Neuronal corrections are notoriously tough to track, but Peter Strick has a trick: He uses the rabies virus. It moves from one neuron to the next in a predictable time span, which makes it handy in Strick’s studies of the connections between brain regions. Here, primate neurons in the cerebellar cortex are infected with rabies virus.
TRACINGS
IN THE BRAIN

RABIES HELPS SCIENTISTS UNDERSTAND
HUMAN DEXTERITY AND PARKINSON’S

BY MELINDA WENNER MOYER

There’s no easy way to peer inside the brain and study its complex, intermingled circuits. But Peter Strick, University of Pittsburgh professor in the Departments of Neurobiology and Psychiatry and codirector of the Pitt–Carnegie Mellon University Center for the Neural Basis of Cognition (CNBC), has a trick: He employs viruses. With their help, he recently discovered a division in the human motor cortex that could explain why we exhibit more finely tuned movements than other animals. He has also uncovered a surprising connection between two brain regions that solves a mystery about Parkinson’s disease.

For decades, neuroscientists studying how neurons connect to one another have had to rely on tracers “that were good, but not optimal,” says Strick. A dye injected into a neuron might reveal the immediate neuron it connects to, but it loses its potency before revealing the next neuron in line. But “that’s not the way the brain works,” Strick explains. “It’s not just who speaks to the neuron and who the neuron then speaks to. These brain areas are parts of circuits.”

Deciphering these circuits, Strick says, is the first step toward understanding how humans acquire complex skills. Our ability to learn to play a Chopin waltz—granted, after lots of practice—“is so fundamental to who we are, and is what differentiates us,” Strick explains. “We start by asking what the [brain’s] road map is. And viruses are a wonderful way of working out that road map.”

Strick’s virus of choice is rabies, which moves from neuron to neuron. What’s useful about rabies is that it replicates in each neuronal cell body, so it doesn’t fade over time. It also moves backwards, tracing a path from, say, a muscle cell, through multiple neurons, eventually reaching the cerebral cortex.

A couple of years ago, Strick and collaborators injected rabies into the shoulder, elbow, and finger muscles of rhesus monkeys and gave the virus enough time to infect neurons two steps back toward the brain. (The virus does not cause these animals any pain or distress.) Strick saw that the virus was able to reach the animals’ brains by traveling through a motor neuron and then a special neuron in the spinal cord. It had infected the motor cortex, the portion of the cerebral cortex responsible for planning and executing movement, but only on the right side. When he injected the virus into the same muscles of different rhesus monkeys and gave the virus enough time to infect not two but three steps back, he also saw the virus in the left half of the motor cortex. It had traveled along two motor neurons on its journey.

Earlier work by other researchers revealed that humans and rhesus monkeys—but not cats and rats—have some brain-to-muscle connections via just one neuron. This finding suggests that these “express” connections emerged more recently in evolutionary history than the less direct ones and might be responsible for complex movements. Strick’s study, published in 2009 in the Proceedings of the National Academy of Sciences (PNAS), suggests that these new and old connections are also physically separated in the brain. The “new” motor cortex most likely coordinates finely tuned movements, and the “old” cortex is probably responsible for crude movements. It’s likely that “the old motor cortex gets our movements within the right range, and then the new motor cortex supplies the elegance and all of the adaptability and flexibility,” he says.

Strick and his colleagues have also used rabies to discover new connections between brain regions. In a May 2010 PNAS paper they showed that the cerebellum, a brain region involved in motor control and motor learning, communicates with the basal ganglia, a group of forebrain regions involved in movement and habit formation. Malfunctions in the basal ganglia are also thought to be responsible for producing tremors in patients with Parkinson’s disease.

Earlier research by other scientists had shown that Parkinson’s patients have abnormal activity in the cerebellum, but no one knew why. When Strick’s colleagues injected rabies into the cerebella of cebus monkeys, he saw that they connected to the basal ganglia by way of two neurons.

“It’s not a direct connection; that’s why it wasn’t seen with conventional tracers,” he says. Strick believes that the basal ganglia are sending abnormal signals to the cerebellum, which in turn is either trying to correct for them or is relaying them. If the latter is true, the cerebellum may be more directly responsible for Parkinson’s tremors, a finding that could have important implications for treatment. The cerebellum “could either be the problem or the solution,” Strick says.
Say you’ve made it through the stress of a doctoral program, postdoctoral work, and a rigorous interview process. You’re almost there, right? Once you land your first faculty position, you can finally enjoy the reward you’ve been working toward: your very own research.

Not so fast. When it comes to starting an independent research career, the first step can be an overwhelming leap.

“Most people ... are expected to generate funding fairly rapidly once they arrive,” explains Michael Butterworth, a PhD assistant professor in the Department of Cell Biology and Physiology in the University of Pittsburgh School of Medicine. “Setting up your own lab is a daunting enough experience—to have to then get a grant [application] out the door in the first few months is very difficult.”

Butterworth officially started his lab in April of 2010. He studies the regulation of channels that transport salt in the body and the mechanisms responsible for trafficking these channels within cells of the kidney and the airway—work that might eventually have implications for hypertension and cystic fibrosis. Butterworth’s transition to independence was slightly smoother than average, thanks to a new award from the National Institutes of Health (NIH).

On average, most PhDs don’t win their first NIH Research Project Grant (aka R01 grant) until age 42; for MDs and MD/PhDs it’s 44. To help up-and-coming investigators start their careers sooner, in 2006 the NIH introduced the Pathway to Independence awards, or K99/R00 awards. The two-phased award serves as a bridge between a candidate’s postdoctoral career and an independent research position.

The first phase, the K99, allows the scientist to work closely with a mentor as a postdoc for up to two years and provides up to $90,000 of funding. The goal is to work with a well-respected senior advisor to develop a solid research plan and, equally important, build confidence and skills as a researcher. At the end, the candidate has the option to apply for the second phase, the R00 award; it provides three years of funding worth up to $250,000 for the candidate’s independent research.

For PhD Michele Okun, one of the first Pathway to Independence grant recipients nationwide, having the time to develop skills before starting her research has been a big help. “It took some of the stress off,” she says. “I thought that was a much better approach.”

A Pitt assistant professor of psychiatry in her second year of the independent phase, Okun studies how disturbed sleep affects the immune system in the early stages of pregnancy. She has already found promising connections that she hopes will eventually help reduce such complications as preeclampsia and preterm birth.

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Among those in Pitt’s growing roster of Pathway to Independence awardees: Youko Ikeda, Heth Turnquist, Brian Hermann, Indrani Halder, and Marsha Cole. Not pictured: Michael Butterworth, Michele Okun, Cory Robinson, Alejandro Soto-Gutierrez, Hong Wang. The cellular images (top, human; bottom, mouse) are from Butterworth’s studies. He’s probing how epithelial sodium cells are regulated in the airway.

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**THE BIG LEAP**

**YOU NG PIT T**

**SCIENTISTS START**

**OUT STRONG**

**BY TIFFANI EMIG**

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Preeclampsia, the most common of the serious pregnancy complications, could be thought of as the Great White of childbirth. Obstetricians and midwives keep a keen eye out for its signs—namely, high blood pressure and protein in the urine after the 20th week of pregnancy—because when preeclampsia strikes, it can spell disaster. It is a major cause of maternal morbidity and mortality worldwide, occurring in about 5 percent of pregnancies. In preeclampsia, the mother’s inability to deliver blood, oxygen, and other nutrients can lead to hypoxia for the fetus. The only known cure is to deliver the baby, either by induced labor or caesarean. Fetuses in these pregnancies have a five-fold increased risk of stillbirth.

The seizures that overcome some preeclamptic women were described in the first century, yet to this day, no one knows exactly what causes preeclampsia, or even if it’s one single disease or several that present with similar symptoms.

“Where the disease starts is really still a mystery,” says Carl Hubel, University of Pittsburgh associate professor of obstetrics, gynecology, and reproductive sciences, and of environmental and occupational health.

Hubel and other Pitt researchers are beginning to piece together what exactly preeclampsia is, in part by looking at what happens to preeclamptic women after they give birth, sometimes decades later.

Pitt’s Janet Catov, assistant professor of obstetrics, gynecology, and reproductive sciences as well as of epidemiology, found mothers who had elevated levels of lipids, a risk factor for heart disease later in life, had a two- to three-fold increase in the risk of preterm birth, which can include preeclampsia.

The link with heart disease stirs up a greater question: Does preeclampsia cause heart disease, or does it simply unmask an underlying deficit in these women? Sorting out this question is complicated by the fact that many of the risk factors for preeclampsia—e.g., obesity and high blood pressure—are the same for heart disease. Chicken, meet egg.

Add to this picture the extreme metabolic changes that occur during pregnancy—a woman’s cholesterol and triglycerides increase by 50 to 300 percent as her body builds new blood vessels and a whole new organ, the placenta. She is slightly insulin-resistant, storing more sugar for the fetus. “At midpregnancy, a woman’s lipids and her glucose and insulin levels look like someone with moderate heart disease—and all of that is totally normal,” says Catov.

So what exactly determines which women will suffer from preeclampsia?

Hubel and his collaborators are looking at the blood of pregnant women to try to find out. Senior scientist and founding director of the Magee-Womens Research Institute James Roberts showed in the 1980s and ‘90s that deficiencies in the vascular endothelium, which helps regulate blood flow, were a major feature of preeclampsia. Hubel has since found preeclamptic women have fewer endothelial progenitor cells (EPCs)—a kind of stem cell that helps replace dying or damaged blood vessel cells—than women experiencing normal pregnancies. These cells are crucial in secreting growth factors and other agents critical for vascular function.

Researchers at Harvard University recently discovered that the placentas of preeclamptic women produce an overabundance of circulating targets for the growth factors that produce EPCs. Normally, the growth factors would bind onto targets on the cell membrane; instead, Hubel and his colleagues theorize that the growth factors bind with circulating targets dispersed throughout the bloodstream. If the growth factors get bound up on dummy targets, these women might not produce enough EPCs, ushering the storm of vascular and placental setbacks that characterize preeclampsia.

He cautions that these developments may be fruit of the same rotten tree—perhaps EPC and growth-factor target levels are a consequence of preeclampsia, not the cause. Bit by bit, however, researchers are beginning to make out the broader outlines of the disease.