

CHAPTER

28

Development of the Nervous System

Alison K. Hall

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INTRODUCTION

It remains crucial to consider neural development in a textbook about neurochemistry for several reasons. First, understanding the mechanisms of development reveals adult structure and function. The functions of the adult brain require that the complex neurochemical cell types and functional interconnections in circuits be laid down early in development and that they mature appropriately with experience. Many anatomical features of the mature brain become apparent early in embryonic development, following closely regulated specification. Further, developmental mechanisms must inform therapeutic approaches. We need to understand and utilize biological regulatory mechanisms discovered by studying neural development in therapeutic approaches for adult neural disease and neurodegenerative disorders, to program stem cells, to restore

functional connections by axons re-growing after injury and to reveal the capacity of our brains as we learn throughout our lives. Finally, experimental embryological approaches are stunning in their beauty and impact. The transplantation of one bit of tissue or a bead with a morphogen has revealed the self-organizational, complex properties of embryos that both humble systems biologists and guide developmental geneticists in new directions.

This chapter will outline the embryology of the nervous system and highlight the general structure of brain regions that arise from the neural tube early in development. The role of adjacent, non-neuronal cells in inducing dorsal and ventral identity to cells along the neuroaxis will be discussed, as well as embryonic organizing centers that specify regions of the brain. Even after regional divisions are laid out, neural activity plays a unique role in development of the brain, revealing a functional

tuning and plasticity not seen in other systems. This area has exploded with new understanding—and new questions—in recent years, so that only general findings about neural development, needed to understand other chapters, are addressed here. The reader is encouraged to read more deeply about this rapidly changing field.

EARLY EMBRYOLOGY OF THE NERVOUS SYSTEM

The human embryo, at about two weeks' gestation, includes the three primary germ layers that will ultimately contribute to the entire embryo. These include the ectoderm (future nervous system and skin), mesoderm (future muscle and bone) and endoderm (future lining of gut and some organs), and even at this early stage some aspects of the future brain have already been laid out. At about three weeks of development in the human, overt signs of the nervous system begin to emerge as the neural plate appears as a long ovoid shape on the dorsal side of the embryo. Along the midline of the neural plate, a groove forms and the outer walls of the groove widen gradually, eventually rounding up and closing along the dorsal surface of the embryo, forming the neural tube. The neural tube closure begins around the back of the neck, and extends in expanding spot-welds, eventually forming a closed tube. Along the neural tube, the future spinal cord forms with modest morphological change, but rostral areas of the tube undergo dramatic expansion to form the cerebral hemispheres of the brain. Failure of the neural tube to completely close in caudal regions results in a range of functional deficits called spina bifida. Failure of the neural tube to close at rostral regions results in profound disruption of brain structures in anencephaly, which is generally not compatible with life. Perhaps half of all neural tube defects can be prevented if women consume a folic acid-containing supplement before and during the early weeks of pregnancy (see [Czeizel & Dudás, 1992](#)). Foodstuffs in the United States, including bread and cereal, are now fortified with this vitamin to prevent these very early neural birth defects.

The CNS arises from the neural tube

The wall of the neural tube is initially composed of the simple columnar cells of the neuroepithelium, but quickly gives rise to zones containing neurons or glial cells of the central nervous system. By contrast, most neurons and glia of the peripheral nervous system (and of several other structures) are derived from the neural crest, composed of migratory cells at either side of the neural tube. Neuroepithelial cells undergo cell divisions that can produce additional precursors, post-mitotic neurons, or astrocyte or oligodendrocyte cells, and some of these cells are stem cells (see Chapter 30). Subsequent maturation of the neural tube leads to the primordial layers of the CNS that are easily appreciated in the relatively simple spinal cord: an ependymal layer adjacent to the central canal, the mantle layer with immature nerve cells that is the future gray matter, and the marginal layer with axonal processes of developing neurons that will become white matter. Within

the gray matter, most dorsal (or alar) neurons are sensory, while ventral (or basal) neurons become motor in function, separated by a sulcus limitans. In more rostral regions of the neural tube, however, the telencephalic expansion that forms the brain means that in the adult, most white matter tracts are deep in the brain, while gray matter is more superficial.

The major divisions of the CNS are identifiable early in development

The neural tube also matures along its length, with rostral portions of the tube undergoing extensive proliferation that causes the walls to form bulges, or vesicles. Initially three brain vesicles (forebrain, midbrain, hindbrain) are apparent. Soon, two structures emerge from the initial forebrain, including the telencephalon that gives rise to the cerebral vesicles, and the diencephalon that gives rise to thalamic structures and the retina of the eye. Thus, at about five weeks of human development, the major divisions of the mature central nervous system are clearly identifiable ([Fig. 28-1](#)). Eventually, the dramatic expansion of the telencephalic vesicles leads to the formation of the cerebral cortex, visible on the outside of the brain that surrounds the diencephalic and midbrain structures located more centrally in the mature brain.

SPATIAL REGIONALIZATION

The simple neural tube differentiates along two orthogonal axes: dorsoventral and rostrocaudal. Cells along the neural tube respond to local, diffusible signals that induce sensory or motor neuron populations within the neuroepithelium, and become biochemically patterned very early in development to express genes reflecting their specific functions in their specific, spatial locations (i.e., forebrain versus hindbrain). The signals in these orthogonal axes that pattern large regions of the brain are discussed in this section.

A dorsoventral pattern arises with signals from adjacent non-neuronal cells

Signals from outside the neural tube induce neural precursors to adopt ventral or dorsal cell fates. The ventral neural tube is patterned by sonic hedgehog (*Shh*) secreted by notochord and floorplate, while the dorsal neural tube receives signals from bone morphogenetic protein (*BMP*) from skin ([Fig. 28-2](#)). Shh signaling in the ventral portion of the neural tube is most notably responsible for the induction of floor plate cells and motor neurons ([Roelink et al., 1995](#)). The ventralizing power of the notochord and the Shh it secretes has been demonstrated using embryological studies. For example, if a second notochord is transplanted to a new location next to the intermediate spinal cord, a new floorplate and new motor neurons develop next to the grafted notochord. By contrast, if the host notochord is removed, motor neurons do not form ([Van Stratten 1985a, b](#)). In tissue culture studies, shh addition at different concentrations results in particular cell types derived from the neural tube. Shh at high concentration induces floorplate cells that normally form at the ventral

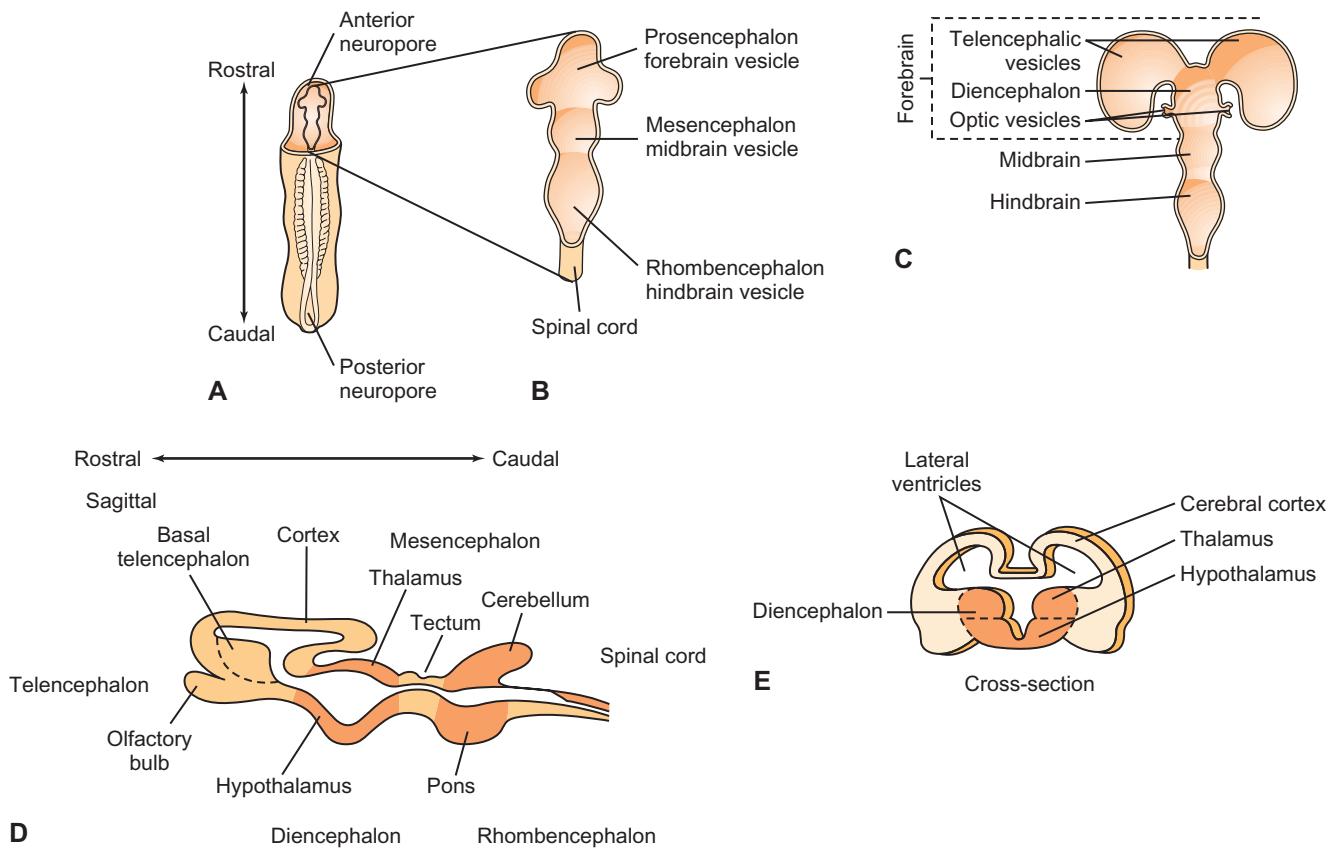


FIGURE 28-1 The neural tube forms enlarged vesicles that correspond to major brain regions. (A) The neural tube as it appears on the dorsal surface. First three and then five major brain vesicles (B) form that give rise to the entire brain. Arrows correspond to (C) sagittal section and (D) cross-section, which reveal major brain regions. (E) Adult spinal cord cross-section reflects simple dorsal alar sensory and ventral basal motor neuron pattern established in the embryo.

midline during the neural fold stage, while at lower concentrations Shh induces motor neurons and interneurons. This kind of concentration dependence suggests that Shh is a morphogen. The ligand Shh binds to the patched receptor, and Shh signaling allows the intracellular Gli transcription factor to signal to the nucleus. Retinoic acid is also required ventrally along with Shh during differentiation of motor neurons.

There is also an active dorsal BMP signal from the skin that establishes another signaling center, the roof plate. BMP seems to act in a concentration-dependent manner in dorsal regions, similar to Shh in the ventral aspect (Liem et al., 1995). BMPs actively induce dorsal cells, slightly later than ventral cell induction. The ligand BMP signals through cell surface BMP receptors and intracellular SMADs that are protein transcription factors translocated to the cell nucleus.

In combination, these molecules induce a distinct combination of transcription factors in neural precursors. Each different progenitor cell domain in the ventral neural tube is established by different classes of homeobox transcription factors grouped into class I and Class II genes (depending on their response to Shh activity) and whose borders are refined by cross-repression. For example, progenitors give rise to motor neurons (or to oligodendrocytes) depending on the activities of the protein Olig2 and the homeobox genes are Pax6, Nkx6.1 and Nkx6.2 (see review by Wilson & Maden, 2005).

The rostrocaudal axis is specified by homeobox-containing genes

The same genetic systems in many animals are used to control regional development of the body plan, and some of those genes specify rostro-caudal domains of the nervous system. For example, the rostrocaudal axis of the fruit fly *Drosophila* is set up initially by factors unequally deposited in the egg that then subsequently regulate genes to be expressed in one but not another region of the developing embryo. A hierarchy of gene actions divides the embryo into smaller and smaller domains with unique identities (involving gap, pair-rule and segment polarity genes). The homeobox-containing or Hox genes control the ultimate identity of a segment. This results in a reproducible, segmented embryo and adult, at least in the fly. A rather spectacular example of the regulatory control imposed by Hox genes described in many textbooks is the genetic mutation of ultrabithorax gene (*Ubx*). *Ubx* normally specifies the third thoracic segment, and mutants lacking this gene are flies that have an additional second thoracic segment and four wings.

Homologous homeobox genes were found in the genomes of mice and humans as well as flies, and much work has been done to learn the functions of homeobox genes in nervous system patterning. Homeobox genes encode transcription factors

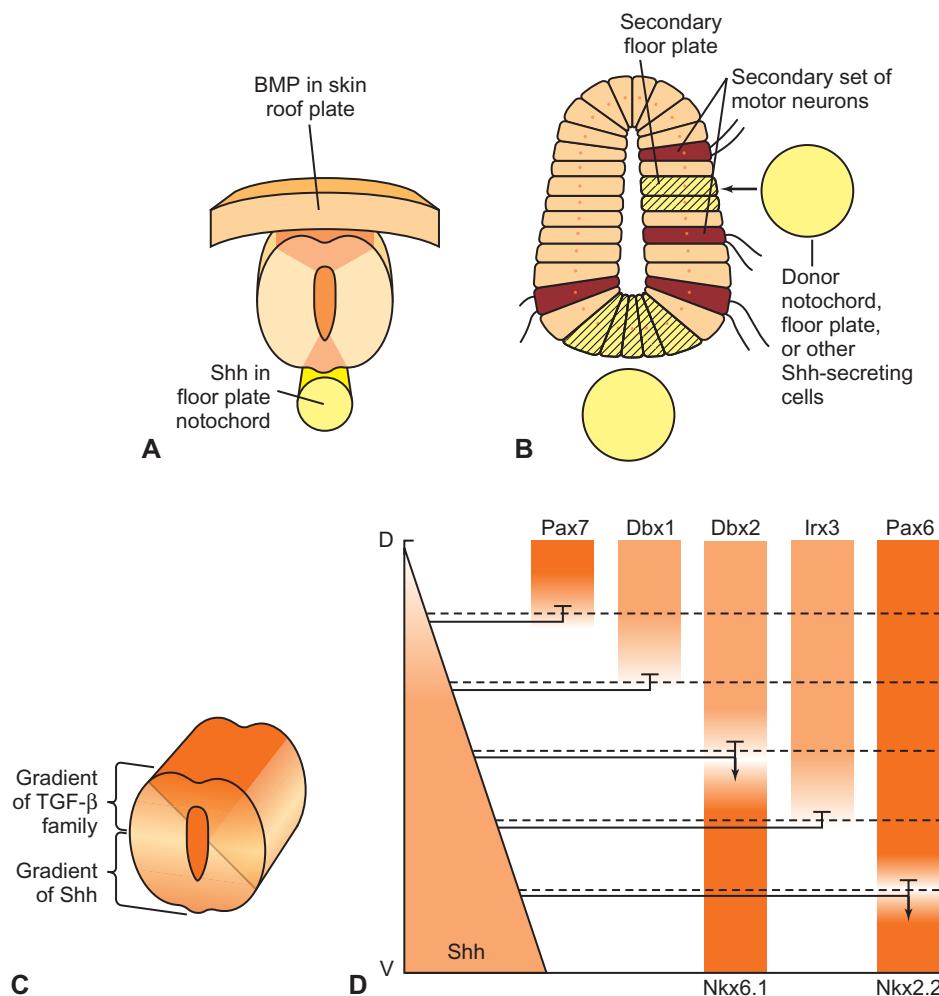


FIGURE 28-2 Dorsoventral patterning of the neural tube. (A) The neural tube is exposed to bone morphogenetic proteins (BMPS) from the dorsal epidermis and sonic hedgehog (*shh*) from ventral notochord. These set up secondary signaling centers in the roofplate and floorplate, respectively. (B) Implantation of a second notochord adjacent to the intermediate neural tube induces a second floorplate and additional adjacent motor neurons. (C) The two signaling centers establish a gradient of signaling that results in (D) differential transcription factor expression, depending on position in the neural tube.

that typically switch on cascades of other genes. Curiously, the rostral border of genes in a cluster are expressed in order along the length of an embryo, often with broad expression of genes at the 3' end, and very precise delineation of the rostral limits of more 5' genes. In mammals, there are four clusters of Hox genes expressed in overlapping domains in the nervous system (Fig. 28-3). The proteins they encode contain 60-amino-acid DNA-binding domains. Different Hox genes also have different sensitivities to retinoic acid: The sensitivity to retinoic acid (RA) matches the order of the genes in a cluster, and matches their expression in the embryo. The Hox genes at the 3' end of clusters are most sensitive to retinoic acid (and thus turn on at low concentrations of RA) and are expressed throughout much of the embryo, and genes at the 5' end of the cluster are least sensitive to retinoic acid (and thus require high concentrations of RA) and are expressed only in the posterior of the embryo. Thus retinoic acid is a “posteriorizing” agent in embryos.

An interesting example of Hox gene function in the vertebrate nervous system is seen in the developing hindbrain. In

the hindbrain, localized swellings of the neural tube called rhombomeres are normally associated with the sensory and motor components of cranial nerves. The anterior boundaries of Hox gene expression also correspond to rhombomere borders, and it seems that the specific group of Hox genes expressed in a rhombomere specifies its identity. For example, *Hoxa2* is the only Hox gene in r2, *Hoxa2* and *Hoxb2* are coexpressed in r3, and r4 expresses multiple Hox genes. One way to study Hox function is to isolate transgenic animals that lack particular Hox genes. When *Hoxa1* (or *Hoxb1*) was deleted, embryos had reduced r4 and r5 rhombomeres. However, *Hoxb1* expression requires *Hoxa1* expression itself, complicating matters. With *Hoxa2* deletion, the r1/r2 boundary is absent, r1 is enlarged and r2/r3 reduced, suggesting at least a partial switch in fate to an r1 phenotype (Gavalas, 1997). In general, the loss of a Hox gene by mutation will cause that part of the embryo to develop structures that are normally only found more anteriorly, although particular gene expression (and cross-regulation) may affect that finding.

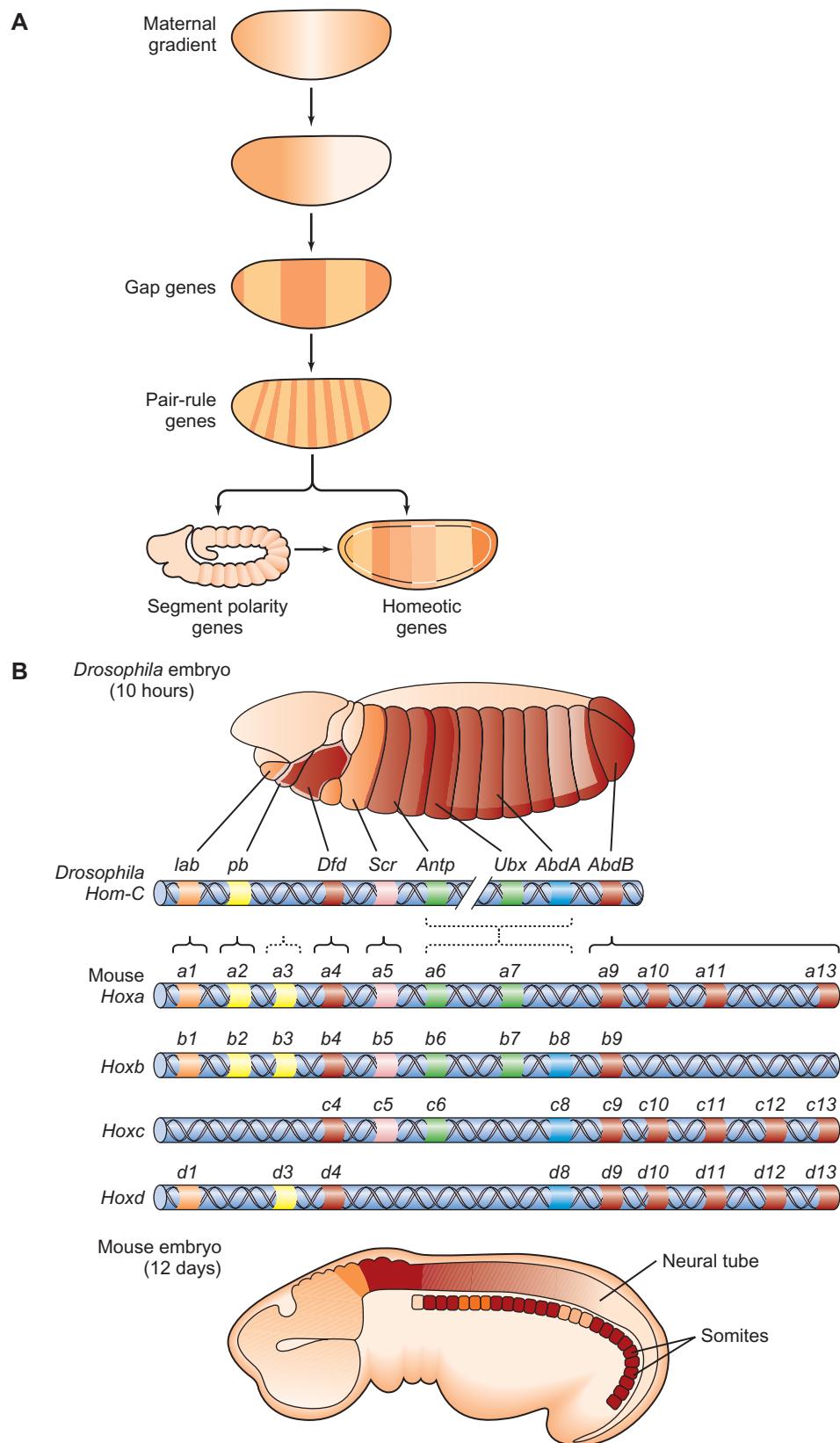


FIGURE 28-3 Homeotic genes regulate rostrocaudal patterns conserved in fly and mammal. (A) The fly embryo is patterned in sequentially smaller domains by maternally derived polarity, gap, pair-rule, segment polarity and homeotic genes. (B) Homeotic genes are conserved in fly Hom-C cluster and mammalian 4 Hox gene clusters. Mouse genes with higher numbers are expressed later and more posteriorly. Retinoic acid levels are high in posterior regions.

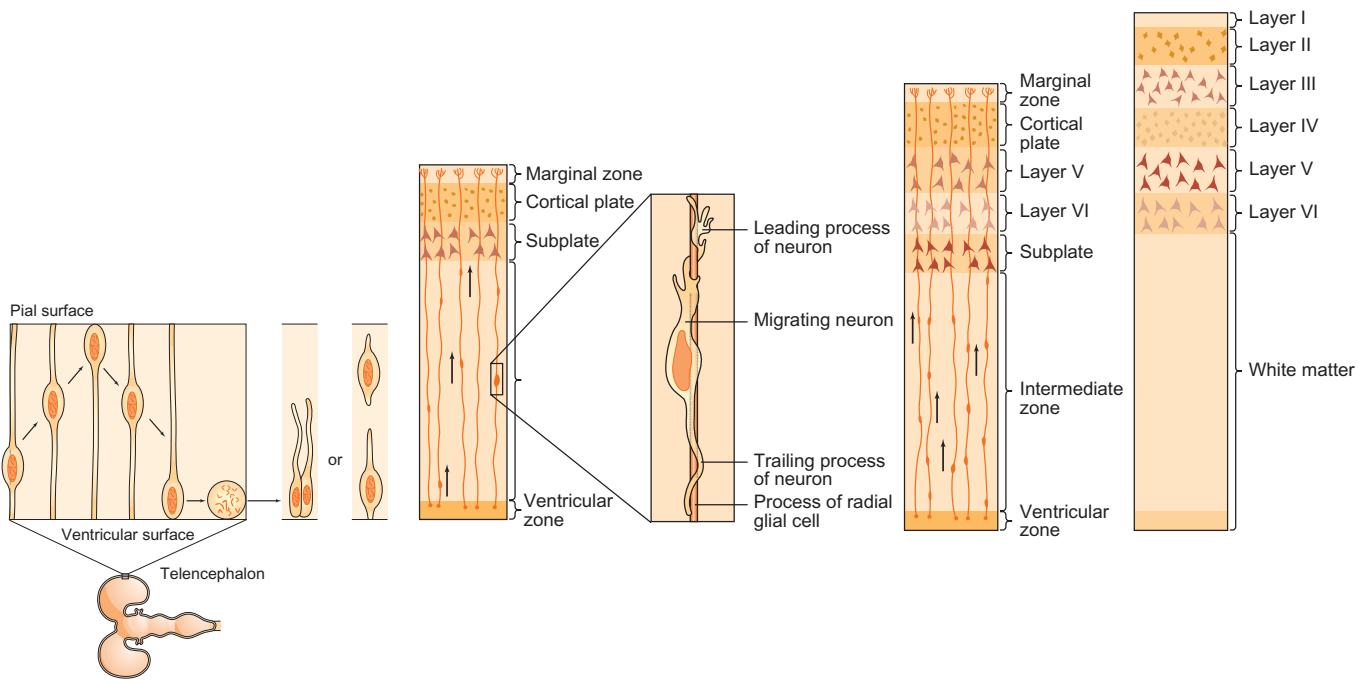


FIGURE 28-4 Cell division in the neural tube gives rise to cortical neuron layers. Symmetric cell division early gives rise to similar cells, while asymmetric cell division gives rise to a precursor and a neuron. Postmitotic neurons migrate on radial glial cells to form layers of distinct cortical neurons.

Specification of motor neurons along the spinal cord also involves Hox genes. Whole columns of motor neurons that innervate the fore- or hind-limb regions are specified by adjacent mesoderm called somites, and expression of Hox proteins is closely aligned with the position in which molecularly defined motor neuron subtypes are generated (Jessell, 2000). That is, expression of Hox6 proteins segregates with forelimb lateral motor column (LMC) neurons, Hox9 proteins with thoracic PGC neurons, and Hox10 proteins with hindlimb LMC neurons.

Embryonic signaling centers organize large regions of the brain

Major CNS areas are specified during development by distinct domains of gene expression established by embryonic signaling centers (Fig. 28-4). Unlike the spinal cord, the most rostral brain regions are underlaid not by notochord, but by pharyngeal endoderm and prechordal mesoderm, which play similar inductive roles but are specified somewhat differently. A secreted factor, Cerberus, present in prechordal mesoderm was shown to induce the most anterior parts of the amphibian brain. Cerberus blocks both BMPs and Xwnt8 (Glinka et al., 1997). It appears that forebrain is made by blocking both Wnt and BMP4.

At the midbrain/hindbrain boundary, a local embryonic signaling center called the isthmus secretes inductive factors that pattern surrounding brain. In chick embryos, if a second isthmus was grafted to a new location in the brain, it induced new midbrain or cerebellar structures and set up the rostrocaudal axis of midbrain as reflected by the expression of engrailed 1 (caudal) and engrailed 2 (rostral) (Alvarado

Mallart, 2005). This suggested that patterning of the midbrain and cerebellum is controlled by the centrally located isthmus, which instructs the cells on one side to become the midbrain and on the other side to become the cerebellum. Wnt1 (homolog of wg wingless) and FGF8 are secreted by isthmus cells and control mesencephalic differentiation. Implantation of FGF8 beads can also duplicate midbrain regions, just as isthmus transplants do (Crossley et al., 1996). FGF8 beads also induce expression of Wnt1, En-2 and FGF8 itself, all genes of the isthmus. The expression of transcription factors GBX2 in the anterior hindbrain and OTX2 in forebrain and midbrain also set up large domains that have a common border at the isthmus. The function of these genes appears to be in forming a boundary (Millet et al., 1999). It may be that this region first establishes the Otx2 and Gbx2 domains, followed by En, Fgf8, and Wnt1 induction by the presumptive “midbrain/cerebellum” domain. OTX2 and GBX2 then restrict Fgf8 and Wnt1 to their appropriate domains to form the organizer.

The forebrain telencephalon gives rise to the cerebral cortex, hippocampus, basal ganglia, olfactory bulb and amygdala, which represent a highly complex and very human region of the CNS and whose general layout is also established by signaling centers. Early in development, similar dorsoventral patterning based on BMP/Wnt dorsal signals and Shh ventral signals specify positional identity. In addition, key embryonic signaling centers define rostrocaudal position, including the rostral patterning center in the septum that secretes FGFs, and a cortical hem that secretes Wnts and BMPs (Fig. 28-5) (O’Leary, 2007). The signaling centers create strong effects locally and diminishing effects distantly. This spatial gradient of induced expression of specific transcription factors initiates regionalization.

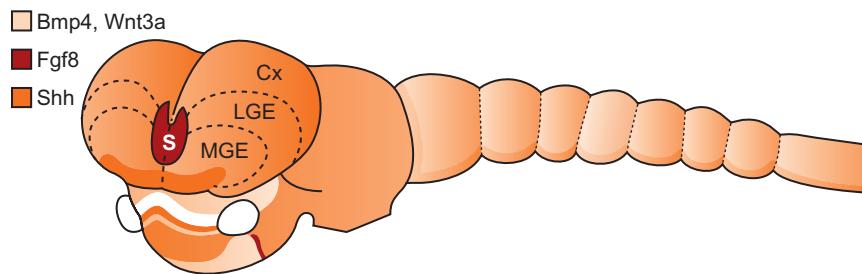


FIGURE 28-5 Embryonic signaling centers in the brain. A rostral patterning center at the septum secretes fgfs; a dorsal center called the cortical hem secretes wnts and bmps, and a mid/hindbrain isthmus secretes fgfs. From Hoch et al., (2009).

Homeobox transcription factors serve essential and reciprocal functions compartmentalizing the forebrain. The forebrain can be divided into prosomeres (akin to rhombomeres) subdivided by longitudinal and transverse boundaries and identified by three families of homeobox genes (Dlx, Otx, Nkx) and several Wnt genes that have restricted patterns of expression that specify the domains (Puelles & Rubenstein, 2003). These regionally enriched transcription factors affect the allocation of areas to specific functions in the adult.

NEUROGENESIS AND GLIOGENESIS

Neurons have a birthdate

From the mammalian neuroepithelium, neurons are usually produced first, and various glial cells later. Initially, neuroepithelial cells undergo cell division accompanied by nuclear migration within the cell, with DNA synthesis occurring near the midpoint, and cytokinesis completion at the ventricular region. Some neuroepithelial cells transition to a radial glial cell that has endfeet at ventricular and pial surfaces. During development, radial glial cells provide the scaffold for migrating cells in the cortical plate. Radial glial cells share some similarities with neuroepithelial cells, but also appear rather heterogeneous and express selective markers GLAST, RC2 and BLBP in varying combinations (Pinto & Götz, 2007). Radial glial cells can also divide asymmetrically to produce a self-renewing radial glial cell and a postmitotic neuron (Götz & Huttner, 2005). Indeed, time-lapse fluorescent imaging and lineage mapping studies confirm that radial glial cells can produce neurons and glia (Noctor et al., 2001; Malatesta et al., 2003). Because neurons are postmitotic in the adult, the embryonic day on which a precursor cell division gives rise to a neuron is called a neuron birthdate. In the cerebral cortex, a neuron birthdate is related to the particular cortical layer position and function that the neuron ultimately acquires. Indeed, early-born neurons migrate to a location just past the subplate, while later-born neurons migrate past them to reside in sequentially outer layers of the cortex (Fig. 28-5).

Reelin and notch signaling contribute to cortical layer organization

Some of the factors that regulate the process of cortical neuron migration have recently been clarified. The extracellular

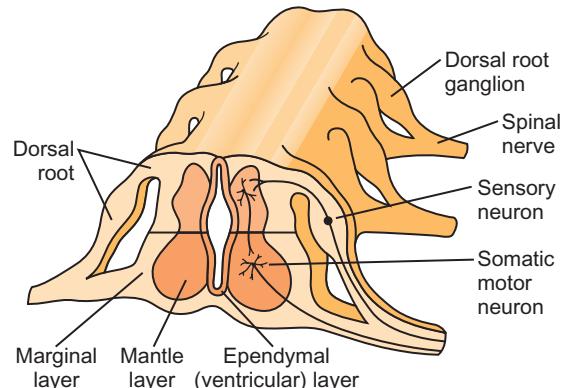


FIGURE 28-6 Development of the spinal cord. The neural tube rounds up with dorsal (alar) regions and ventral (basal) regions, separated by the sulcus limitans. This early neural tube structure is largely unchanged morphologically in the adult spinal cord, with layers of ventricular (ependymal), mantle (neuron cell bodies)- and marginal (axonal) layers. In the telencephalon, some precursors migrate tangentially from the ganglionic eminences and also give rise to cortical neurons.

matrix-associated glycoprotein reelin is secreted by Cajal-Retzius cells in the developing cerebral cortex. Reelin is essential for the stereotyped “inside-out” layering seen in development. Mice with a deficiency in reelin (Reeler) have an inverted lamination of the neocortex. The human reelin (RELN) mutation has been linked to lissencephaly and autism (Hong et al., 2000). Reelin interacts with Notch signaling in neuronal migration. Reelin-deficient mice have reduced levels of the Notch signaling molecule, and loss of Notch signaling itself in migrating neurons results in migration defects. Overexpression of Notch signaling molecule repairs the laminar and morphological abnormalities of migrating neurons in Reeler (Hashimoto-Torii et al., 2008).

Neuronal migration can also take circuitous routes. Telencephalic neurons arise not only from cortical neuron generation at the ventricular zone, but also with precursor migration from ventral sources. In particular, the vast majority of GABAergic interneurons and projection neurons are generated in the lateral and medial ganglionic eminences of the telencephalon and they migrate tangentially in the lateral cortical stream (Fig. 28-6).

TUBEROUS SCLEROSIS

Alison K. Hall

Tuberous sclerosis complex (TSC) is a group of multi-system tumor disorders characterized by benign tumors in the brain, kidneys, lungs, heart or skin. When affecting the brain, TSC can be accompanied by seizures, mental retardation and behavior problems. Patients with TSC are often diagnosed in the first few years of life, may undergo surgical removal of tumors, and often require continued management throughout life. The severity of symptoms vary, with some individuals enjoying relatively normal lives, and others bearing more serious complications.

Two genes have been identified that underlie TSC, and only one needs to be affected for the disorder to be present (Leung et al., 2007; Inoki et al., 2005). The TSC1 gene located on chromosome 9q34 codes for the protein hamartin, while TSC2, located on chromosome 16p13 codes for the protein tuberin. Hamartin and tuberin proteins form a complex inside cells that turns out to be a molecular switch for cell growth. Hamartin-tuberin puts the brakes on a key kinase, mammalian Target of Rapamycin (mTOR), which normally activates S6K and drives cell growth (see figure). When either of these genes is defective and the tuberin-hamartin complex malfunctions, the mTOR pathway is constitutively activated and tumors can form.

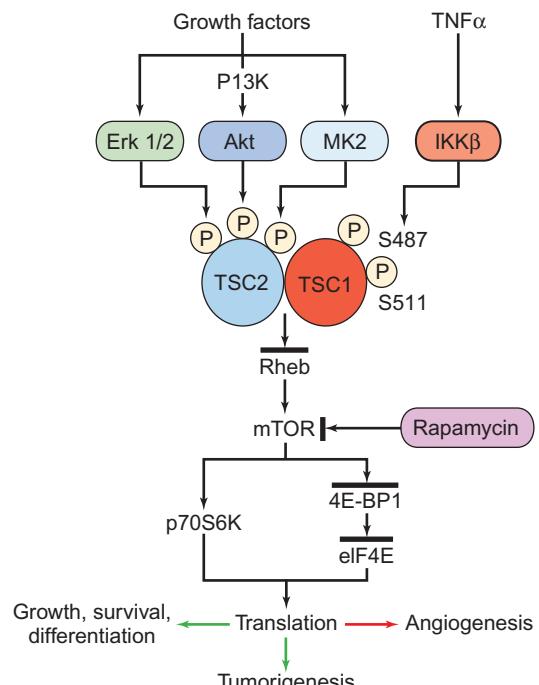
The assumption has been that the neurobehavioral deficits seen in some patients were directly related to the number of tubers present in the brain, or repeated seizure activity, but resolution of the molecular pathway suggests a third explanation. For years, there were surprising findings that some patients with many brain tubers functioned normally, while others with few tubers had significant cognitive challenges, so tuber load wasn't the clear explanation. The mTOR pathway also controls the neuronal actin cytoskeleton and synaptic contacts, and defects in these processes might also underlie the cognitive deficits.

Drugs to control the mTOR pathway are already in use and may control TSC. Initially, a mouse model for TSC was useful in understanding the disorder. Mice with an inactivating mutation in *Tsc2* gene show cognitive deficits, and brief treatment with the mTOR inhibitor rapamycin in adult mice rescued the behavioral deficits. Rapamycin is already in use as an immunosuppressant, particularly after kidney transplant, and clinical trials are now studying its effects in controlling aspects of TSC (Bissler et al., 2008). In late 2010, the FDA approved a rapamycin-based drug for TSC patients with subependymal giant cell astrocytoma who

cannot be treated with surgery. Initial reports with rapamycin-based drugs have been encouraging, but there is concern that tumors may re-grow when treatment stops.

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Neuronal specification involves proneural and neurogenic gene functions

As large regions of the neuroaxis adopt regional fates, within them, individual cells acquire distinct cellular identities. One of the important “decisions” made by these cells is whether to become neuronal or glial. Many of the genes important in neuronal specification were initially identified in model organisms such as *Drosophila*. In flies, the neurogenic region is ventrally located (rather than dorsally as in mammals) and during development, some cells in this region delaminate and

give rise to neuroblasts that proliferate and differentiate into motor neurons. The cells in the neurogenic region that do not give rise to neurons differentiate into non-neuronal support cells. In the fly neurogenic region, the production of neurons and non-neuronal cells is determined by proneural genes, which define the population of cells that are competent to, or might adopt, a neural fate. Proneural genes are transcriptional regulators that code for helix loop helix (HLH) proteins and they are expressed in clusters of cells in the neurogenic region from which individual precursors will be singled out. Proneural genes include the achaete-scute complex and atonal.

In flies, when a proneural gene is deleted, there are no neuroblasts, and by contrast, if a proneural gene is overexpressed, there are extra neurons and sense organs (see review by Urbach & Technau, 2004).

Vertebrate homologues to proneural genes include Mash1 related to the achaete-scute family and others related to atonal (Math 1, Neurogenin and NeuroD). Many of these proneural genes are capable of promoting ectopic neuronal differentiation (including *Xenopus* Xash3, chicken Cash 4, mouse Ngn1). Some, however, are normally expressed too late *in vivo* to play such a role (i.e., NeuroD) and likely play roles in controlling exit of a progenitor from the cell cycle.

In *Drosophila*, the number of neurons that eventually come from the cluster of cells that express proneural genes is determined by the action of neurogenic genes. Early hints from embryological studies showed that when presumptive neuroblasts were ablated, they were replaced by other adjacent cells. It seems that any of the competent cells in a proneural cluster might become a neuron, but lateral inhibition mediated by the neurogenic genes suppresses other cells of the proneural cluster from becoming neurons. When one cell becomes determined as a neuron, it inhibits the others in the cluster. In *Drosophila*, the inhibitory signal between cells is the Delta ligand, and neighboring cells express the receptor Notch (or jagged, or serrate receptors). During lateral inhibition, one cell acquires more Notch activity than its neighbors, and the two cells adopt different fates. Local cell interactions mediated by Notch restrict expression of AS-C factors to the one neuroblast, while the surrounding epithelial cells do not express achaete-scute genes. In *Drosophila* deficient in Notch, all cells express achaete-scute genes, there is no lateral inhibition and all become neuroblasts (the lethal "neurogenic" phenotype).

In mammals, these genes also regulate neuronal specification and have additional functions. The mammalian Notch signaling pathway includes four receptors and five ligands in the Delta and Jagged families. Activation of notch receptor signals activates HES (hairy and enhancer of split related) genes that are negative regulators of proneural proteins Mash and Neurogenins. Notch signaling results in accumulation of b-HLH proteins and antagonizes proneural genes, preventing cells from becoming neural, and thus they become glia. Notch signaling also appears to actively promote glial fate (Gaiano & Fishell, 2002).

During CNS development, neural progenitor cells give rise in succession to neurons and glia, including astrocytes and oligodendrocytes. Mature astrocytes contribute to homeostasis at synapses, nodes of Ranvier, the blood-brain barrier, and structural elements at the pial surface. Astrocytes mount fibrotic responses to injury, and appear to be morphologically and biochemically heterogeneous. Astrocytes are derived from neural precursors both in shared lineages with neurons and from lineages with oligodendrocytes.

Development of the brain requires control over precursor proliferation and differentiation to produce appropriate populations of neurons. Multiple signaling pathways, including FGF and wnts as well as notch and sonic hedgehog pathways (discussed above), can regulate neural progenitor number. A recent study evaluated the central role of GSK-3 in neurogenesis. When both isoforms of GSK-3 were conditionally deleted

in precursors using a nestin promoter, a dramatic increase in neural progenitors and decrease in postmitotic neurons was observed (Kim et al., 2009), suggesting GSK-3 may be a common signal regulating neural homeostasis through multiple signaling pathways in development.

PNS DEVELOPMENT AND TARGET INTERACTIONS

Unlike the CNS, which develops from a neuroepithelium and forms characteristic layers, the peripheral nervous system is derived from migratory multipotent cells of the neural crest. The peripheral nervous system consists of autonomic (sympathetic and parasympathetic) and sensory neurons as well as associated peripheral glial cells, and is organized in groups of cells called ganglia.

The neural crest gives rise to PNS derivatives by induction

The neural crest is a transient, highly migratory population of precursor cells that divide rapidly and give rise to many different cell types. Neural crest cells migrate through the anterior portion of the somite and along stereotyped pathways toward their peripheral sites. We know that extracellular matrix molecules like fibronectin and laminin are on crest cell pathways, but these molecules seem to be permissive in action. Interestingly, the posterior portion of the somite appears to exclude crest cell migration by expressing inhibitory ephrins. Thus, it is likely that neural crest migration is allowed by cell substrate adhesion molecules and restrained by repellent factors. Elegant embryological studies by Le Douarin using chick-quail transplants have shown that different neural crest cell populations along the neuraxis give rise to particular mature derivatives in response to local tissue cues (Le Douarin 2011 video).

The neural crest has proven useful in understanding the differentiation of specific cellular phenotypes in the peripheral nervous system. A compelling example comes from the adrenergic sympathetic neurons derived from neural crest precursors by local BMP signaling (Fig. 28-7). Neural crest cells that migrate near the dorsal aorta coalesce as sympathetic ganglia and differentiate into adrenergic neurons. Cells in the dorsal aorta normally express BMP2/4, and thus BMPs were thought to be important in adrenergic induction. Indeed, overexpression of BMP4 near this region results in the ectopic development of TH-positive, DBH-positive cells *in vivo* (Reissmann et al., 1996). Conversely, implantation of beads that release a BMP inhibitor near the dorsal aorta prevents the development of sympathetic neurons (Schneider et al., (1999)). These data point to an important role of BMPs in adrenergic neuron specification. Similarly, neural crest stem cells *in vitro* when exposed to BMPs may differentiate into cells with autonomic neuron markers Mash1 and Phox2a. When challenged with neuregulin, however, gliogenesis is enhanced (Shah et al., 1994; Morikawa et al., 2009).

Sensory neuron differentiation is also regulated by transcriptional mechanisms including Runx genes. Runx1 is expressed

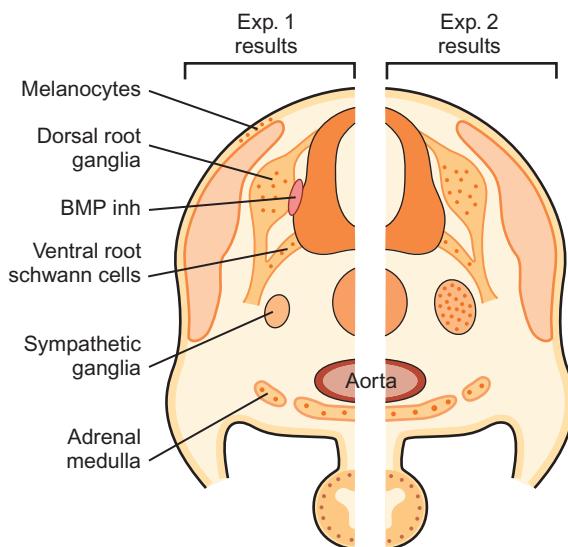


FIGURE 28-7 Neural crest cell progeny are affected by tissue signals. The neural crest gives rise to sensory dorsal root ganglion neurons and glia, peripheral nerve Schwann cells, and autonomic sympathetic and parasympathetic neurons, among other derivatives. The dorsal aorta expresses BMPs early in development that are important for adrenergic differentiation (left). If a bead with a BMP inhibitor is implanted, adrenergic neurons do not form in sympathetic ganglia (right). If BMP is overexpressed, many more neurons are adrenergic.

by the TrkA+ population of cells that contacts skin, while Runx3 is expressed by the TrkC+ cells that contacts muscle. Runx1 is required for the expression of a variety of proteins critical for nociceptor function (Chen et al., 2006). A complex interplay of intrinsic genetic programs and extracellular signals appears to regulate phenotypic determination in the PNS.

AXON GUIDANCE CONTRIBUTES TO CORRECT CONNECTIONS

As neurons mature, they extend axons to make appropriate functional connections during axon outgrowth. Young neurons extend processes with growing tips called growth cones for pathway selection (see Chs. 7, 32). The growth cone is specialized with flat lamellipodia and finger-like filopodia that appear to probe the environment, and ultimately consolidate to direct the growth cone. Growing axons express large cell surface integrins that bind to extracellular matrix molecules like laminin in many general pathways in the developing nervous system. Axons travelling in the same direction may also become closely connected by fasciculation, a process enhanced by cell adhesion molecules (see Ch. 9).

More specific guidance cues including chemoattractants can alter growth cone trajectory. Tessier-Lavigne and colleagues identified the chemoattractant netrin, which is secreted by midline neurons in the spinal cord (Moore et al., 2007). The resulting gradient of netrin attracts dorsal horn neuronal axons with netrin receptors to grow toward and then to cross the midline, ultimately forming the spinothalamic tract. The midline also contains a second repellent

molecule, *slit*. Interestingly, dorsal horn neurons initially lack slit receptors called *robo* until they pass the midline, where *robo* is then upregulated (and the resulting axons are repelled from returning to the midline).

Ephrins and their Eph receptors often play crucial roles, often in chemorepulsion in axon guidance (see Ch. 26). For example, in the visual system, retinal ganglion cells (RGCs) project first to the optic chiasm and then to the tectum/superior colliculus with a careful topographical map, and this system is strongly affected by ephrins. Eph receptors are present on the neuronal growth cone, and graded expression of both receptors and ligands contributes to this topographical map (Birgbauer et al., 2001). In the retina, repulsive ephrin-B drives RGCs into the optic disc. At the optic chiasm, repulsive EphB segregates ventrotemporal RGCs to the correct side of the brain for setting up binocular vision. Finally, in the superior colliculus, attractive EphB forward signaling guides RGCs to their proper termination sites.

Naturally occurring cell death eliminates cells and synapses

Throughout the vertebrate nervous system, about half the neurons originally generated in embryogenesis die during normal development. The number of neurons present in the adult depends heavily on the target tissues they contact. The effects of target removal suggest that neurons must find suitable targets producing specific factors in order to survive. Such factors are called neurotrophic ("trophic" means "to nourish"), and they are present in a limited supply. Thus, normally occurring cell death results from the failure of some neurons to compete for the small amount of the trophic factor.

The role of target was established in embryological studies performed in Victor Hamburger's laboratory, where the extension of motor neurons to their targets in hindlimbs was explored. Early in development, many motor neurons developed initially, but about half that number was present in normal adults. If a hindlimb was removed early in development, even fewer motor neurons were present on the corresponding side of the spinal cord. If an extra hindlimb was grafted onto the embryo, many more neurons were present on that side of the spinal cord in the mature animal.

Much of the early information on neurotrophic factors was obtained from investigating nerve growth factor (NGF) (see Ch. 29). In experiments on neuronal survival by Hamburger and Levi-Montalcini, implantation of a mouse sarcoma was found to increase the size of nearby sensory and sympathetic ganglia. Later, snake venom and male mouse salivary gland were shown to possess a similar neurotrophic activity, and the agent responsible was ultimately identified as NGF. NGF does not affect all kinds of neurons; its survival activity is limited to sympathetic chain and sensory ganglia of neural crest origin and some cholinergic neurons in the CNS. Other neurotrophins have been identified, including brain-derived neurotrophic factor and neurotrophins-3 and -4, which confer survival on different types of neurons. Glial cell line-derived neurotrophic factor (GDNF) is a survival factor for parasympathetic neurons, while a family of molecules—including neurturin, artemin and persephin—appears important for parasympathetic neuron survival. Trophic factors and their

receptors have been identified with selective and often overlapping functions in neurons of the CNS and PNS (see Ch. 29).

Neurotrophic factors were initially thought to promote survival by stimulating metabolism (hence their name). Instead, these factors appear to suppress a latent suicide program in neurons that leads to apoptosis. Apoptosis is described in detail in Chapter 37. The first evidence that lack of a neurotrophin kills neurons resulted from studies in which protein or RNA synthesis was inhibited in sympathetic neurons. These neurons required NGF for survival. However, if one removed the growth factor and inhibited protein synthesis, the neurons lived. This result suggested that there was an active death program that required protein synthesis. We now know of many genes in the apoptotic program, including Bax, Apaf-1 and caspase genes, which are pro-apoptotic, and we know that the binding of neurotrophins to their cognate receptors leads to promotion of Bcl-like anti-apoptotic activity, or to inhibition of caspase activity. These cell death genes cause neurons to die by apoptosis, leading to the systematic dismantling of the cell, while neurotrophins switch off the cell death program.

SYNAPSE FORMATION

The neuromuscular junction between motor neurons and muscle cells

The neuromuscular junction formed between motor neuron axons and target muscle cells has proven a rich research model system in which to explore how specific synapses form. The neuromuscular junction is the best-characterized synapse, in large part because it is simple and accessible. In this synapse, when the motor neuron depolarizes, synaptic vesicles are mobilized and fuse with the plasmalemma, releasing the neurotransmitter acetylcholine (Ach), which travels across the synaptic cleft where it reaches acetylcholine receptors (AChR) on the postsynaptic plasma membrane of the myofiber. As the AChRs open ion channels, the myofiber membrane depolarizes, resulting in muscle contraction (see Chs. 13, 44).

Motor neurons and muscle fibers initially develop independently of each other in the embryo, but they each modify the other during synapse formation (Fig 28-8). Motor neuron growth cones contact the myotube and can initially generate very weak synaptic transmission, which improves with consolidation of ACh receptors. Initially, the axon of the motor neuron releases the proteoglycan agrin into the extracellular space at the point of contact. Agrin sets off interactions first that activate a muscle-specific kinase (MuSK) receptor in the postsynaptic muscle membrane, and this in turn leads to downstream activation of the cytoplasmic protein Rapsyn. Rapsyn contains domains that allow for AChR association and multimerization, and it is responsible for AChR clustering in the postsynaptic membrane. Thus, with synapse maturation, AChRs become locally concentrated in the plasma membrane of the myotube in postsynaptic densities. Additional components are recruited into these densities in the mature synapse. Correspondingly, the presynaptic axon grows in size and increasing numbers of synaptic vesicles cluster at the active zones. These presynaptic changes are

thought to be mediated by neurotrophin secreted by muscle cells and cell adhesion molecules on the postsynaptic membrane. These and other aspects are discussed in a recent review (Sanes & Yamagata, 2009).

The formation of central glutamatergic synapses involves similar developmental events but somewhat different specific players (McAllister, 2007). Axodendritic contacts may be facilitated by cell adhesion molecules, but are also influenced by neuroligins and neurexins. Signals from the postmitotic cell, likely involving the neurotrophin BDNF, activate TrkB influence over synapse formation. Deficits in one or more of these complex signals may underlie psychiatric or neurodevelopmental disorders.

The study of synapse formation has benefited from some truly novel imaging approaches over the years, including fluorescent imaging of axons in living animals over time (McCann et al., 2008) and imaging afforded with molecular tools. The novel “Brainbow” mouse transgenic lines were developed to visualize neuromuscular form, and their physiology can be extended to study neural circuits throughout the nervous system (Livet et al., 2007). Genomes of the Brainbow mice contain a transgenic insert consisting of randomly arranged, fluorescent protein genes separated by loxP sites and driven by neural/glial-expressing Thy1 promoter. After Cre recombination, the transgene undergoes excision events that position a different fluorescent protein gene adjacent to the promoter, resulting in its expression so that each neuron expresses one of a wide variety of possible fluorescent protein combinations.

ACTIVITY AND EXPERIENCE SHAPE LONG-LASTING CONNECTIONS

Unlike early neural developmental events, synaptic rearrangement during late synapse formation requires neural activity. Some rearrangement occurs as the result of spontaneous activity during embryogenesis, but the majority of synaptic rearrangement happens after birth and as a result of sensory experience or motor activity. Crucial studies elucidating general principles of postnatal nervous system development were performed by Torsten Weisel and David Hubel who, along with Roger Sperry, shared the Nobel Prize in 1981 for their work on the development of the visual system.

In the mammal, axons from the retina of the eye grow in specific patterns to each lateral geniculate nucleus (LGN), which is similar to the tectum of other animals. In the LGN, axons from each eye initially spread out in the nucleus, but in the adult, axons from the two eyes segregate into eye-specific domains. Blocking neural activity with tetrodotoxin prevents the segregation, indicating that neural activity must be important.

Segregation appears to require synaptic stabilization of coordinately active cells. In the simplest sense, only retinal presynaptic axons that are active at the same time as the postsynaptic LGN target neuron “win” and are retained, while asynchronous neuronal inputs “lose.” Donald Hebb proposed this mechanism for synaptic modification, suggesting that when a retinal wave drives an LGN neuron to fire, because activity does not occur in all the presynaptic neurons at the

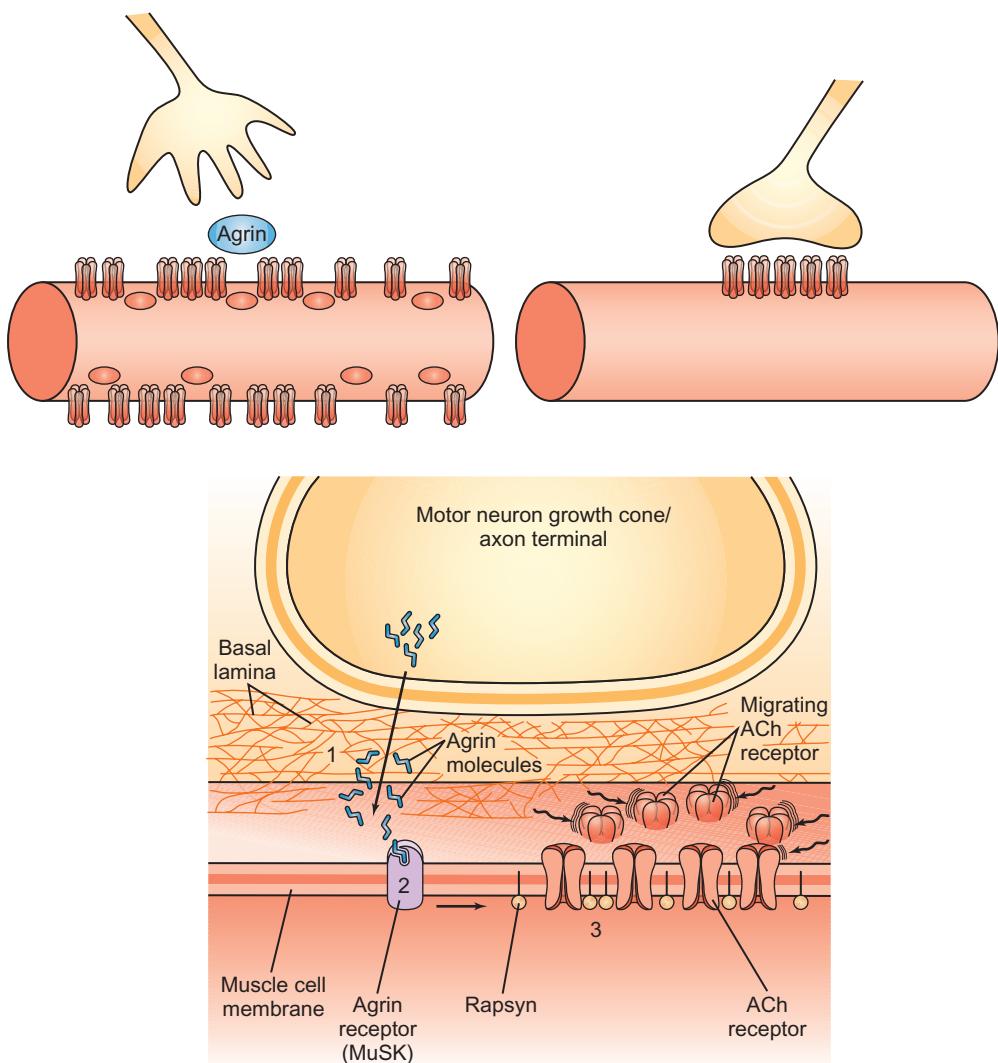


FIGURE 28-8 Synapse formation at the neuromuscular junction. Motor neurons secrete agrin at contact points with the myofiber and can cluster agrin receptors and MuSK, which in turn can cluster AChRs via the action of rapsyn at those sites.

same time, eventually the active presynaptic axons “win” at synapses and are reinforced.

In the LGN of monkeys and cats, the eye-specific domains are reflected in their projections to layer IV of the cortex in ocular dominance columns that form before birth. However, these cortical domains are still “plastic” and can change with experience. In classical studies, Hubel and Weisel closed one eyelid experimentally so that eye had no meaningful visual experience. When such monocular deprivation is started after birth, the “open-eye” columns expand as axons take over territory, while “closed eye” columns shrink. The process can be reversed by opening the previously shut eye and closing the open one, and again, the resulting axonal territories from each eye expand or contract to reflect the amount of activity in the respective eye. These synaptic rearrangements are not only dependent on activity, but are also dependent on experience, reflecting the quality of the visual experiences of the open eye. However, this remarkable plasticity does not remain forever; when monocular deprivation is initiated later in life, the

cortical domains do not change and more connections appear fixed.

Ultimately, the layer IV information from each eye must be recombined in cortical layer III to produce binocular vision. Again, visual activity is important for correct formation of these connections. Unlike formation of eye-specific domains in layer IV that requires asynchronous activity, the formation of connections in layer III for binocular vision requires synchronous activity from both eyes. Experience and synaptic plasticity in learning and memory are discussed further in Chapter 56.

SUMMARY

Experimental embryological approaches have revealed the remarkable self-assembly that occurs in neural development, and have led to our understanding of the cellular and molecular mechanisms that regulate these processes. Indeed, the major regulatory events that control the formation of the

major structures and cells in the brain have largely been elucidated in the last decade or so. In a way, however, explaining the structure of the brain is only part of the story, as the brain is notorious for allowing us to learn throughout our lifetimes. There is a close relationship between the experience-dependent brain development that we just discussed and the learning and memory that go on throughout adulthood. Processes of development and learning probably are based on similar neural mechanisms, but occur at different times in the life of the organism and involve different brain areas. In this way, experience continues to alter brain circuitry throughout life.

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