President Clinton named Karl Kandler one of this country's elite young researchers. He started out his scientific career obsessed with animal behavior. Eight-year-old Zia Kandler inherited her dad's zeal.

OF DUCKS AND DYSLEXIA

BY EDWIN KIESTER JR.

THE BRAKES OF THE BRAIN

Karl Kandler's journey to the White House started in a small town in Bavaria amid a noisy, lively, and ever-changing menagerie of animals. "Birds, chickens, ducks, fish, turtles, water insects, I brought them all home," he recalls with a smile. "I drove my mother to distraction."

Flash forward several decades to another continent. The little boy in lederhosen has grown into a slim, trim University of Pittsburgh assistant professor of neurobiology who still speaks with the precise consonants of his native land. It's October 2000, and at the invitation of President Bill Clinton's administration, Kandler stands in the executive mansion with 11 other researchers to receive the Presidential Early Career Award for Scientists and Engineers (PECASE). The PECASE is the highest honor the government gives to young scientists, and honorees' work must be deemed exceptionally promising and contributory. The executive citation

PHOTOGRAPHY | CAMI MESA

praises Kandler for "outstanding contributions" in explaining the cellular basis for disorders and dysfunctions like "speech perception, language development and dyslexia."

"Hot" and "exciting" are words used by Lynn Luethke, program director for hearing in the National Institute on Deafness and Other Communication Disorders to describe Kandler's research in relation to dyslexia. "It's very basic but with important implications for clinical problems," Luethke says. "Understanding the causes can lead to the development of treatments, and, more important, to the means for preventing such disorders."

Kandler is elucidating confounding disorders like dyslexia by pursuing a more fundamental, and seemingly obscure, field of research. He is figuring out how the brain organizes its complex network of neurons during the early stages of development—and how that neuronal organization both turns on and turns off actions and feelings.

His interest traces back to a boyhood in the small town of Schrobenhausen, amid the rolling Bavarian country northwest of Munich.

"There is nothing to report about this town except that they grow lots of white asparagus," a German delicacy, Kandler says.

But the area turned out to be an ideal environment for a budding neurobiologist. Karl the schoolboy detoured on his daily walk home to visit creeks and ponds, where he tried to capture fish with his bare hands, watching how their instincts enabled them to elude him. When he did catch them—"never by being faster, only by tricking them," he says—he brought them home as "guests" to the aquarium in his bedroom. The fish shared the room with a colony of brown and black mice Kandler was breeding to see how the color is passed on: "My mom made me quit when I had over 60 of them." She allowed a tortoise, a chameleon, and snakes in the room as well, but banished the ducks and chickens outside.

The boy fished, too, but reeling in a catch was never the main goal. He was really interested in taking in what was going on around him. A "successful fishing day" for Kandler might mean seeing a snake and some birds and then getting an eyeful of bluegills building their underwater nests.

The antics of the nine-spine stickleback, a common small fish of Bavarian waterways, particularly intrigued him. A male stickleback builds an elongated nest on the creek bottom, then attempts to lure females while vigorously defending it against other males. He follows a strict sequence of courtship and defense behavior that scarcely varies from stickleback to stickleback. Clearly, the young Kandler could see, stickleback brains had to be similarly programmed to explain this complex but stereotyped behavior.

"It was a big deal for me at the time," he says.

Between Bavaria and the Beltway, Kandler's early days in academe had him examining such abstruse subjects as the predatory nature of tree snakes on Guam and the come-hither messages of moths. (He learned why the wily sticklebacks so often eluded him when an exchange student in Colorado: "Fish have a special fast escape-behavior circuit, basically a three-synapse circuit with just one single neuron, called the Mauthner cell . . . specialized for speed at many different levels.")

As an undergraduate at Bavaria's University of Regensburg, Kandler recalls a professor who gave a lecture about pheromones—the chemicals released by insects to, among other things, attract mates. "A single molecule of the pheromone on the antenna of a male moth is enough to arouse him sexually," he says.

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"Until then, I had been observing animal behavior, *why* animals did what they did. Now I wanted to know *how* they did it, what was happening in the nervous system that enabled them to act as they acted."

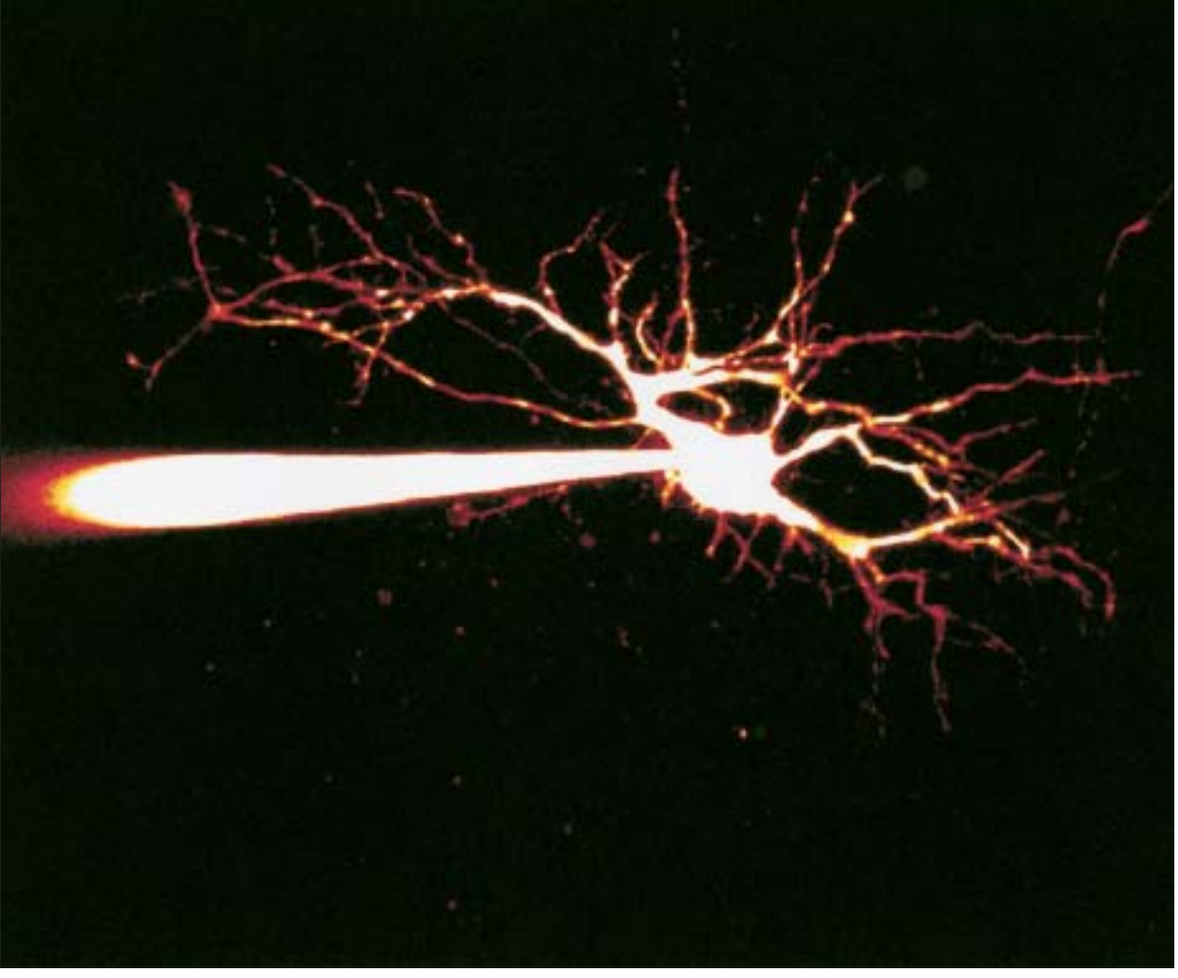
Questions about the wiring of the brain fascinated him more and more. His search for answers led him into neurobiology. After Regensburg, Kandler went on to the University of Tübingen, where he obtained his doctorate, then to Duke University as a postdoctoral student. He joined the faculty of the University of Pittsburgh School of Medicine four years ago as an assistant professor of neurobiology.

In Pittsburgh, he has focused on the development of the poorly understood "inhibitory network" of nerve cells. To oversimplify, inhibitory neurons serve to keep the nervous system in a precise, delicate balance; they counteract neuronal impulses turned on in response to sensory input. When, for example, we touch a hot surface, neurons of the "excitatory network" fire to convey the message to the brain. Remove the hand, and inhibitory neurons tell the others to stop firing. The alarm is over. "It's like the brakes of the brain," Kandler explains.

This is where dyslexia and learning disorders enter into the picture. To read or understand speech, one must develop "phonemic awareness," recognition of the tiny individual fragments of spoken sound, or phonemes, that blend one into another to make comprehensible speech. The brain does this assembly and interpretation for us, whether the phonemes are uttered aloud or printed on the page. It is important, though, for each "puh" or "fuh," as Kandler illustrates with puffs of the lips, to switch off quickly to make way for the next phoneme. Switching off is the task of inhibitory neurons. Some suggest that people who are dyslexic process auditory signals slowly, and Kandler theorizes that an underdeveloped or abnormally organized inhibitory network may be at fault. For instance, to a dyslexic person, Kandler's own quick, sharp consonant sounds might not be interpreted swiftly enough for the following phoneme to be understood.

And abnormalities of the excitatory-inhibitory balance might partly explain other disorders. Epilepsy, for instance. Perhaps seizures, especially in the first year or two of life, are caused by the failure of inhibitory neurons to "inhibit" motor neurons controlling movement, so that the neurons keep firing and firing and produce the classic excitatory overload characteristic of a seizure. "That could be the worst-case effect," Kandler says, pointing out that up to 15 percent of infants may have seizures in the first months of life, while the neuronal networks are still developing.

Other researchers have speculated that autism and schizophrenia may result from an abnormal network balance. And some



Kandler's team records electrical and chemical responses from a living neuron in the brain of a newborn mouse. The researchers are investigating how immature synapses (nerve connections) are eliminated or stabilized within the mysterious inhibitory neuronal network. LEFT: What looks like a ray of light on the left is a pipette filled with fluorescent calcium-sensitive dye penetrating a living neuron. ABOVE: A strand of optical fiber, narrower than a human hair, looks much bigger as this "miniature flashlight" shines.

LEFT: COURTESY KANDLER. RIGHT: CAMI MESA

believe depressed inhibitory neurons may explain puzzles like the "phantom pain" amputees sometimes feel in an absent limb.

Much mystery remains about the inhibitory neuronal network, though its existence is taken as a given; after all, some system has to prevent excitation from running on unchecked. What blocked more detailed knowledge was that the presumed inhibitory neurons seemed inextricably intertwined with their excitatory counterparts, so that they were difficult to study individually. And there seemed to be no easily studied animal model.

Kandler, however, has identified a part of the auditory system that can be used as a model. The lateral superior olive (LSO), a question-mark shaped area in the brain stem of mammals, is much simpler in structure, easier to access, and more independent in its function than other areas of the brain. The LSO helps us to localize sound. When a sound is "heard," neurons in the ear nearest the noise become more excited than those in the opposite ear. LSO neurons receive excitatory input from one ear and inhibitory input from the other. Together, they let us know that that clanging is over there to the left, not to the right.

To visit Kandler's lab on the 14th floor of the Biomedical Science Tower is to step into a Lilliputian world of miniatures and "clever and innovative" approaches, as Robert Malenka and Roger Nicoll of the University of California at San Francisco wrote in reviewing Kandler's research in *Nature Neuroscience*. (Kandler's 600-square-foot laboratory is sort of miniature, too. "You have three or four people in here, stumbling into each other, and the most common words are 'Excuse me,'" he notes.)

Kandler's white-coated postdoc, Deda Gillespie, demonstrates a study method usually performed by lab colleague Gunsoo Kim. She bends over a microscope, peering into a small chamber. The chamber holds a bath medium with finely prepared living slices from the LSO of a newborn mouse, each a mere 20th of a millimeter thick. Gillespie manipulates a pipette, not much bigger than a broomstraw, and an optical fiber, thinner than a human hair. Intermittently, there is a quick, bright flash from the tip of the fiber.

The LSO is laced with a "caged compound," which may sound like a confinement area for miscreants, but is actually an almost infinitesimal amount of glutamate covered with a special coating. Glutamate, a neurotransmitter that triggers neuronal activity, is rendered inert by the "cage," or coating, this very effective one developed by Brigitte Schmidt of Carnegie Mellon University. An ultraviolet, or UV, flash from the optical fiber breaks the cage and frees the glutamate, allowing the neurons in the tiny illuminated area to fire and activate the neurons to which they are attached. Neuron by neuron, Gillespie traces those connections. At the same time, she is able to record the strength of each connection: Her pipette is filled with salt solution that conducts electrical impulses from receiving neurons, reading their signals with sensitive amplifiers.

Kandler likens this approach to mapping computer wires: "We determine not only where the wires run but also what voltage they are using, how effective the wires are."

"You shine it up here, see?" Kandler explains, pointing to a dendrite in a diagram he has drawn on a chalkboard. "Like a miniature flashlight. And then up here. . . and up here. . . and up here. . . and you can bring this cell to fire." He points to axons, the sites of the neuron's outputs, as well as dendrites, the sites of its inputs.

"You can ask the neuron, with whom are you connected? Which other neurons?"

"Use it or lose it" is Kandler's favorite mantra for how neuronal activity organizes the brain during the early stages of excitatory development. The neuro community has shown many times over that excitatory connections go through a pruning process: Those that are most utilized are reinforced and strengthened. Those with less purpose fall away. But little was known about inhibitory network development until Kandler was able to show, with the help of fiberoptic UV flashes, those connections are trimmed as well—in the LSO of rodents at least.

By the age of two weeks, when a rat is capable of hearing, three out of four of its LSO connections have been eliminated. Using a series of age-graded samples from the LSO of animal models within the first two weeks of development, Kandler recorded sources of inhibitory inputs.

"Let's say I'm a neuron," he says. "I receive inhibitory inputs from the Cathedral, from Scaife Hall, from McDonald's—almost every building in Oakland. A week later, we see the connection from Scaife is gone, from McDonald's is gone. Only the Starbucks connection remains."

Once a rat starts hearing, Kandler discovered, its inhibitory LSO connections have been functionally pruned, meaning the connecting "wires" may still exist but aren't used. Whether the "use it or lose it" dictum applies in inhibitory cases is not at all clear.

"At this point we don't know whether the ones used most often are maintained," says Kandler. "Though this is believed to be the case for excitatory connections, we have no idea whether this concept also applies to inhibitory connections.

"The next step is to find out why certain connections are preferred over others—what tells the cells to keep some connections and not the others."

"Here's the best part" of his LSO studies, Kandler reports, barely containing his enthusiasm: As part of his PhD work, he found that inhibitory neurons start out behaving excitatory. In a rat's first week, LSO neurons defined as inhibitory by their chemical structure (emitting neurotransmitters that are inhibitory in adults, GABA and glycine) act in ways indistinguishable from excitatory connections. A few scientists had reported this developmental paradox in other brain areas, but Kandler was skeptical. He needed to see for himself: "I was surprised. I didn't expect to see this."

This phenomenon may explain why children and animals are more prone to epilepsy as newborns. "It's a very dangerous time, I think—like a wolf in sheepskin," says Kandler. "The brakes of the brain act like the accelerator."

He notes that if there's an insult to the brain, a stroke perhaps, as the brain rewires itself, inhibitory connections act excitatory again. This excitatory mimickery may make cells more plastic, Kandler conjectures. But again, he has no confirmed explanations. Not yet anyway.

Using the same caged compound and miniature flashlight method, Kandler has settled another question that has divided the research community for years: When synaptic connections in the hippocampus are strengthened or weakened by activity, what's happening? On what end does the change take place, the pre- or postsynaptic side? This highly technical mouthful sounds like an "inside baseball" controversy, of interest to only a handful of researchers, but the hippocampus is the seat of learning and memory. Knowing more about how it puts itself together could not only benefit learning and education but perhaps lead to better understanding of the memory losses of, say, Alzheimer's disease.

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"The question is whether the changes in the cell are caused by the strength of the input, or by the cell itself becoming more sensitive to the input," says Lawrence Katz of Duke University, who was Kandler's mentor and collaborated with him on the research.

"It's like you and I are talking on the phone," continues Katz, "and at first I can't hear you, but then I can hear you better. Is that because you are talking louder, or because my ear is picking up the sounds better? We said it was the 'ear,' the change was greater sensitivity in the neuron itself. And we were right."

Kandler managed to set the record straight by stimulating the postsynapse artificially, with a fiber optic UV flash. Since the optical fiber was a constant, any change in the strength of this semiartificial connection must have been on the postsynaptic end, how the cell received it, Kandler deduced.

"The novel approach of Kandler and colleagues has shed a bright light on a persistently vexing problem," Malenka and Nicoll remarked in their review, "the eventual solution to which will go a long way in clarifying some of the most fundamental properties of synaptic communication."

"Karl is very skilled and very passionate about his research," Katz says of his one-time postdoc. "He was fearless about making new things. He had great technical skills and fantastic hands. We shared many things. Neuroscience, of course, but also a love for fishing and for Alfa Romeos."

When Katz bought a new Alfa, he offered to sell Kandler the older one parked in his garage. "It was covered with dust and had no license plates," Katz recalls, "but Karl wanted to test-drive it. So I said, 'Sure, let's take it around the block.' He was horrified. He said, 'Without license plates? We could never do that in Germany.' So we started out, and we hadn't gone 200 feet before a cop whistled us over. He not only gave us tickets but said, 'You *cannot* drive that car on the streets. So we had to *push* it back to the garage." Kandler bought the car but now has a different Alfa, a 1974 model, which he treats lovingly:

"I never wanted to fix cars, but once I started to work on the Alfa, I liked it because it is basically the same question I ask in the lab: 'How does it do it?' And the Alfa does it in a very elegant way."

Kandler has hiked over the Alps from Munich to Italy, crossed the Pyrenees, and trekked in Kashmir and Nepal. He spends less time in the outdoors and a lot of time in the lab these days. Now, he says, his major pastime is his family: Xaver's soccer games and Zia's menagerie. Following in her father's footsteps, Zia Kandler, 8, has collected three birds, a chinchilla, a rat who recently died, a dog, fish, caterpillars, praying mantises, an Eastern king snake, and most recently, a species of chicken that lays blue eggs. Her mother, Dinnie Goldring, sounds resigned. "How, in this family, could I object?" she asks. ■