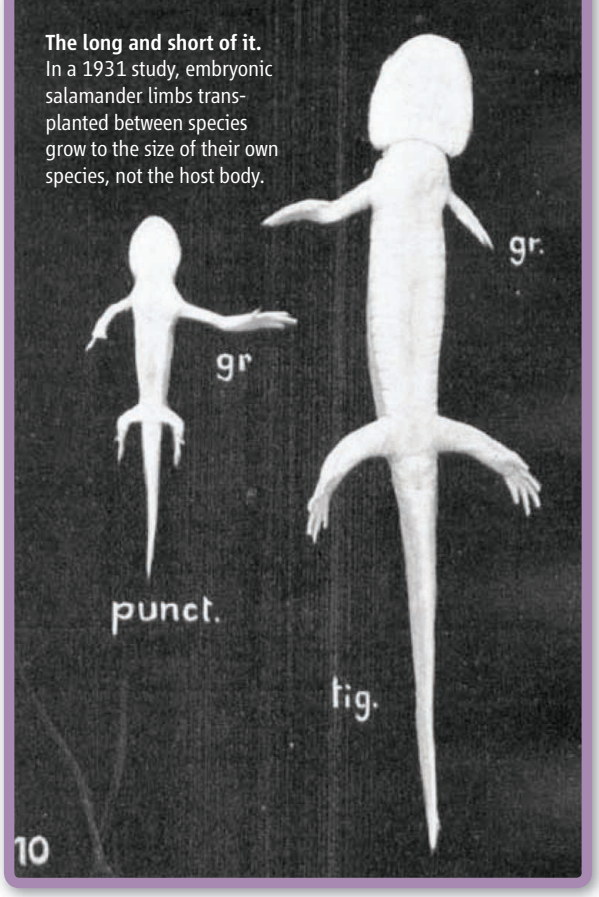


**The long and short of it.** In a 1931 study, embryonic salamander limbs transplanted between species grow to the size of their own species, not the host body.

grown heads have a wrinkled surface that resembles a hippopotamus hide.) Disruptions in the Hippo signaling pathway can lead to overgrowth, suggesting that it plays some role in controlling size. But recent studies suggest that it probably isn't the whole answer, says developmental biologist Georg Halder of the KU Leuven in Belgium.

Another set of genes that help with size sensing are ones that produce proteins called morphogens. These molecules originate from a single source in an embryo and diffuse across cells. They are best known for helping determine patterns during development. But some also influence the size of organs, tissues, and limbs. One theory is that when a morphogen gradient is steep—that is, there is a large difference in its concentration from one cell to another—then cells continue to divide. As cells divide, the gradient becomes more gradual. Once it has flattened out to a particular level, cells stop dividing.

There is also evidence that a cell's sense of which direction is up—called planar cell polarity—helps control growth. When certain genes involved in determining cell polarity are disrupted or missing, body parts tend to grow larger, suggesting that they can't sense when they should stop. "It's no coincidence that some of these polarity genes may act as tumor suppressors," Grewal says, and are suspected of playing a role in some cancers.

Many researchers suspect that a developing organ somehow senses the mechanical forces on its growing and dividing cells. One theory is that relative crowding and stretching of cells helps determine whether a cell continues to divide or stops.

The size of an organ depends not only on how many cells it has, but also how big those cells are. Some developing organs—plant leaves, for example, and fruit fly wings—can compensate when fewer cells are available by making the individual cells larger. How a leaf knows when to expand its cells is also unclear, says Hirokazu Tsukaya, a developmental biologist at the University of Tokyo who was among the first to characterize the phenomenon in leaves. He and his team have evidence that some sort of cell-to-cell communication drives the process. Here, too, the evidence suggests that a plant doesn't count cells but can somehow assess the overall size of a leaf, says plant biologist Beth Krizek of the University of South Carolina in Columbia. "But the mechanism of how that works is another mystery."

The size of tissues, and ultimately an overall organism, also clearly depends on signals from the environment, which researchers call extrinsic factors. Those size control systems are connected to, but different from, the intrinsic systems that help ensure an organism is correctly proportioned. In plants, growth can be especially sensitive to such outside factors, Krizek notes, because they can't move. Plants growing in shade, for example, concentrate on stem growth—to reach the sun—instead of leaf development. In animals, the amount of nutrition available can strongly influence the final size of some organs. One dramatic instance is the horn on a rhinoceros beetle. The horn is a sexually selected trait; males with bigger horns get access to more females. Recent studies have shown that the size of the horn is particularly sensitive to insulin signaling, which is related to the beetle's nutrition. That, in turn, signals the animal's overall fitness (*Science*, 27 July 2012, p. 408).

The problem of size control is still a fundamental one for developmental biologists, says Peter Lawrence of the University of Cambridge in the United Kingdom. Together with shape, size "is the material that evolution largely works on." But the field is still mostly in the dark. Despite hundreds of papers on what happens when the Hippo signaling pathway is interrupted, Lawrence notes, what scientists really need to understand is what it does when it is working properly. "That is not something we know." **–GRETCHEN VOGEL**

# Why Do So Many Neurons Commit Suicide During Brain Development?

With a few notable exceptions, the roughly 100 billion neurons we have at birth are the only ones we'll ever have. Unlike skin and immune cells, which continuously self-renew, once a neuron has differentiated from its parent stem cell it will never divide again. Given this finite supply, why do so many neurons—more than half in some brain regions—kill themselves during embryonic brain development?

For roughly 50 years, many scientists focused on a single explanation for this rampant cellular suicide. Their hypothesis was rooted in research on the peripheral nervous system, which connects the nerves of the brain and spinal cord to limbs, organs, and sensory systems. To survive in the developing brain, researchers thought, neurons must compete for limited quantities of a chemical "trophic" factor released by the targets they aim to innervate. Without this signal, the cells self-destruct in a process known as programmed cell death, or apoptosis. Called the neurotrophic hypothesis, the concept neatly explained how an overabundance of neurons could attach where needed, or be culled.

"We were all carried away by this observation," says neuroscientist Yves-Alain Barde of the University of Basel in Switzerland. Inspired by the discovery of nerve growth factor in the 1950s, a protein essential to the growth and survival of sensory and motor neurons in the peripheral nervous system, Barde hunted for a single "survival" molecule for neurons in







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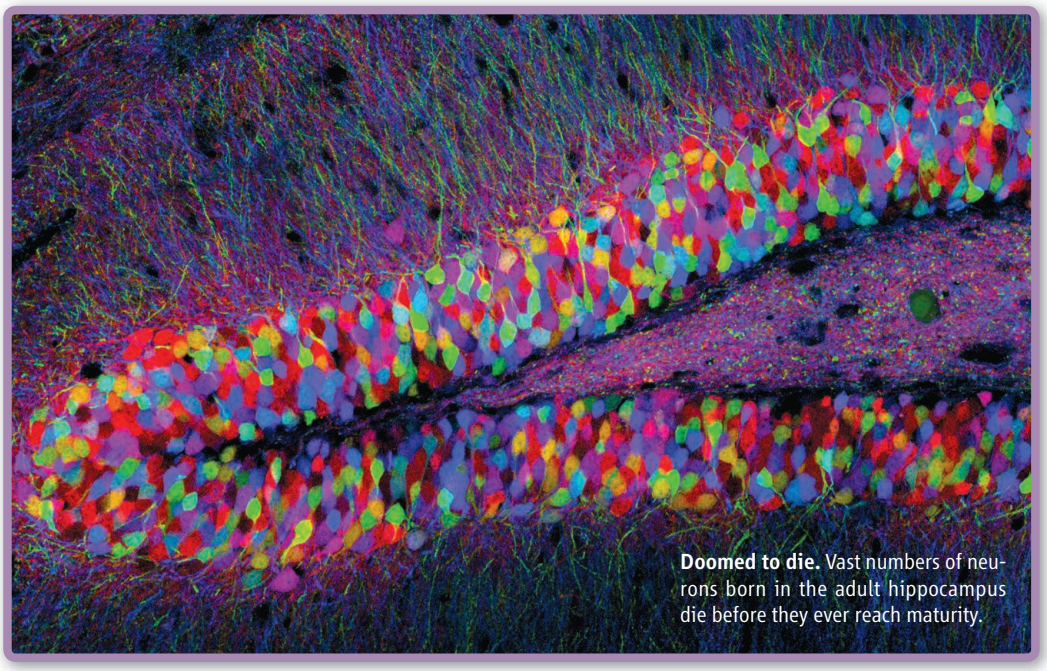

**Doomed to die.** Vast numbers of neurons born in the adult hippocampus die before they ever reach maturity.

the brain. Although he hoped that his discovery in the 1980s of brain-derived neurotrophic factor would be that molecule, "this turned out not to be the case," he says. The protein does promote neuron survival in some parts of the brain, but it is more widely involved in stimulating their growth and shaping their connections.

Today, researchers recognize that the neurotrophic hypothesis alone cannot explain why so many cells die in the brain. "There's a complexity that we didn't appreciate," says Kevin Roth, a neuroscientist at the University of Alabama, Birmingham, School of Medicine. From the moment a neuron is born, he says, the cell is influenced not just by trophic factors, but also by a barrage of environmental and genetic cues that will ultimately determine whether it ever becomes a mature neuron.

Thanks to the invention of antibodies that can detect neurons as they self-destruct, scientists have identified at least two, and possibly three, waves of neuronal cell death in the embryonic mammalian brain during development, each tightly regulated by different enzymatic pathways. The first wave strikes down cells before they are fully differentiated neurons. Evidence suggests that this stage of cell death helps sculpt the size and shape of the nervous system, says neuroscientist Rae Nishi of the University of Vermont in Burlington.

Interfering with this process can have serious consequences. For example, by blocking the activity of caspases, a family of enzymes that aids in killing off young neurons, "you get really ugly, big brains," and the embryos soon die, Nishi says. Some caspases appear to target embryonic neurons with extra or missing chromosomes, suggesting that the programmed cell death culls abnormal cells, according to recent work from neuroscientist Jerold Chun at the University of California, San Diego.

A second wave of cell suicide occurs after neurons have begun to differentiate and extend their axons to make contact with other cells. Although some researchers have tried to "shoehorn" the die-off at this later stage into the neurotrophic hypothesis, Nishi says, she believes her group's work on neurons that innervate eye muscles suggests that lack of a survival molecule is not what triggers death at this time. Supporting this idea are new studies on cells in the cerebral cortex called inhibitory interneurons. Derek Southwell, a neuroscientist at Stanford University in Palo Alto, California, and colleagues transplanted a group of developing mouse interneurons into tissue where similar cells had already established themselves. In mice, 40% of inhibitory interneurons normally die during development after they migrate out of their birthplace in the forebrain to the cortex. Precisely 40% of the transplanted cells also died, suggesting that they acted of their own accord and not in response to their environment, Southwell says. "It seems there is some kind of developmental clock that times this decision about survival versus death," he concludes.

This strictly programmed cell death could prevent abnormal cells from being cancerous or remove cells that served some transient role. The cells might also require direct stimulation from other cells to survive and die off if they are isolated, Southwell suggests. "The truth of the matter is that we have no idea."

Such massive neuronal suicide could also have no purpose. In some cases, "blocking cell death doesn't have any dramatic consequences," Barde says. In 2006, for example, neuroscientist Ronald Oppenheim at the Wake Forest School of Medicine in Winston-Salem, North Carolina, was chagrined at the result of creating mice with absolutely no programmed cell death after neuronal differentiation. (He and his colleagues blocked the activity of a gene necessary for apoptosis.) "The most striking thing was that despite having all these tens of thousands of excess neurons throughout the nervous system, [the mice] seemed really quite normal," he says. "That was an embarrassing revelation. I thought, why have I been spending most of my career studying this?"

Although the excess cells look like motor neurons and have axons, they don't work. "They just hang around," Oppenheim says. "You might call them undead." He suggests that the developing nervous system compensates for the extra cells by simply not linking them in to neural circuits. Another possibility is that the effects of excess cells are too subtle to easily detect, Nishi says, adding that similar studies that have prevented normal cell death during the brain's development in mice have uncovered heightened anxiety and defensiveness.

Understanding the brain's profligate ways has become paramount. Overturning conventional wisdom, scientists now know that adults generate new neurons throughout their lives in a few select brain regions, such as the hippocampus. Many of these newcomers have short lifespans. "Of the immature neurons born in the hippocampus [during adulthood], the vast majority die before they're ever integrated into any kind of circuitry," Roth says. "Any time you have neurogenesis, the counter side is cell death." For now, however, why these processes are so deeply intertwined remains a mystery.

**–EMILY UNDERWOOD**

**Online**
**sciencemag.org**
Podcast interview with editor John Travis (http://scim.ag/pod_6137).

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