Above: Santiago Ramón y Cajal’s sketches of neurons informed his ideas about the neural system as a fragmented network. A century later, Zuo-Zhong Wang uses a simplified model—a single muscle cell—to study neurotransmission. Right: An axon (green) branches out to control a muscle cell. Acetylcholine receptors (red) cluster near the tip of each axon. How this happens is a mystery of neuroscience that Wang is beginning to crack.
In 1906, two men stepped onstage to receive their shared Nobel Prize. In their acceptance speeches, they presented utterly opposing ideas. Camillo Golgi said that the nervous system was one continuous network, which ran unbroken throughout the body. Santiago Ramón y Cajal argued that the network was fragmented into a billion separate pieces—individual cells that were not continuous one with the next.

Cajal often sketched the nerve cells he'd observed under the microscope. His drawings show knobs with branches at one end and a single long straight tendril running from the other. He guessed that the end with the branches received signals from other cells, while the lone protruding thread sent signals. Cajal was right, of course. But the story of how nerve cells communicate across the gaps that divide them is still being written today. A recent finding by Zuo-Zhong Wang, an assistant professor of neuroscience at the University of Pittsburgh, adds a new twist to the evolving tale.

In the decades since Golgi and Cajal offered their disparate views, scientists have established the basics. Electricity generated in the body of the cell travels down a single long protrusion (the axon). At the end of the axon are many tiny sacs of chemicals. When stimulated by electricity, the sacs spill their contents into the space between one cell and the next. The chemicals go to receptors on the branches (dendrites) of the next cell, where they may stimulate the production of a new electrical signal.

Today, researchers know that most mental illnesses are linked to problems with the transmission of signals between neurons. Many toxins—including the gas sarin, some kinds of snake venom, and the poison that causes botulism—disrupt communication between nerve cells; this can lead to paralysis, and even death. A better understanding of how neurotransmission works could lead to better treatments—so scientists strive to work out, at the molecular level, how signals travel from one nerve cell to the next.

But the brain is not easy to study. It contains 200 billion nerve cells. Each cell has hundreds or thousands of dendrites, branching like trees, and its spindly axon can extend for as long as a foot. The brain is a tangled web of crisscrossing dendrites and axons. It is difficult to distinguish which hairlike protrusion belongs to which knobby cell body or where a given signal begins and ends. To add to the complexity, a single neuron, at a single instant, can receive chemical messages from as many as 10,000 other cells. And there are more than 30 different chemicals that traverse between cells. Because of the brain's extraordinary intricacy, Wang decided to study muscle, which offers a simplified version of the connection between nerve cells.

Nerves in the spinal cord control our muscles. One axon goes from the spine to each cell in the muscle, forming a neuromuscular junction or synapse. Here there is no thicket of neuronal parts, but a single axon and a single chemical, acetylcholine, to carry the nerve's signal. There are no dendrites, but acetylcholine receptors become clustered near the axon.

Wang, a PhD neurobiologist, is interested in how these synapses form as an embryo grows. During development, before the axon reaches out from the spine, the acetylcholine receptors are spread out all over the surface of the muscle cell. Once the axon arrives, however, the receptors move until they are all clustered at the site of the axon, which rests just over the cell. If the axon is cut or the nerve is destroyed, the receptors will once again spread out all over the surface of the cell.

What brings the receptors to the axon (the process is called aggregation) and keeps them there? Wang certainly would like to find out. “We believe that the principles derived by studying the neuromuscular junction can be applied directly to the central nervous system synapses, because structurally they’re very similar,” he says.

To uncover proteins that are likely to be involved in the aggregation process, Wang and his lab did a three-month-long experiment. After transfecting yeast cells with mouse muscle DNA, they discovered that out of the 20,000 muscle proteins in mice, at least 14 interact directly to the central nervous system synapses, because structurally they’re very similar,” he says.

And yet he never expected to find the protein playing a role in neurotransmission. The APC protein was discovered about 10 years ago when researchers were studying a family with a rare form of inherited colon cancer. The gene that was mutated in this family, and which caused their disease, expressed for a single protein, the adenomatous polyposis coli—associated protein, or APC protein. It would be easier to study than lesser-known proteins, because so much work had already been done on it.

The APC protein is found in nearly every cell in the human body. What was its role in non-colonic cells? No one knew. “Nobody has ever thought that it’s involved in neurotransmission,” says Wang. His paper linking the APC protein to receptor aggregation was published in Nature Neuroscience in October.

“I’m excited that we are at the stage that we have the ability to solve something that was a mystery to people probably a couple of decades ago,” says Wang.
MIGRAINE-RELATED GENES UNEARTHED

By Cara J. Hayden and Dottie Horn

To the naked eye, the transparent worms look like dust motes made visible by a beam of sunlight. Under the microscope, as he watches them wriggle, squirm, and slink, Miguel Estevez can see through their skin to their organs. A Pitt assistant professor of neurology, Estevez can see that these worms are uncharacteristically sluggish because they suffer from a worm equivalent of migraine that impairs their motor coordination.

As Estevez observes the lethargic worms, a white flaming light flashes in his right eye. The light jags and squiggles, crawling along his retina like the magnified worms, and spreads across his vision. He is experiencing the visual aura that often precedes a migraine attack. For him, the auras are an annoyance, a common occurrence when he is stressed and sleep deprived. For others, the auras warn of days of pain. “According to my mother, she has never had a day when she was free of headaches,” says Estevez. (Migraines affect a quarter of the world’s population.)

Scientists know that migraine is caused by multiple genetic defects; and it’s difficult to locate the particular genes that contribute to the disease. However, in the ‘90s, the Dutch Migraine Genetics Group studied a family with an extremely rare form of the disease, called familial hemiplegic migraine (FHM), which is caused by a defect in a single gene. People with FHM not only have headaches, but also lose coordination or become paralyzed during attacks of the disease. In 1996, the Dutch group identified the gene responsible for the family’s disease—it codes for the CACNA1A calcium channel in the brain.

Identifying the calcium channel, says Estevez, was a “first insight” into what goes wrong in all migraine.

For decades, scientists have known that in most adults migraine is caused by a deficiency of serotonin, a brain chemical involved in mood and memory, but they have not known what causes low serotonin levels. After the 1996 discovery, researchers explored the molecular steps leading to low serotonin levels. Understanding those steps would likely direct them to other genes involved in the disease.

To dig deeper, Estevez turned to the transparent worms known as Caenorhabditis elegans. (In a sense, this worm has won a Nobel. The 2002 prize went to three scientists who used the worm as a model to study the genetic regulation of organ development and programmed cell death.) Estevez chose the worms because of their genetic similarity to humans and rapid life cycle (they grow from egg to adulthood in two days). His first step was to create a worm model of FHM—he knocked out the worm equivalent of the gene defective in the FHM family. Although it is impossible to say whether the worms have headaches, they definitely have low serotonin levels and coordination problems. Estevez quantifies a worm’s level of coordination by prodding it and timing how long it takes for it to complete one S-shaped crawl. Normal worms are five times faster than the knockout worms.

In the next step, Estevez took 50,000 knockout worms and treated them with a toxic chemical (ethyl methane sulphonate). The majority of the worms would be unaffected, but in some, the chemical would induce a random mutation in a single gene. He then let the 50,000 worms (they are hermaphrodites) reproduce. Each worm has 200 to 300 offspring, so Estevez wound up with some 12 million worms. About 1,200 of them had random single mutations.

Estevez then screened the mutants and identified about 20 mutations of interest. These were mutations that caused the knockout worm, which once had an abnormally pokey crawl, to slink faster and also to have improved serotonin levels. In other words, the mutation caused the worm’s FHM symptoms to improve. This indicated that the mutated gene was able to compensate for the knocked-out calcium channel that otherwise would have led to a low serotonin level. It appears that the mutated gene might also be defective in some people who suffer from migraine.

Out of those 20 odd mutations of interest, one was of a TGF-beta (a growth factor) receptor gene. Estevez’s additional experiments confirmed that, in worms, the receptor is involved in the process by which the CACNA1A calcium channel affects serotonin levels. “[It] is intriguing, since human families with mutations in a TGF-beta receptor gene can actually have migraine with aura.

“We’ve used the worm for the invertebrate studies, which are relatively easy and quick, to give us a short list of candidate genes.”

Next for Estevez: Begin searching for the human equivalent of these genes in families with migraine.
Every year, hundreds of thousands of pregnant women in the United States go into premature labor. Their water breaks, or they realize that the backache they’ve been nursing with Tylenol is actually labor, and they are rushed to the hospital, where they give birth three weeks or more short of the normal 40 weeks of gestation. Those who give birth before 28 weeks have babies who often either die or have huge health risks—they may be born blind or with cerebral palsy, respiratory distress syndrome, or other conditions.

No one knows what causes most premature births, though they account for 12 percent of live births and are the leading cause of morbidity and mortality for babies in the United States. However, clinicians at Magee-Womens Hospital and a group of other academic hospitals have finally found a treatment that helps to prevent premature labor in the most susceptible of the child-bearing population—women who’ve had a previous preterm birth.

For years, researchers have known that progesterone helps maintain pregnancy in animals. More than 40 years ago, studies involving periodic blood tests showed that progesterone levels steadily drop as animals approach their delivery dates. When this was discovered, researchers assumed that the same hormonal pattern would apply in humans, but daily blood tests in pregnant women didn’t show the same ebb of progesterone before delivery. Despite this, a few studies in the 1960s tried treating pregnant women susceptible to early labor with progesterone. The studies showed promise, but they were small and largely discounted.

Then, in the early 1980s, Congress mandated that a portion of federal research dollars be directed to clinical studies, as some members felt that basic science labs weren’t delivering enough results that applied directly to human health. The National Institute of Child Health and Human Development decided to spend this money to create a research network of clinicians from various medical centers, called the Maternal Fetal Medicine Units Network. The network would include hospitals serving large numbers of pregnant women with complications, to ensure they would get sample sizes that were scientifically stringent, explains Steve N. Caritis. Caritis (Res ’73) is a professor of obstetrics, gynecology, and reproductive sciences at the University of Pittsburgh School of Medicine and director of maternal-fetal medicine at Magee.

In the late 1990s, network researcher Paul Meis from Wake Forest University proposed a randomized placebo-controlled trial that would revisit the tantalizing promise of progesterone hinted at in those trials in the 1960s. Eventually, the new trial involved Magee, 18 other medical centers, and 463 pregnant women who’d had at least one previous preterm birth. The women were enrolled when they were between 16 and 20 weeks gestation. Two-thirds received weekly intramuscular injections of progesterone, and one-third received injections of a placebo. The results were dramatic: The women receiving shots of progesterone were a third less likely to go into premature labor. Further, the infants born to women receiving progesterone weighed more on average and were less likely to require supplemental oxygen.

“This is the first treatment that has ever reduced the rate of preterm birth,” says Caritis, one of the investigators on the study, which was published June 12 in The New England Journal of Medicine. “Nobody knows why it works.” Physicians around the country are now giving progesterone to pregnant women who have had a previous preterm delivery.

Caritis and Dwight Rouse from the University of Alabama at Birmingham are beginning a follow-up study this fall. Fourteen centers will test progesterone on another group at high risk for preterm labor: pregnant women carrying twins and triplets.

For Caritis, the progesterone breakthrough highlights the power of these networks of clinical researchers. “This study could never have been done at any one center,” he explains. “Even at Magee, with 8,000 deliveries a year, it would have taken me 20 years to recruit 463 women.”