

## **Secrets of the Human Brain Revealed**

### **Development, Disease & the Zika Virus**

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## **BREAKTHROUGHS IN BIOSCIENCE / SECRETS OF THE HUMAN BRAIN REVEALED: DEVELOPMENT, DISEASE & THE ZIKA VIRUS**

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### **ON THE COVER »**

Radial glial cells (gray) infected by Zika virus (orange)  
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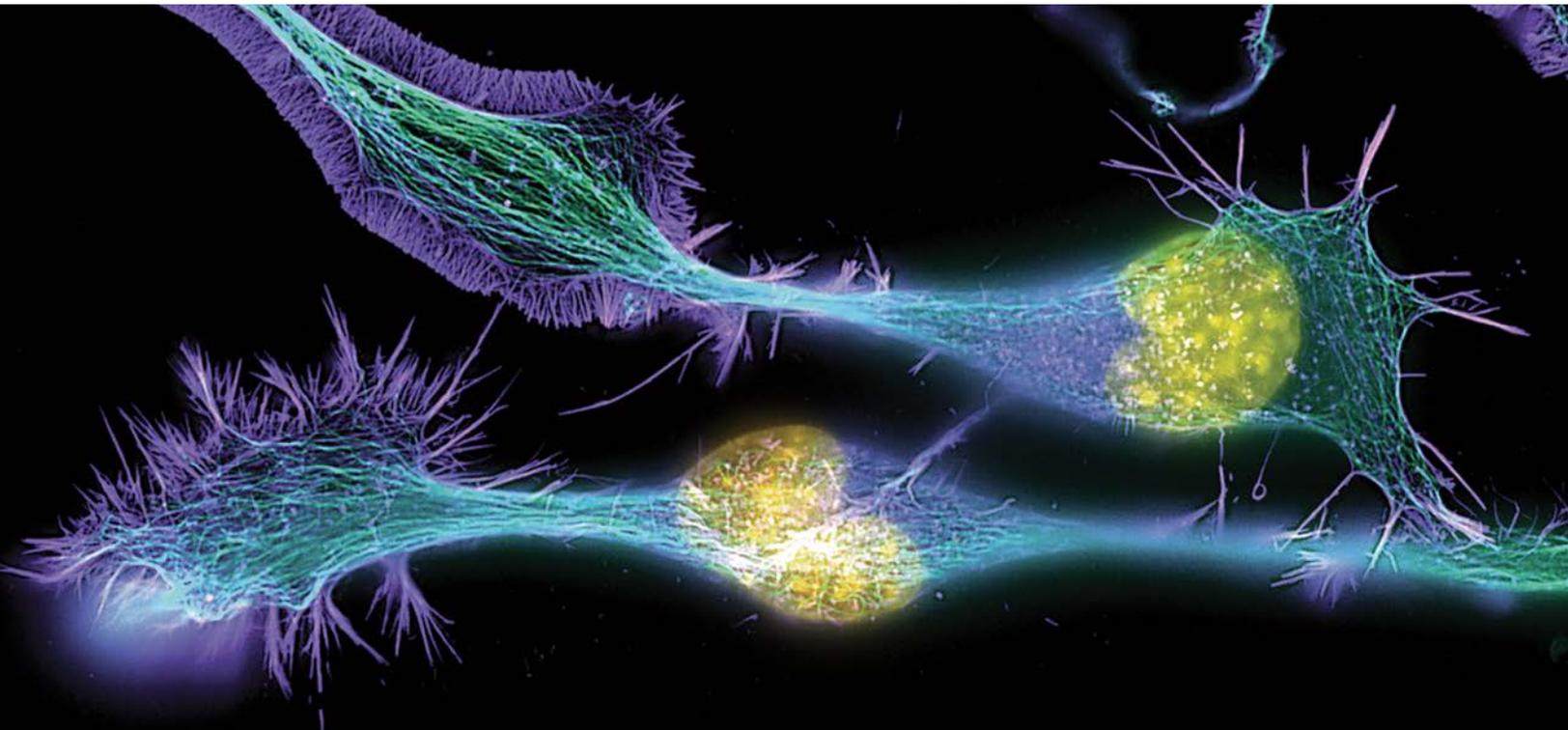
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# Secrets of the Human Brain Revealed

## Development, Disease & the Zika Virus



**IN THE SUMMER OF 2016,** many professional golfers, including the top-ranked in the world, decided not to participate in the Rio Olympic Games over fear of the Zika virus. The world's number one golfer at the time, Jason Day, stated, "The reason for my decision is my concerns about the possible transmission of the Zika virus and the potential risks that it may present to my wife's future pregnancies and to future members of our family." Other athletes used different strategies, including using mosquito netting, wearing mosquito repellent track suits, and even freezing sperm in advance of the games.

All of those measures seemed prudent because of a virus that, until early in 2015, was pretty much unknown. The mosquito-transmitted Zika virus, named for the forest in Uganda where it was first identified in 1947, is not usually serious. For most people, infection either causes no symptoms or leads to a rather mild illness

with a fever, skin rash, muscle aches, and conjunctivitis.

But after the virus started spreading across Brazil in 2015, doctors there noticed an uptick in the number of babies born with a condition called microcephaly. Microcephaly is a disorder in which babies are born with unusually small heads, and it is associated with

**DEVELOPING NEURONS IN MICE** » Neurons (blue) isolated from the mouse brain, with a nucleus (yellow) surrounded by a cell body and thin branching structures called dendrites (purple). *Image credit: Torsten Wittmann, University of California, San Francisco*

developmental delays, brain damage, and even death. In these cases, the babies did not just have small heads; the scale and severity of the brain damage was drastic. Large portions of the developing brain were shrunken and some parts did not form at all.

In studying newborns, as well as fetuses from miscarriages and stillbirths with microcephaly, researchers detected Zika virus in the amniotic fluid, the placenta, the umbilical cord, the fetal blood, and the fetal brain tissue. In 2016, both the Centers for Disease Control and Prevention (CDC) and the World Health Organization declared that there was enough evidence to support the conclusion that Zika infection in pregnant women causes microcephaly in infants. Further, CDC assessed over 2,500 pregnancies in women who lived in US territories from January 2016 to April 2017. They found that 1 in 20 women who became infected with the Zika virus during pregnancy had a baby with Zika-associated birth defects.

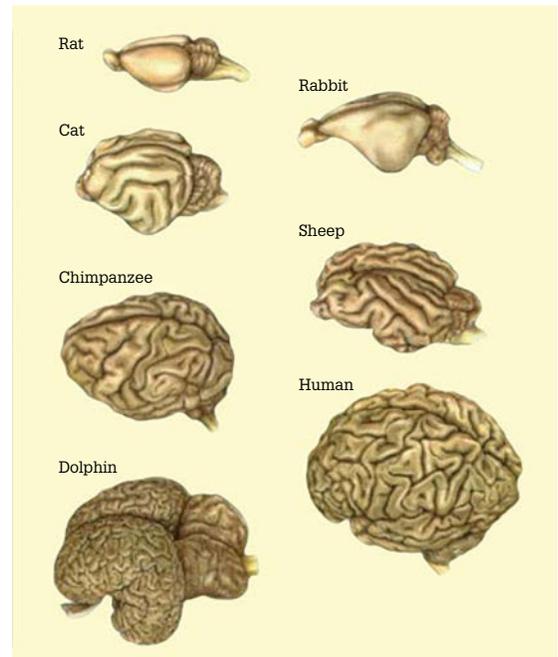
The Zika microcephaly crisis highlights what we know, and what we still have to learn, about how the human brain develops – as well as why this knowledge is so important to human health.

### THE HUMAN BRAIN

The human brain is estimated to have grown in size three-fold over the last two to three million years of evolution. Most of this increase is due to an expanded neocortex, a brain structure unique to mammals and especially enlarged in humans (Figure 1).

The neocortex supports advanced cognitive functions, including short-term memory, planning, and problem solving. The convolutions or folds of the brain that give it its wrinkly appearance allow more cortical tissue to fit into our skulls. This expansion in cortical surface area is associated with a dramatic increase in the number of neurons, the working cells of the brain.

The human brain uses a lot of energy. Even though the brain comprises about two percent of a person's total body weight, it is estimated to use 20 percent of the oxygen flow. The human brain is arguably the most complex structure on the planet weighing in at only three pounds. It consists of approximately 100 billion neurons (the information-processing cells of the brain) and probably five to ten times as many glial cells (the functional support cells of the brain). Each neuron can make connections with more than 1,000 other neu-

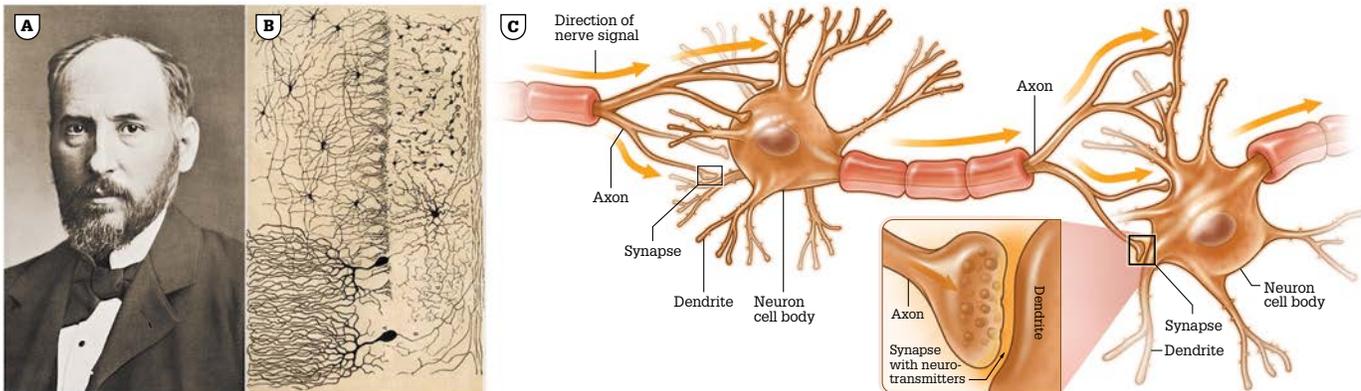


**FIGURE 1 / COMPARATIVE ILLUSTRATIONS OF CEREBRAL DEVELOPMENT IN DIFFERENT MAMMALS** (Brains are not to scale) Image credit: [www.thebrain.mcgill.ca](http://www.thebrain.mcgill.ca)

rons, so the adult brain is estimated to have more than 60 trillion neuronal connections.

Santiago Ramón y Cajal, a Spanish histologist and often called the “father of neuroscience,” studied these neuronal connections in depth. He used a special stain originally developed by Camillo Golgi, an Italian physician, to examine the brains of small birds, cats, and humans under the microscope. What he saw changed the long-held dogma concerning the structure of the neuronal network within the brain.

Scientists once thought the cells in the central nervous system formed a continuous network. Using the Golgi stain, Cajal demonstrated that the nervous system was made up of individual, separate cells and that information is passed from neuron to neuron through extensions of their cell bodies (Figure 2). These extensions lengthen and become the two major types of fibers or branches that allow neurons to communicate with other neurons – axons and dendrites. Axons send signals from neurons, while dendrites receive input from other neurons. Cajal and Golgi were awarded the 1906 Nobel Prize in Physiology or Medicine “in recognition of their work on the structure of the nervous system.”



**FIGURE 2 / THE BASIC ANATOMY OF THE NEURON »** (A) Santiago Ramón y Cajal, a Spanish histologist and often called the “father of neuroscience.” (B) One of Cajal’s many drawings of neurons using the Golgi stain. This drawing shows neurons from a chick’s brain. (C) Structure of a neuron. A typical neuron is made up of dendrites, which receive information from a neighboring cell and transmit it to the cell body; a cell body, which contains the organelles needed to keep the cell alive; and an axon, which transmits information to the next neuron. Synapses are tiny junctions between neurons where chemical and electrical signals are passed from one cell to the next. *Image credits: (A,B) Wikimedia Commons, (C) Illustration: © Mike Linkinhoker, Link Studio, LLC.*

Through their dendrites and axons, neurons communicate with each other via specialized sites known as synapses. Usually, the end of an axon forms a synapse with either dendrites or a neuron’s cell body. In most synapses, small amounts of chemicals called neurotransmitters are released, carrying messages from one neuron to the next.

Development of the interconnected nature of the nervous system begins around the third week after conception and extends into adolescence and even early adulthood. So complex are the challenges involved in growing a brain, that at least half of the genes in the human genome are put to work for brain development. However, genes cannot do the job alone. They need environmental influences to work with them to build the brain. This is why sensory stimulation is so important to babies’ development – why we talk to babies and make faces at them, even before they can understand us.

Over the past several decades, scientists all over the U.S. and the world have made huge advances in our understanding of how a baby’s genes, brain cells, and environment interact to build the brain, and how disruptions in these same factors can go awry to cause a wide variety of brain malformations and impairments.

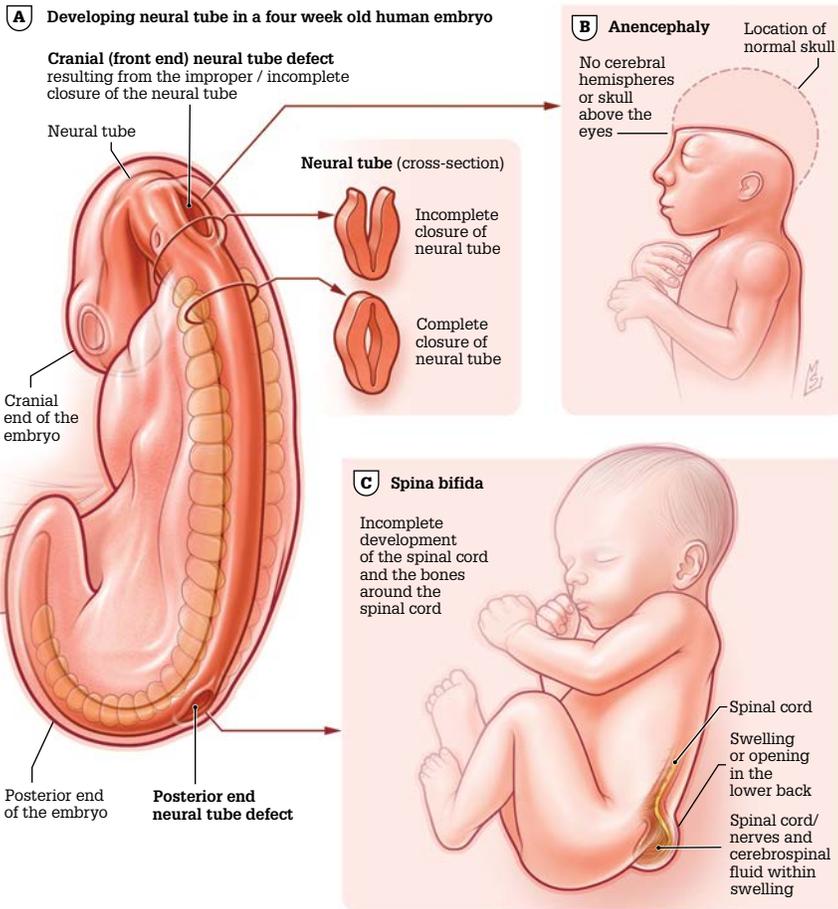
### EARLY NERVOUS SYSTEM DEVELOPMENT

Brain development proceeds in a series of major stages. These include the birth and differentiation of

neurons, migration of immature neurons from their birthplaces to their final positions, the growth of fibers between communicating neurons, the generation of synaptic connections, the naturally occurring death of many neurons, and the refinement of connections between neurons.

The first well-defined neural structure, the neural tube, forms during the third week of gestation. The process starts with the formation of a neural plate, which contains neural progenitor cells (NPCs) – a special class of stem cells that give rise to all the different cell types of the brain and spinal cord. The first sign of neural tube development is the appearance of two ridges or folds that form along the sides of the neural plate. Over the course of several days, the entire embryo lengthens as the ridges rise, fold inward, and fuse to form a hollow tube, like a straw. By the end of the fourth week of gestation, the open ends of the neural tube close off.

The failure of either end of the neural tube to close can lead to lethal deformities or lifelong disabilities. If the front end of the neural tube does not close properly, the result can be incomplete development of the cerebral hemispheres or brain stem. One devastating result of this can be anencephaly in which a child is born without cerebral hemispheres and often without any skull above the level of the eyes. Sadly, these infants usually die within a few days or weeks of birth. An incomplete closure of the posterior end of the neural tube leads to spina bifida, a birth defect of varying severity that caus-



**FIGURE 3 / ABNORMALITIES DURING NEURAL TUBE DEVELOPMENT »** Abnormalities can result in birth defects, such as anencephaly and spina bifida. *Image credit: Illustration: © Mike Linkinhoker, Link Studio, LLC.*

es problems in the spinal cord and the bones of the spine. In the most severe cases, the posterior portion of the spinal cord is totally or partially undeveloped and the entire lower back may be open. While some cases of spina bifida are treatable with surgery, others prove fatal for the infant (Figure 3).

**NEURONAL BIRTH & DIFFERENTIATION**

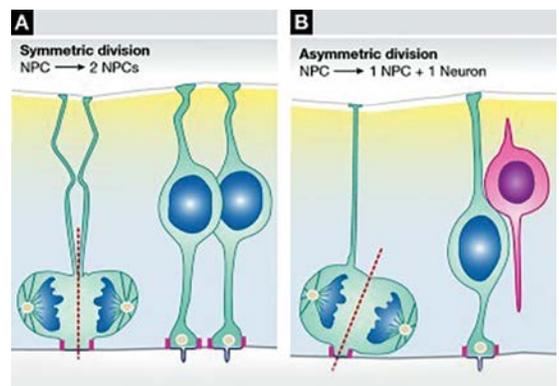
The production of neurons begins six weeks after conception, and the NPCs drive brain growth. In addition to its role in producing progenitor cells, the neural tube morphs into a series of interconnected cavities that form the brain’s ventricular system. During brain development, the ventricle walls are the site of most neuronal birth. As NPCs divide, decisions are made about the resulting cells’ fates. Some will become neu-

rons or glial cells, while others will continue to divide and produce more progenitor cells. Neurons and glia are differentiated cells, meaning their fate has been determined, and they cannot divide again to produce more NPCs.

There are two specific types of division – symmetric and asymmetric (Figure 4). In symmetric division, a cell divides producing two similar cell types (e.g., a NPC divides to produce two additional NPCs), while asymmetric division produces two different cell types (e.g., a NPC divides to produce another NPC and a neuron). Before the sixth week gestation, the population of NPCs divides by symmetric cell division. This expands the population of progenitor cells that will eventually produce the central nervous system. Beginning with the sixth week of gestation, however, the mode of cell division shifts to asymmetric. The new progenitor cell remains in the ventricular zone and continues to divide, while the neuron travels to take its place in the developing brain.

**NEURONAL MIGRATION**

Neurons may travel long distances to self-organize into all the structures of the brain. The process by which newly generated neurons migrate from their birthplace to their final position in the brain is known as neuronal migration. Much of what we know about this process comes from the work of Dr. Pasko Rakic, who was born



**FIGURE 4 / TYPES OF NEURAL STEM CELL DIVISION »** A neural stem cell can divide by symmetric division (A) in which it splits into two neural progenitor cells (NPC), or it can divide by asymmetric division (B) whereby the cell produces one NPC and one neuron. *Image credit: Reprinted from EMBO Reports, Judith TML Paridawn and Wieland B Huttner, Neurogenesis during development of the vertebrate central nervous system, Copyright (2014), with permission from Wiley.*

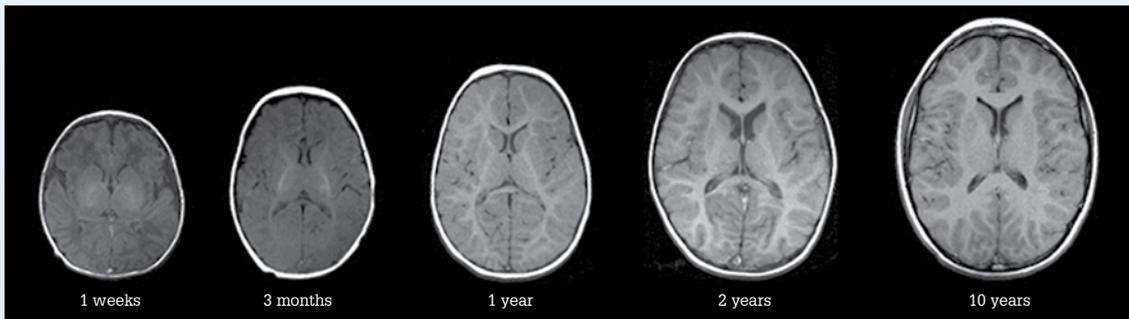
## INFLUENCE OF ENVIRONMENT ON BRAIN DEVELOPMENT

**THE PROCESSES THAT GUIDE** brain development involve ongoing interactions between genetic and environmental factors. Genetic programs played out within individual neurons underlie processes such as the birth of neurons, neuronal migration, and the guidance of axons to their targets.

The environment plays a larger role after axons

In addition to modifying synaptic connections, experience also affects the organization of the cortex. Maturation of the cortex requires diverse forms of input after birth, and the specific experiences of the individual shape the organization of the brain.

Both enrichment and deprivation dramatically affect the structure and function of developing brains.



**MRI SCANS SHOWING BRAIN DEVELOPMENT FROM BIRTH THROUGH YEAR 10.** Image credit: [https://pediatricmri.nih.gov/nihpd/info/image\\_gallery.html](https://pediatricmri.nih.gov/nihpd/info/image_gallery.html)

reach their target areas. Environmental input can come from a variety of sources, including the placenta, the mother's body, the physical environment, and sensory experiences.

Neural activity modifies and prunes synapses for an extended period after birth during which the brain continues to develop. During the pre-school ages, the brain experiences a four-fold increase in size, and by age six, it has reached about 90 percent of its adult volume. The number of synapses we possess in infancy far exceeds the number we ultimately end up with as fully-grown adults. Through experience, some synaptic connections are strengthened, while others wither away.

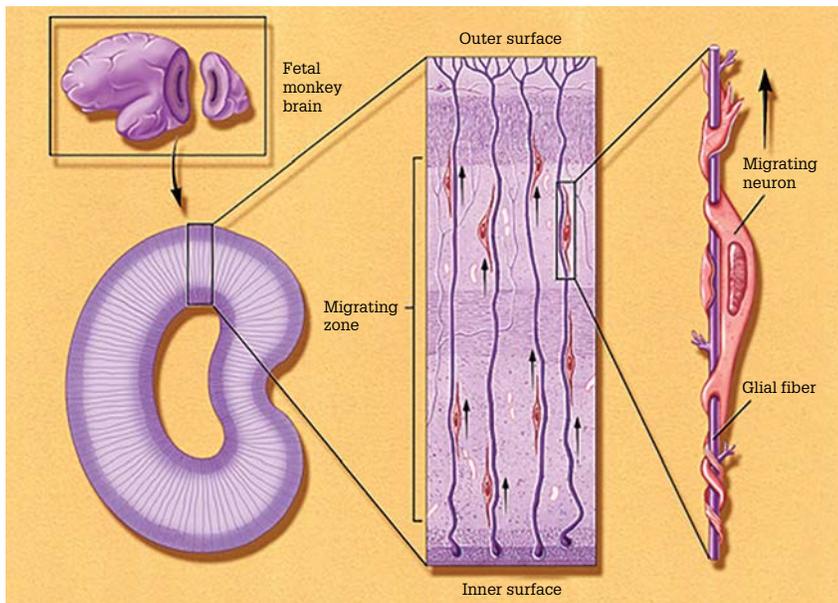
Studies have shown that animals reared in complex environments have denser cortical synapses and an increase in the number of glia, suggesting widespread effects of experience on the complexity and function of the developing brain. Sensory deprivation, on the other hand, has more selective effects, targeting particular cortical sensory systems. For instance, Drs. David Hubel and Torsten Wiesel, while studying at Johns Hopkins University and Harvard Medical School, showed that depriving cats of vision in one eye shortly after birth can alter basic patterns of organization within the primary visual area of the cortex. Thus, deprivation of one type of sensory input results in reorganization within the part of the brain associated with that particular sensory system. This holds true in humans, as well. If a baby's eye is injured or they wear an eye patch too long, their visual cortex will not be wired properly.

in the former Yugoslavia and worked at both Harvard University and Yale University.

In a major experiment funded by the National Institutes of Health (NIH), Dr. Rakic traced the birth and migration of neurons in the brains of fetal macaque monkeys during different periods of development. At the same time, he also studied these processes in

mice and humans in order to assess the similarities as well as the differences between organisms. These experiments led Dr. Rakic to discover that cerebral cortical neurons originate in the subventricular zone (an area adjacent to the ventricular zone where there is extensive neural progenitor activity) and migrate to their final positions.

The earliest born neurons do not have that far to travel. The neuron just stretches an extension of its cell body a little beyond the edge of the ventricular zone into the outer region of the brain compartment. Then the nucleus of the cell moves through the cytoplasm



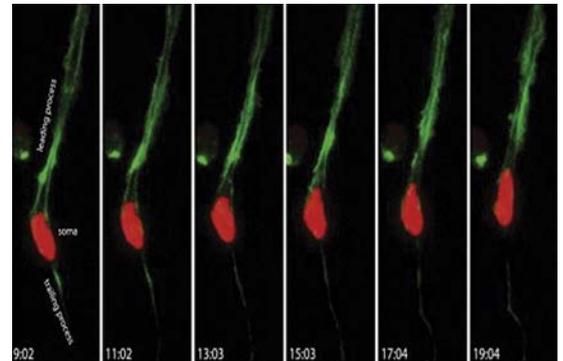
**FIGURE 5 / RADIAL GLIAL CELLS »** Neurons migrate from their birth places near the ventricle to their final destinations in the brain using radial glial cells as a type of “rope ladder.”  
Image credit: [www.brainfacts.org](http://www.brainfacts.org)

of the extension, out of the ventricular zone, and into the developing cortex. At later stages of development, the brain becomes larger, and the distances that a neuron must migrate to reach its final destination become longer. The 19th-century Swiss anatomist Wilhelm His studied the developing human cortex in great detail. From his observations, he suggested that a population of cells he called spongioblasts form a cytoplasmic bridge through which immature neurons migrate. Later, these cells were renamed radial glial cells (RGCs) (Figure 5).

RGCs are a special population of cells in the ventricular zone that guide neuronal migration by acting as scaffolds. RGCs form a sort of “rope ladder” configuration along which neurons can migrate. To do this, RGCs send a long arm-like extension out of the ventricular zone, while their cell bodies, stay in the ventricular zone. The migrating neurons attach themselves

to the vertical radial glial guide, and move along the cellular scaffold and out into the developing cortex. Many neurons can use the same glial guide cells, following one another as they move along the glial rope ladder and out of the ventricular zone.

With support from NIH, Dr. Mary Hatten and her colleagues at The Rockefeller University developed a novel approach for viewing the movements of neurons along the RGCs of mice using real time visual analyses. She and her colleagues demonstrated that specific proteins called F-actin and myosin II are localized in the leading edge of the neuronal cell body. Specifically, Dr.



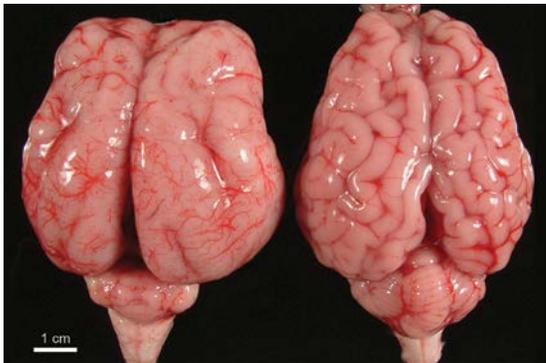
**FIGURE 6 / PROTEIN MOTORS PULL NEURONS ALONG RADIAL GLIAL CELLS »** Dr. Mary Hatten and colleagues at The Rockefeller University investigated how neurons move along radial glial cells to get to their final destination. They discovered that specific motor proteins congregate at the leading edge of the neuron (shown in green) pulling the rest of the cell along the glial cells. The nucleus of the cell is shown in red. Time difference between each panel is approximately two minutes. Image credit: *Reprinted from Neuron, 63 / 1, David J. Solecki, Niraj Trivedi, Eve Ellen, Govek, Ryan A. Kerekes, Shaun, S. Gleason, and Mary E. Hatten, Myosin II Motors and F Actin Dynamics Drive the Coordinated Movement of the Centrosome and Soma during CNS Glial Guided Neuronal Migration, p. 63-80., Copyright (2009), with permission from Elsevier.*

Hatten discovered that myosin II, a type of protein motor, was responsible for “pulling” the neuronal cell body along the RGC (Figure 6).

As neurons migrate away from the ventricular zone, they move outward eventually becoming the cerebral cortex. The cerebral cortex lies at the surface of the brain and controls higher level mental activity, including perception, cognition, language, and decision-making. The migration of neurons into the developing cortex results in the formation of an orderly,

six-layered structure. For the most part, the earlier migrating neurons form the deepest layers while the later migrating neurons contribute to successively superficial layers. Thus, each group of migrating neurons passes through the existing layers already formed by earlier born neurons, generating the cortex in an inside-out manner. Once the migrating neurons have reached their final positions, they begin to make synaptic connections with other neurons, establishing information processing networks.

When things go awry during neuronal migration, a rare disorder called lissencephaly can result (Figure 7). In

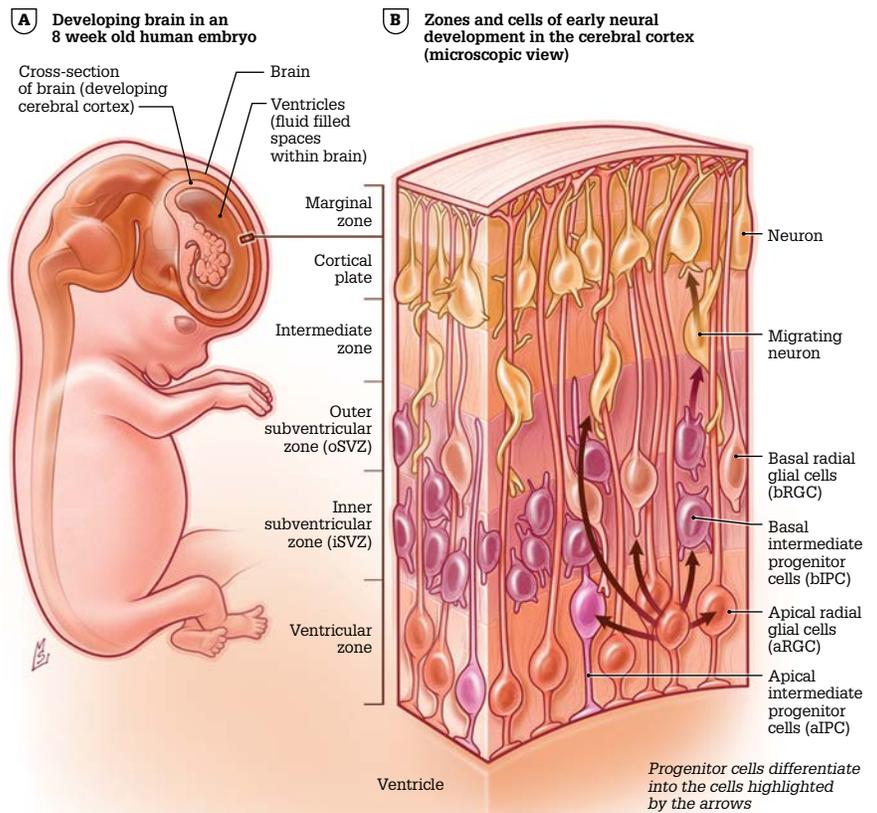


**FIGURE 7 / LISSENCEPHALY »** A lissencephalic (smooth) lamb brain (left) compared to a normally-folded lamb brain (right). Image credit: Pérez et al. *Hereditary lissencephaly and cerebellar hypoplasia in Churra lambs. BMC Veterinary Research. 2013, 9:156.*

lissencephaly, the brain is smooth, rather than folded and wrinkly, and the cortex is thicker than usual. This thickening is caused by defective neuronal migration, making the cortex disorganized. Depending on the severity, lissencephaly can lead to intellectual impairment and other neurological problems. Mutations (alterations in DNA code) in the genes, *lissencephaly-1 (LIS1)* and *doublecortin (DCX)*, account for the majority of lissencephaly cases. These genes affect neuronal migration from the ventricular zone to the developing cortex.

### THE CELLS THAT PRODUCE NEURONS

NPCs proliferate (increase rapidly in number) in the ventricular zone of the developing cortex, and principal among these cells are RGCs. While they were first identified as being the scaffolds for neuronal migration, in the early 2000s, scientists dis-

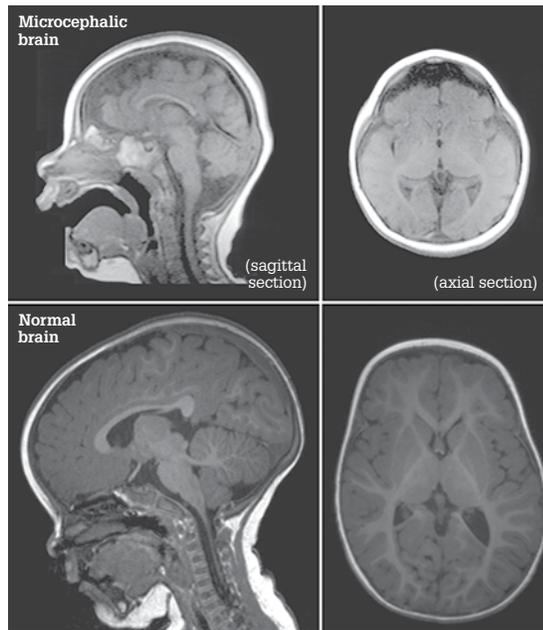


**FIGURE 8 / NEURAL PROGENITOR CELLS »** Zones of the developing brain and location of neural progenitor cells. The cells in the cross section are not to scale. Illustration: © Mike Linkinhoker, Link Studio, LLC.

covered something completely unexpected: RGCs are also neuronal progenitors.

Progenitor cells fall into two distinct groupings based on their location in the expanding brain – apical or basal. Apical progenitor cells include apical radial glial cells (aRGCs) and apical intermediate progenitor cells (aIPCs). These cells undergo division and proliferation within the ventricular zone and remain in contact with the ventricle. Basal progenitor cells consist of basal radial glial cells (bRGCs) and basal intermediate progenitor cells (bIPCs). Basal progenitor cells do not come into contact with the ventricle, and they undergo cellular division and proliferation in the subventricular zone (SVZ), which is adjacent to the ventricular zone of the brain (Figure 8).

In the SVZ, bIPCs divide symmetrically to produce a pair of NPCs. In this way, each individual progenitor cell



**FIGURE 9 / MICROCEPHALY IN THE HUMAN BRAIN »** Magnetic resonance imaging (MRI) showing different views of a microcephalic brain (top) and a normal brain (bottom) in one-year old children. Image credit: Shen et al. *Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. Nat Genet. 2010, 42:3*

is ultimately capable of producing many neurons. Primates and humans have a larger number and a greater variety of RGCs and intermediate progenitor cells compared to other vertebrates like fish and birds. Thus, it has been suggested that the abundance of these cells may contribute to the evolutionary expansion of the human brain and to the increased thickness of the human cortex. When there are deficiencies in the proliferation of NPCs, the result can be microcephalic brains (e.g., babies infected with the Zika virus) (Figure 9).

Many types of microcephaly are caused by mutations in genes involved in cell division. Even though these genes work in many tissues of the body, mutations affect the brain most severely. This is because NPCs only have a narrow window of time to complete the large number of division cycles necessary to give rise to all the cell types of the brain.

Some genetic mutations, such as those that occur in the genes *WDR62* and *NDE1*, have been implicated in microcephaly through their involvement in neural progenitor proliferation in the ventricular zone. Aside from genetic causes, microcephaly can result from

infections of the fetus during pregnancy (e.g., toxoplasmosis, cytomegalovirus, rubella, and Zika virus); exposure to drugs, alcohol, or toxins during pregnancy; or severe malnutrition. Children with microcephaly typically have developmental delays, including problems with speech and movements. In addition, they can suffer from seizures, intellectual disabilities, impaired vision, and hearing loss. Some cases of microcephaly, however, can be mild, with affected children having no symptoms other than a smaller than usual head.

### GENETIC FACTORS IN BRAIN DEVELOPMENT

More than half of all human genes are involved in supporting brain development. Mutations can affect any of these genes and may contribute to disorders affecting the size or organization of the brain. In addition to brain malformations like lissencephaly and microcephaly, which are visible by MRI, examples of more subtle neurodevelopmental disorders include attention deficit/hyperactivity disorder, autism spectrum disorder, and learning and intellectual disabilities.

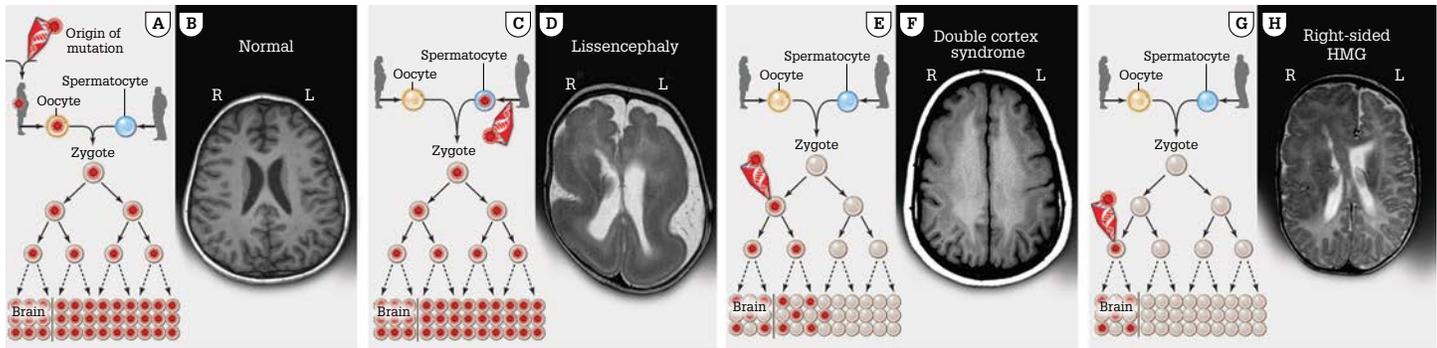
The past few years have seen significant advances in genetic tools and DNA sequencing. Advanced technology that allows processing of multiple DNA sequences at the same time has made it possible to search for mutations in all of the genes of the human genome in a single experiment. This has greatly expanded our understanding of the genetics of brain development.

Most genetic mutations are inherited from one's parents and are present in all the cells of the affected individual's body. These are termed germline mutations, as the change in DNA sequence was passed from grandparent to parent to child. In contrast, *de novo* mutations are mutations in DNA that are present in a child but not detectable in either parent. In some cases, this happens because *de novo* mutations arise in the sperm or egg that produced the child, but are not present in other tissues of the parent. For example, one disorder that can be caused by *de novo* germ cell mutation is lissencephaly (Figure 10, C-D).

In other cases, *de novo* mutations arise not in the sperm or egg, but later during a baby's development, generating "somatic" mutations that only affect some tissues and not others. Somatic mutations occur in any cell other than the sperm or eggs and are not passed down to offspring. Double cortex syndrome, for example, can be caused by a *de novo* somatic mutation in fetal brain tissue (Figure 10, E-F).

These *de novo* somatic mutations lead to individuals who are “mosaic,” meaning only a subset of their cells harbor the mutation. Such a mutation in brain cells may affect cell proliferation or function and contribute to neurological diseases. Somatic mutations in several genes are behind a brain malformation called hemime-

mutations exhibit autism-like behavioral characteristics, including anxiety, repetitive behavior, and changes in social interactions. Neurodevelopment was also delayed in mouse embryos with the mutation. The researchers concluded that mutations in *CHD8* are a risk factor for autism spectrum disorders.



**FIGURE 10 / EXAMPLES OF *DE NOVO* MUTATIONS AND HOW THEY CAN INFLUENCE BRAIN DEVELOPMENT »**

(A) Mutations in the female’s egg can lead to offspring with epilepsy even if the brain looks normal (B). (C) Mutations in the *LIS1* gene may arise in the male’s sperm resulting in a brain with lissencephaly (D). (E) *de novo* mutations that arise early in development can result in only a subset of cells harboring the mutation. An example of this type of mutation is double cortex syndrome (F). (G) Mutations that occur later in development sometimes affect only one tissue. In this case, the brain is affected with a gene mutation causing hemimegalencephaly (H). *Image credit: From Science, 341/6141, Annapurna Poduri, Gilad D. Evrony, Xuyu Cai, and Christopher A. Walsh, Somatic mutation, genomic variation, and neurological disease, p. 43-51, Copyright (2013). Reprinted with permission from AAAS.*

galencephaly (Figure 10, G-H). In this disorder, one cerebral hemisphere is enlarged and malformed, leading to epilepsy. Studies of brain tissue from patients led to the identification of somatic mutations in a gene that is active in the developing brain during cortical formation. Surprisingly, enlargement of an entire half of the cerebral cortex can occur even when only 8-35 percent of brain cells carry the mutation. This suggests that a minority of brain cells with a somatic mutation can disrupt the function of widespread cortical circuits.

Several studies using DNA sequencing have identified a high rate of *de novo* mutations in neurological, psychiatric, and cognitive disorders such as autism, schizophrenia, and intellectual disability. One of the genes frequently found to have *de novo* mutations in children with autism spectrum disorder is *CHD8*. Since the probability of incurring *de novo* mutations increases with age, some hypothesize that children born of late-age parents may have increased risk of autism.

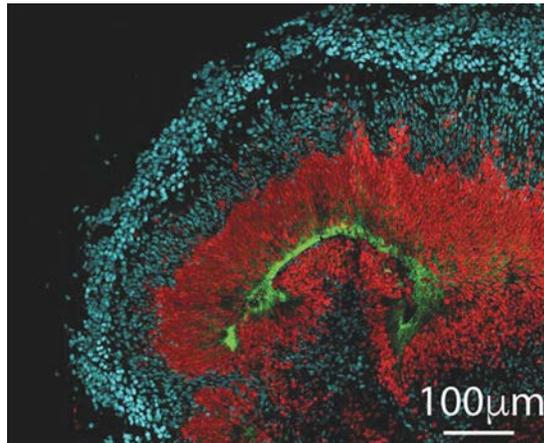
Dr. Yuta Katayama and colleagues from Kyushu University in Japan recently showed that mice with *CHD8*

## ZIKA, MICROCEPHALY & BRAIN DEVELOPMENT

Developmental and genetic studies reveal that the formation of the brain is a highly coordinated and delicate process that can be disrupted at several steps. As noted previously, many external factors can adversely affect brain development, including malnutrition, environmental toxins, drugs, and infections like the Zika virus.

Scientists are trying to understand how a virus that was identified nearly 70 years ago is now posing such a grave risk to developing babies. One possibility is that mutations in the Zika virus made it more virulent or easily transmissible as it moved from Africa to South America. It’s also possible that serious complications went mostly unnoticed in poorer countries that lack effective disease tracking. A third possibility is that co-infections of Zika with other viruses, such as dengue and chikungunya, may work together to produce microcephaly.

Understanding the Zika virus is so important because it exerts devastating effects on the developing brain. Many affected infants die before delivery or shortly after



**FIGURE 11 / ZIKA VIRUS AND THE AXL RECEPTOR** » Experiments demonstrated that the Zika virus uses the AXL receptor (green) to gain entry into neural progenitor cells (red) in the developing brain. Neurons at the outermost region of the developing brain are blue. *Image credit: Reprinted from Cell Stem Cell, 18 /5, Tomasz J. Nowakowski, Alex A. Pollen, Elizabeth Di Lullo, Carmen Sandoval-Espinosa, Marina Bershteyn, and Arnold R. Kriegstein, Expression Analysis Highlights AXL as a Candidate Zika Virus Entry Receptor in Neural Stem Cells, p. 591-596, Copyright (2016), with permission from Elsevier.*

birth. Those that survive face a challenging future and may suffer from impaired eyesight, hearing loss, rigid limbs, and frequent seizures. There is no treatment for the brain damage caused by the Zika virus, only palliative therapies to help mitigate its consequences.

Since the Zika outbreak in Brazil began, scientists have discovered important clues as to how Zika interferes with the developing brain. NPCs are susceptible to Zika infection, while mature neurons are not as vulnerable. RGCs appear to be especially targeted.

Some of the first insights came from two mouse studies published in May 2016. Together, these experiments presented the first conclusive evidence that Zika infection during pregnancy can cause microcephaly in an unborn fetus. With support for his research from the São Paulo Research Foundation, the Tooth Fairy Project, and NIH, Dr. Alysson Muotri and his colleagues at the University of California, San Diego infected pregnant mice with Zika virus. They found that the virus crosses the placenta and causes birth defects like microcephaly in newborn mice.

Dr. Muotri's team showed that Zika virus is particularly likely to infect brain cells, replicating inside of them until they burst releasing more viral particles that can infect

other brain cells. What's more, the brain cells susceptible to Zika infection are NPCs, the fast-multiplying cells that are responsible for building the brain. If a fetus loses just a small percentage of these cells, a portion of its brain will never develop properly, resulting in an abnormally small brain.

A collaboration between Dr. Xu Zhiheng at the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences and Dr. Qin Cheng-Feng at the Beijing Institute of Microbiology and Epidemiology confirmed the finding that the Zika virus destroys NPCs after they injected the virus directly into the brains of mouse embryos in the womb.

Dr. Muotri and colleagues also looked at the effect of Zika infection on human brain organoids, three-dimensional masses of cultured brain cells. These organoids are composed of different types of neurons structured in layers, mimicking those found in the intact human brain. These cultures are sometimes referred to as "minibrains." Using these "minibrains" the researchers showed that the virus disrupts its organization and kills the NPCs within it.

Dr. Arnold Kriegstein's laboratory, along with two others from the University of California, San Francisco, exposed brain tissue samples to the Zika virus for 24 hours and then added a fluorescent tag to look for a specific receptor called AXL. Kriegstein's lab had been studying AXL receptors in the developing brain, and they knew that AXL drives cell growth, proliferation, and survival. They also knew that these receptors form a thick coat around NPCs. What they did know is whether AXL receptors played a role in Zika infection. To study this, the researchers looked for which types of neurons in the brain tissue became infected with the virus and whether they also had AXL receptors.

They found that AXL is present throughout the developing cortex, particularly in the region containing dividing progenitor cells. When the researchers added a molecule that blocked AXL receptors to a dish of brain cells, it prevented the Zika virus from entering these cells. These results suggest that the Zika virus takes advantage of the AXL receptor to gain entry into NPCs in the developing brain (Figure 11).

Progenitor cells may not be Zika's only target, however. In a separate experiment funded by NIH, Drs. Michael Diamond and Indira Mysorekar at Washington University in St. Louis injected pregnant mice with the Zika virus. They found the virus not only damaged the brain, but

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## THE BRAIN INITIATIVE

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To further understand the human brain, in April of 2013, the White House announced the launch of the BRAIN Initiative (Brain Research through Advancing Innovative Neurotechnologies). This program aims to support the development of new technologies with the hope of learning more about the human brain. Five governmental agencies – NIH, the Defense Advanced Research Projects Agency (DARPA), the National Science Foundation (NSF), the Intelligence Advanced Research Projects Activity (IARPA) and the Food and Drug Administration (FDA) – are working together to achieve this goal. Knowledge

gained through the BRAIN Initiative will aid in our quest to treat some of humanity's most devastating brain disorders, including Alzheimer's disease, Parkinson's disease, autism, depression, and traumatic brain injuries.

Of the initiative, President Obama stated, "There is an enormous mystery waiting to be unlocked, and the BRAIN initiative will change that by giving scientists the tools they need to get a dynamic picture of the brain in action and better understand how we think and how we learn and how we remember. And that knowledge could be – will be – transformative."

also attacked the placenta. This reduced the nutrient and blood exchange that normally occurs between the mother and the fetus, slowing down the fetus's growth.

Further, Dr. Carolyn Coyne of the University of Pittsburgh and her team showed that the Zika virus must cross the placenta in order to affect fetal brain cells. They discovered that the Zika virus is able to infect the placenta during the first and second trimesters, but late-stage placentas can withstand Zika infection. This could be related to the activity of AXL receptors, which are more common in placental cells earlier in pregnancy.

Together, these experiments might explain why the developing brain is particularly susceptible to Zika virus infection and how infection during pregnancy produces microcephaly. After crossing the placenta, the virus might enter the developing brain via the bloodstream or cerebrospinal fluid. Once in the brain, the virus preferentially destroys NPCs, the population of cells that normally generates all the cell types in the cortex, resulting in microcephaly.

Future research will almost certainly focus on just how the virus uses the AXL receptor to break into brain cells, as this could provide an important clue for designing effective drugs against Zika infection. However, Zika might use other receptors, as well. Therefore, other drugs that could enhance the survival of NPCs during Zika infection could be helpful. Confounding factors such as the mother's immune health and genetic makeup might contribute to the likelihood of passing the infection on to

the developing fetus. More research is needed to figure out how the virus crosses the placenta and exactly how it triggers the death of NPCs.

Although many questions remain, the use of human cerebral organoids, genetic sequencing, and studies in mice are allowing researchers to gain a more sophisticated understanding of the mechanisms of Zika infection. In addition, further epidemiologic studies could reveal whether interactions of Zika infection with other factors determine the severity of the outcomes.

Insights into the effects of the Zika virus on the developing brain were made possible by the rapid response of NIH, CDC, and the research community. Funding allocated by NIH has supported scientists who are researching the natural history, transmission, and pathogenesis of the Zika virus; developing accurate diagnostic tests; and devising and testing experimental vaccines. In fact, one DNA vaccine is undergoing a multi-site clinical trial to protect against the virus, and was developed by scientists at the National Institute of Allergy and Infectious Disease (part of NIH). This vaccine had shown promise in human clinical trials.

### DEVELOPMENT, DISEASE & FUTURE RESEARCH

Brain development is a complex constellation of processes that begins a few weeks after conception and for some neurons, continues throughout our lifetime. The interplay of genetic and environmental factors underlies brain development, from the formation of the early nervous system, to the birth and migration of neurons, to the establishment of neuronal connections.

Sometimes these processes go awry, leading to specific brain disorders and diseases. By studying abnormal brain development, scientists are learning more about how normal brain development proceeds. Cutting-edge genomics, studying “minibrain” organoids in a dish, and using animal models, as well as other techniques, are

allowing scientists to shed light on the intricate interplay of genetic and environmental factors that affect human brain development. The hope is that with our increasing understanding of these processes in healthy and abnormal brains we will learn how to prevent devastating neurodevelopmental disorders. 🌐

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### ADDITIONAL SUGGESTED READING

National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development  
**“Neural Tube Defects (NTDs)”**

<https://www.nichd.nih.gov/health/topics/ntds/conditioninfo/Pages/default.aspx>

Society for Neuroscience: BrainFacts **“Brain Development”**

<http://bfresp.sfn.org/brain-basics/brain-development/>

Johns Hopkins School of Education, *New Horizons*

**“Embryological Development of the Human Brain”** by Arnold B. Scheibel

<http://education.jhu.edu/PD/newhorizons/Neurosciences/articles/Embryological%20Development%20of%20the%20Human%20Brain/index.html>

Howard Hughes Medical Institute: Making Your Mind: Molecules, Motion, and Memory

**“Development of the Human Embryonic Brain”**

<http://www.hhmi.org/biointeractive/development-human-embryonic-brain>

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doi: 10.1007/s11065-010-9148-4.

Fernández, V., Llinares-Benadero C., and Borrel V. (2016). **Cerebral cortex expansion and folding: what have we learned?**

*EMBO J.* 35(10):1021-44. doi: 0.15252/embj.201593701.

Florio, M. and Huttner, W. B. (2014). **Neural progenitors, neurogenesis, and**

**the evolution of the neocortex**. *Development* 141: 2182-2194. doi: 10.1242/dev.090571.

National Public Radio May 11, 2016 **“How the Zika Virus Damages The Brain”** by Michaeleen Doucleff

<http://www.npr.org/sections/goatsandsoda/2016/05/11/477648872/how-the-zika-virus-damages-the-brain>

Science News March 4, 2016 **“Zika virus kills developing brain cells”** by Gretchen Vogel

<http://www.sciencemag.org/news/2016/03/zika-virus-kills-developing-brain-cells>



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