Marta Zlatic is as capable of portraying the Greek heroine Hecuba as she is of pulling back the curtain on the neural circuitry of fruit fly larvae. At both pursuits, she's won wide acclaim.

All the World's a Stage

BY SARAH GOFORTH

PHOTOGRAPHY BY ELI MEIR KAPLAN

It's hard to imagine two settings more dissimilar than the vast rotunda of a Greek theater and a small, windowless microscopy room at HHMI's Janelia Research Campus. But Group Leader Marta Zlatic is equally at home in both spaces. At this moment, her microscope's narrow beam is illuminating a *Drosophila* fruit fly larva on its own stage, the semitransparent creature wriggling in a way barely perceptible to the naked eye. To Zlatic, the larva's movements represent a nuanced performance fine-tuned by millions of years of evolution – and a foundation for understanding the neural basis of behavior.

Fifteen years ago, while she was an undergraduate at the University of Cambridge in England, Zlatic herself was in a spotlight onstage – as Hecuba, the ill-fated queen in the Euripides tragedy, *The Trojan Women*. A student of linguistics and neuroscience, Zlatic knew ancient Greek and had attended the audition for the play out of curiosity. Lacking acting experience, she was assigned a role in the chorus. When the lead actress quit halfway through rehearsals, however, the director, impressed by Zlatic's fluency with the language and text, asked her to step in. Audiences and critics cheered the performance, and Zlatic went on to land a string of roles known for their complexity: Electra, Medea, Lady Macbeth, even Oedipus Rex.

"Anyone who has seen a play should wonder: How can this wonderful system, the brain, produce so many different emotions and behaviors?" Zlatic says today, seated in her sparsely decorated office upstairs from the microscope room in Janelia's east wing. Three empty champagne bottles, one for each major paper published by her lab in its five years of existence, sit next to her computer. The bottles, along with the humble model organism she has chosen as her life's work, make a quiet statement about the patience required of scientists who study such ambitious questions. To go big, you must often start small.

Zlatic leans forward. The human brain, she explains, has 100 *billion* neurons, roughly equivalent to the number of stars in the Milky Way. Fruit flies have only 100,000, and a fruit fly larva just 10,000. Reduce a nervous system to that relative simplicity, and suddenly it becomes possible to study it comprehensively – not only to describe what it looks like (map it visually), but also to determine which cells connect to one another (map its wiring, or "connectome") and to figure out how those networks listen and respond to signals from the outside world (map its behavior). Zlatic's team, with a constellation of collaborators at Janelia and around the world, is building something akin to Google Maps for the larval brain, placing these layers of information on top of each other like satellite images overlaid on roadways. They are now beginning to outline, with neuron-by-neuron precision, the neural circuits that determine how a larva responds to its environment and decides what actions to take.

It is no frivolous exercise. "We can look at this much simpler system, and its smaller range of behaviors, and see that many of the things we learn are the same across species," Zlatic explains. For example, *Drosophila* larvae have the same sensory modalities as most animals – sound, light, odor, touch, and heat. Furthermore, they're able to process this information, remember it, and make decisions based on it. How does a nervous system make behavioral choices based on previous experience and a multitude of sensory inputs? What can understanding this simple system tell us about the human brain and our capacity for language, art, and the pursuit of knowledge?

Zlatic wants to know the answers to those questions. By all accounts, her team is already taking steps toward constructing a model for a brain atlas – one goal of the multimillion-dollar BRAIN (Brain Research through Advancing Innovative Neurotechnologies) Initiative that President Barack Obama announced in 2013.

"We are trying to understand what's happening in the brain during sensing and behaving," says Tihana Jovanic, a postdoctoral researcher in Zlatic's lab. "Gaining a better understanding of how the brain works will help uncover insights into how different neural processes are integrated so an organism can function in an ever-changing environment."

A Renaissance Mind

Zlatic was born in Croatia, the only child of a theoretical physicist and a philosophy major who worked at the Ministry of Culture. Her parents were gregarious and ecumenical in their thinking, and she grew up immersed in lively conversation about art and politics, often in mixed languages. Throughout her youth, the family was as likely to be traveling abroad – her father's research career took the family to Germany, England, and California for months at a time – as to be at home in Zagreb.

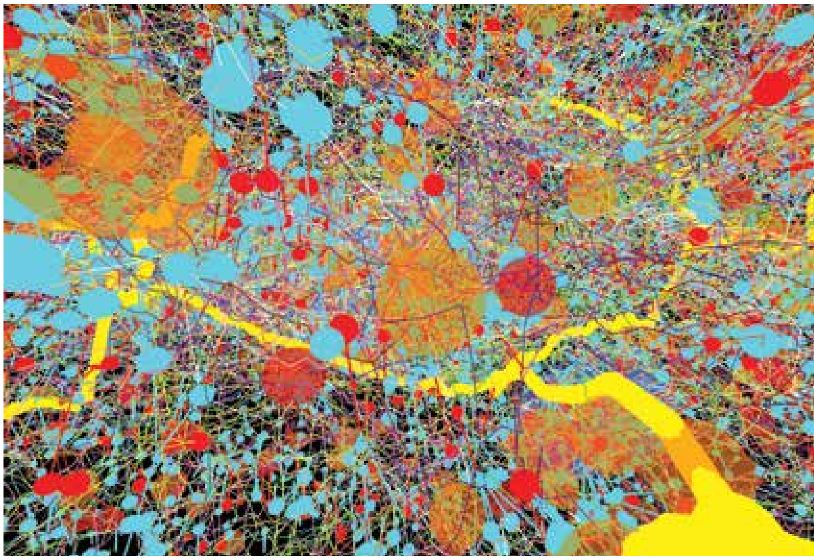
"I grew up with the idea that I wanted to do biology, because I was fascinated by living beings – especially animals and their behavior," Zlatic reflects. "I also loved literature and art. I always saw these things as complementary." She had both the talent and the opportunity to pick up languages, adding English, French, and German to Croatian. By age 12, she was also studying Latin and ancient Greek in school. At age 30, she spoke six languages, in addition to being literate in Latin and ancient Greek.

"I loved grammar and the rules of language," says Zlatic, "but I was also interested in biology because I wanted to understand how the brain was capable of creating and learning these systems." In high school, she held "the naïve view" that she could someday understand in detail how the brain creates language, and she set that as a goal for herself. She studied linguistics and Russian at the University of Zagreb in Croatia before earning a full scholarship to Trinity College at the University of Cambridge, where she pursued neuroscience. Every summer, she would return to Zagreb to continue her language studies.

"To me, it was like a vacation, to return to Zagreb every summer," says Zlatic. "It didn't occur to me that I was doing something unusual."

In her last year as an undergraduate, Zlatic was inspired by the lectures of Cambridge neuroscientist Michael Bate, who was working to understand how neural circuits form in *Drosophila* embryos. She found irresistible the idea that one could watch how a nervous system comes

This digital "map" of a fruit fly larva neural circuit highlights a neuron (yellow) that's able to sense vibrations.



into being, could actually witness neurons finding their partner cells and forming connections, known as synapses. She secured a PhD-track position in Bate's lab to study the earliest steps in that process – her idea being that by studying how the nervous system develops, she would gain insights into its function and, ultimately, into the biological roots of behavior.

Zlatic focused her attention on sensory neurons in the larvae, studying how they form the extensions, called axons and dendrites, that allow them to communicate with other neurons. Once in place, an axon branches at its tip and connects with another neuron's dendrite, forming part of a circuit. Neurons responsible for different senses, such as the detection of heat or touch, send axons to the region of the fly's nervous system specific to that sensation. Zlatic wanted to understand what was guiding their choice of target. Were the axons being guided by positional cues to terminate at a particular location – a specific "address" in the nervous system – and just connecting with whatever cell they found at that location? Or were they seeking a particular partner, irrespective of the address? "The answer is position, because altering axons' ability to sense positional cues resulted in their overshooting the address where their partners are normally found. They did not appear to care where their partners were," she says. "But what we couldn't tell was whether their partners cared about *them*," she adds, because no one had pieced together a neural circuit for the *Drosophila* nerve cord.

Outside Bate's lab, she remained an avid thespian. Onstage, Zlatic was "the absolute star," remembers her friend Julian Huppert, then a fellow PhD student at Cambridge and now a member of the British Parliament. "She was just spellbindingly good."

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—ALBERT CARDONA

She was no less talented in the lab. With Huppert and two other friends, she won funding in an innovation competition to form a biotech start-up aimed at filling a need apparent from her research – for a "virgin collector," a way, that is, to more easily collect virgin female fruit flies. The company developed a synthetic pheromone that, in theory, would prevent the insects from mating. The compound suppressed mating most of the time, but not all the time, and the company failed. Zlatic took the bump in stride, says Huppert. "She was trying to fill a need, to solve a problem, and she knew that what she learned would make her better at solving other problems," he says.

About the time that Zlatic and Huppert were establishing their company, developmental neuroscientist Jim Truman, then at the University of Washington, visited the Bate lab on sabbatical. Truman was studying how stem cells develop into different kinds of neurons in the larval brain – work that would later provide a foundation for Zlatic's brain-behavior mapping. Zlatic, Truman says, stood out: "She was full of pointed questions, tireless in her research and all of her activities. I remember thinking it was exhausting just watching her."

In 2006, Julie Simpson, one of Janelia's first group leaders, invited Zlatic to join her lab as part of the campus's visiting scientist program. Simpson was using the genetic tools developed by Janelia Executive Director Gerry Rubin and others to study the neural and genetic basis of adult fruit fly behavior. Zlatic – who by then was completing a visiting postdoctoral appointment at Columbia University on a prestigious Trinity College fellowship – joined Simpson's group and began piecing together the first larval behavior map. "I came here with the idea of trying to map circuits and find the function and behavior of the neurons I used to study," she says. "I quickly realized this was the place to be for the kinds of questions I was interested in – the relationship between structure and function of the nervous system. At that time, no one else at Janelia was working on the larva, but Julie just let me play with larvae anyway."

A year later, she earned a lab head position at Janelia and started her own group.

Cardona lab

The label "MET" on the fruit fly specimens below refers to methoprene-tolerant protein, which controls metamorphosis in the developing flies.

A Marriage of Minds

By that time, Janelia had become a bustling hub for the study of the *Drosophila* brain. Albert Cardona, a young Spanish developmental biologist and neuroscientist with a wide smile and enthusiastic manner, came to the campus for Janelia's first fly brain conference, organized by Simpson.

"The whole field was excited about the fly lines coming out of Gerry Rubin's lab," he remembers, "and there was an energy about what these genetic tools would make possible. But what I remember about that meeting is only Marta." Two encounters stand out: In one, Zlatic was seated by herself in Janelia's darkened auditorium before a talk, and she patted the seat next to her. "She said, 'Sit here,' and I did," Cardona laughs. In the other, he had joined her in Janelia's pub, Bob's, to discuss data coming out of her lab. "I remember that as we were sitting there together, this guy who was a big-shot conference attendee was there also, talking with us. I remember just thinking, *Go away*."

Cardona joined Simpson's lab as a visiting scientist on a pilot project that allowed him to take hundreds of electron microscope images of a segment of the fly larval nerve cord. This was proof of concept that it was possible to reconstruct what a neural circuit looks like in an insect nervous system at the level of single neurons and their synaptic connections. Janelia later committed to completing this wiring diagram for the full larva, under Cardona's leadership.

Jim Truman, who had come to Janelia in 2007, was by then using light microscopy to image Rubin's fly strains, each of which had been engineered to allow researchers to study small sets of neurons. The result of both efforts combined was that Zlatic could now manipulate individual neurons and study their effect on behavior. Her ambitious aim was to study not only how neurons affected behavior, but also how they work together to integrate many kinds of information – pain, temperature changes, and odors, for example – and then select a behavior in the animal's best interest.

"To understand this," she explains, "we need to know three things: circuit structure; physiological properties of neurons, which we can understand at the level of single neurons; and the causal relationship between neurons and behavior."

Meanwhile, Cardona was formulating his own dream project – to lay out the fruit fly larva "connectome," a map of neural connectivity in the brain. Then he got a job in Zurich. But by that time their relationship was far from just a meeting of scientific minds. They managed to not only stay in touch from afar, but also to get together periodically, with each working as a visiting scientist at the other's institute, until Cardona was hired as a group leader at Janelia in 2011. They got married – and also cemented their research collaboration. "Some people play sports, other people study," says Cardona. "Marta studies. If she is interested in something, usually she is intensely interested in it."

And, notes their colleague Jim Truman, "Albert's approach to science is very different from Marta's, in a complementary way. Without his abilities to reconstruct the nervous system of the larva, basically, we would be shooting in the dark."

A Larva's Life

Spend much time with Zlatic or anyone on her team today, and you may never look at a maggot the same way. Under the microscope, its wriggles become less random, even purposeful: Out in the world, larvae will sensibly try to approach food and pleasant aromas, notes Zlatic – but at the same time, they have to avoid a range of threatening situations. There are many ways to escape from danger, depending on the context and the animal's previous experience. In the same way that some mammals can walk, trot, or gallop away, larvae have different means of escape. Some of these means are fast but costly in terms of energy expenditure; others may be slower but less costly. Often, they need to string together sequences of behavior.

Zlatic wanted to understand how the animal's nervous system chooses among these various behaviors. This is a problem faced by all nervous systems, but in the *Drosophila* larva it is particularly tractable. Her team used a genetic toolkit, developed by Rubin, to activate different sets of neurons and watch what the larvae did in response, like flipping switches in a circuit breaker to test the wiring behind the walls.

Rubin's lab had developed more than a thousand mutant fly strains with a gene called *GAL4* inserted into specific clusters of neurons. Zlatic's team crossed those strains with other fly strains that had been engineered to produce, in the presence of GAL4, a light-sensitive protein called channelrhodopsin. This gave Zlatic access to the switchboard. By shining light on larvae from each hybrid strain, she could selectively activate whatever neurons had been engineered to include *GAL4*.

Then she and Tomoko Ohyama, a research specialist in her lab, started flipping switches, videotaping the groups of larvae as they performed. Right away, they gleaned some surprising insights, says Ohyama, because they saw variation between individual larvae when the same neurons were stimulated. That indicated to the scientists that even the most discrete behaviors are probabilistic and not 100-percent predictable. Still, clear patterns began to emerge. Zlatic and Ohyama enlisted the help of a team of machine learning specialists and statisticians in the Johns Hopkins University lab of Carey Priebe, who developed an algorithm to classify the behaviors with greater precision than the human eye could discern. In the spring of 2014, the scientists published their work, widely deemed a new framework for mapping behavior to individual classes of neurons, in the journal *Science*. In all, they had analyzed the movements of nearly 40,000 larvae.

Zlatic calls this achievement "just the beginning," as the paper describes only which neurons evoke a given behavior and not the circuit mechanisms by which it occurs. The next step is to relate this knowledge to the connectome and a neural activity map. "Marta has been a spark plug to keep this going; again, it is her enthusiasm that really makes larval systems work in this cohesive way," says Truman.

Marta Zlatic manipulates neurons in fruit fly larvae to study their effect on behavior.

It is also a feat that few scientists can pull off, given that it requires marrying disciplines as diverse as neurobiology, electron microscopy, and machine learning, says Rubin. "A lot of smart people talk themselves out of doing things because they can't do it all themselves or be in total control," he says. "That is not Marta's style. She says, 'I have this big problem I want to solve, and I know I can't do it all. I need to get this piece from that person, and this other piece from another person, and together we'll make it work.' That's a skill and a talent – like being a good field general. It's an approach to science I admire."

In the meantime, Zlatic and Cardona have also embarked on what she calls the "ultimate act of creativity" – parenthood. Their son, David, now three, is poised to match his mother's linguistic abilities.

"Marta speaks Croatian to David, and he speaks Catalan or English to me. I understand a bit of Croatian, though, and when I speak it to David he corrects me," laughs Cardona, a native of Spain's Catalonia region. The demands of two active laboratories plus a toddler keep the family busy, but they make it work; Zlatic takes the "morning shift," waking up with David and making him breakfast, while Cardona heads to the campus at sunrise. She works late while he makes dinner, and then the family has an hour or two together before bed. "It works for us," says Cardona, "because we know what our priorities are."

And so Zlatic has given up acting in favor of her greater loves – science and family. "What I love most about science is ideas," she says. "You look at the data, it inspires ideas, then you test them, then you lose them, then you try out other ones. The creative aspect of science is coming up with ideas in dialogue with the past. That's very beautiful to me."

"There's something about her that I think is true of a lot of the really good scientists I've seen in my career," says Rubin. "They're happy. They're having fun. They say that to be a successful scientist you have to maintain your childlike curiosity. She exudes that." ■

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—GERRY RUBIN

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