

**Induction and patterning of the nervous system**

Reading: (review first part of chapter on neurulation if you choose)

9e: stem cells pp 323-331; Chp 9 333-345

8e: Chp 12: 380-391

Learning Goals Be able to:

Design experiments that indicate the importance of certain molecules for D/V patterning of the neural tube

Explain the overlying principles of neural fate specification and commitment to different fates.

Distinguish between intrinsic differences and signaling interactions that lead cells to their eventual fate in the nervous system

Design experiments that prove both mechanisms are involved in the above.

The ectoderm differentiates into two major body components -- the epidermis, which is the outer layer of the skin, and the nervous system. Ectodermal cells are epithelial. Although the highly branched, specialized nerve cells do not resemble a layer of simple epithelial cells, these neuronal cells do retain some of the characteristic properties of the epithelial cells from which they originate (for example, a clear polarity). The development of the nervous system is incredibly complex, but can be broken down into roughly 4 phases: 1) competence and segregation (or induction) of neuronal cells from ectoderm (discussed in class 9), 2) regional specification and differentiation of different kinds of neuronal cells (class 9 and today), 3) axonal pathfinding to establish connections, and 4) maintenance of connections among neuronal cells. We will discuss phases 3 and 4 later in the course.

We've gotten up to the point so far of describing the commitment of cells within the neural ectoderm and the many signals involved. One more general set of patterning events takes place before we can think about the differentiation of individual fates within the nervous system, and that is the patterning of the Dorsal-Ventral axis of the nervous system. These patterning events take place just after neurulation is complete.

**Please review the notes from class 6 on the mechanical details of neurulation.**

**D/V patterning**

The dorsal/ventral differences of the neural tube are most evident in the spinal cord, which is organized into distinct dorsal and ventral regions with different functions (10.14, 10.12).

--Dorsal region (**sensory**). These dorsal regions contain primarily interneurons that are synapsed on by axons from sensory neurons located in the peripheral nervous system. The dorsal-most region of the alar plate is called the roof plate.

--Ventral region (**motor**). Gives rise to the cell bodies of motor neurons that carry information away from the spinal cord to the muscles. The ventral-most region of the basal plate is called the floor plate.

How is this D/V patterning of neural tube established?

--Grafting experiments established that:

1) there are ventralizing signals from notochord and floorplate. When the notochord is missing, no motor neurons are produced.

--This signal is Sonic hedgehog (Shh), a vertebrate homolog of the Drosophila secreted signaling molecule hedgehog. Shh is expressed in a concentration gradient from ventral to dorsal. The level of Shh determines (by controlling expression of transcription factors) what kinds of neurons differentiate (10.15).

-- Shh induces the expression of ventrally required genes, and in turn represses certain transcription factors, restricting them to the dorsal side of the neural tube.

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2) There are dorsalizing signals from the roof plate (top portion of the neural tube) and overlying epidermis. These signals are TGF-beta family members, including BMP-4. They act by countering the ventral signal (Shh) from the floorplate and notochord. The fact that these same signals (BMPs) served to ventralize the mesoderm and ectoderm during gastrulation should not phase you. Part of what's happened is re-organization—the “ventral” epidermis now also lies above or dorsal to the neural ectoderm. But, this is a theme you will see again—the same signaling molecules are used over and over in the development of an organism. Sometimes a single molecule can have multiple functions that seem contradictory, but remember that time is passing as the embryo develops, and additional molecules are expressed, turned off, or interact as time goes on.

### Cell fate determination in the brain

Our goal now is to understand how cells within the neural tube decide to stop dividing and migrate out to their final destination as part of the brain or spinal cord, and how these cells know where to go. Are they committed? Or do they receive further signals before/during or after they migrate that determine what they differentiate into?

### "Birth" of neurons

In all regions of the developing vertebrate brain, proliferation takes place in the so-called ventricular zone, which is immediately adjacent to the lumen (see ppt slides). At this time, the neural tube is composed entirely of neuronal stem cells called neuronal progenitors. As the cells divide, the cell bodies undergo an interesting migration from the apical to basal surface of the tube. This migration, although not fully understood, appears critical for production of the correct number of neurons--if it is prevented, fewer neurons are ultimately produced. **Asymmetric divisions** are often the hallmark of daughter cells taking on different fates. Commonly, asymmetric divisions result in one daughter containing a cytoplasmic component, and the other not. The segregation of these components then ultimately results in the cells taking on different fates. In the ventricular zone of the neural tube, asymmetric division separate one daughter cell from the ventricular surface (that daughter that is “born” and migrates away), whereas symmetric divisions leave them both in contact with the ventricular zone, and both cells continue to divide.

**Neuronal progenitors are stem cells. A neuron is born when it leaves the mitotic cycle. This process, and subsequent determination of fate depends on many molecules.**

### An example of fate specification: organization of the cortical plate of the cerebral cortex

#### Organization of the laminae of the cortex

The cortex is what allows mammals to “process” information. The neurons executing most of the action are located in the cortical plate of the neocortex. The neurons get to this location by migrating along glial processes that extend from the ventricular zone all the way out to the cortical plate. They are organized into 6 layers, and differentiate in a sequential fashion

#### **How are the fates of these cells determined (*ie*, how do they know which layer to go to)?**

These questions were addressed with two basic techniques (that are NOT covered well in your book, sorry!)

- 1) Clonal analysis : allows identification of the types of cells that a single dividing cell can make. Thymidine labeling experiments (replicating DNA takes up the radioactively labeled T) demonstrated that the first layer of neurons to form is the deepest (layer 5/6) (figs 10.10, 10.11). In other words, these layers are formed by an “inside-out” pattern of migration: the cells that leave the ventricular zone first migrate the shortest distance, to layer 5/6, while cells that are born later migrate out to the more superficial layers, 1/2/3.
- 2) Transplantations: heterochronic: different time (same place) and heterotopic: different place (same time).

In the cortex, the transplantation experiments were heterochronic, and demonstrated several features of laminar determination (see ppt slides):

*1. EARLY: "birthdate" determines fate.*

--When progenitor cells that would normally migrate to deep layers were transplanted into the same area, but into an older host, these cells were able to take on the fate of the cells surrounding them (changed their fate to migrate to superficial layers). Oddly, this was only possible **if** they were transplanted before their final S phase. If they completed S phase in their native environment, they appeared committed. Thus, this time of final DNA synthesis before final division was important for determining cell fate.

--So, there appeared to be two critical factors: changes executed (most likely at the DNA chromatin level) during final S phase, and different signals present in early vs. later stage ventricular zones.

*2. LATER, the possible fate of the cells becomes restricted.*

--When late stage progenitors that would normally migrate to superficial layers are transplanted into earlier stage hosts [ P1 (post-natal day 1) cells transplanted to an E29 (embryonic day 29) host], the transplanted cells cannot change their fate, even if they undergo their final S phase in the new environment.

--They are irreversibly committed to migrate to superficial layers.

Take home: Fate specification of cerebral cortex neurons depends on both intrinsic (time of birth; changes within cell based on time alone) and extrinsic factors (cell contacts and environment). Fate is restricted over time.

**What signals are involved in this progressive fate determination? Is there a "default" fate of the cells that is overridden over time?**

To test this, cells from early stage ventricular zone (E29) were isolated in culture and put into different environments while they were undergoing their final S phase. They were placed in low density (no contacts), pellet (dissociated and then allowed to re-associate; contacts present, but different from usual), and explants (a chunk of tissue in which all normal architecture preserved). After 6 hours in culture, cells that were not already dissociated were dissociated, and the cell suspension transplanted into late stage host (P1). The finding was quite interesting: when not in contact with each other, E29 cells were unable to take on deep layer fate (low density and many of the pellet cells). Thus, the deep layer fate was not something that was intrinsic and lost over time, but rather a fate that had to be maintained by signaling. The deep layer fate was not the default! This experiment did not prove that the superficial layers were default, but did demonstrate that the fate change taken by E29 cells when put into a later stage host could have been due both to LACK of signals from the normal early stage cells, and/or different signals present at the late stage that helped to signal superficial fate. A recent paper (Mutch 2009) suggests that b-catenin could be one of the signaling molecules that specifies deep layer fate.

Thus, in conclusion, signaling is obviously important for cell fate commitment. Progenitor cells in the nervous system, in general, are not committed to their fate. However, their fates can be restricted over time, and this restriction may be due to a change in the environment, or a change within the cell (ie, the production of certain transcription factors simply falling off to a low or absent level).

**Review questions**

1. Where do the dorsal and ventral signals that help the spinal cord take on either sensory or motor fates come from, and how do they pattern the D/V axis?
2. How can BMPs be involved in dorsalizing the neural tube when they are ventralizing molecules in the gastrulating embryo?
3. What is a stem cell, and how do neuronal progenitors in the ventricular zone act as stem cells?
4. How and in what order are the laminae established in the cerebral cortex, and what experiments demonstrated this order?
5. What tells neurons from the ventricular zone where to migrate to and what to differentiate into? Are

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these neuroblasts pluripotent, or are they already specified to take on a particular cell fate?