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## Neuroembryology

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## Neuroembryology

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### Abstract

How is it that some cells become neurons? And how is it that neurons become organized in the spinal cord and brain to allow us to walk and talk, to see, recall events in our lives, feel pain, keep our balance, and think? The cells that are specified to form the brain and spinal cord are originally located on the outside surface of the embryo. They loop inward to form the neural tube in a process called neurulation. Structures that are nearby send signals to the posterior neural tube to form and pattern the spinal cord so that the dorsal side receives sensory input and the ventral side sends motor signals from neurons to muscles. In the brain, stem cells near the center of the neural tube migrate out to form a mantle zone, and a set of dividing cells from the mantle zone migrate further to produce a second set of neurons at the outer surface of the brain. These neurons will form the cerebral cortex, which contains six discrete layers. Each layer has different connections and different functions.

### Keywords

Brain; nervous system; development; induction; neurulation; spina bifida

## INTRODUCTION

Right now, reading this article, you are using cells in your brain—neurons—to understand words, react to thoughts, and record memories. When you were an embryo, all of the cells in your body were identical. But as time passed, some cells developed into neurons while others developed into skin cells or hair cells or muscle cells.

How is it that some cells become neurons? And how is it that neurons become organized in the spinal cord and brain to allow us to walk and talk, to see, recall events in our lives, feel pain, keep our balance, and think?

### SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Video S1. Neurulation in the chick embryo. This animation shows the movements of the back or dorsal side of the chick embryo, which contains the prospective epidermis (skin, blue), neural tube (central nervous system, purple), and neural crest cells (peripheral nervous system, pigment; teal). The central portion folds and forms the neural tube; the epidermis covers the neural tube, and the prospective neural crest cells form a link between the epidermis and the neural tube. These neural crest cells then migrate away, leaving the neural tube disconnected from the epidermis. Animation courtesy of Dr. Yoshiko Takahashi, Kyoto University, Japan.

The answers to these questions help us understand not just how we develop from an embryo into a full-grown person, but also how our body and brain constantly adapt—throughout our entire lives—to the environment.

## **SPECIFICATION: HOW CERTAIN CELLS BECOME SPECIALIZED FOR THE BRAIN AND SPINAL CORD**

Let's begin with the early stages of the vertebrate embryo, when the cells have not yet become specialized. We will use the chick embryo as an example, since its early anatomy approximates human development, is relatively simple, and has been well studied. These embryos begin as a flat disc of cells that will transform itself in three dimensions. Each cell is unspecified—that is to say, the cells are not yet committed to become any particular type of cell. But each cell has a particular location relative to the center and outside of the disc. This location is critical as it will influence each cell's future development. This is a major principle of developmental biology: What a cell becomes is in large part specified by interactions with its neighbors.

After a number of cell divisions, a group of cells in the midline of the disc starts moving from the disc into the space underneath it (Figure 1A). We call this movement “gastrulation” and the region where the movement takes place is called the “primitive streak.” Gastrulation gives the cells new neighbors and new positions. The relationships of the cells with their new neighbors causes changes in their gene and protein expression. These changes allow them to move from the surface of the disc, through the primitive streak, and out to the rest of the embryo.

The first migrating cells are those that will eventually produce the most anterior (“throat”) portion of the gut tube. (These cells are sometimes called the anterior endoderm). Then a layer of cells migrates through the primitive streak in between the gut layer and the top layer. This layer is called mesoderm, and the midline of this mesoderm layer of cells forms the *notochord*, a rod along the head-to-tail axis. This notochord, the mesodermal rod extending from head to tail, is a defining feature of all chordates (Figure 1B, C), the phylum to which all vertebrate animals (amphibians, reptiles, fish, birds, and mammals) belong. The notochord produces chemical signals that allow nearby cells to start becoming neurons. The formation of the notochord defines the axes of the individual. Specifically, it defines the dorsal-ventral (back-belly) axis, since the tissue above it (dorsal) will be brain and spinal cord, and those below it (ventral) will be gut endoderm. It also specifies the anterior-posterior (head-tail) axis, since at one end of the rod the migrating cells will form head structures (anterior) and at the other end the migrating cells will form tail structures (posterior). Finally, the notochord establishes the left-right axis, since it forms an early ‘spine’ of the embryo, dividing it into right and left sides. Many experiments over many decades have concluded that all vertebrate organisms studied so far share similar processes of gastrulation and notochord formation, as well as other organizational strategies for generating the early body plan<sup>1</sup>.

In the early 1900s, Hans Spemann showed that the specification of the nervous system took place at gastrulation. Using salamander embryos that had just started gastrulation, Spemann

showed that when a region of prospective neural cells was transplanted into a region of another embryo where epidermal (skin) tissue would have normally formed, the transplanted cells gave rise to epidermal tissue. In other words, they differentiated in accordance with their *new* location because they responded to the local chemical signals produced there. However, when the same transplantation experiments were performed with cells from embryos near the end of gastrulation, Spemann obtained completely different results. Rather than differentiating in accordance with their new location, the transplanted cells became neurons, even in the middle of the epidermis, presumably because they had already been specified by signals from their pre-transplantation environment. Within the time separating early and late gastrulation, these cells lost their capacity to change. Something caused them to become specified as neurons.

Spemann's next set of experiments showed that the region of the embryo that generates the notochord, called the *organizer*, was responsible for this specification. In one of the most famous experiments in embryology, Spemann and his student Hilde Mangold placed early organizer cells from the back of one salamander embryo into the belly of another early salamander embryo. What happened was astonishing. The embryo with the organizer graft developed two anterior-posterior axes—one induced by signals from the original organizer and one induced by signals from the grafted one. Each of the organizers generated a notochord and specified the tissue above them to become neural. The embryos formed two body axes resulting in conjoined twin salamanders. For this work, Spemann won the Nobel Prize in 1935.

More recent experiments have shown that the organizer secretes proteins into the nearby environment. These proteins block the action of signaling proteins coming from the skin and allow the cells situated between the organizer and the skin to be specified as neurons.

## NEURULATION: HOW CELLS ARE TRANSFORMED TO BECOME THE NEURAL TUBE

The cells that are specified to form the brain and spinal cord are originally located on the outside surface of the embryo, continuous with the future epidermis. How do they move inside where the brain and spinal cord are ultimately found? The cells have to move inwardly and move together. To see this, hold a piece of paper at its two long edges, a few inches in from either side. Now, slowly bring your hands together toward the center of the paper and make the middle of the paper loop down. That is what happens at the back of the embryo (Figure 2A–D; Video S1). The cells that are going to become the epidermis begin moving together toward the mid-back of the embryo and 'push' the neural plate in the midline into the inside of the embryo to form the neural tube. The cells in the neural tube also participate in the action by changing shape and pulling the epidermal cells up and over their top to meet in the midline<sup>2</sup>. As a result, the neural tube forms right under the dorsal midline of the embryo, and it is connected to the dorsal epidermis by a stalk of cells. Its closure into a tube starts at the "top" or head end of the embryo, and zippers its way down toward the tail end (Figure 2E). This process of moving future neurons into the embryo is called neurulation.

The structure formed in this way is called the neural tube. The notochord and anterior endoderm cells beneath it, and skin cells above it, cause the neural tube to become organized into brain and spinal cord, which comprise the central nervous system. The epidermis will form the outer layer of the skin, and the stalk between the epidermis and neural tube forms the *neural crest*, a transient structure of cells that will migrate away, thereby creating separation between the neural tube and epidermis. The neural crest cells migrate throughout the body to become the cells of the peripheral nervous system (which relays messages to and from the heart, stomach, and other organs), and many other structures including the bones of the face and neck, and the pigment cells of the skin. The mesodermal cells adjacent to the neural tube (on the right and left sides) and under the epidermis will become the bones of the spine in addition to the dermis of the skin, the back muscles, blood vessels, and connective tissue.

The closure of the neural tube is a very complex process. It involves coordination between nuclear genes, cytoplasmic contractile proteins (that change the shape of cells and allow them to migrate), and proteins outside the cells that link the cells together (Figure 3). Numerous genes have to be active at the right time for the neural tube to form properly, and several dietary factors, such as cholesterol and folic acid (Vitamin B9), must be present as well. In 1 of every 1000 human births, this process does not continue to completion in a medically significant way. Failure to close the most anterior part of the neural tube causes anencephaly, a lethal condition where the brain fails to develop. Failure to close more posterior parts of the neural tube results in spina bifida, the severity of which depends on how much spinal cord is exposed. As many as 20% of all people may have the mildest form of spina bifida, which typically requires no treatment. In fact, this is the developmental condition that gives the Rhodesian ridgeback dog its ridge.

## NEURAL DIFFERENTIATION: BECOMING THE REGIONS OF THE BRAIN AND SPINAL CORD

The early development of most vertebrate brains, including humans', is similar. The early neural tube is a straight structure (Figure 2E, 4A). However, even before the posterior portion of the tube has formed, the most anterior portion of the tube undergoes drastic changes (Figure 4). In the anterior region, the neural tube balloons into the three primary vesicles: (1) the forebrain, which forms the cerebral hemispheres, thalamus, hypothalamus, and retina; (2) the midbrain, which forms part of the brainstem and includes the tectum (for visual and auditory processing) and the motor pathways of the basal ganglia; and (3) the hindbrain, which includes the cerebellum (for balance and coordination of movement) and the medulla oblongata (to regulate breathing and other basic functions). The neural tube in the neck and trunk form the spinal cord, a complex structure in its own right that (among other things) receives sensory input from and sends motor signals to the periphery.

The neural tube (and therefore the brain and spinal cord) differs with respect to its dorsal and ventral halves. In the spinal cord, the dorsal side receives sensory input; for example, a touch-sensitive receptor in the skin of your finger will connect to the dorsal part of the spinal cord. In contrast, motor signals issue from neurons in the ventral spinal cord to activate the

muscles. Within the spinal cord are also numerous interneurons that relay information between the sensory and motor neurons and create neural circuits. Spinal neural circuits perform a variety of simple and complex computations. Among the simpler ones is the spinal reflex by which you can pull your hand quickly to safety when your finger touches something hot. Such spinally mediated behaviors don't require you to think about what you're doing, although you can modulate your response using your brain so that you don't drop your Aunt Daisy's favorite teacup when it turns out to be hotter than you expected.

The dorsal-ventral axis of the neural tube is modified (or *induced*) by signals coming from the immediate environment (Figure 5)<sup>3</sup>. Proteins released by cells in the notochord (the same tissue that, as it forms induces the central surface cells of the embryo to become the neural tube) influence the neural tube directly above it to become the ventral motor region of the spinal cord. If a bit of notochord from a chick embryo is removed and placed against the side of the neural tube in another chick embryo, that second embryo will form a neural tube with two ventral regions, one normal, and one induced on the side by the graft<sup>4</sup>. Both of these regions will make motor neurons. In contrast, the overlying epidermis specifies the dorsal or sensory part of the neural tube. In combination, these signals from above and below specify the precise identities of neurons in the neural tube and, ultimately, the spinal cord.

## NEURAL STEM CELLS: HOW THE BRAIN FORMS ITSELF

So far, we've been focusing on how *other* parts of the body help induce and organize the central nervous system. We mentioned that the organizer cells specify certain outer (peripheral) cells of the embryo to become neural, how cells specified to become epidermis push these neurally specified cells to make the neural tube, and how neighboring cells (such as those in epidermis, anterior endoderm, and notochord) give the neural tube cells protein cues to become specialized brain and spinal cord cells.

Again, what we are discussing here for nervous system development is a basic principle that applies to all of developmental biology: The destiny of a cell is in large part specified by interactions with its neighbors. However, neural cells that develop later also contribute to their own form. What is established in the neural tube is an array of *neural stem cells*. In the anterior neural tube, these cells now start dividing and migrating in ways that will produce the specific anatomies of the forebrain, midbrain, and hindbrain.

A stem cell is a type of cell that divides into two different types of cells. One of its progeny typically remains in the same location, replacing its parent with an identical stem cell. But the second of its progeny is capable of changing into a different kind of cell; this cell often migrates away and proliferates to make more cells, each of which differentiates in accordance with its new position in the embryo.

The neural stem cells stay near the center of the neural tube, lining the inner cavity that will later be filled with cerebrospinal fluid and called a ventricle. In the regions that are to become the hindbrain, daughter cells from the neural stem cells migrate away from the center and divide to produce a second cellular layer—the mantle layer—comprised of neurons and glial cells. (Glial cells, which are actually more numerous than neurons and

have long been known to serve a number of “housekeeping” functions, are increasingly being viewed as equal partners in the computational functions of the nervous system.) The mantle layer cell bodies have a grey color to them, thus explaining its designation as “grey matter.” The cells in the mantle layer extend processes called axons, which are coated with fatty white myelin to insulate them. Hence, where the myelinated axons travel together they are the “white matter” of the central nervous system.

In the brain, the cerebellar cortex and cerebral cortex modify this early structure of stem cells, axons, and neural cell bodies. For instance, in the cerebrum (Figure 6), dividing cells from the mantle layer migrate out to produce a third set of cell bodies at the outer surface of the brain. These neurons form the first layer of the cerebral cortex, which will generate six discrete layers through additional cell division and migration. Each layer makes different connections and has different functions.

The layers of the cerebral cortex form in an “inside-out” manner (Figure 6). Those neurons that form the first group of cells dividing and migrating from the cortex travel the shortest distance. Those neurons formed next migrate through this first group to form the next layer. Once they arrive at their destination, the neurons produce adhesion molecules that clump them together into functional units such that their location, layer and connections determine their function.

Until the end of the last century, it was thought that once the brain had been formed, no new neurons were produced. However, around 2000<sup>5,6</sup>, scientists found that in adults there are stem cells lining the ventricles that continue to divide, producing thousands of new neural precursor cells each day. These cells act very much like the stem cells of the embryonic brain, in that they renew themselves as well as produce additional neural precursor cells. These adult stem cells have not been found in many locations, but the locations where they have been found suggest that they may, among other things, be responsible for the regulation of new memories<sup>7</sup>.

## CONCLUSION

Gregor Eichele wrote: “What is perhaps the most intriguing question of all is whether the brain is powerful enough to solve the problem of its own creation.” In the formation of the central nervous system, we see that cell specification depends both on the source of the cell within the embryo (lineage) and what signals the cell receives from its neighbors (induction). We have seen that gastrulation is the time when the fate of many vertebrate cells is determined, and that coordination of cytoskeleton, gene expression, and extracellular proteins is required for such complicated processes as neurulation. Finally, we have seen how stem cells spur the emergence of cells required for the development of the central nervous system. These and many other scientific discoveries have made it clear that the nervous system is a masterpiece of embryology.

## Supplementary Material

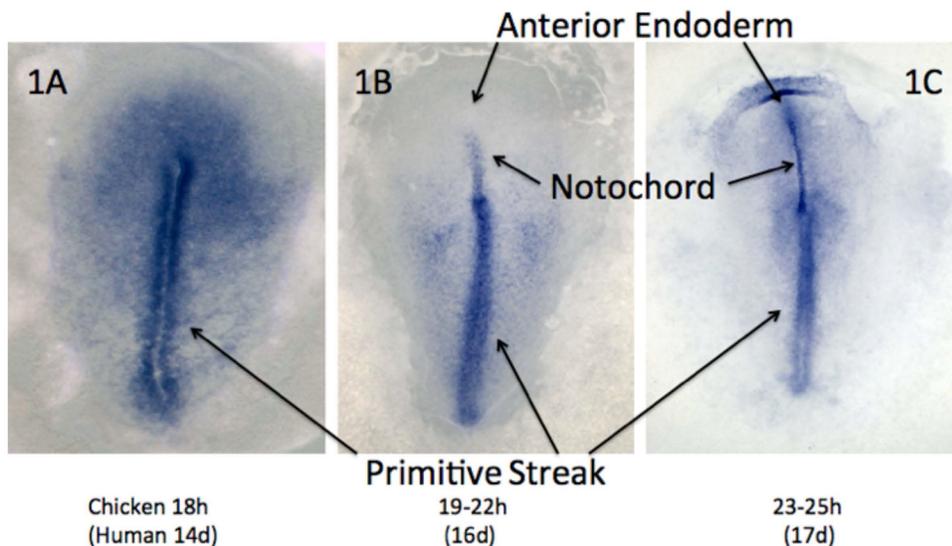
Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

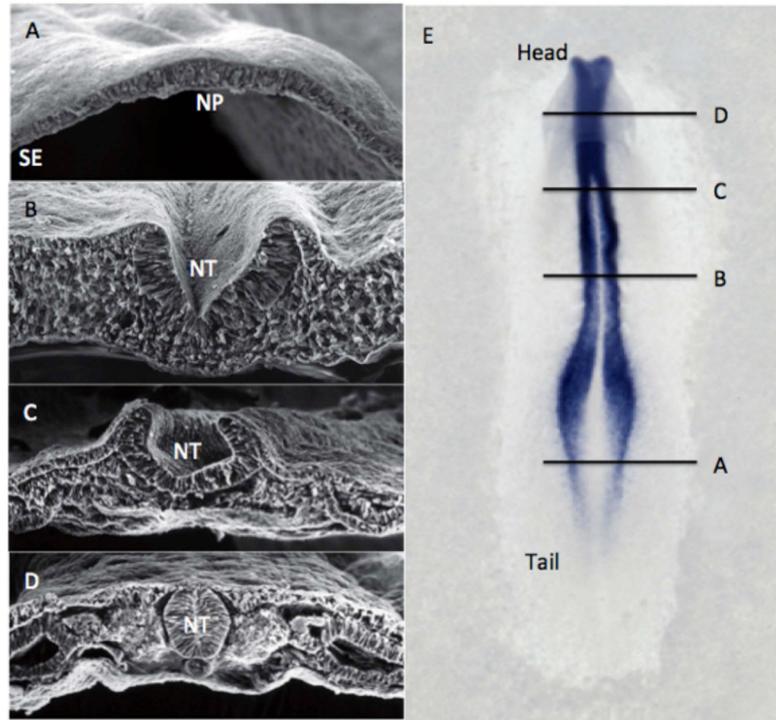
We gratefully acknowledge the following groups or scientists for allowing us to use their images and videos (In order of appearance): GEISHA (Parker Antin's lab group), Katherine Tosney, M. Angeles Rabadán and Elisa Martí Gorostiza, Gary Schoenwolf, and Yoshiko Takahashi. Grants from NIH/NICHHD (1P41HD064559-01) and NSF (IOS 1457177) helped to support the authors during the preparation of this review.

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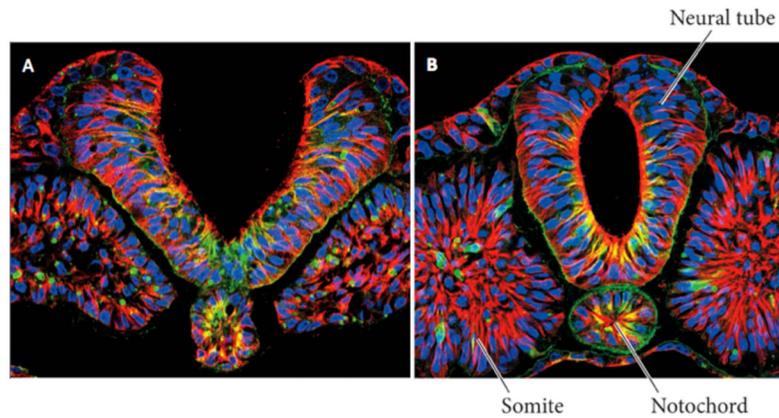
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**Figure 1.** Gastrulation in the chicken embryo as a model for early human development. Cell movements of gastrulation begin very early (6–19 hours for chicken and 14–16 days for human). (A) The primitive streak forms in the central portion of the embryo. (B–C) The first cells migrating through the primitive streak migrate underneath the covering layer (epiblast) and become the anterior endoderm and the notochord. The anterior endoderm and notochord instruct the epiblast cells above them to become neural tube rather than skin epidermis (19–23h/16–21d). Photographs of RNA accumulation during these stages of development: (A, MIR130B, a micro RNA expressed in the primitive streak; B, ARHGAP8, encodes an enzyme found in the primitive streak and notochord; C, SNX30, encodes a cytoplasmic protein found in the notochord and anterior endoderm) courtesy of the GEISHA database, University of Arizona, Tucson, AZ, URL: <http://geisha.arizona.edu>.<sup>8</sup>

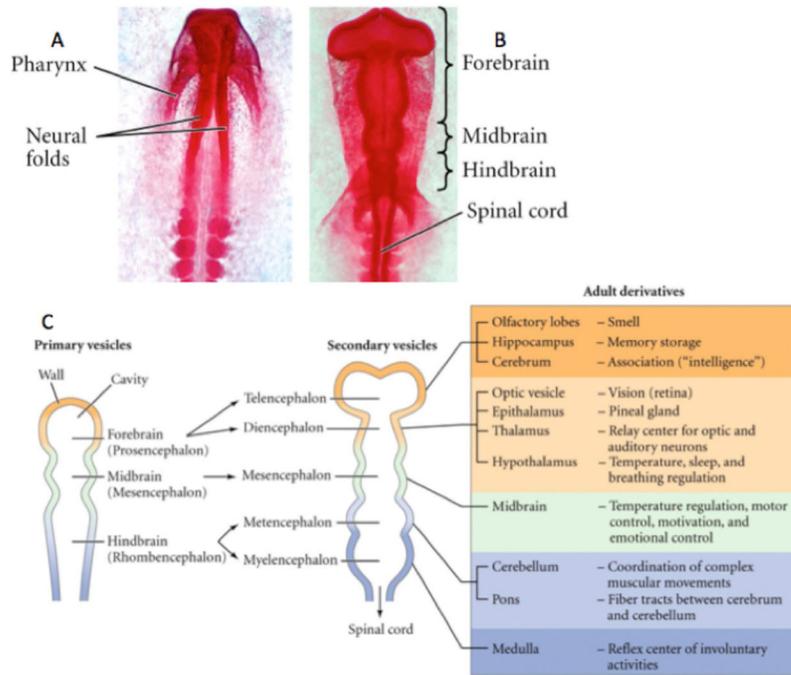


**Figure 2.** Neurulation in the chick embryo. (A–D) Three-dimensional photographs taken by a scanning electron microscope, which allows one to see individual cells. (A) Cells of the neural plate (NP) elongate, becoming taller than the more lateral cells that are going to become the skin epidermis (SE). (B) The bending of the neural tube (NT) begins. (C) Prospective skin epidermal cells from the sides migrate toward the center of the embryo, causing the neural plate to start forming a neural tube (NT). (D) The epidermal cells come together over the top of the neural tube (NT). (E) An over-view, looking down at a chick embryo (33 hours) labeling a gene product ((OLFM1 in blue) expressed in the neural plate and tube. The neural tube has started closing, beginning in the head region. Because the embryo develops in a head to tail progression, all phases of neural plate and tube closure can be seen at different axial levels in an embryo of this stage. Labels A–D on this embryo correspond approximately with the neural development shown in panels A–D. (A–D) courtesy of Dr. Kathryn Tosney, University of Miami, USA, E (OLFM1) courtesy of the GEISHA database, University of Arizona, Tucson, AZ.<sup>8</sup>



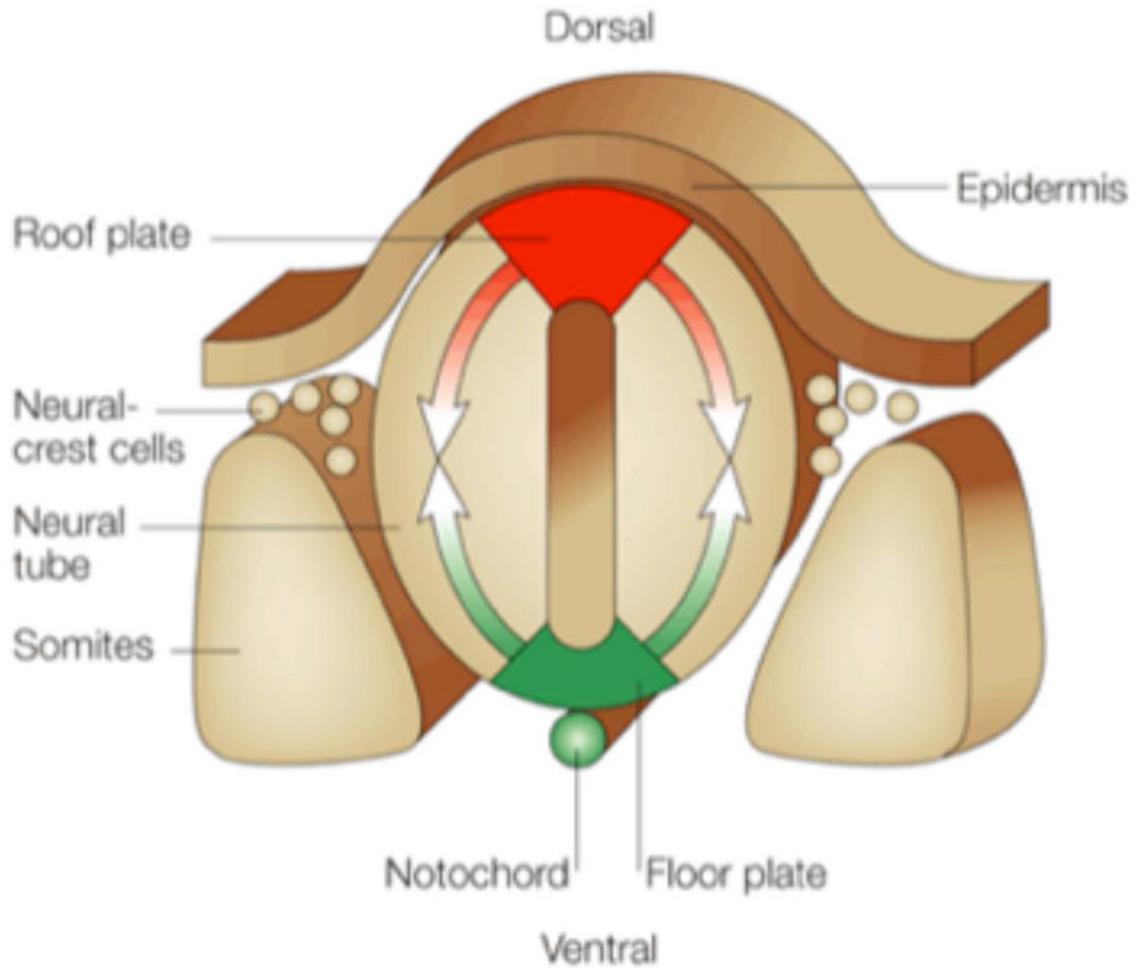
**Figure 3.**

Closing the neural tube demands coordination among nuclear genes, cytoplasmic proteins, and extracellular matrix proteins. (A) The neural plate cells fold as a result of cell shape changes. The nuclei are stained blue; the cytoplasmic contractile proteins are stained red; and the extracellular matrix proteins connecting the cells are stained green. The rod-like notochord can be readily identified beneath the folding neural plate cells by its round cross-section. It serves as an anchor, giving a pivot upon which the folding takes place. The clumps of cells to the side of the neural tube and notochord are the somites, which will form structures including the back muscles, dermis of the skin, and the vertebral bones that will surround the neural tube. (B) The moment of neural tube closure. In this part of the embryo, the tips of the neural folds reach out toward each other and form a tube, as the prospective epidermis covers it. Photographs courtesy of Drs. M. Angeles Rabadán and Elisa Martí Gorostiza, Institute of Molecular Biology of Barcelona, Spain.



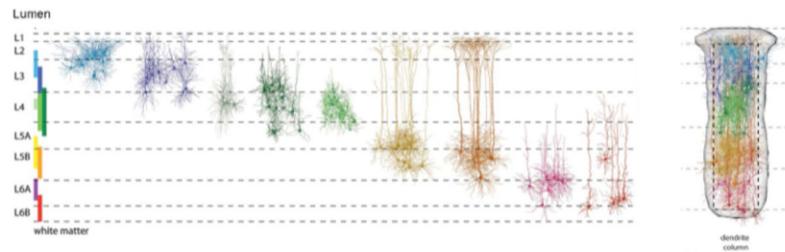
**Figure 4.**

Formation of the first chambers of the chick brain. (A) At about 28h in the chick (~21 days in human) the neural tube closes in the head. (B) The neural tube begins to expand into the early brain vesicles, forebrain, midbrain and hindbrain anterior to the spinal cord. By 40h in chick (~24d in human) the neural vesicles become further specialized; the forebrain into the telencephalon and diencephalon (which includes the two bulging regions that will become the eyes), the midbrain or mesencephalon, and the hindbrain into the met- and myelencephalon. (C) In the human the three primary brain vesicles become similarly subdivided as development continues and become functionally different from each other. Photographs courtesy of Gary Schoenwolf, University of Utah<sup>9</sup>



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**Figure 5.** Specification of the dorsal (upper) and ventral (lower) portion of the neural tube. In the trunk of the embryo, the epidermis instructs the uppermost cells at the neural tube to secrete compounds that turn the nearby cells into sensory neurons. These neurons will receive touch, pain and temperature input from the skin and other peripheral organs. In the lower portion of the neural tube, the notochord secretes proteins that instruct the ventral-most cells of the neural tube to secrete proteins that instruct the nearby cells to become motor neurons. The motor neurons innervate the muscles. Adapted from<sup>10</sup>



**Figure 6.**

“Birthdays” of neurons. The neural stem cells are located in the lowest layer of the neural tube, the inside layer, bordering the fluid-filled lumen, which will become the ventricles. As neurons are generated (‘born’) from the stem cells, they migrate through the already-existing neurons to join their age-appropriate peers. In the cerebral cortex in human and mouse (shown), there are six major layers. The connections of some of these nerves are shown in the figure at the right. Adapted from<sup>11</sup>