TECHNICAL WIZ

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BY JOHN CAREY

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PHOTOGRAPHY BY SUSANA RAAB

FOR HER PHD research, Gwyneth Card needed to master an unusual skill—fly wrangling. Card, at the California Institute of Technology (Caltech), wanted to know how fruit flies flee from predators.

To find out, Card painstakingly herded flies down a tiny tunnel and manually opened a gate to release them one at a time onto a platform. She then played the role of "predator," pulling a string to send a threatening black disc zooming down a rod toward the fly. "I was the apparatus," she says. "It was like a one-woman fly show."

Card's fly legerdemain, combined with high-speed photography, revealed that fruit flies don't always flee with just a simple jump. Instead, they often perform a more complex sequence of movements, coordinating the position of their legs and preparing their wings. But that discovery raised new questions about what's going on in the tiny fly brains. To answer them, Card knew she had to move far beyond manual fly herding. "We needed to observe thousands of flies a day," she says. Only then could she test flies with a vast number of genetic variations and pin down the nerve circuits involved in the escape behavior.

So in 2010, Card moved to HHMI's Janelia Farm Research Campus and began to dream of an automated fly-scaring apparatus. "I had this whole elaborate plan of all the crazy things I wanted to build," she says.

Card came to the right place. Janelia was explicitly created to tackle areas of science that, if well-funded, had the potential to transform science in the next 10-30 years. After holding a series of workshops and consulting with advisors, HHMI leadership tightened its focus to two complementary areas: identifying how neural circuits process information, and developing imaging technologies and computational methods. "HHMI did an analysis and asked, What is holding the field of neuroscience back?" says Reed George, now senior director of Scientific Services. "The most obvious answer was instrumentation." As a result, Janelia was established with a focus not just on neurobiology, but also on the development of imaging systems, reagents, computer algorithms, and other tools.

Ryan Williamson, Gwyneth Card, Brian Coop, and Tanya Tabachnick (l–r) created a "virtual flyswatter" to study nerve circuits involved in escape behavior.

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—ROIAN EGNOR

HHMI hired microscope builder Eric Betzig and software experts, and brought in Executive Director Gerry Rubin's treasure trove—a library of more than 7,000 genetically distinct strains of fruit fly. It's been a successful strategy. The cutting-edge microscopes from Betzig's and others' groups, the software algorithms, and the reagents that have been developed are already advancing science both within and outside of Janelia.

But Reed George and others realized that the individual scientists also needed a broad range of specific, customized tools. So in the windowless rooms along Janelia's service corridor, deep in the heart of the building, HHMI built a high-tech machine shop and then hired a machinist and an engineer. In the years since Janelia's opening in 2006, that original shop has grown under George's guidance into a state-of-the-art facility. Called Instrument Design & Fabrication (ID&F), the facility has giant machines for cutting delicate parts from hunks of metal, 3-D printers, and 17 engineers and fabrication experts who have a passion for science. "ID&F is an awesome resource," says Janelia Lab Head Anthony Leonardo, who studies dragonflies. "I came here in part because of ID&F; these guys are more skilled than anyone I've worked with in the past."

A Test of Ingenuity

Those skills are now more important than ever. Many of today's experiments, such as recording from the brains of flies as they move freely in response to threats or other stimuli, just can't be done with commercially available equipment, says Michael Dickinson, a neuroscientist at the University of Washington, who recognized early on the value of bringing engineers into his lab.

The grants that many university scientists get, however, don't usually include funds for hiring engineers. A few other research labs have skilled equipment and tool builders, "but it's not on the same scale as at Janelia," says Leonardo. "It gives the scientists a huge advantage," says Dickinson, who was Card's graduate advisor at Caltech and

is now a regular visitor to Janelia.

And it's a rare scientist who can build his or her own sophisticated equipment. "I understand the biological questions, but I would have no clue how to make a door go up and down," says Lab Head Ulrike Heberlein.

When Card brought her ambitious fly wrangling plans to the engineers, they "were game for it," she recalls. "They love to challenge themselves." Lead mechanical engineer Tanya Tabachnik, who had built machinery capable of packaging virtually any product in cardboard or plastic wrap before coming to Janelia, devised tiny tunnels for flies to traverse from their vials to the test platform. Working with members of Card's lab, Brian Coop (who had previously designed, among other devices, prosthetic arms for a company that supplies veterans with artificial limbs) created a trap door that automatically releases one fly at a time onto the platform. He also constructed a virtual reality system that "attacks" the fly. "It's a lot of work, just to scare a fly," says Coop.

It also takes the right touch. The trap door often shut on a fly instead of allowing it through. Splat! "It squished a lot of flies," Coop recalls. The fly juice damaged sensors and made a mess. So Coop figured out how to adjust the timing so the door closes more slowly and the flies are, at worst, momentarily pinned down instead of annihilated.

Card's lab is now using the intricate apparatus, dubbed FlyPez, to startle 300 flies a day, and hopes to triple that number. So far, Card has learned that identical threats can trigger either the hardwired jump to safety or the more complex sequence of movements. Now her lab group is busy charting the neural circuits involved, which will add another piece to the puzzle of how the brain works.

The other 42 lab heads at Janelia are pursuing similar advances in knowledge—and many are also relying on tools, instruments, and devices fashioned by the ID&F team. The engineers have designed tiny tweezers to grab inch-long fish without harming a fin, and devices to gently hold flies by their necks. They've customized microscopes to image the entire head of a mouse and devised powerful strobe lights to capture precise images of dragonflies in flight. They've built microdrives for inserting electrodes into mouse and fly brains with amazing accuracy and saved researchers from anesthesia-caused headaches during animal surgeries by sucking the fumes away from the operating table.

The engineers' ingenuity is constantly being tested. Before coming to Janelia, instrument design specialist Jason Osborne had built parts for the space shuttle. But when Lab Head Vivek Jayaraman asked ID&F for a customized treadmill for fruit flies, Osborne faced a constraint that hadn't come up in the space program: delicate insect legs. "You don't want to break the fly's little legs if he's cruising along and then has to stop all of a sudden," he says. The solution ID&F and the lab came up with was modernizing an old idea: suspending a little foam ball on a column of air for the fly to run on. The ball responds quickly, with little inertia, to the fly's every move, and the ball's precise motion can be monitored. The treadmill has helped Vivek's lab group learn how flies perceive and respond to motion—and Osborne and colleague Gus Lott earned slots as co-authors on a 2010 *Nature Methods* paper describing the technique and its results.

Engineer Jason Osborne (r) is helping Roian Egnor develop video trackers and microphone arrays to monitor social behavior in mice.

Applied Wizardry

ID&F's work tends to fall into a few main categories. "There's the stuff we [scientists] couldn't possibly do technically, and the stuff I could do but would take me 100 times longer," says Lab Head Roian Egnor. Another important chunk of work involves making simple pieces, like brackets and cables, to boost the scientists' productivity.

The common thread in these projects is using technical wizardry to make researchers' lives easier and to advance the science. To study how odors affect behavior, for instance, Matt Smear, a research specialist in Egnor's lab group, designed a device to blow smells toward mice running on treadmills. But even after weeks of fine-tuning,

Engineer Jeff Jordan and postdoc Huai-Ti Lin are devising a new way to serve up "fly bait" for hungry dragonflies.

the odor plume wasn't wafting correctly to the mice's noses. "So I call Jason," says Egnor. "He comes up and in literally five minutes finds a solution."

Just down the hall, in Anthony Leonardo's lab, researchers are studying how dragonfly brains orchestrate the complex flight maneuvers needed to nab prey. His lab team has built tiny backpacks that capture and transmit signals from neurons. But once a dragonfly is fitted with nerve probes and a backpack, the window for testing its behavior is short–and there's no guarantee that the dragonfly will be interested in chasing a live bug. "With real prey, the odds are small that predator and prey will meet in that intricate ballet," says Leonardo. "So we want to have prey at the time the animals are interested in behaving."

The best answer seemed to be a fake bug that would fly around and reliably trigger dragonfly attacks. But how to create such a robot? Leonardo handed the problem to ID&F's Jeff Jordan. Realizing that a tiny, radio-controlled fly would be too expensive and challenging, Jordan says, "We had to think out of the box." He came up with the idea of a bead at the intersection of two strings. It works, to a point. Dragonflies attack until they learn that the whole reason for predation–a meal–is missing. So Jordan is now working on a more sophisticated multi-string system in which the "prey" will be a tasty morsel–perhaps even a real fruit fly–mounted on a tiny barbed rod.

And somebody's got to sort the fruit flies. Normally, that job would fall to graduate students. But Janelia's lab groups are small, and they don't have large numbers of graduate students. "A lot of things that might be done by throwing more labor at them, we can't do here," explains Saul Kravitz, who recently joined Janelia as senior director for Advanced Computation and Technology and now oversees ID&F. Besides, this sort of labor is tedious. Mistakes are made. So ID&F systems engineer Peter Polidoro, working with Janelia's Applied Physics and Instrumentation Group, has been developing a sophisticated fruit fly sorter. Starting with 10 vials of flies on a rack, the system chills the insects to put them to sleep, tips the vials' contents onto a ramp, and jiggles the flies down the ramp and past a camera, which uses image processing software to tell the flies apart by gender. Then, little puffs of air blow males into one container and females into another.

"I really like the idea of building machines to do things and collect data automatically to free up the scientists," says Polidoro. And, as with most of the tools devised at Janelia, ID&F makes all the hardware and software available open source so that researchers outside of Janelia can put them to use.

The Sweet Spot

The ID&F tasks that most excite both engineers and scientists, though, are the ones that open the door to new avenues of research. "There's a sweet spot for Janelia projects," says Egnor. "If it seems logical that experiments should be done, but no one is doing them because they're

For a glimpse into the world of ID&F at Janelia, see the slideshow at www.hhmi.org/bulletin/winter2014.

hard, those are Janelia projects." Egnor is tackling one such effort—exploring the brain circuitry that enables animals to navigate complex social environments. "We need two pieces of technology that are hard," she explains. First, they need to detect exactly what an animal is doing, "not just that George is sniffing another animal, but that he's sniffing the left haunch of Fred, and Fred is the subordinate male, and Fred and George just had a fight," she says. "We need the whole social context." With ID&F's help, her lab is developing video trackers and microphone arrays surrounding a special cage to accurately record and map every animal's behavior and vocalizations. "We're getting close," says Egnor.

The second requirement may be even tougher: measuring activity from neurons without affecting the animals' behavior. It's possible to implant electrodes in mouse brains to record activity. But if an animal looks implanted, any other self-respecting mouse will attack it. "It thinks, you've got that funny thing on your head and you're moving slow, and I'm going to beat you up," says Egnor. So ID&F lead electrical engineer Steven Sawtelle worked with the lab team to design a chip, smaller and thinner than a fingernail, that can be slipped under the skin on a mouse's head. It beams signals from electrodes in the brain to a receiving computer for analysis. Adam Taylor and Ben Arthur in Janelia's Scientific Computing group are helping Egnor's lab develop advanced software for synchronizing the flood of data from the video trackers, microphones, and neurons.

Egnor would never have attempted the complex effort without ID&F and the software team, she says. "Just knowing they are there means that when a crazy thought floats across my mind, I don't dismiss it."

Speeding Science

These success stories don't mean that interactions between scientists and ID&F are always wrinkle free. With multiple requests to juggle, engineers find that projects sometimes take longer than scientists expected. Scientists, in turn, sometimes move the goal posts, dragging out project timelines. And even with 17 engineers, there's far more demand for ID&F's services than can be met. "The biggest challenge is simply scheduling all the requests," says Coop.

Yet, to a person, the engineers say they thrive on the pressure and love being able to contribute to the science. "I'm so passionate and so excited about the research here," says Osborne. Jordan turned down a higher paying job designing satellite-tracking backpacks because, he says, the "mission and goal here are much more of a draw than money." The engineers are content to have their contributions only briefly mentioned in papers or presentations. "I work on something I think is extremely complicated and cool and amazing, and the scientists give a talk and you realize it was a very small piece," says Sawtelle. "But the scientists are the artists. We are the tool builders."

At the same time, they also say they have more to offer. "We want the scientists to use us more for things that are really challenging and less for just cables and brackets," says Tabachnik. Her suggestion to the researchers: "Bring us in at the beginning of a project so we can dream with you."

Another suggestion, which Janelia's management is exploring, involves making individual projects more broadly applicable. "We are building a portfolio of reusable components that can really accelerate the science, as opposed to everyone using his or her own very specialized solutions and starting from scratch every time," explains Kravitz.

Such an approach could also boost scientific progress outside of Janelia. Both scientists and engineers hope that sophisticated tools such as the automated fly sorter, Card's fly frightener, or the "fly bar"—a technological tour de force being used by Heberlein to study the neurobiology of alcohol addiction and motivation—will be replicated in other labs or offered by companies as commercial products. "The goal is always to create something the whole field can adopt," says Card.

And in fact, just like Betzig's microscopes and Rubin's genetic libraries, a few of ID&F's creations, such as a custom two-photon laser scanning microscope, have begun to find homes with researchers elsewhere. More widespread adoption, however, may require more time—and more scientific discoveries at Janelia. The onus is on the scientists, explains Parvez Ahammad, a junior fellow who studies neural circuits in fruit flies. "We have to show that something is worth studying," he says. "If we find interesting biological insights, people will want to replicate what we did and would need the same designs and technologies."

For now, Janelia's scientists mainly see ID&F as one of the crucial shared resources that speed up their research and boost the chances of new discoveries. "If I had one thing I needed to make and had a year to do it, I probably could get it done painfully," says Lab Head Albert Lee. "But when I have many things and want them quickly, and want the best, that's when I need ID&F." ■

Steven Sawtelle applies his expertise in electrical engineering to design microcircuits and specialized chips.