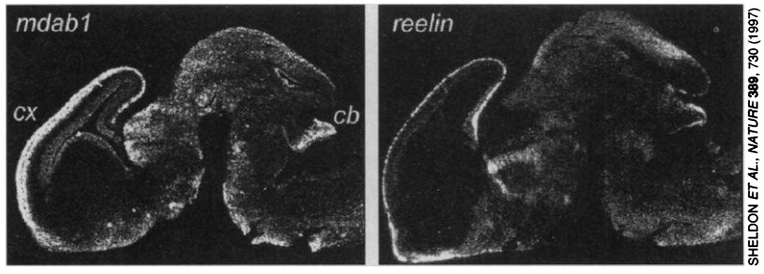
NEUROBIOLOGY

# Researchers Find Signals That Guide Young Brain Neurons

Building a brain during embryonic development is no easy matter. Like fans searching for their seats in an overcrowded football stadium, newborn nerve cells trying to find their final destinations in the complex layers of the brain have to navigate a tortuous route, traveling through hoards of other neurons while constantly staying on the alert for the signposts that will direct them to their own sections and seats. Neurobiologists had few clues to the molecular machinery guiding these neuron migrations until 2 years ago, when they discovered a protein that seems to act as a signpost for some neurons. Now, they have found another protein that may help the neurons read that sign.




SHELDON ET AL., NATURE 389, 730 (1997)

**A dab here and there.** mDab1 is made in migrating neurons in the cerebellum (cb) and cortex (cx) in developing mouse brains. Reelin is made at their destinations.

The apparent signpost is the product of a gene called *reelin*, recently identified as the mutant gene in a strain of mice dubbed *reeler* because of their reeling gait. The mutated *reelin* gene causes the mice to have partially scrambled brains in which many neurons don't make it to their proper destinations. Because Reelin, the protein product of the gene, is released near the destination of those migrating neurons, researchers hypothesized that its normal job is to somehow help them reach their final home in the brain.

In the new work, reported by Chris Walsh of Harvard Medical School in Boston and his colleagues in the August issue of *Neuron*, and by teams headed by Tom Curran of St. Jude Children's Research Hospital in Memphis, Tennessee, and by Jonathan Cooper at the Fred Hutchinson Cancer Research Center in Seattle in this week's *Nature*, researchers have uncovered a protein that may work in partnership with Reelin. The protein, called mDab1, is made by a gene that is mutated in mice known as *scrambler*, which have a behavior and brain disorganization similar to those of *reeler* mice.

That resemblance between the two mouse strains, plus the results so far with mDab1, raise the possibility that the new protein is part of a signaling pathway triggered by Reelin. Researchers have found, for example, that mDab1 interacts with the well-known intracellular signaling enzymes known as tyrosine kinases. And the protein is located in the right cells: the migrating brain neurons that respond to Reelin.

Neurogeneticist Karl Herrup of Case Western Reserve University in Cleveland cautions that it's far from certain that Reelin and mDab1 work together in the same cascade of molecular signals. Still, he says, the findings are "extraordinarily exciting," because they provide "genetic handles on the process of moving neurons." Those handles could ultimately help researchers decipher the cellular biochemistry that controls the movements of young neurons—and perhaps understand how those movements are disrupted in certain rare human genetic conditions that result in a seriously disorganized brain (*Science*, 15 November 1996, p. 1100).

The path to the current findings began nearly 50 years ago, with the discovery of the *reeler* mutation in mice. Studies of the brains of *reeler* mice revealed that neurons in both the cerebral cortex and the cerebellum—a brain area important for motor coordination—pile up in jumbles rather than migrating to their proper locations. Researchers were clueless as to the molecular cause of this defect until 1995, when Curran and his colleagues identified the mutant gene.

Analysis of the normal gene's protein product, which they called Reelin, revealed that it is made and secreted by cells in the vicinity of where the abnormal neurons should end up in the brain. Apparently, under normal conditions the protein helps guide the movements of the traveling neurons. But beyond that, Reelin gave no hints about how it exerts its effects. "We couldn't connect it with any biochemistry," Curran says.

So researchers turned to another mouse mutation, *scrambler*, discovered a few years ago by Muriel Davisson at Jackson Laboratory in Bar Harbor, Maine. Mice with the *scrambler* mutation behave like *reeler* mice, and their brains look the same too, with piled-up neurons that never reach their destinations. Perhaps, researchers hoped, the protein product of the mutant gene would turn out to be the Reelin receptor or some other molecule that the migrating neurons require to follow Reelin's command. Curran's team, as well as Walsh's group in collaboration with Andre Goffinet of the FUNDP School of Medicine in Namur, Belgium, independently set out to find the *scrambler* gene, using a technique called "positional cloning," in which genetic crosses between mice are used to identify ever smaller chromosome regions containing the mutant gene. By early this year, after much hard work, both teams were close.

But then, in a serendipitous discovery, Brian Howell, a postdoc with Cooper at the Hutchinson Center, learned that a gene he was studying is in fact the one mutated in *scrambler* mice. Howell was not looking for the *scrambler* gene, but instead was searching for mouse proteins that bind to the tyrosine kinase Src, an enzyme that transmits signals inside the cell. He found one such protein, encoded by the mouse version of a fruit fly gene called *disabled* (*dab*), which causes flies to have defective nervous systems.

To see whether the mouse gene, which they called *mdab1*, might also affect neural development, Cooper's team created mutant mice missing the gene. Surprisingly, the animals behaved like *reeler* mice, and had similarly scrambled brains, making the team wonder whether the loss of mDab1 protein could somehow block Reelin production. But tests showed that the animals make normal amounts of Reelin. "That led us to the more exciting model that mDab might be acting downstream of Reelin," Howell says.

Aided by news of the Cooper team's finding, postdoc Mike Sheldon in Curran's group, which had begun a collaboration with Cooper's, and a separate team including graduate students Marcus Ware and Jeremy Fox in Walsh's group, soon identified *mdab1* as the mutant gene they were closing in on with their positional cloning. Additional evidence for the role of mDab1 in *scrambler* mice came when Sheldon, collaborating with Katsuhiko Mikoshiba's team at RIKEN in Tsukuba, Japan, and the University of Tokyo, found a defect in *mdab1* RNA in the brains of mice with a mutation called *yotari* (for a Japanese word describing a drunken gait) that Mikoshiba's lab had identified. The Japanese group had already shown by mating experiments that the gene affected by the *yotari* mutation is the gene mutated in *scrambler* mice.

To learn more about how the gene influences neural migration, the Curran and Cooper teams investigated which brain cells make the mDab protein. The results were gratifying: "mDab is in the cell types affected in the *reeler* and *mdab* mutants," says Howell—the cells that normally respond to Reelin. "Therefore it is possible that [mDab] is acting in those cells to relay a signal."

Researchers now hope they can trace the pathway in which the mDab1 protein apparently acts. The protein's structure and ability to bind to Src suggest it is a "docking protein" that can link a tyrosine kinase like Src to another protein in a signaling pathway. The pathway may be triggered when Reelin binds to an unidentified cell surface receptor, but there's no evidence for that so far.

But even if mDab is not activated by Reelin, it is involved in neuronal migration, says Howell, and so may help researchers answer the question of what goes on in the migrating neurons to keep them on target, or, in the case of mutations, throw them off. "What is going wrong isn't that well understood," Howell says. "Having this protein that appears to be working [in the affected neurons] may be very helpful."

**–Marcia Barinaga**

ARCHAEOLOGY

# The Balance of Power in Ancient Ireland

**DUBLIN**—According to ancient texts, before Christianity came to Ireland about A.D. 400, the country was dominated by three principal kingdoms, the most powerful of which at any one time was the home of the "high" king or queen of Ireland. Their centers of power—at Navan Fort in what is now County Armagh, Northern Ireland; Tara, in County Meath, near Dublin; and Rathcroghan, in County Roscommon in the west—date back as far as 2500 B.C. The kingdoms' struggles for power and prestige are the stuff of Celtic legends that are deeply embedded in the modern Irish consciousness.

Archaeologists have focused most of their attention on Tara and Navan Fort. Rathcroghan, in contrast, has long been considered something of a poor relation. Many archaeologists thought it was less significant than the other two, and was built around a mound formed by nature, rather than by human excavation. Recent geophysical studies of Rathcroghan may, however, change perceptions about the balance of power in ancient Ireland. In its heyday, Rathcroghan may have been more impressive than its two rivals.

KEVIN BARTON/UCG

**Bull's-eye.** Magnetic survey shows ring structure measuring 20 meters across; fainter rings may have been earlier workings.

A 3-year study by researchers from University College Galway (UCG) has shown that the broad, flat, 7-meter-high mound appears to have been built for ritual purposes, and the enclosure surrounding it is in fact larger than those at Tara and Navan Fort. "Rathcroghan mound is spectacular. It is 90 meters across, and within the mound there are three concentric rings that may represent ring fort settlements from the early Christian period," says John Waddell, one of the team leaders. "Rathcroghan is the [royal] site we know least about. ... It is a spectacular site in terms of the mound and the structures that have been found," says Jim Mallory of Queens University Belfast, who has worked on the Navan Fort site.

The UCG project is the first study of Rathcroghan since archaeologist Michael Herity surveyed its topography in the 1960s. Rather than embarking on excavations straightaway, UCG geophysicist Kevin Barton carried out a detailed subsoil survey. Barton and his colleagues used standard geophysical techniques, including ground-probing radar and magnetic gradiometry, which measures the magnetic properties of subsoil materials, as well as a new tool in the surveyor's armory, electrical tomography. "We were the first in Ireland to use this technique," says Barton.

To carry out electrical tomography, the team placed metal electrodes into the ground and passed a current between them through the subsoil, measuring its resistivity, which varies depending on what it is made of. Using a large number of such measurements taken in different directions and at various depths, the team used computer modeling to construct vertical "slices" of the subsurface and then built these up into a three-dimensional image of the interior of the mound.

The geophysical survey produced a wealth of new information about Rathcroghan: evidence of ditches, walls, postholes indicating structures and fences, and different phases of building in the mound. "Geophysics has completely changed our interpretation of Rathcroghan mound," says Waddell. "We now know that it is a very complicated site with a prolonged history of human activity. ... The mound is, without doubt, [humanmade] ... indicating that a large amount of labor was invested here, suggesting an organized society with an element of leadership." Eoin Grogan, an archaeologist working for the Irish government's Discovery Programme who has studied the Tara site, says, "We now know that geophysics works. This is an impressive piece of research that has produced exciting evidence."

The size of the Rathcroghan complex was a big surprise. It is 370 meters from the middle of the central mound to a circular enclosure where the team found postholes, indicating the presence of a wooden perimeter fence. This is almost double the size of the 200-meter enclosures at Tara and Navan Fort.

Many of the team's discoveries are reminiscent of features found at Tara and Navan Fort and support archaeologists' earlier conclusions that these sites were used for important rituals, such as the inauguration or burial of kings and queens. For example, the team found, through the use of magnetic techniques, what they believe to be repeated burnings on and around the mound and also linear earthworks leading into the mound—similar to the "ritual roadways" found at Tara. In contrast to other large Celtic sites, there is no evidence of settlement within the enclosure in the pre-Christian period, suggesting that the huge area of open ground between the mound and the enclosure was used for large-scale rituals in pagan times. "We are now asking, 'What is the significance of the sheer complexity of Rathcroghan?' " says Waddell. "This will be one of the hardest nuts to crack. How do we interpret the beliefs of ancient peoples?"

This month, the team will present a final report on the study to the Irish Heritage Council, which funded the work, identifying areas for future digs. But Waddell says the prevailing mood is against rushing into excavations, as there is still much to be learned with geophysical techniques. Says geophysicist Andrew David of the British conservation organization English Heritage: "If these techniques are used judiciously in the future, I believe that Ireland has tremendous potential for new discoveries."

**–Sean Duke**

*Sean Duke is a science writer in Dublin.*