creates with this information show the electrical signal moving across the heart as a wave.

Using this technology, Salama has demonstrated how a myocardial infarction—a heart attack that results in a mass of dead tissue in the heart—creates a barrier to this wave. When it encounters the barrier, the wave can split into two, potentially setting off an arrhythmia. The heart might survive these extra beats—there's a period between beats when waves seem to die off rather than propagate. Or the irregular rhythm could set off a chain reaction of continuing uncontrolled and uncoordinated beats. This is fibrillation, the sight of which has been described as a heart writhing like a can of worms.

"Guy sort of sits astride two different disciplines," says Michael Kotlikoff, professor and chair of biomedical sciences at Cornell University's College of Veterinary Medicine.

"He's very unusual in that he's a physicist and a biomedical engineer on one side and a biologist on the other … a superb cardiac physiologist and also a superb optical physicist."

Kotlikoff and Salama are collaborating on another novel means of imaging a beating heart in the lab. Kotlikoff's lab genetically engineered a mouse to express a protein that fluoresces in the presence of calcium. With Salama's imaging expertise, the researchers are able to observe the movement of calcium ions in the heart of a living animal—ions that change the polarity of heart cells as they move in an out of the cell.

As the combined work of these friends of Salama advances, cardiologists may one day be able to identify more precisely who is at risk for experiencing a fatal arrhythmia, know why they are at risk today more than they were last week, and take steps to reduce that risk.

Salama also intends to find out the difference between an arrhythmia that your heart can handle and one that is life threatening.

Many heart attacks are mechanical malfunctions of the pump that result from blocked vessels and necrotic tissue. Salama and his colleagues have, through calcium mapping, identified purely electrical malfunctions of the heart that lead to arrhythmias.

These actually happen all the time, and the heart usually has no problem with them.

Yet, sometimes, they become a big problem, perhaps because of genetic mutations in the ion channels. A fraction of the people who take various drugs that affect the behavior of the ion channels can also experience problems. Essentially, these hearts develop a prolonged interval during which the cells become depolarized—that is, they accumulate a positive charge at a time when the cell usually has a negative charge. The heart then acts like an overheated circuit, because it has more electrical juice than it can handle. The cells overload and spawn spontaneous extra beats. Drugs that may contribute to this include methadone and certain antibiotics and antiarrhythmics. But the good news is that Salama's discoveries may point the way to lessening the risk of arrhythmia in people who take such medications or who have a genetic predisposition to these electrical disasters.

Back when Salama was a grad student querying the photographic industry for voltage-sensitive dyes, others were exploring similar questions. One was Alan Waggoner, a young chemist at Yale University. He was part of a group making dyes by cooking, combining, and distilling chemical compounds to fashion custom molecules. Today, he's a molecular craftsman extraordinaire in his own right, known for cooking up dyes that can make the essential and minuscule operations of cells visible to mere mortals. By coincidence, Waggoner and Salama both have been in Pittsburgh for some years now, Waggoner at Carnegie Mellon University's Molecular Biosensor and Imaging Center, which he directs, and Salama at Pitt. For the past four years, it has been possible to find them at opposite ends of a conference table as they collaborate on the next generation of dyes.

Waggoner is the synthesizer—the dyes are cooked and created in his lab. This willowy, laid-back scientist with a white beard might be seen with an unlit, partially chomped cigar lying on a table in front of him. Salama is the implementer—his lab takes the dyes and puts them through their paces in beating hearts. His salt-and-pepper beard is as meticulously trimmed as Waggoner's is shaggy.

In 2006, these two, with colleagues, published a description of seven new dyes—the Pittsburgh Dyes, as they call them—in the *Journal of Membrane Biology*. Salama and Waggoner hope that these dyes will help them achieve a first: 3-D imaging of voltage changes in the heart.

The fluorescence of these dyes is several orders of magnitude greater than those Salama started with long ago. With the right imaging technology—currently being developed and tested in Salama's lab—it should be possible to study voltage changes deep within the heart tissue as well as on its surface.

With this information, says Salama, he would like to construct a 3-D map of the electrophysiology of the heart—a map that would show the electrical and chemical changes that sweep through all layers of the heart with each beat. Such a map would be an enormous step forward in learning exactly what goes wrong to create arrhythmias, Salama believes. He points out that today cardiologists often know when a person is at risk for an arrhythmia.

"What they don't know is why did this person experience a fatal arrhythmia on Wednesday and not Tuesday? What pattern of ion channels must we pay attention to in order to understand the transition between vulnerable and not vulnerable?"

"It's discovery science, too," Waggoner chimes in. "It's like a game. ... Could we make this tool useful? Can we make it useful for studying heart disease? Those are big challenges—that makes it interesting! And there are very high stakes if you're successful." ■