

The Role of Organizers in Patterning the Nervous System

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Abstract

The foundation for the anatomical and functional complexity of the vertebrate central nervous system is laid during embryogenesis. After Spemann's organizer and its derivatives have endowed the neural plate with a coarse pattern along its anteroposterior and mediolateral axes, this basis is progressively refined by the activity of secondary organizers within the neuroepithelium that function by releasing diffusible signaling factors. Dorsoventral patterning is mediated by two organizer regions that extend along the dorsal and ventral midlines of the entire neuraxis, whereas anteroposterior patterning is controlled by several discrete organizers. Here we review how these secondary organizers are established and how they exert their signaling functions. Organizer signals come from a surprisingly limited set of signaling factor families, indicating that the competence of target cells to respond to those signals plays an important part in neural patterning.

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INTRODUCTION

Although it remains astounding, even to the experienced neurobiologist, that a structure as complex as the human brain can arise from a single cell, work in different vertebrate model organisms has started to reveal a network of tissue and genetic interactions that engineer this extraordinary feat. During embryogenesis, the primordial neuroepithelium progressively subdivides into distinct regions in a patterning process governed by small groups of cells that regulate cell fate in surrounding tissues by releasing signaling factors. These local signaling centers are called organizers to reflect their ability to confer identity on neighboring tissues in a nonautonomous fashion.

SPEMANN'S ORGANIZER AND EARLY NEURAL PATTERNING

In 1935 Hans Spemann received the Nobel Prize in Medicine for his work with Hilde Mangold showing that transplantation of a small group of cells from the dorsal blastopore lip of a donor embryo to the ventral side of a host embryo is sufficient to induce a secondary body axis (reviewed in De Robertis & Kuroda 2004, Niehrs 2004, Stern 2001). Differently pigmented salamander embryos were used as donors and hosts, allowing for an easy distinction between cells of graft and host origin. Surprisingly, most tissues in the induced second axis were derived from the host, suggesting that the graft had induced surrounding tissue to form axial structures. Thus, Spemann named

Dorsal blastopore

lip: a group of dorsal mesodermal cells of the amphibian embryo where the involution of mesoderm and endoderm starts, marking the onset of gastrulation

the dorsal blastopore lip the organizer, and tissues with comparable inductive activity have since been identified in all vertebrate model organisms and more recently also in some nonvertebrates (Darras et al. 2011, Meinhardt 2006, Nakamoto et al. 2011). Nowadays the term organizer is used more widely to describe groups of cells that can determine the fate of neighboring cell populations by emitting molecular signals.

The ectopic twin induced in Spemann's experiment contained a complete CNS that was properly patterned along its anteroposterior (AP, head-to-tail) and dorsoventral (DV, back-to-belly) axes, indicating that the organizer harbors both neural-inducing and neural-patterning activities. More recently, a large number of factors that are expressed in Spemann's organizer have been identified, and several were found to be secreted inhibitors of bone morphogenetic proteins (BMPs). In combination with other findings in frog and fish embryos, this led to a model whereby Spemann's organizer induces the neural plate in the dorsal ectoderm by inhibiting BMPs, whereas the ventral ectoderm forms epidermis because it remains exposed to BMPs (De Robertis & Kuroda 2004, Muñoz-Sanjuán & Brivanlou 2002). Experiments in chick embryos have since added complexity to this default model for neural induction by implicating other signaling proteins such as fibroblast growth factors (FGFs) and Wnts as additional neural inducers (Stern 2006).

During gastrulation, the organizer region stretches out and gives rise to the axial mesendoderm (AME), which comes to underlie the midline of the neural plate along its AP axis. Otto Mangold found that different AP regions of the AME induced different parts of the embryonic axis when grafted into host embryos, leading to the idea of regionally specific inductions by the organizer (Niehrs 2004). This model was challenged in the 1950s when Nieuwkoop and others proposed that the CNS is patterned by a gradient of a transformer that travels within the plane of the neural plate and induces different neural

fates in a dose-dependent manner such that forebrain, midbrain, hindbrain, and spinal cord form at increasing levels of this transformer (Stern 2001). FGFs (Mason 2007), retinoic acid (Maden 2007), and Wnts all posteriorize neuroectoderm dose-dependently, but Wnts appear to be the best candidates to fulfill this role in a manner consistent with Nieuwkoop's model (Kiecker & Niehrs 2001a). Spemann's organizer also secretes inhibitors of Wnts, in addition to BMP antagonists, and these factors remain expressed in the anterior AME but are absent from the posterior AME during gastrulation (Kiecker & Niehrs 2001b). Thus, the Spemann-Mangold model of regionally specific inductions and Nieuwkoop's gradient-based model turn out to be two sides of the same coin: The anterior AME induces the forebrain by acting as a sink for posteriorizing Wnts.

DORSOVENTRAL PATTERNING

The ectoderm that surrounds the neural plate expresses BMPs while Spemann's organizer and the extending AME express BMP inhibitors. Experiments in zebrafish embryos have suggested that this generates a gradient of BMP activity that defines mediolateral positions within the neural plate (Barth et al. 1999). Hence, a Cartesian coordinate system of two orthogonal gradients is established—a Wnt gradient along the AP axis and a BMP gradient along the mediolateral axis—and the AME defines the origin of this system by secreting BMP and Wnt antagonists (**Figure 1a**) (Meinhardt 2006, Niehrs 2010). It is clear that such global mechanisms can establish only a crude initial pattern, and we argue below that this pattern is increasingly refined through the establishment of local (or secondary) organizers in the neuroepithelium.

Gastrulation is followed by neurulation during which the lateral folds of the neural plate roll up and fuse to form the neural tube (**Figure 1b,c**). Thus, the initial mediolateral pattern is transposed into DV polarity: Cells that are medial in the plate end up ventral in

AP: anteroposterior

DV: dorsoventral

Bone morphogenetic protein (BMP):

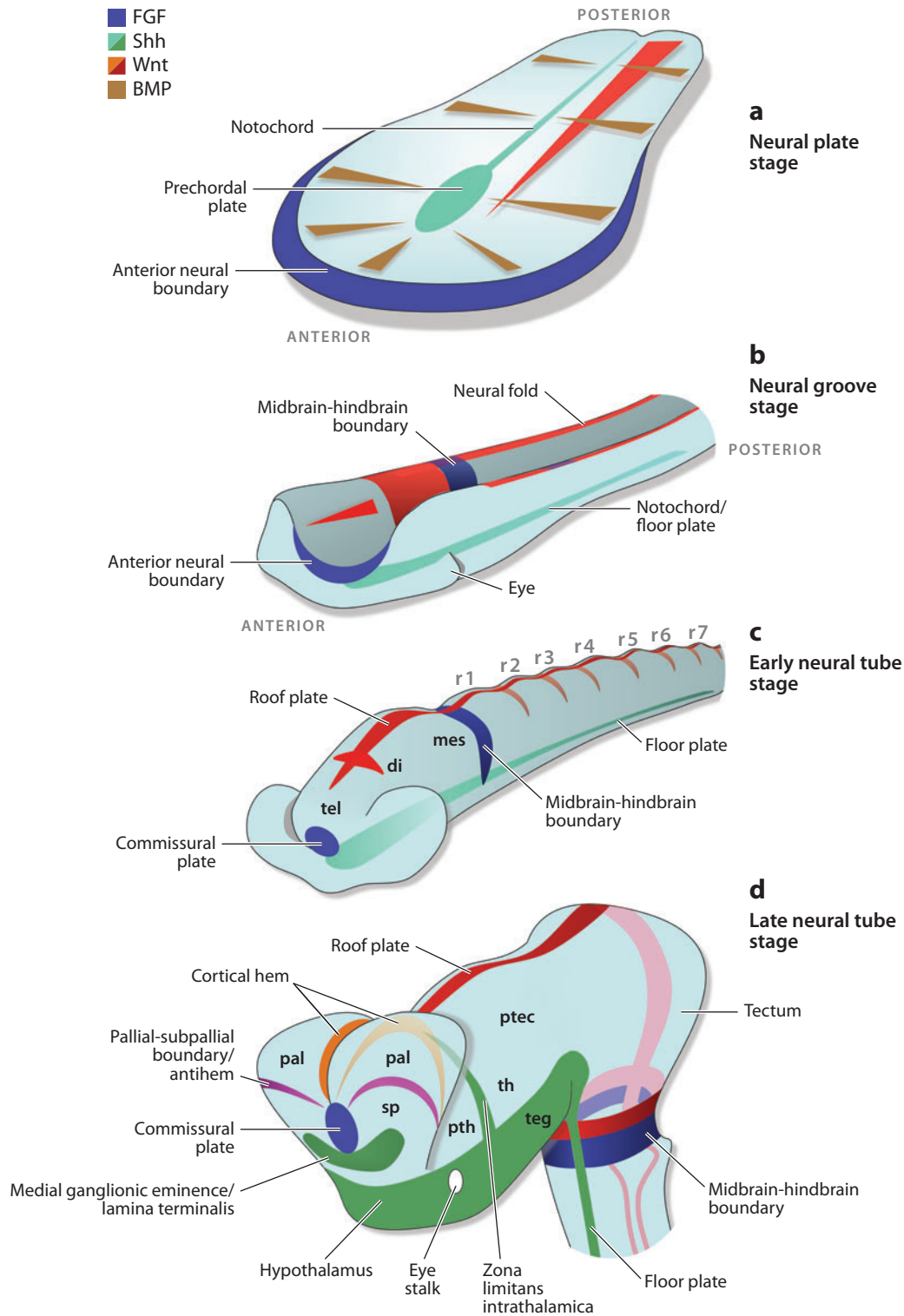
subfamily of the transforming growth factor β superfamily of secreted signaling factors; initially identified by their promotion of bone and cartilage formation

Fibroblast growth factor (FGF):

secreted signaling molecules that signal via tyrosine kinase receptors

Wnts: secreted lipid-modified glycoproteins that regulate multiple aspects of embryogenesis and adult homeostasis by activating several different signaling pathways

AME: axial mesendoderm



the tube, whereas lateral neural plate cells end up dorsal.

The Notochord

During gastrulation and neurulation a large portion of the AME narrows to a thin rod, the notochord, which underlies the midline of the neural plate and later most of the neural tube (Figure 1*a-c*). The prechordal plate, the anterior end of the AME that lies beneath the prospective anterior forebrain, is a bit wider than the notochord. The ventralmost cells of the neural tube that reside directly above the notochord form the floor plate on either side of which form motor neurons and various types of ventral interneurons.

Although not a neural structure, the notochord was one of the first tissues shown to act as a local organizer of CNS development. Microsurgical experiments in chick embryos revealed that the notochord is both necessary and sufficient to induce ventral neural identity: Sections of the spinal cord from which the notochord had been removed developed without a floor plate or motor neurons; conversely, the transplantation of pieces of notochord beneath the lateral neural plate resulted in the induction of an ectopic floor plate and motor neurons above the graft (Placzek et al. 1990, van Straaten et al. 1985).

A breakthrough in understanding this action of the notochord came with the finding that *Sonic hedgehog* (*Shh*), a vertebrate ortholog of the *Drosophila* segment polarity gene *hedgehog*, is expressed in the notochord and a bit later also in the floor plate. *Shh* encodes a secreted signaling factor and is thus a prime candidate for the inductive signal released by the notochord.

Overexpression of *Shh* in mouse, zebrafish, and frog, or coculture of rat neuroectoderm with *Shh*-expressing cells result in ectopic floor plate, motor neuron, and ventral interneuron induction, indicating that *Shh* mimics the effect of notochord grafts (Echelard et al. 1993, Krauss et al. 1993, Roelink et al. 1994). Conversely, mice carrying a mutation in the *Shh* gene fail to form a floor plate and lack multiple ventral neural cell types (Chiang et al. 1996). Taken together, these data strongly suggest that *Shh* mediates the organizer function of the notochord.

The Floor Plate

The floor plate is a strip of wedge-shaped glial cells along the ventral midline of the neural tube. Like the notochord, the floor plate expresses *Shh* and is therefore likely to contribute to the induction of ventral cell identities. Fate-mapping studies in the chick embryo in combination with detailed examinations of cellular morphologies and marker gene expression have revealed that the floor plate consists of different cell populations along both its AP and mediolateral axes (Placzek & Briscoe 2005). For example, whereas the floor plate is devoid of neural progenitors in the spinal cord and hindbrain, dopaminergic neurons are generated in the floor plate of the midbrain (Ono et al. 2007, Puelles et al. 2004).

The origin of the floor plate remains controversial. The ablation and grafting experiments described above strongly suggest that it is induced by *Shh* signaling from the notochord; however, others have argued that the floor plate and notochord originate from a common precursor population in Spemann's organizer

Motor neurons:
efferent neurons that control muscle activity

Interneurons:
neurons that connect afferent and efferent neurons in multisynaptic pathways

***Shh*:** a secreted signaling factor; its active form is lipid modified and proteolytically processed

Figure 1

Main stages of neural development in a schematized amniote embryo. (a) Neural plate stage. Mediolateral gradients of BMP (*brown wedges*) and *Shh* (*green*) activity together with an anteroposterior gradient of Wnt activity (*red wedge*) establish a quasi-Cartesian coordinate system of positional information across the neural plate. (b) Neural groove stage. The interplay between Wnts and ANB-derived Wnt inhibitors patterns the area between the presumptive forebrain and midbrain (*red wedge*). (c) Early neural tube stage. (d) Late neural tube stage. Colors: FGF expression (*blue*), *Shh* expression (*green*), BMP expression (*brown*), Wnt expression (*orange/red*). Abbreviations: di, diencephalon; mes, mesencephalon; pal, pallium; ptec, preteectum; pth, prethalamus; r, rhombomere; sp, subpallium; teg, tegmentum; tel, telencephalon; th, thalamus.

Morphogen: factor that is released locally, forms a concentration gradient within a tissue, and induces different cell fates dose-dependently

and that floor plate cells are inserted into the midline of the neural plate as the AP axis of the embryo extends (Le Douarin & Halpern 2000). These views are likely not entirely incompatible: The common lineage of notochord and floor plate may endow them with shared properties, and both tissues are capable of inducing homeogenetic responses in neuroepithelium.

Ventral Neural Patterning by Shh: A Paradigm for Morphogen Signaling

The specification of multiple cell types in the ventral neural tube is arguably one of the most thoroughly studied examples of neural patterning. Considerable evidence gathered over the past 15 years indicates that Shh functions as a true morphogen in this process; i.e., it is released from a local source (notochord, floor plate) and forms a concentration gradient that specifies different cell fates in a dose-dependent fashion (Dessaud et al. 2008, Lupo et al. 2006). In mouse embryos that were genetically engineered to express fluorescently labeled Shh protein (Shh-GFP) from the *Shh* locus, a declining ventral-to-dorsal gradient of fluorescence is detectable within the ventral neural tube (Chamberlain et al. 2008).

The morphogen model is intuitively appealing because it explains a complex process pattern formation, drawing on a simple chemical activity—the diffusion of a single substance from a localized source. However, trying to understand the cellular mechanism of morphogen signaling raises a number of difficult issues. For example, how are different concentrations of a morphogen translated into distinct cell fates? In vertebrates, Shh activates the transcriptional activators Gli1 and Gli2 and antagonizes the repressor Gli3. Thus, the extracellular gradient of Shh is translated into opposing gradients of intracellular Gli1/2 and Gli3 activity along the DV axis of the neural tube (Fuccillo et al. 2006, Lei et al. 2004, Stamatakis et al. 2005). These overlapping activities regulate two classes of transcriptional control genes: Class I genes such as *Pax6*, *Pax7*, and *Irx3* are repressed, and class II genes, including *Foxa2*, *Nkx2.2*,

Olig2, *Nkx6.1*, *Dbx1*, and *Dbx2*, are induced by Shh-Gli signaling. Different thresholds of Shh signaling are required for the repression or activation of individual class I and class II genes, resulting in a nested expression pattern of these genes along the DV axis of the spinal cord. Furthermore, several class I and class II genes cross-repress each other, resulting in a sharpening of the boundaries between their expression domains (Briscoe et al. 2000). Ultimately, the combinatorial expression of class I and class II genes at a specific DV location determines which type of neural progenitor will form.

Dessaud et al. (2008) recently suggested that the duration of exposure, in addition to the extracellular concentration of Shh, determines the fate of the receiving cell. This model is likely to reflect the gradual buildup of the Shh gradient in vivo better than would a static gradient model.

Ventral Patterning in the Hindbrain and Midbrain

Early in its development, the hindbrain becomes subdivided into a series of seven to eight segments called rhombomeres (r1–8) (reviewed in Kiecker & Lumsden 2005). Nevertheless, the topological organization of neurons along the DV axis of the hindbrain is similar to that of the spinal cord. The induction of hindbrain motor neurons (which contribute to the IVth–XIIth cranial nerves) also depends on Shh signaling from the notochord/floor plate and on the nested expression of various class I and class II genes (Osumi et al. 1997, Pattyn et al. 2003, Takahashi & Osumi 2002).

The ventral part of the midbrain (and of the posterior forebrain) gives rise to the tegmentum where neural progenitors are organized in a series of morphologically visible arcs that are characterized by periodic gene expression patterns (Sanders et al. 2002). Gain- and loss-of-function experiments in chick embryos have provided evidence that this pattern is also controlled by Shh signaling (Agarwala et al. 2001, Bayly et al. 2007).

Organizers of Ventral Forebrain Development

Like the notochord, the prechordal plate expresses *Shh*, and grafts of this AME tissue to the lateral forebrain result in ectopic expression of the ventral forebrain marker *Nkx2.1* in chick embryos. However, this effect cannot be mimicked by implanting Shh-producing cells, suggesting that the inductive capacity of the prechordal plate reaches beyond mere secretion of Shh (Pera & Kessel 1997). A good candidate factor to mediate this difference is BMP7, which is expressed in the prechordal mesoderm but not in the posterior notochord and synergizes with Shh in inducing ventral forebrain identity (Dale et al. 1997). Furthermore, potent Wnt inhibitors are expressed in the prechordal plate, and these likely contribute to forebrain induction and ventralization (Kiecker & Niehrs 2001b).

The vertebrate forebrain becomes divided into the telencephalon anteriorly and the diencephalon posteriorly (Figure 1c). The dorsal part of the telencephalon, the pallium, gives rise to the hippocampus and the cerebral cortex (or functionally equivalent structures in non-mammalian species), whereas the ventral part, the subpallium, gives rise to the basal ganglia. The earliest distinction between pallial and subpallial identity is established at the neural plate stage and is mediated by Shh from the prechordal plate (Gunhaga et al. 2000). However, prechordal plate-derived Shh also induces a new domain of *Shh* expression in the most ventral part of the subpallium, the presumptive medial ganglionic eminence (MGE), in a manner that is very similar to the induction of the floor plate by Shh from the notochord. This telencephalic *Shh* domain is likely to act as a secondary organizer that refines the DV pattern of the telencephalon (Figure 1d) (Sousa & Fishell 2010).

The Roof Plate

Another specialized cell population, the roof plate, forms along the dorsal midline of the entire neural tube. Similar to the floor plate,

the roof plate is induced by inductive signals from a nonneural tissue—in this case by BMPs and Wnts expressed in the ectoderm flanking the neural plate (Chizhikov & Millen 2004b). Neural tube closure brings the roof plate progenitors from either side of the neural plate together to form the dorsal midline of the neural tube. Identifying a specific genetic pathway for roof plate formation has been complicated by the fact that two other cell groups, the neural crest and some dorsal interneuron progenitors, also arise from the dorsal midline.

However, there is good evidence that the roof plate functions as an organizer of dorsal patterning in the spinal cord. Six populations of dorsal interneurons (dI1–6) are generated in the dorsal half of the spinal cord, and the coculture of roof plate and naive neural plate tissue leads to the induction of at least two of those (Liem et al. 1997). Genetic ablation of the roof plate in mice results in the lack of dI1–3 and expansion of dI4–6 interneurons, confirming that the roof plate is not only sufficient but also required for dorsal spinal cord patterning (K.J. Lee et al. 2000). *Lmx1a* mutant mice in which roof plate formation is disrupted show a similar, if somewhat milder phenotype (Millonig et al. 2000). Overexpression of *Lmx1a*—which encodes a LIM homeodomain transcription factor—in the chick spinal cord results in ectopic induction of dI1 at the expense of dI2–6 interneurons in the vicinity of the electroporated cells, suggesting that a diffusible signal is secondarily induced (Chizhikov & Millen 2004a).

Identification of the signal that mediates the organizer function of the roof plate has been more problematic than identifying that of the floor plate. The roof plate expresses several Wnts and a large number of BMPs, and the genetic inactivation of individual factors often results in entirely normal spinal cord development, probably owing to compensation by other family members. The BMP-type factor GDF7 is an exception because *Gdf7*^{-/-} mutant mice lack dI1 interneurons (Lee et al. 1998). Zebrafish mutants with defects in the BMP pathway fail to form Rohon-Beard neurons,

Boundary: an interface between two adjacent tissues that prevents intermingling between cells from either side of the boundary

indicating that BMPs are required for the formation of at least some dorsal cell types (Nguyen et al. 2000). *Wnt1/Wnt3a* double mutant mice show a severe reduction of dI1–3 and a concomitant expansion of dI4 and dI5 interneurons and are therefore phenotypically similar to roof plate–ablated mice, although less severely so (Muroyama et al. 2002). However, the reduction of dI1–3 interneurons could be due to defective proliferation rather than patterning because Wnts act as mitogens in gain-of-function experiments in chick (Megason & McMahon 2002).

Do roof plate signals act in a dose-dependent fashion, similar to Shh on the ventral side of the neural tube? Some evidence points toward graded effects by both BMPs and Wnts in the dorsal spinal cord (Liem et al. 1997, Megason & McMahon 2002, Timmer et al. 2002); however, the picture is far less conclusive than for Shh and it remains possible that qualitative as well as quantitative mechanisms are at work (i.e., individual BMPs or Wnts may specifically induce certain subpopulations of dorsal interneurons).

Dorsal Patterning in the Anterior Hindbrain

The dorsal part of the anterior hindbrain (r1) undergoes a series of complex morphological changes that result in the formation of the cerebellum. Granule cells, the most prevailing cell type in the cerebellum, are generated from the rhombic lip, a germinal zone at the interface between the roof plate and the r1 neuroepithelium (Hatten & Roussel 2011, Wingate 2001). The roof plate of r1, like that of the spinal cord, expresses several BMPs, and these are sufficient to initiate granule cell formation when added ectopically to r1 neuroepithelium (Alder et al. 1999). The locus coeruleus, the major noradrenergic nucleus of the brain, is also induced in the dorsal half of r1 before its neurons migrate ventrally to reach their final destination in the lateral floor of the IVth ventricle. Application of BMP antagonists to the anterior hindbrain of chick embryos results in the disappearance of or a dorsal shift of locus coeruleus neurons,

suggesting that the role of BMPs in the induction of these neurons may be dose dependent (Vogel-Höpker & Rohrer 2002).

The Cortical Hem

In the telencephalon, the roof plate sinks between the two cortical hemispheres and gives rise to the monolayered choroid plexus medially and to the cortical hem laterally. Immediately adjacent to the hem, which expresses BMPs and Wnts, the hippocampus is induced and the cerebral cortex forms next to that. Genetic ablation of the telencephalic roof plate in mice results in a severe undergrowth of the cortical primordium, suggesting a nonautonomous effect of the hem on cortical specification (Monuki et al. 2001).

Ectopic application of BMPs to the developing chick telencephalon leads to holoprosencephaly (a failure to separate the cerebral hemispheres), but this is likely to be a result of increased cell death rather than a change in patterning (Golden et al. 1999). A requirement for BMP signaling in telencephalic patterning has been tested by genetically disrupting a BMP receptor gene in the mouse forebrain. These mice fail to differentiate the choroid plexus, but all other telencephalic subdivisions develop normally, arguing against a role for BMPs as an organizer signal (Hébert et al. 2002).

Wnt signaling specifies dorsal identity at the earliest stages of telencephalic development (Backman et al. 2005, Gunhaga et al. 2003), and the Wnt antagonists secreted by the prechordal plate likely help to set up early DV polarity by protecting the ventral forebrain from the dorsalizing activity of Wnts. At later stages, Wnt signaling is required for hippocampus differentiation (Galceran et al. 2000, S.M. Lee et al. 2000, Machon et al. 2003). The boundary between the pallium and the subpallium (PSB) begins to express Wnt inhibitors, raising the possibility that a gradient of Wnt activity is established across the pallium between the hem and the PSB (which has also been called the antihem) (Assimacopoulos et al. 2003, Frowein et al. 2002). However, there is little evidence

for a later patterning function for Wnts beyond hippocampus induction (Chenn & Walsh 2002, Hirabayashi et al. 2004, Hirsch et al. 2007, Ivaniutsin et al. 2009, Machon et al. 2007, Muzio et al. 2005). The PSB also expresses several members of the epidermal growth factor family, transforming growth factor α and FGF7, but their roles in telencephalic development remain unknown (Assimacopoulos et al. 2003).

In summary, two major signaling centers organize DV patterning of the neural tube: the roof plate dorsally, which secretes BMPs and Wnts, and the floor plate ventrally, which secretes Shh. Both are induced by the same sets of molecular signals from the epidermis and the notochord, respectively. The notochord itself is an organizer that can mediate ventral neural patterning. In the telencephalon, an additional potential organizer, the PSB, is located midway along the DV axis.

ANTEROPOSTERIOR PATTERNING

In contrast with the DV axis, which is patterned by two signaling centers at opposite poles of the neural tube, the AP axis is patterned by several discrete local organizers.

The Midbrain-Hindbrain Boundary

The boundary between the midbrain and hindbrain (MHB) is characterized by a morphological constriction of the neural tube and is therefore also called the isthmus (**Figure 1c,d**). The first experimental evidence that the MHB functions as an organizer came from microsurgical studies conducted in chick embryos: Transplantation of anterior hindbrain or posterior midbrain tissue into the posterior forebrain of a host embryo induced the formation of an ectopic isthmus and ectopic midbrain tissue around the graft (Bloch-Gallego et al. 1996, Gardner & Barald 1991, Martinez et al. 1991). Experimental rotation of the entire midbrain vesicle formed a double-posterior midbrain and induced ectopic midbrain and

cerebellar structures in the posterior forebrain (Marín & Puelles 1994). Demonstrating a requirement for the MHB in the patterning of the surrounding tissues by microsurgery turned out to be less feasible because the isthmus organizer rapidly regenerates after surgical removal (Irving & Mason 1999).

Two signaling factors, Wnt1 and FGF8, are expressed on the anterior and posterior sides of the MHB, respectively. Both factors are required for midbrain-hindbrain development as *Wnt1* mutations in the mouse, and mutations in *Fgf8* in both mouse and zebrafish embryos result in defects in midbrain patterning and cerebellum formation (McMahon et al. 1992, Meyers et al. 1998, Picker et al. 1999, Reifers et al. 1998, Thomas et al. 1991). However, in gain-of-function experiments only FGF8 can mimic MHB organizer function (Crossley et al. 1996, Irving & Mason 2000, Lee et al. 1997, Martinez et al. 1999), whereas Wnt1 seems to promote cell growth and proliferation without affecting patterning (Panhuysen et al. 2004). Thus, FGF8 is the main organizer factor secreted from the MHB.

What determines the AP position of MHB formation? Many studies have uncovered a genetic module, including FGF8, Wnt1, and several transcription factors that stabilize MHB gene expression in a network of positive maintenance loops and mutually repressive interactions (Liu & Joyner 2001, Wurst & Bally-Cuif 2001). In particular, the homeodomain transcription factors *Otx2* and *Gbx2* play a central role in MHB positioning. *Otx2* is expressed in the forebrain and midbrain and *Gbx2* in the anterior hindbrain, and the interface between the two expression domains presages the position of the MHB from neural plate stages onward. Various gene targeting experiments in the mouse have demonstrated that an experimental shift of this interface always results in a concomitant repositioning of the MHB (Acampora et al. 1997, Broccoli et al. 1999, Millet 1999, Wassarman et al. 1997). Knockdown and cell transplantation experiments in the fish have revealed that the *otx/gbx* interface is regulated by Wnt8 at the

MHB: midbrain-hindbrain boundary

ANB: anterior neural border

ZLI: zona limitans intrathalamica

neural plate stage (Rhinn et al. 2005). Thus, the position of the MHB is directly defined by the early gradient of Wnt signaling that establishes the initial AP polarity of the neural plate. Similar to the mutual repression of class I and class II genes in the spinal cord, *Otx2* and *Gbx2* repress each other, thereby stabilizing the binary cell fate choice around the MHB (Liu & Joyner 2001, Wurst & Bally-Cuif 2001).

The Anterior Neural Boundary/Commissural Plate

Elegant cell ablation and transplantation experiments in zebrafish revealed an organizing function of the anterior border of the neural plate (ANB) (Houart et al. 1998). A Wnt inhibitor of the secreted Frizzled-related protein family is expressed in the ANB, and overexpression and depletion of this factor phenocopy the effects of ANB transplantation and removal, respectively (Houart et al. 2002). *Wnt8B* is expressed in the presumptive midbrain and posterior forebrain at the stages when ANB signaling is required and is therefore likely to be the main antagonist of the ANB. Thus, after the global gradient of Wnt activity has established general AP polarity in the neural plate, Wnts regulate AP identity in a more localized fashion in the prospective forebrain-midbrain region (**Figure 1b**).

In the mouse, a role for Wnt inhibition from the ANB has yet to be demonstrated; however, FGF8, which is also expressed there, can mimic the anteriorizing effects of ANB in explants in vitro (Shimamura & Rubenstein 1997). Studies in both mouse and zebrafish embryos have demonstrated a need for FGFs in forebrain patterning (Meyers et al. 1998, Walshe & Mason 2003).

After neural tube closure, the ANB becomes a patch of cells at the anterior end of the neural tube that will eventually form the commissural plate (CP), a scaffold for the formation of forebrain commissures at later stages. The CP continues to express FGF8, and impressive in utero electroporation experiments in the mouse have revealed that FGF8 promotes anterior at the expense of posterior cortical fates. An ectopic

source of FGF8 at the posterior end of the cortex resulted in a partial mirrored duplication of anterior cortical areas (Fukuchi-Shimogori & Grove 2001). These experiments identified the CP as an organizer of cortical patterning via its secretion of FGF8. Toyoda et al. (2010) recently showed that FGF8 acts directly and at a long range during this process, i.e., as a true morphogen.

The Zona Limitans Intrathalamica

The zona limitans intrathalamica (ZLI) is a narrow stripe of *Shh*-expressing cells in the alar plate of the diencephalon, transecting the neuraxis between the presumptive prethalamus and the thalamus (Kitamura et al. 1997, Shimamura et al. 1995, Zeltser et al. 2001). Gain- and loss-of-function experiments in chick, zebrafish, and mouse embryos have revealed that the ZLI acts as an organizer of diencephalic development by secreting Shh (Kiecker & Lumsden 2004, Scholpp et al. 2006, Vieira et al. 2005, Vue et al. 2009). At least in the thalamus, the activity of Shh appears to be dose dependent, with higher levels of signaling inducing the gamma-aminobutyric acid (GABA)-ergic rostral thalamus and lower levels inducing the glutamatergic caudal thalamus (Hashimoto-Torii et al. 2003, Vue et al. 2009; but see Jeong et al. 2011).

Fgf8 is expressed in a small patch in the dorsal ZLI and contributes to regulating the fate decision between the rostral and caudal thalamus (Kataoka & Shimogori 2008). Furthermore, a plethora of *Wnts* shows sharp borders of expression at the ZLI, suggesting that Wnt signaling may also be involved in regulating the regionalization and/or proliferation of the diencephalic primordium (Bluske et al. 2009, Quinlan et al. 2009).

The apposition of any neural tissue anterior to the ZLI with any tissue between the ZLI and the MHB results in induction of *Shh*, indicating that planar interactions are sufficient to induce the ZLI organizer (Guinazu et al. 2007, Vieira et al. 2005). The ZLI forms at the interface between the expression domains

of two classes of transcription factors: zinc finger proteins of the *Fez* family anteriorly and homeodomain proteins of the *Irx* family posteriorly (Hirata et al. 2006, Kobayashi et al. 2002, Rodríguez-Seguel et al. 2009, Scholpp et al. 2007). Expression of *Irx3* in chick is induced by Wnt signaling, suggesting that, as for *Gbx2* at the MHB, the early Wnt signal that posteriorizes the neural plate directly positions the ZLI (Braun et al. 2003).

Rhombomere Boundaries

Segmentation of the hindbrain into rhombomeres is controlled by graded retinoic acid signaling and by the reiterated and nested expression of tyrosine kinases and transcription factors, many of which are vertebrate orthologs of *Drosophila* gap and *Hox* genes (Kiecker & Lumsden 2005, Maden 2007). In zebrafish, the boundaries between rhombomeres express several Wnts (**Figure 1c**), and the knockdown of these factors results in disorganized neurogenesis adjacent to the boundaries, suggesting that they may function as organizers, although no patterning defects have been demonstrated within the rhombomeres of such embryos (Amoyel et al. 2005, Riley et al. 2004).

COMMON FEATURES OF NEUROEPITHELIAL ORGANIZERS

As discussed above, organizers influence cell fate in surrounding tissues by secreting diffusible signaling factors that often act in a morphogen-like fashion—that is, they induce different responses in receiving cells at different distances from the source. In addition to this defining feature of organizers, several other commonalities have been observed regarding the establishment, maintenance, and signaling properties of neuroepithelial organizers.

Organizers Form Along Cell Lineage Restriction Boundaries

One of the hallmarks of hindbrain segmentation is the formation of cell lineage–restricted

boundaries that prevent cells from moving between adjacent rhombomeres (Fraser et al. 1990, Jimenez-Guri et al. 2010). This finding prompted a search for boundaries in other parts of the neural tube, which led to the discovery that such boundaries often coincide with organizers (Kiecker & Lumsden 2005): Cell lineage restriction at the MHB has been demonstrated by sophisticated time-lapse imaging in zebrafish embryos and genetic fate mapping in the mouse (Langenberg & Brand 2005, Zervas et al. 2004); the ZLI is flanked by boundaries on either side (Zeltser et al. 2001); and signaling functions have been reported for rhombomere boundaries (see above). Lineage restriction has not been tested at the ANB, but it seems unlikely that cells intermingle freely across the neural-epidermal border. All three DV organizers—floor plate, PSB, and roof plate—also show some degree of lineage restriction (Awatramani et al. 2003, Fishell et al. 1993, Fraser et al. 1990, Jimenez-Guri et al. 2010).

The molecular mechanisms underlying boundary formation in the neural tube are not well understood. However, specific signaling pathways have been implicated: Eph-ephrin signaling is essential for segmentation in the hindbrain (Cooke et al. 2005, Kemp et al. 2009, Xu et al. 1999), and the Notch pathway appears to be involved in boundary formation at the ZLI, at rhombomere boundaries, and at the MHB (Cheng et al. 2004, Tossell et al. 2011, Zeltser et al. 2001).

Cell lineage restriction at organizers probably serves a dual function. First, boundaries tend to minimize contact between flanking cell populations, which may help to keep organizers in a straight line, facilitating the generation of a consistent diffusion gradient. Second, cells on either side of the organizer are kept in separate immiscible pools, thereby stabilizing a pattern after it has been induced.

Positive Feedback Maintains Organizers

Another feature shared by several neuroepithelial organizers is that their maintenance

Neurogenesis: the process by which proliferating neural progenitors exit the cell cycle and differentiate into functional neurons

depends on the signal they produce. Both the floor plate and the roof plate are induced by their own signals, BMP and Shh; the ZLI depends on ongoing Shh signaling (Kiecker & Lumsden 2004, Zeltser 2005); and MHB integrity depends on FGF signaling (Sunmonu et al. 2011, Trokovic et al. 2005). Alan Turing's classical model for pattern formation postulated a chemical network of local self-enhancement and long-range inhibition, and the autoinduction of neuroepithelial organizers fits the local self-enhancement component of this model rather well (Meinhardt 2009).

Intrinsic Factors Regulate Differential Responses to Organizer Signals

FGF signaling from the MHB establishes the tectum anteriorly and the cerebellum posteriorly, and Shh from the ZLI induces prethalamic gene expression anteriorly and patterns the thalamus posteriorly. How can one signal induce such asymmetric responses on either side of an organizer? Two orthologs of the *Drosophila* competence factor *iroquois*, *Irx2* and *Irx3*, are expressed posterior to the MHB and ZLI, respectively. Ectopic expression of these factors anterior to the organizer results in a conversion of tectum into cerebellum and of prethalamus into thalamus (Kiecker & Lumsden 2004, Matsumoto et al. 2004). These effects are dependent on the organizer signals FGF and Shh, suggesting that *Irx2* and *Irx3* are not patterning factors themselves but that they convey a prepattern that determines the competence of different subdivisions of the neural tube to respond to secreted signals.

FGF-soaked beads induce ectopic mid-brain and hindbrain structures to form from posterior forebrain tissue, but in the anterior forebrain FGFs anteriorize the pallium. Similarly, Shh from the ventral midline induces the hypothalamus marker *Nkx2.1* anteriorly, whereas it induces *Nkx6.1* posteriorly. The limit between these two regions of differential competence to respond to FGFs coincides with the ZLI, and the homeobox genes *Irx3* and *Six3* were shown to mediate posterior

versus anterior competence (Kobayashi et al. 2002).

Taken together, intrinsic factors establish a prepattern in the developing CNS that regulates the cellular response to organizer signals. These factors are often induced by the earliest signals that pattern the neural plate—for example, *Irx3* is induced and *Six3* is repressed by posteriorizing Wnt signaling (Braun et al. 2003)—thereby linking early and late stages of neural patterning. This does not mean that organizer signals are merely permissive triggers that determine the timing and extent of regional specialization, the identity of which is prepatterned; they are also responsible for evoking different responses within the same field (as exemplified by the induction of GABAergic versus glutamatergic neurons by different doses of Shh within the *Irx3*-positive thalamus).

Hes Genes Prevent Neurogenesis in Organizer Regions

Organizers typically coincide with boundaries that are characterized by slower proliferation and a delay or absence of neurogenesis (Guthrie et al. 1991, Lumsden & Keynes 1989). Transcription factors of the *Hes* family that mediate Notch signaling are required to inhibit neurogenesis at the MHB of zebrafish and frog embryos (Geling et al. 2004, Ninkovic et al. 2005, Takada et al. 2005). All neural progenitors express *Hes* genes, but they usually become downregulated when cells undergo neurogenesis. An analysis in mouse embryos has revealed that in boundary regions *Hes* genes remain expressed and that it is this strong persistent expression that sets boundaries apart and allows organizer regions to form (Baek et al. 2006).

NEUROEPITHELIAL ORGANIZERS ALSO REGULATE PROLIFERATION, NEUROGENESIS, AND AXON GUIDANCE

Many organizer signals also function as mitogens, suggesting that growth, in addition to

patterning, is modulated by organizers. For example, *Shh* mutant mice show not only patterning defects, but also a structural lack of many ventral neural tissues (Chiang et al. 1996). *Wnt1* promotes growth in the MHB region (Panhuysen et al. 2004), and *Wnts* from the roof plate and cortical hem are known to regulate proliferation of the spinal cord and pallium (Chenn & Walsh 2002, Ivaniutsin et al. 2009, Megason & McMahon 2002, Muzio et al. 2005). FGFs from the MHB promote growth of the midbrain and cerebellum (Partanen 2007), but they also serve as survival factors in the midbrain (Basson et al. 2008). Similarly, FGFs secreted from the CP prevent apoptosis and promote growth in the telencephalon (Paek et al. 2009, Thomson et al. 2009). Contrary to the proliferative effects of FGFs, *Shh*, and *Wnts*, the BMP pathway often induces apoptosis when ectopically activated (Anderson et al. 2002, Lim et al. 2005, Liu et al. 2004).

To complicate the picture even further, some organizer factors promote cell cycle exit and neurogenesis (Fischer et al. 2011, Hirabayashi et al. 2004, Machon et al. 2007, Munji et al. 2011, Xie et al. 2011). These seemingly contradictory effects of the same classes of signals may be explained by temporal changes in the competence of the target cells (Hirsch et al. 2007); however, in some cases different members of the same protein family exert opposing effects on the balance between proliferation and differentiation (Borello et al. 2008, David 2010). Thus, by releasing growth-promoting and growth-inhibiting cues from localized sources, organizers help to mold the increasingly complex shape of the neural tube and coordinate the temporal progression of neurogenesis in defined subdivisions of the neural tube (Scholpp et al. 2009).

Once their regional identity has been established, differentiated neurons need to wire up precisely to form functional networks. Organizers also play a role at this stage of CNS formation, for example, by expressing axon guidance factors such as the chemoattractant netrin, which is secreted by the floor plate to guide commissural axons (Dickson 2002,

Tessier-Lavigne & Goodman 1996). More recently, many of the classical morphogens that are secreted by organizers have been found to double as axon guidance molecules at later stages (Charron & Tessier-Lavigne 2005, Osterfield et al. 2003, Sánchez-Camancho et al. 2005, Zou & Lyuksyutova 2007). *Shh* from the floor plate cooperates with netrin in attracting commissural axons, whereas BMPs from the roof plate repel them (Augsburger et al. 1999, Charron et al. 2003). After these axons have crossed the midline, *Shh* signaling repels them via Hedgehog-interacting protein (Bourikas et al. 2005). *Wnts* are expressed in an AP gradient in the floor plate and guide the same axons anteriorly after they have crossed the midline (Lyuksyutova et al. 2003), whereas corticospinal axons are directed posteriorly by a repulsive interaction between *Wnts* and the atypical *Wnt* receptor *Ryk* (Liu et al. 2005). Thus, organizers influence CNS formation not only at early patterning stages, but also at later stages when functional circuits are established.

NEUROEPITHELIAL ORGANIZERS IN EVOLUTION

Vertebrates possess the most complex of all brains; even their closest relatives, the tunicates and hemichordates, have relatively simple nervous systems (Meinertzhagen et al. 2004). Sets of transcription factors that mark AP and DV subdivisions of the neural tube are conserved far beyond the chordate phylum (Irimia et al. 2010, Lowe et al. 2003, Reichert 2005, Tomer et al. 2010, Urbach & Technau 2008), and several orthologs of AP marker genes are even found along the head-to-foot axis of the coelenterate *Hydra*, suggesting an ancient origin of the genetic modules that regulate neural patterning (Technau & Steele 2011). By contrast, local organizers appear to be far less conserved: For example, an equivalent of the MHB is present in the urochordate *Ciona* (Imai et al. 2009) but not in the cephalochordate *Amphioxus* (Holland 2009). Both *Ciona* and *Amphioxus* seem to lack an equivalent of the ZLI, whereas a comparable region has been identified in the

Commissural axons:
nerve fibers that cross
the midline of the
nervous system

hemichordate *Saccoglossus* (C. Lowe, personal communication). Some organizers are even missing in lower vertebrates; no *hedgehog* expression or MGE-like differentiation has been found in the telencephalon of the lamprey, suggesting that this ventroanterior organizer is a gnathostome invention (Sugahara et al. 2011).

These observations indicate that local organizers are more recent innovations than the basic AP/DV patterning network and that they show some evolutionary flexibility that may provide a driving force for morphological change. This idea is supported by the recent finding that differences in forebrain morphology among cichlid fishes from Lake Malawi are correlated with subtle changes in signal strength, timing of signal production, and the position of forebrain organizers (Sylvester et al. 2010). Similarly, loss of eyesight in a cave-dwelling morph of the tetra *Astyanax* was shown to be caused by changes in the forebrain expression of *fgf8* and *shh* (Pottin et al. 2011). Thus, although the basic subdivisions of the brain are likely to have developed a long time ago, organizers are a more recent acquisition that may have been imposed on the underlying

pattern and allow evolutionary adaptation to ecological niches.

CONCLUSIONS

Almost 90 years have passed since Spemann discovered the amphibian gastrula organizer; however, the organizer concept is more topical than ever, in particular in the developing vertebrate CNS where multiple organizers regulate patterning, proliferation, neurogenesis, cell death, and axon pathfinding. Neural organizers are generated by inductive signaling events between neighboring tissues, and they often form along, or are stabilized by, cell lineage restriction boundaries. The pattern induced by an organizer results in the formation of different cell populations that can potentially form further organizers at their interfaces, thereby subdividing the neuroepithelium into increasingly more specialized regions. In many ways, neural development can be regarded as a self-organizing process: Once initial polarity has been established, all the interactions necessary to form a functional CNS occur within the neuroepithelium itself.

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LITERATURE CITED

- Acampora D, Avantaggiato V, Tuorto F, Simeone A. 1997. Genetic control of brain morphogenesis through *Otx* gene dosage requirement. *Development* 124:3639–50
- Agarwala S, Sanders TA, Ragsdale CW. 2001. Sonic hedgehog control of size and shape in midbrain pattern formation. *Science* 291:2147–50
- Alder J, Lee KJ, Jessell TM, Hatten ME. 1999. Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells. *Nat. Neurosci.* 2:535–40
- Amoyel M, Cheng YC, Jiang YJ, Wilkinson DG. 2005. Wnt1 regulates neurogenesis and mediates lateral inhibition of boundary cell specification in the zebrafish hindbrain. *Development* 132:775–85
- Anderson RM, Lawrence AR, Stottmann RW, Bachiller D, Klingensmith J. 2002. Chordin and noggin promote organizing centers of forebrain development in the mouse. *Development* 129:4975–87

- Assimacopoulos S, Grove EA, Ragsdale CW. 2003. Identification of a Pax6-dependent epidermal growth factor family signaling source at the lateral edge of the embryonic cerebral cortex. *J. Neurosci.* 23:6399–403
- Augsburger A, Schuchardt A, Hoskins S, Dodd J, Butler S. 1999. BMPs as mediators of roof plate repulsion of commissural neurons. *Neuron* 24:127–41
- Awatramani R, Soriano P, Rodriguez C, Mai JJ, Dymecki SM. 2003. Cryptic boundaries in roof plate and choroid plexus identified by intersectional gene activation. *Nat. Genet.* 35:70–75
- Backman M, Machon O, Mygland L, van den Bout CJ, Zhong W, et al. 2005. Effects of canonical Wnt signaling on dorso-ventral specification of the mouse telencephalon. *Dev. Biol.* 279:155–68
- Baek JH, Hatakeyama J, Sakamoto S, Ohtsuka T, Kageyama R. 2006. Persistent and high levels of *Hes1* expression regulate boundary formation in the developing central nervous system. *Development* 133:2467–76
- Barth KA, Kishimoto Y, Rohr KB, Seydler C, Schulte-Merker S, Wilson SW. 1999. Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* 126:4977–87
- Basson MA, Echevarria D, Ahn CP, Sudarov A, Joyner AL, et al. 2008. Specific regions within the embryonic midbrain and cerebellum require different levels of FGF signaling during development. *Development* 135:889–98
- Bayly RD, Ngo M, Aglyamova GV, Agarwala S. 2007. Regulation of ventral midbrain patterning by Hedgehog signaling. *Development* 134:2115–24
- Bloch-Gallego E, Millet S, Alvarado-Mallart RM. 1996. Further observations on the susceptibility of diencephalic prosomeres to *En-2* induction and on the resulting histogenetic capabilities. *Mech. Dev.* 58:51–63
- Bluske KK, Kawakami Y, Koyano-Nakagawa N, Nakagawa Y. 2009. Differential activity of Wnt/ β -catenin signaling in the embryonic mouse thalamus. *Dev. Dyn.* 238:3297–309
- Borello U, Cobos I, Long JE, McWhirter JR, Murre C, Rubenstein JL. 2008. FGF15 promotes neurogenesis and opposes FGF8 function during neocortical development. *Neural Dev.* 3:17
- Bourikas D, Pekarik V, Baeriswyl T, Grunditz A, Sadhu R, et al. 2005. Sonic hedgehog guides commissural axons along the longitudinal axis of the spinal cord. *Nat. Neurosci.* 8:297–304
- Braun MM, Etheridge A, Bernard A, Robertson CP, Roelink H. 2003. Wnt signaling is required at distinct stages of development for the induction of the posterior forebrain. *Development* 130:5579–87
- Briscoe J, Pierani A, Jessell TM, Ericson J. 2000. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. *Cell* 101:435–45
- Broccoli V, Boncinelli E, Wurst W. 1999. The caudal limit of *Otx2* expression positions the isthmus organizer. *Nature* 401:164–68
- Chamberlain CE, Jeong J, Guo C, Allen BL, McMahon AP. 2008. Notochord-derived Shh concentrates in close association with the apically positioned basal body in neural target cells and forms a dynamic gradient during neural patterning. *Development* 135:1097–106
- Charron F, Stein E, Jeong J, McMahon AP, Tessier-Lavigne M. 2003. The morphogen sonic hedgehog is an axonal chemoattractant that collaborates with netrin-1 in midline axon guidance. *Cell* 113:11–23
- Charron F, Tessier-Lavigne M. 2005. Novel brain wiring functions for classical morphogens: a role as graded positional cues in axon guidance. *Development* 132:2251–62
- Cheng YC, Amoyel M, Qiu X, Jiang YJ, Xu Q, Wilkinson DG. 2004. Notch activation regulates the segregation and differentiation of rhombomere boundary cells in the zebrafish hindbrain. *Dev. Cell* 6:539–50
- Chenn A, Walsh CA. 2002. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* 297:365–69
- Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, et al. 1996. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* 383:407–13
- Chizhikov VV, Millen KJ. 2004a. Control of roof plate formation by *Lmx1a* in the developing spinal cord. *Development* 131:2693–705
- Chizhikov VV, Millen KJ. 2004b. Mechanisms of roof plate formation in the vertebrate CNS. *Nat. Rev. Neurosci.* 5:808–12
- Cooke JE, Kemp HA, Moens CB. 2005. EphA4 is required for cell adhesion and rhombomere-boundary formation in the zebrafish. *Curr. Biol.* 15:536–42
- Crossley PH, Martinez S, Martin GR. 1996. Midbrain development induced by FGF8 in the chick embryo. *Nature* 380:66–68

- Dale JK, Vesque C, Lints TJ, Sampath TK, Furley A, et al. 1997. Cooperation of BMP7 and SHH in the induction of forebrain ventral midline cells by prechordal mesoderm. *Cell* 90:257–69
- Darras S, Gerhart J, Terasaki M, Kirschner M, Lowe CJ. 2011. β -catenin specifies the endomesoderm and defines the posterior organizer of the hemichordate *Saccoglossus kowalevskii*. *Development* 138:959–70
- David MD. 2010. Wnt-3a and Wnt-3 differently stimulate proliferation and neurogenesis of spinal neural precursors and promote neurite outgrowth by canonical signaling. *J. Neurosci. Res.* 88:3011–23
- De Robertis EM, Kuroda H. 2004. Dorsal-ventral patterning and neural induction in *Xenopus* embryos. *Annu. Rev. Cell Dev. Biol.* 20:285–308
- Dessaud E, McMahon AP, Briscoe J. 2008. Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. *Development* 135:2489–503
- Dickson BJ. 2002. Molecular mechanisms of axon guidance. *Science* 298:1959–64
- Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, et al. 1993. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* 75:1417–30
- Fischer T, Faus-Kessler T, Welzl G, Simeone A, Wurst W, Prakash N. 2011. Fgf15-mediated control of neurogenic and proneural gene expression regulates dorsal midbrain neurogenesis. *Dev. Biol.* 350:496–510
- Fishell G, Mason CA, Hatten ME. 1993. Dispersion of neural progenitors within the germinal zones of the forebrain. *Nature* 362:636–38
- Fraser S, Keynes R, Lumsden A. 1990. Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. *Nature* 344:431–35
- Frowein J, Campbell K, Götz M. 2002. Expression of *Ngn1*, *Ngn2*, *Cash1*, *Gsb2* and *Sfrp1* in the developing chick telencephalon. *Mech. Dev.* 110:249–52
- Fuccillo M, Joyner AL, Fishell G. 2006. Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. *Nat. Rev. Neurosci.* 7:772–83
- Fukuchi-Shimogori T, Grove EA. 2001. Neocortex patterning by the secreted signaling molecule FGF8. *Science* 294:1071–74
- Galceran J, Miyashita-Lin EM, Devaney E, Rubenstein JL, Grosschedl R. 2000. Hippocampus development and generation of dentate gyrus granule cells is regulated by LEF1. *Development* 127:469–82
- Gardner CA, Barald KF. 1991. The cellular environment controls the expression of engrailed-like protein in the cranial neuroepithelium of quail-chick chimeric embryos. *Development* 113:1037–48
- Geling A, Plessy C, Rastegar S, Strähle U, Bally-Cuif L. 2004. Her5 acts as a prepattern factor that blocks *neurogenin1* and *coe2* expression upstream of Notch to inhibit neurogenesis at the midbrain-hindbrain boundary. *Development* 131:1993–2006
- Golden JA, Braciclovic A, McFadden KA, Beesley JS, Rubenstein JL, Grinspan JB. 1999. Ectopic bone morphogenetic proteins 5 and 4 in the chicken forebrain lead to cyclopia and holoprosencephaly. *Proc. Natl. Acad. Sci. USA* 96:2439–44
- Guinazu MF, Chambers D, Lumsden A, Kiecker C. 2007. Tissue interactions in the developing chick dien-cephalon. *Neural Dev.* 2:25
- Gunhaga L, Jessell TM, Edlund T. 2000. Sonic hedgehog signaling at gastrula stages specifies ventral telen-cephalic cells in the chick embryo. *Development* 127:3283–93
- Gunhaga L, Marklund M, Sjödal M, Hsieh JC, Jessell TM, Edlund T. 2003. Specification of dorsal telen-cephalic character by sequential Wnt and FGF signaling. *Nat. Neurosci.* 6:701–7
- Guthrie S, Butcher M, Lumsden A. 1991. Patterns of cell division and interkinetic nuclear migration in the chick embryo hindbrain. *J. Neurobiol.* 22:742–54
- Hashimoto-Torii K, Motoyama J, Hui CC, Kuroiwa A, Nakafuku M, Shimamura K. 2003. Differential ac-tivities of Sonic hedgehog mediated by Gli transcription factors define distinct neuronal subtypes in the dorsal thalamus. *Mech. Dev.* 120:1097–111
- Hatten ME, Roussel MF. 2011. Development and cancer of the cerebellum. *Trends Neurosci.* 34:134–42
- Hébert JM, Mishina Y, McConnell SK. 2002. BMP signaling is required locally to pattern the dorsal telen-cephalic midline. *Neuron* 35:1029–41
- Hirabayashi Y, Itoh Y, Tabata H, Nakajima K, Akiyama T, et al. 2004. The Wnt/ β -catenin pathway directs neuronal differentiation of cortical neural precursor cells. *Development* 131:2791–801

- Hirata T, Nakazawa M, Muraoka O, Nakayama R, Suda Y, Hibi M. 2006. Zinc-finger genes *Fez* and *Fez-like* function in the establishment of diencephalon subdivisions. *Development* 133:3993–4004
- Hirsch C, Campano LM, Wöhrle S, Hecht A. 2007. Canonical Wnt signaling transiently stimulates proliferation and enhances neurogenesis in neonatal neural progenitor cultures. *Exp. Cell Res.* 313:572–87
- Holland LZ. 2009. Chordate roots of the vertebrate nervous system: expanding the molecular toolkit. *Nat. Rev. Neurosci.* 10:736–46
- Houart C, Caneparo L, Heisenberg C, Barth K, Take-Uchi M, Wilson S. 2002. Establishment of the telencephalon during gastrulation by local antagonism of Wnt signaling. *Neuron* 35:255–65
- Houart C, Westerfield M, Wilson SW. 1998. A small population of anterior cells patterns the forebrain during zebrafish gastrulation. *Nature* 391:788–92
- Imai KS, Stolfi A, Levine M, Satou Y. 2009. Gene regulatory networks underlying the compartmentalization of the *Ciona* central nervous system. *Development* 136:285–93
- Irimia M, Piñeiro C, Maeso I, Gómez-Skarmeta JL, Casares F, Garcia-Fernández J. 2010. Conserved developmental expression of *Fezf* in chordates and *Drosophila* and the origin of the zona limitans intrathalamica (ZLI) brain organizer. *EvoDevo* 1:7
- Irving C, Mason I. 1999. Regeneration of isthmus tissue is the result of a specific and direct interaction between rhombomere 1 and midbrain. *Development* 126:3981–89
- Irving C, Mason I. 2000. Signalling by FGF8 from the isthmus patterns anterior hindbrain and establishes the anterior limit of *Hox* gene expression. *Development* 127:177–86
- Ivaniutsin U, Chen Y, Mason JO, Price DJ, Pratt T. 2009. *Adenomatous polyposis coli* is required for early events in the normal growth and differentiation of the developing cerebral cortex. *Neural Dev.* 16:3
- Jeong Y, Dolson DK, Waclaw RR, Matise MP, Sussel L, et al. 2011. Spatial and temporal requirements for sonic hedgehog in the regulation of thalamic interneuron identity. *Development* 138:531–41
- Jimenez-Guri E, Udina F, Colas JF, Sharpe J, Padron-Barthe L, et al. 2010. Clonal analysis in mice underlines the importance of rhombomeric boundaries in cell movement restriction during hindbrain segmentation. *PLoS One* 5:e10112
- Kataoka A, Shimogori T. 2008. Fgf8 controls regional identity in the developing thalamus. *Development* 135:2873–81
- Kemp HA, Cooke JE, Moens CB. 2009. EphA4 and EfnB2a maintain rhombomere coherence by independently regulating intercalation of progenitor cells in the zebrafish neural keel. *Dev. Biol.* 327:313–26
- Kiecker C, Lumsden A. 2004. Hedgehog signaling from the ZLI regulates diencephalic regional identity. *Nat. Neurosci.* 7:1242–49
- Kiecker C, Lumsden A. 2005. Compartments and their boundaries in vertebrate brain development. *Nat. Rev. Neurosci.* 6:553–64
- Kiecker C, Niehrs C. 2001a. A morphogen gradient of Wnt/ β -catenin signalling regulates anteroposterior neural patterning in *Xenopus*. *Development* 128:4189–201
- Kiecker C, Niehrs C. 2001b. The role of prechordal mesendoderm in neural patterning. *Curr. Opin. Neurobiol.* 11:27–33
- Kitamura K, Miura H, Yanazawa M, Miyashita T, Kato K. 1997. Expression patterns of *Brx1* (*Rieg* gene), *Sonic hedgehog*, *Nkx2.2*, *Dlx1* and *Arx* during zona limitans intrathalamica and embryonic ventral lateral geniculate nuclear formation. *Mech. Dev.* 67:83–96
- Kobayashi D, Kobayashi M, Matsumoto K, Ogura K, Nakafuku M, Shimamura K. 2002. Early subdivisions in the neural plate define distinct competence for inductive signals. *Development* 129:83–93
- Krauss S, Concordet JP, Ingham PW. 1993. A functionally conserved homolog of the *Drosophila* segment polarity gene *hb* is expressed in tissues with polarizing activity in zebrafish embryos. *Cell* 75:1431–44
- Langenberg T, Brand M. 2005. Lineage restriction maintains a stable organizer cell population at the zebrafish midbrain-hindbrain boundary. *Development* 132:3209–16
- Le Douarin NM, Halpern ME. 2000. Discussion point. Origin and specification of the neural tube floor plate: insights from the chick and zebrafish. *Curr. Opin. Neurobiol.* 10:23–30
- Lee KJ, Dietrich P, Jessell TM. 2000. Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. *Nature* 403:734–40

- Lee KJ, Mendelsohn M, Jessell TM. 1998. Neuronal patterning by BMPs: a requirement for GDF7 in the generation of a discrete class of commissural interneurons in the mouse spinal cord. *Genes Dev.* 12:3394–407
- Lee SM, Danielian PS, Fritsch B, McMahon AP. 1997. Evidence that FGF8 signalling from the midbrain-hindbrain junction regulates growth and polarity in the developing midbrain. *Development* 124:959–69
- Lee SM, Tole S, Grove EA, McMahon AP. 2000. A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development* 127:457–67
- Lei Q, Zelman AK, Kuang E, Li S, Matise MP. 2004. Transduction of graded Hedgehog signaling by a combination of Gli2 and Gli3 activator functions in the developing spinal cord. *Development* 131:3593–604
- Liem KF Jr, Tremml G, Jessell TM. 1997. A role for the roof plate and its resident TGF β -related proteins in neuronal patterning in the dorsal spinal cord. *Cell* 91:127–38
- Lim Y, Cho G, Minarcik J, Golden J. 2005. Altered BMP signaling disrupts chick diencephalic development. *Mech. Dev.* 122:603–20
- Liu A, Joyner AL. 2001. Early anterior/posterior patterning of the midbrain and cerebellum. *Annu. Rev. Neurosci.* 24:869–96
- Liu Y, Helms AW, Johnson JE. 2004. Distinct activities of Msx1 and Msx3 in dorsal neural tube development. *Development* 131:1017–28
- Liu Y, Shi J, Lu CC, Wang ZB, Lyuksyutova AI, et al. 2005. Ryk-mediated Wnt repulsion regulates posterior-directed growth of corticospinal tract. *Nat. Neurosci.* 8:1151–59
- Lowe CJ, Wu M, Salic A, Evans L, Lander E, et al. 2003. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* 113:853–65
- Lumsden A, Keynes R. 1989. Segmental patterns of neuronal development in the chick hindbrain. *Nature* 337:424–28
- Lupo G, Harris WA, Lewis KE. 2006. Mechanisms of ventral patterning in the vertebrate nervous system. *Nat. Rev. Neurosci.* 7:103–14
- Lyuksyutova AI, Lu CC, Milanesio N, King LA, Guo N, et al. 2003. Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. *Science* 302:1984–88
- Machon O, Backman M, Machonova O, Kozmik Z, Vacik T, et al. 2007. A dynamic gradient of Wnt signaling controls initiation of neurogenesis in the mammalian cortex and cellular specification in the hippocampus. *Dev. Biol.* 311:223–37
- Machon O, van den Bout CJ, Backman M, Kemler R, Krauss S. 2003. Role of β -catenin in the developing cortical and hippocampal neuroepithelium. *Neuroscience* 122:129–43
- Maden M. 2007. Retinoic acid in the development, regeneration and maintenance of the nervous system. *Nat. Rev. Neurosci.* 8:755–65
- Marín F, Puelles L. 1994. Patterning of the embryonic avian midbrain after experimental inversions: a polarizing activity from the isthmus. *Dev. Biol.* 163:19–37
- Martinez S, Crossley PH, Cobos I, Rubenstein JL, Martin GR. 1999. FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on *Otx2* expression. *Development* 126:1189–200
- Martinez S, Wassef M, Alvarado-Mallart RM. 1991. Induction of a mesencephalic phenotype in the 2-day-old chick prosencephalon is preceded by the early expression of the homeobox gene *en*. *Neuron* 6:971–81
- Mason I. 2007. Initiation to end point: the multiple roles of fibroblast growth factors in neural development. *Nat. Rev. Neurosci.* 8:583–96
- Matsumoto K, Nishihara S, Kamimura M, Shiraiishi T, Ootoguro T, et al. 2004. The prepatterning transcription factor *Irx2*, a target of the FGF8/MAP kinase cascade, is involved in cerebellum formation. *Nat. Neurosci.* 7:605–12
- McMahon AP, Joyner AL, Bradley A, McMahon JA. 1992. The midbrain-hindbrain phenotype of *Wnt-1⁻/Wnt-1⁻* mice results from stepwise deletion of *engrailed*-expressing cells by 9.5 days postcoitum. *Cell* 69:581–95
- Megason SG, McMahon AP. 2002. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. *Development* 129:2087–98

- Meinertzhagen IA, Lemaire P, Okamura Y. 2004. The neurobiology of the ascidian tadpole larva: recent developments in an ancient chordate. *Annu. Rev. Neurosci.* 27:453–85
- Meinhardt H. 2006. Primary body axes of vertebrates: generation of a near-Cartesian coordinate system and the role of Spemann-type organizer. *Dev. Dyn.* 235:2907–19
- Meinhardt H. 2009. Models for the generation and interpretation of gradients. *Cold Spring Harb. Perspect. Biol.* 1:a001362
- Meyers EN, Lewandowski M, Martin GR. 1998. An *Fgf8* mutant allelic series generated by Cre- and Flp-mediated recombination. *Nat. Genet.* 18:136–41
- Millet S. 1999. A role for *Gbx2* in repression of *Otx2* and positioning the mid/hindbrain organizer. *Nature* 401:161–64
- Millonig JH, Millen KJ, Hatten ME. 2000. The mouse *Dreber* gene *Lmx1a* controls formation of the roof plate in the vertebrate CNS. *Nature* 403:764–69
- Monuki ES, Porter FD, Walsh CA. 2001. Patterning of the dorsal telencephalon and cerebral cortex by a roof plate-Lhx2 pathway. *Neuron* 32:591–604
- Munji RN, Choe Y, Li G, Siegenthaler JA, Pleasure SJ. 2011. Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors. *J. Neurosci.* 31:1676–87
- Muñoz-Sanjuán I, Brivanlou AH. 2002. Neural induction, the default model and embryonic stem cells. *Nat. Rev. Neurosci.* 3:271–80
- Muroyama Y, Fujihara M, Ikeya M, Kondoh H, Takada S. 2002. Wnt signaling plays an essential role in neuronal specification of the dorsal spinal cord. *Genes Dev.* 16:548–53
- Muzio L, Soria JM, Pannese M, Piccolo S, Mallamaci A. 2005. A mutually stimulating loop involving *emx2* and canonical wnt signalling specifically promotes expansion of occipital cortex and hippocampus. *Cereb. Cortex* 15:2021–28
- Nakamoto A, Nagy LM, Shimizu T. 2011. Secondary embryonic axis formation by transplantation of D quadrant micromeres in an oligochaete annelid. *Development* 138:283–90
- Nguyen VH, Trout J, Connors SA, Andermann P, Weinberg E, Mullins MC. 2000. Dorsal and intermediate neuronal cell types of the spinal cord are established by a BMP signaling pathway. *Development* 127:1209–20
- Niehrs C. 2004. Regionally specific induction by the Spemann-Mangold organizer. *Nat. Rev. Genet.* 5:425–34
- Niehrs C. 2010. On growth and form: a Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes. *Development* 137:845–57
- Ninkovic J, Tallafuss A, Leucht C, Topczewski J, Tannhäuser B, et al. 2005. Inhibition of neurogenesis at the zebrafish midbrain-hindbrain boundary by the combined and dose-dependent activity of a new *hairy/E(spl)* gene pair. *Development* 132:75–88
- Ono Y, Nakatani T, Sakamoto Y, Mizuhara E, Minaki Y, et al. 2007. Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. *Development* 134:3213–25
- Osterfield M, Kirschner MW, Flanagan JG. 2003. Graded positional information: interpretation for both fate and guidance. *Cell* 113:425–28
- Osumi N, Hirota A, Ohuchi H, Nakafuku M, Iimura T, et al. 1997. *Pax-6* is involved in the specification of hindbrain motor neuron subtype. *Development* 124:2961–72
- Paek H, Gutin G, Hébert JM. 2009. FGF signaling is strictly required to maintain early telencephalic precursor cell survival. *Development* 136:2457–65
- Panhuisen M, Vogt Weisenhorn DM, Blanquet V, Brodski C, Heinzmann U, et al. 2004. Effects of Wnt1 signaling on proliferation in the developing mid-/hindbrain region. *Mol. Cell. Neurosci.* 26:101–11
- Partanen J. 2007. FGF signalling pathways in development of the midbrain and anterior hindbrain. *J. Neurochem.* 101:1185–93
- Pattyn A, Vallstedt A, Dias JM, Sander M, Ericson J. 2003. Complementary roles for *Nkx6* and *Nkx2* class proteins in the establishment of motoneuron identity in the hindbrain. *Development* 130:4149–59
- Pera EM, Kessel M. 1997. Patterning of the chick forebrain anlage by the prechordal plate. *Development* 124:4153–62

- Picker A, Brennan C, Reifers F, Clarke JD, Holder N, Brand M. 1999. Requirement for the zebrafish mid-hindbrain boundary in midbrain polarisation, mapping and confinement of the retinotectal projection. *Development* 126:2967–78
- Placzek M, Briscoe J. 2005. The floor plate: multiple cells, multiple signals. *Nat. Rev. Neurosci.* 6:230–40
- Placzek M, Tessier-Lavigne M, Yamada T, Jessell T, Dodd J. 1990. Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science* 250:985–88
- Pottin K, Hinaux H, Rétaux S. 2011. Restoring eye size in *Astyanax mexicanus* blind cavefish embryos through modulation of the Shh and Fgf8 forebrain organising centres. *Development* 138:2467–76
- Puelles E, Annino A, Tuorto F, Uziel A, Acampora D, et al. 2004. *Otx2* regulates the extent, identity and fate of neuronal progenitor domains in the ventral midbrain. *Development* 131:2037–48
- Quinlan R, Graf M, Mason I, Lumsden A, Kiecker C. 2009. Complex and dynamic patterns of Wnt pathway gene expression in the developing chick forebrain. *Neural Dev.* 4:35
- Reichert H. 2005. A tripartite organization of the urbilaterian brain: developmental genetic evidence from *Drosophila*. *Brain Res. Bull.* 66:491–94
- Reifers F, Böhlh H, Walsh EC, Crossley PH, Stainier DY, Brand M. 1998. *Fgf8* is mutated in zebrafish *acerebellar* (*ace*) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. *Development* 125:2381–95
- Rhinn M, Lun K, Luz M, Werner M, Brand M. 2005. Positioning of the midbrain-hindbrain boundary organizer through global posteriorization of the neuroectoderm mediated by Wnt8 signaling. *Development* 132:1261–72
- Riley BB, Chiang MY, Storch EM, Heck R, Buckles GR, Lekven AC. 2004. Rhombomere boundaries are Wnt signaling centers that regulate metamer patterning in the zebrafish hindbrain. *Dev. Dyn.* 231:278–91
- Rodríguez-Seguel E, Alarcón P, Gómez-Skarmeta JL. 2009. The *Xenopus Irx* genes are essential for neural patterning and define the border between prethalamus and thalamus through mutual antagonism with the anterior repressors *Fezf* and *Arx*. *Dev. Biol.* 329:258–68
- Roelink H, Augsburger A, Heemskerck J, Korzh V, Norlin S, et al. 1994. Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of hedgehog expressed by the notochord. *Cell* 76:761–75
- Sánchez-Camacho C, Rodríguez J, Ruiz JM, Trousse F, Bovolenta P. 2005. Morphogens as growth cone signalling molecules. *Brain Res. Brain Res. Rev.* 49:242–52
- Sanders TA, Lumsden A, Ragsdale CW. 2002. Arcuate plan of chick midbrain development. *J. Neurosci.* 22:10742–50
- Scholpp S, Delogu A, Gilthorpe J, Peukert D, Schindler S, Lumsden A. 2009. *Her6* regulates the neurogenetic gradient and neuronal identity in the thalamus. *Proc. Natl. Acad. Sci. USA* 106:19895–900
- Scholpp S, Foucher I, Staudt N, Peukert D, Lumsden A, Houart C. 2007. *Otx11*, *Otx2* and *Irx1b* establish and position the ZLI in the diencephalon. *Development* 134:3167–76
- Scholpp S, Wolf O, Brand M, Lumsden A. 2006. Hedgehog signalling from the zona limitans intrathalamica orchestrates patterning of the zebrafish diencephalon. *Development* 133:855–64
- Shimamura K, Hartigan DJ, Martinez S, Puelles L, Rubenstein JL. 1995. Longitudinal organization of the anterior neural plate and neural tube. *Development* 121:3923–33
- Shimamura K, Rubenstein JL. 1997. Inductive interactions direct early regionalization of the mouse forebrain. *Development* 124:2709–18
- Sousa VH, Fishell G. 2010. Sonic hedgehog functions through dynamic changes in temporal competence in the developing forebrain. *Curr. Opin. Genet. Dev.* 20:391–99
- Stamatata D, Ulloa F, Tsoni SV, Mynett A, Briscoe J. 2005. A gradient of Gli activity mediates graded Sonic hedgehog signaling in the neural tube. *Genes Dev.* 19:626–41
- Stern CD. 2001. Initial patterning of the central nervous system: how many organizers? *Nat. Rev. Neurosci.* 2:92–98
- Stern CD. 2006. Neural induction: 10 years on since the ‘default model’. *Curr. Opin. Cell Biol.* 18:692–97
- Sugahara F, Aota S, Kuraku S, Murakami Y, Takio-Ogawa Y, et al. 2011. Involvement of Hedgehog and FGF signalling in the lamprey telencephalon: evolution of regionalization and dorsoventral patterning of the vertebrate forebrain. *Development* 138:1217–26
- Sumnonu NA, Li K, Guo Q, Li JY. 2011. *Gbx2* and *Fgf8* are sequentially required for formation of the midbrain-hindbrain compartment boundary. *Development* 138:725–34

- Sylvester JB, Rich CA, Loh YH, van Staaden MJ, Fraser GJ, Strelman JT. 2010. Brain diversity evolves via differences in patterning. *Proc. Natl. Acad. Sci. USA* 107:9718–23
- Takada H, Hattori D, Kitayama A, Ueno N, Taira M. 2005. Identification of target genes for the *Xenopus* Hes-related protein XHR1, a prepattern factor specifying the midbrain-hindbrain boundary. *Dev. Biol.* 283:253–67
- Takahashi M, Osumi N. 2002. *Pax6* regulates specification of ventral neurone subtypes in the hindbrain by establishing progenitor domains. *Development* 129:1327–38
- Technau U, Steele RE. 2011. Evolutionary crossroads in developmental biology: Cnidaria. *Development* 138:1447–58
- Tessier-Lavigne M, Goodman CS. 1996. The molecular biology of axon guidance. *Science* 274:1123–33
- Thomas KR, Musci TS, Neumann PE, Capecchi MR. 1991. *Swaying* is a mutant allele of the proto-oncogene *Wnt-1*. *Cell* 67:969–76
- Thomson RE, Kind PC, Graham NA, Etherson ML, Kennedy J, et al. 2009. Fgf receptor 3 activation promotes selective growth and expansion of occipitotemporal cortex. *Neural Dev.* 4:4
- Timmer JR, Wang C, Niswander L. 2002. BMP signaling patterns the dorsal and intermediate neural tube via regulation of homeobox and helix-loop-helix transcription factors. *Development* 129:2459–72
- Tomer R, Denes AS, Tessmar-Raible K, Arendt D. 2010. Profiling by image registration reveals common origin of annelid mushroom bodies and vertebrate pallium. *Cell* 142:800–9
- Tossell K, Kiecker C, Wizenmann A, Lang E, Irving C. 2011. Notch signalling stabilises boundary formation at the midbrain-hindbrain organiser. *Development* 138:3745–57
- Toyoda R, Assimacopoulos S, Wilcoxon J, Taylor A, Feldman P, et al. 2010. FGF8 acts as a classic diffusible morphogen to pattern the neocortex. *Development* 137:3439–48
- Trokovic R, Jukkola T, Saarimaki J, Peltopuro P, Naserke T, et al. 2005. Fgfr1-dependent boundary cells between developing mid- and hindbrain. *Dev. Biol.* 278:428–39
- Urbach R, Technau GM. 2008. Dorsoventral patterning of the brain: a comparative approach. *Adv. Exp. Med. Biol.* 628:42–56
- van Straaten HW, Hekking JW, Thors F, Wiertz-Hoessels EL, Drukker J. 1985. Induction of an additional floor plate in the neural tube. *Acta Morphol. Neerl. Scand.* 23:91–97
- Vieira C, Garda AL, Shimamura K, Martinez S. 2005. Thalamic development induced by Shh in the chick embryo. *Dev. Biol.* 284:351–63
- Vogel-Höpker A, Rohrer H. 2002. The specification of noradrenergic locus coeruleus (LC) neurones depends on bone morphogenetic proteins (BMPs). *Development* 129:983–91
- Vue TY, Bluske K, Alishani A, Yang LL, Koyano-Nakagawa N, et al. 2009. Sonic hedgehog signaling controls thalamic progenitor identity and nuclei specification in mice. *J. Neurosci.* 29:4484–97
- Walshe J, Mason I. 2003. Unique and combinatorial functions of Fgf3 and Fgf8 during zebrafish forebrain development. *Development* 130:4337–49
- Wassarman KM, Lewandowski M, Campbell K, Joyner AL, Rubenstein JL, et al. 1997. Specification of the anterior hindbrain and establishment of a normal mid/hindbrain organizer is dependent on *Gbx2* gene function. *Development* 124:2923–34
- Wingate RJ. 2001. The rhombic lip and early cerebellar development. *Curr. Opin. Neurobiol.* 11:82–88
- Wurst W, Bally-Cuif L. 2001. Neural plate patterning: upstream and downstream of the isthmus organizer. *Nat. Rev. Neurosci.* 2:99–108
- Xie Z, Chen Y, Li Z, Bai G, Zhu Y, et al. 2011. Smad6 promotes neuronal differentiation in the intermediate zone of the dorsal neural tube by inhibition of the Wnt/ β -catenin pathway. *Proc. Natl. Acad. Sci. USA* 108:12119–24
- Xu Q, Mellitzer G, Robinson V, Wilkinson DG. 1999. In vivo cell sorting in complementary segmental domains mediated by Eph receptors and ephrins. *Nature* 399:267–71
- Zeltser LM. 2005. Shh-dependent formation of the ZLI is opposed by signals from the dorsal diencephalon. *Development* 132:2023–33
- Zeltser LM, Larsen CW, Lumsden A. 2001. A new developmental compartment in the forebrain regulated by *Lunatic fringe*. *Nat. Neurosci.* 4:683–84
- Zervas M, Millet S, Ahn S, Joyner AL. 2004. Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. *Neuron* 43:345–57
- Zou Y, Lyuksyutova AI. 2007. Morphogens as conserved axon guidance cues. *Curr. Opin. Neurobiol.* 17:22–28



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Errata

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