A GUT FEELING

M. LOVETT

When most people look at lobsters, they see dinner. Eve Marder saw a key to the theoretical underpinnings of animal behaviour. **Ishani Ganguli** reports.

Larry Abbott was a theoretical particle physicist at Brandeis University when he first met Eve Marder at an off-campus retreat to celebrate the induction of a new centre for neuroscience. It was the late 1980s and he was just starting to dip his toes into the statistical mechanics of neural networks. But he wasn't sure what to expect from Marder, who had already made a name for herself as an iconoclast in the field. Abbott talked about his mathematical models, and Marder told him he needed to see a 'real' neural network in person, so she invited him to visit her lab.

A few weeks passed back at the Brandeis campus in Waltham, Massachusetts, and Abbott's student needled him about the invite. "I finally listened," he says, and he was blown away. Along a bench, tiny grape-like clusters of nerve cells pulsed away in Petri dishes. Their activities weren't directly visible, but the rhythmic discharges between nerves ticked away on a chart recorder attached to the cells by electrodes.

"It was so fascinating — what was going on — that I thought, 'I should become a neuroscientist,'" says Abbott. And, with help from Marder, he did. Marder spent hours with him, meeting daily to answer his questions and later to discuss theories that would define both of their careers. Something for which Abbott, now a long-time collaborator of Marder and a professor of theoretical neuroscience at Columbia University in New York, is grateful: "I was a nobody," he says. "She could have easily given me a quick tour and forgotten about it. But she didn't. She's quite remarkable that way in not giving up on unlikely sorts of people."

> **"It was so fascinating that I thought I should become a neuroscientist."**
> — Larry Abbott

For nearly four decades, Marder has taken on all kinds of unlikely sorts in the hope of forging a path of her own design in neuroscience. The 59-year-old has crafted a tremendous body of work on — of all things — the stomachs of lobsters and crabs bought from local fish markets, using the creatures' gastric wiring as an investigational model to launch several fields of inquiry and dozens of careers. Her work has explained how even simple neural networks can create diverse functional repertoires, and how the homeostatic mechanisms within allow these networks to produce reliable behaviour patterns despite the constant turnover of nerve-cell components.

Marder first found herself at Brandeis as a teenager in autumn 1965. Any scientific aspirations she had before going to college withered in the political heat of the time, she says. Demonstrations and voter-registration drives led her to dream of becoming a civil-rights lawyer. She declared a major in politics.

But in her junior year, Marder followed a room-mate into a course on abnormal psychology that changed everything for her. These were the days when the blame for schizophrenia and many other psychological ills was placed squarely on parents. Marder was intrigued by the idea, then heretical, that schizophrenia had a biochemical cause and that dysregulation of neurotransmitters — which relay electrochemical signals from nerve to nerve — could vastly influence a person's grip on reality. So she holed herself up in the Brandeis library and read every book she could find on how the brain seemed to use the nerve signal-dampening inhibitory neurotransmitter, gamma-aminobutyric acid (GABA) to keep itself in check. This first introduction to neurotransmitters sparked a lifelong curiosity about nerve-cell communication. She switched her major to biology and never looked back.

In 1969, Marder started graduate school at the University of California, San Diego. It was there, under Allen Selverston, that she

first encountered the lobster stomatogastric-ganglion (STG) system. This neural network comprises just 30 neurons that control the gut muscles of lobsters and other crustaceans — allowing them to grind food using gastric 'teeth' and then pass it down their digestive tract. It has become the best-studied example of a central-pattern generator, the same type of rhythmic neural circuit that controls breathing and chewing in humans. Excised and secured on a Petri dish, this network will fire rhythmically to the beat of its own drum for hours, without any outside input. The STG's large neurons, easy to identify and record from the preparation, provide a ready glimpse into the tiny but powerful circuit.

The big picture

Whereas Marder says that most people in neurobiology were studying single neurotransmitters such as serotonin and GABA in isolation, she had broader questions in mind. As many as eight neurotransmitters had been identified by then, and she wanted to know why so many were required in the brain.

As she was discovering the system that would define her research career, Marder was also working to define her values as a scientist. As a graduate student, she watched fellow neuroscientists scramble to purify the nicotinic acetylcholine receptor — a critical part of nerve and neuromuscular signalling — and quickly learned something about herself. "[With] a consensus problem that everybody thinks is interesting, you have a built-in audience and built-in peer group approval. But I realized I would never choose to work that way," she says. Her own vision would matter little, Marder says, in an "enterprise where the goal was so well defined that it was going to get done no matter what".

So she went after the circuitry of the STG system. Glutamate had already been established as the neurotransmitter at play at many of the synapses — the connections between nerve cells. With painstaking measurements of electrical activity in the 30 cells of the ganglion, and by searching for biochemical clues, she discovered that it wasn't alone. Acetylcholine — the neurotransmitter of choice in the nerve to muscle interfaces of vertebrates — was also at play in half of the neuromuscular junctions in this arthropod system. She found that like glutamate, acetylcholine could excite or inhibit depending on context, adding to a growing body of evidence that a neurotransmitter's effect could vary based on its targets[1].

> **"Marder has an amazing ability to come up with ways to get at big questions that are relevant to neuroscience."**
> — Adam Taylor

The findings earned her her first publication — in *Nature* — and a PhD. Marder continued her pursuit of neurotransmitters over four years of postdoctoral training at the University of Oregon in Eugene and the École Normale Supérieure in Paris, France, where she honed her biochemistry, electrophysiology and biophysics techniques. In 1978, Marder turned down a tenure track position at Cornell University in Ithaca, New York, to return to her alma mater as an assistant professor, and has not left since.

Neuroscientists had always treated networks as electrical systems with fixed connectivity. But an offhand observation Marder had made as a graduate student led her to question this dogma. In the process of figuring out which neurotransmitters the ganglion cells use to communicate, Marder had dumped candidate after candidate onto the system. She had noticed at the time that many of these molecules had an effect on the output of her tried and true lobster preparation, even those that weren't acting as neurotransmitters. But she hadn't known what to make of this at the time. Newly situated at Brandeis, she began to investigate with her budding lab team.

What she soon began to realize was that these substances — acting on a time course of hundreds of milliseconds to hours, a glacial pace in the firestorm of STG signalling — were 'neuromodulators'. This was heresy. "People working in vertebrate systems still thought that vertebrate neurons ... were very simple on–off figures." But modulators meant that the systems didn't have to be hard wired. Neurons can release one or several neuromodulators — some of which are also neurotransmitters, such as serotonin, and others, such as the peptide proctolin. Like hormones, they bind to receptors on other neurons, triggering long-lasting changes in how the neurons respond to the fast-acting neurotransmitters that allow cells to communicate. "They can do all sorts of interesting things," she says, such as alter the intrinsic excitability of cells, the amount of neurotransmitter released each time a neuron 'fires', or the firing patterns as a whole. She began to parse out which neuron types have which receptors for these functions[2].

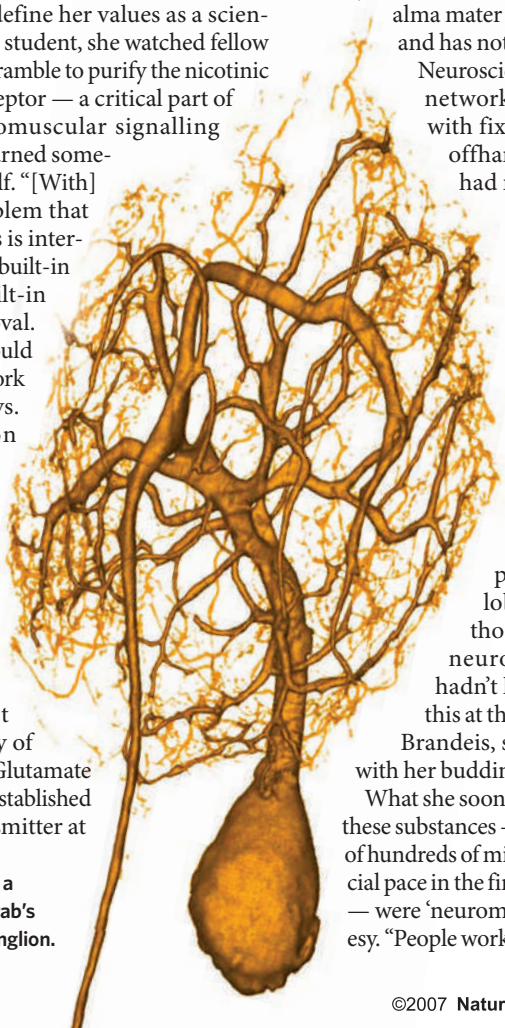
New antibodies against potential modulators eased the process of searching intact tissue for these modulators through the late 1980s and early 90s. "Every year or two would add one or two more," says Marder. Mass spectroscopy accelerated the search in the late 1990s. Within the next 10 years, she says, the full cast of 50-odd characters in the neuromodulation story should be assembled — by her lab and others. At that point, she says, they will get a clearer picture of how all the parts interact.

From lobsters to humans

The applicability of her work on crustaceans to human neuroscience is a point that animates her. Neuromodulators have been found in all animal brains. "If you don't think they're doing the same thing, you're just silly," she says. "As far as I'm concerned, evolution never throws away a good mechanism." Although experts were not open to the neuromodulators' snail-paced mode of communication between neurons, Marder considered it a logical step. "It was so obvious to me that this is the way it was working that I was always surprised when people didn't think it worked that way."

Abbott, at least, had no reason to doubt her, and months after they met, their collaboration spawned the birth of the 'dynamic clamp': a neurophysiological method that allows researchers to simulate finely tuned neural networks using computer-manipulated nerve cells. Scientists can adjust different parameters, tweaking conductance through ion channels triggered by ligands or voltage changes, and see the effects on the circuit in real time[3]. The clamp is now used worldwide in diverse applications such as simulating heart muscle cells.

At the same time, her collaboration with Abbott led her to ask new questions about the



E. MARDER/D. BUCHER/A. TAYLOR

Impression of a neuron from a crab's stomatogastric ganglion.

neurons she had studied for decades. The neurons were resourceful in modifying their patterns, but how did they manage to keep their basic performance intact over time? Marder had been working with lab members to build a model of the lateral pyloric motor neuron, an important controller of the stomatogastric system. "I found it frustrating beyond belief," Marder recalls. Coordinating the individual current contributions of ion channels in each membrane to predict the system's overall behaviour proved unwieldy. "I said if the model is so sensitive to variations of each type that it takes a smart postdoc months and months to play with, how does the neuron get it right?"

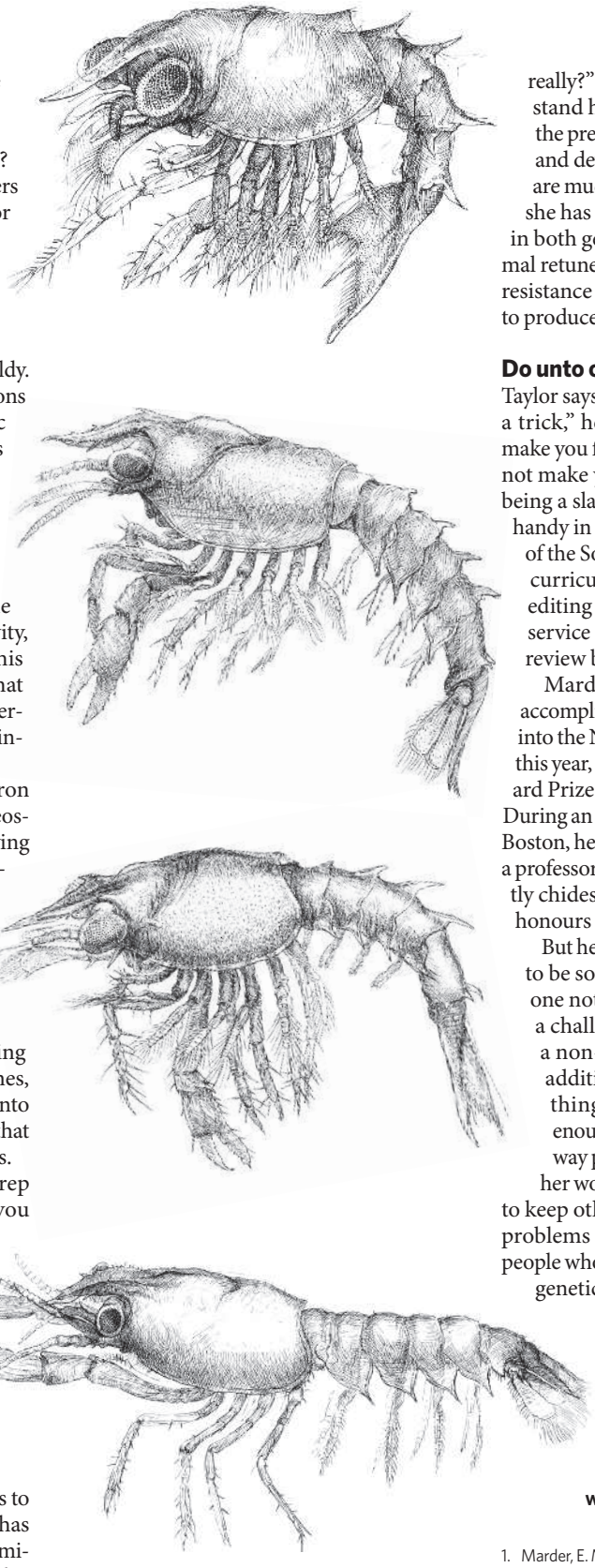
"There have got to be some simple rules that let the neuron self-tune even though ion channels are turning over continuously over time," she remembers venting to Abbott. It turns out there were. The independent variable was the output activity, not the number of channels mediating this activity. Marder and Abbott discovered that neurons were remarkably resilient when perturbed, changing their conductances to maintain a given output[4].

What are these rules by which the neuron gets its activity right? This question of homeostasis continues to drive her work. She is trying to figure out how cells maintain stable network performance over long periods of time despite the ongoing replacement of ions and ion channels.

Today, as when she started, Marder says that about a dozen labs are working on the stomatogastric ganglion system. The niche field suits Marder's style: turning unexpected questions into mainstream ones, then passing the pursuit of their answers onto researchers working on model organisms that can best handle them, such as mice and flies.

"One of the dangers of working on a prep such as the STG is if you're not careful, you can fall into studying the system for the system's sake," says Adam Taylor, a postdoctoral fellow in Marder's lab. "She has an amazing ability to come up with ways to get at big questions that are relevant to neuroscience writ large within the stomatogastric ganglion of crustaceans."

And there are many more such questions to answer, says Marder. For example, her lab has shown that different mechanisms produce similar output patterns, but to what extent does this concept extend to more complex systems? "Your respiratory system keeps you breathing, mine keeps me breathing. How different are they really?" she asks. She is also trying to understand how this built-in variability fits into the preservation of circuits as animals grow and develop. The neurons of adult lobsters are much bigger than those of juveniles, yet she has measured the same motor patterns in both generations, suggesting that the animal retunes properties such as cell-membrane resistance or the distance between nerve cells to produce the same effect.



B. MARDER

Larval stages of the Maine lobster (*Homarus americanus*), the subject of much of Eve Marder's work on crustacean stomatogastric ganglia.

Do unto others

Taylor says Marder leads by example. "It's quite a trick," he says, how she "manages both to make you feel like you should work harder and not make you feel depressed about your boss being a slave-driver". Such skills may come in handy in November when she adds president of the Society for Neuroscience to her extracurricular activities, which already include editing the *Journal of Neurophysiology* and service on several advisory committees and review boards.

Marder has a habit of downplaying her accomplishments — which include induction into the National Academy of Sciences earlier this year, and winning the 2005 Ralph W. Gerard Prize from the Society for Neuroscience. During an interview at her home in down-town Boston, her husband — Arthur Wingfield, also a professor of neuroscience at Brandeis — gently chides her for her reticence as he lists her honours and responsibilities.

But her spotlight-shyness is part of a desire to be so far out on the leading edge that no one notices her. At least not right away. It's a challenge she revels in. "If you work on a non-consensus problem, you have the additional burden of having to do something that's interesting enough, novel enough or articulate enough to change the way people think," Marder says. Certainly her work has articulated enough questions to keep others busy. "There are parts of these problems that will be much better solved by people who can work on mice and flies and real genetic organisms."

For her own part, though, Marder's model loyalty is firm. It even extends beyond the bench. "I don't like to eat lobsters anymore," she says, "because I find I just feel bad for them." ■

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