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Review

Developmental mechanisms and experimental models to understand forebrain malformative diseases

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The development of the central nervous system can be divided into a number of phases, each of which can be subject of genetic or epigenetic alterations that may originate particular developmental disorders. In recent years, much progress has been made in elucidating the molecular and cellular mechanisms by which the vertebrate forebrain develops. Therefore, our understanding of major developmental brain disorders such as cortical malformations and neuronal migration disorders has significantly increased. In this review, we will describe the major stages in forebrain morphogenesis and regionalization, with special emphasis on developmental molecular mechanisms derailing telencephalic development with subsequent damage to cortical function. Because animal models, mainly mouse, have been fundamental for this progress, we will also describe some characteristic mouse models that have been capital to explore these molecular mechanisms of malformative diseases of the human brain. Although most of the genes involved in the regulation of basic developmental processes are conserved among vertebrates, the extrapolation of mouse data to corresponding gene expression and function in humans needs careful individual analysis in each functional system.

Keywords: Brain malformations, cortical development, forebrain regionalization, prosomeric model, secondary organizers

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The vertebrate central nervous system (CNS) originates from the embryonic ectoderm. Differentiation of the neural ectoderm from the ectoderm and the formation of the neural plate correlate with an early phase of the complex gastrulation process, which culminates with neural tube formation (neurulation) and, subsequently, the establishment of the anteroposterior (AP) and ventrodorsal axes of the CNS (for review, see Rubenstein *et al.* 1998; Shimamura *et al.* 1995; Smith & Schoenwolf 1997).

These initial patterning events are controlled by vertical and planar inductive interactions between the neuroectoderm and the surrounding blastodermal derivatives. At neural plate and neural tube stages, local signaling centers in the neuroepithelium, known as secondary organizers start to refine the initially rough AP specification and regulate the generation of various AP neural territories at the hind-midbrain border and in the prosencephalon (for review, see Echevarria *et al.* 2003).

The prenatal period of mammalian development extends from fertilization of the ovum to 19 days of gestation in mice or 280 days of gestation in humans. This period can be subdivided into smaller temporal segments: pre-embryonic (ovum), embryonic and fetal periods (Fig. 1).

During the first days after fertilization, the unicellular embryo develops into the multicellular blastocyst that ingresses in the uterine endometrium to form with its outer cells the trophoblastic placenta, while the inner cell mass of blastomeres progresses to the next embryonic periods (1–16 days in humans and 1–7 days in mice). The study of these initial stages and their developmental alterations are generating an important body of knowledge because these data represent the experimental basis for assisted fertilization therapies in humans and can be analyzed in mouse and humans by '*in vitro*' assays. Moreover, given that gastrulation is an important overall embryonic cellular process precisely regulated by complex genetic interactions (the most important time of our life – Lewis Wolpert), mutations in the genes active during this period tend to produce severe developmental alterations that frequently end with spontaneous abortion. To detect a lethal condition originated by alterations in the genes regulating these primordial stages in the mouse, it is required a detailed analysis of the offspring in relation to the inheritance models of the studied mutation. Therefore, the analysis of the phenotypic characteristics in these mutants must start very early in development.

After neurulation, the principal regions of the brain are already specified in the neural epithelium. A precise fate map of the presumptive brain regions at this stage has been correlated to gene expression studies and experimental embryology data (revised in Cobos *et al.* 2001; García López *et al.* 2004; Rubenstein *et al.* 1998). The normal development of each region depends on molecular mechanisms that

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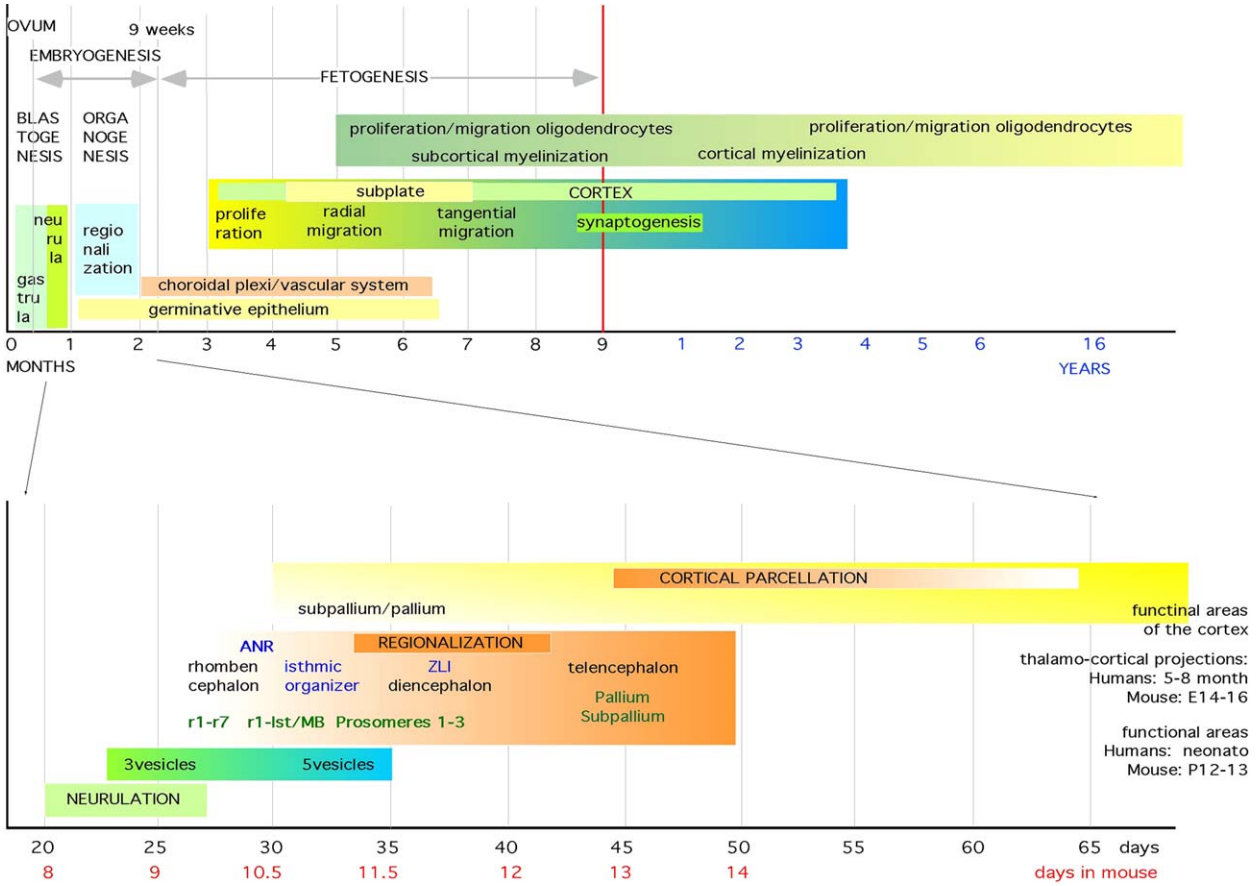

Figure 1: Comparative diagram of human and mouse development. The time periods when the main events of brain development occurred are represented by gray bars. Inside the bars developmental process are given. Neurulation and brain regionalization stages have been enlarged to give an extensive comparative view of each event in relation to human (upper black numbers) and mouse (red lower numbers) developmental times.

underlie differential proliferation and morphogenetic movements or deformation of neuroepithelial cells and, later, neurogenesis, cell differentiation and maturation in specific neural regions. In addition to this genetic mechanism to generate cell diversity, epigenetic influences play a role by means of post-transcriptional control of genetic expressions, permitting an optimal adaptation of the developing brain system to normal or pathologic circumstances.

Molecular patterns and regionalization of the neural tube

The early neural tube is an elongated, increasingly bent tubular structure (Fig. 2) in most of the vertebrates. However, even before the posterior portion of the tube has closed, the most anterior portion of the tube is undergoing drastic shape changes. In this region, the neural tube balloons into three primary vesicles: forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon) (Figs 1 and 2; Martinez & Puelles 2000). By the time the posterior end of the neural tube closes, the optic vesicles have evaginated laterally from each side of the developing forebrain. At this early stage of development (three-vesicle stage), the bending of the length axis, already observed at the late neural plate stages, increases considerably, leading to the cephalic and cervical flexures of the neural tube (Fig. 2).

As development proceeds (five-vesicle stage; Fig. 2), the prosencephalon becomes subdivided into the anterior forebrain (including telencephalon and hypothalamus) and the more caudal diencephalon.

For almost a century, neuroembryologists have used morphological information to suggest that the brain is segmented (revised in Puelles 1995, 2001). The basis for this argument is the transient embryonic appearance of repeated transverse constrictions present in the forebrain, midbrain and hindbrain that subdivide the neural tube into transverse sectors called segments or neuromeres. The formation of these neuroepithelial segments obeys to complex genetic regulation of neuroepithelial cellular processes such as relative surface growth (higher at the canters of segments, lower at the limits), clonal properties (cell–cell adhesivity, intercalation and ionic intercommunication through gap junctions) and

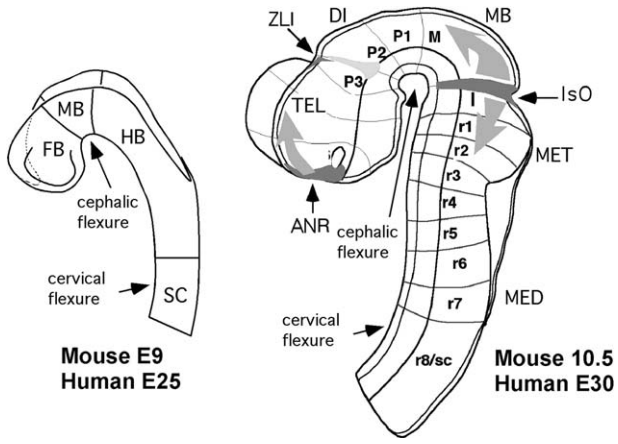

Figure 2: Neural tube regionalization. Neural tube regionalization is represented at two developmental stages. Early neurula stage (E9 in mouse and E25 in human embryo) represents the three-vesicle stage, when forebrain (FB), midbrain (MB) and hindbrain (HB) are differentiated from the spinal cord (SC) in the anterior part of the embryo. At stage E10.5 in mouse (E30 in human embryo), the regionalization of the neural tube is almost complete, then the brain vesicles (five-vesicle stage) and neuromeres are identifiable. Secondary organizers ANR, ZLI and IsO have been also identified: Arrows show the direction of the morphogenetic activity. DI, diencephalons; I, isthmus; TEL, telencephalon; MB, midbrain or mesencephalon, MED, medulla; MET, metencephalon; P1-P3, prosomeres; r1-r7, rhombomeres; r8/sc, pseudorhombomeres and spinal cord.

planar AP propagation or restriction of gene expression patterns. The analysis of gene expression patterns in the neural tube provides much direct or indirect evidence on these mechanisms.

Gene expression maps in the anterior neural tube

The discovery that putative regulatory genes are expressed in regionally restricted patterns in the developing forebrain has provided new tools for defining, at higher resolution, histogenic domains and their boundaries. Based on gene expression patterns as well as on embryological, histological and morphological information, two models have been used to interpret neural plate and tube regionalization: a regional topographic model, largely aimed at saving the classic concept of sulcal division of the diencephalon in the four longitudinal columnar zones of Herrick (Álvarez-Bolado *et al.* 1995) and a segmental-topologic model, called 'prosomeric model' (Puelles & Rubenstein 1993; Rubenstein *et al.* 1994), aimed to be consistent with emergent morphologic, molecular and experimental data on the bent longitudinal forebrain axis, contradictory to the zones of Herrick.

Although today there are still some studies that do not use the segmental paradigm to interpret gene expression patterns in the developing neural tube, most current authors follow the prosomeric model because it has shown more topographic and anatomic accuracy, easily lends itself to comparative analysis and clearly displays higher predictive capacity than other alternatives.

The prosomeric model hypothesizes that the embryonic forebrain, like the rhombencephalon, is a neuromeric structure subdivided into a grid-like pattern of histogenic domains by longitudinal (columnar) and transverse (segmental) boundaries (Puelles & Rubenstein 2003; Puelles *et al.* 2004; Rubenstein *et al.* 1994). The longitudinal boundaries segregate columns of cells with similar histogenic properties that are differentially specified by dorsoventral (DV) patterning mechanisms, equivalent to the lateromedial patterning mechanisms acting at the neural plate stage. The interactions that occur during DV patterning of the neural plate and tube originate four major longitudinal columnar territories, which are called, from ventral to dorsal, floor plate, basal plate, alar plate and roof plate. These territories are reproduced consecutively in each segment along the AP axis of the neural tube, thus maintaining a metameric (iterated) distribution of fundamental neuroepithelial domains throughout the neural primordium. Patterning along the AP axis leads to differential genetic identities of the diverse segments, irrespective of their common fundamental DV pattern.

Transverse boundaries accordingly subdivide the brain into segments (neuromeres), especially the prosencephalon whose segments are called prosomeres (p1-6; Puelles & Rubenstein 1993, or, in a simplified schema, p1-p3 and the secondary prosencephalon; Puelles & Rubenstein 2003) and the rhombencephalon, whose segments are called rhombomeres (r1-r7) and pseudorhombomeres (r8-r11) (Cambronero & Puelles 2000; Marin & Puelles 1995) (Fig. 2).

Segmental anatomy, which in an extended sense analyzes brain and head development in terms of mutual topological relations and commonality of molecular genetic effects at different embryonic stages, has resulted to be of special relevance for practical clinicians because it permits to establish ontogenetic relationships between the brain and the surrounding tissues (Carstens 2004). Moreover, it is interesting that observed causal relationships between the gene expression patterns and the formation and ulterior development of identified developmental units of the neuroepithelium have been a key element to propose integrative morphological and genetic classifications of CNS malformations (Barkovich *et al.* 2005; Sarnat & Flores-Sarnat 2004).

Mediolateral patterning (DV patterning)

It has been established that within the posterior neural plate mediolateral (ML), regional identities are specified in part by molecules produced by adjacent nonneural tissues. At the spinal cord level of the neural plate, medial cell fates are specified by the notochord (for review, see Tanabe & Jessell 1996). Both gain-of-function and loss-of-function experiments show that medial signaling is regulated by Sonic Hedgehog (Shh) produced by the axial mesendoderm. Shh is first produced by the notochord, and later its expression is induced in the overlying median neural plate. However, gain-of-function experiments and gene expression data support the idea that lateral signaling is regulated by members of the transforming growth factor-beta superfamily, such as bone morphogenic

proteins 4 and 7 (BMP4 and BMP7), produced by nonneural ectoderm as well as by dorsalin and activin (Liem *et al.* 1995).

Because the notochord does not underlie the anterior forebrain (the anterior end of the notochord ends at the level of prosomere 3, underlying the prethalamic basal plate), it has been unclear whether patterning of the medial (ventral) forebrain is regulated by mechanisms distinct from those active in more posterior regions. Anterior to the notochord, there is an axial mesendodermal structure named prechordal plate. Several lines of molecular and genetic evidence do suggest that medial/ventral specification of the forebrain is regulated by the prechordal plate and involves molecular mechanisms similar to those in more posterior CNS regions. The analysis of mice lacking a functional *Shh* gene shows that Shh is essential for medial patterning of the entire CNS (Chiang *et al.* 1996). Shh mutations in mice and humans have been described as a cause of holoprosencephaly (HPE) (Chiang *et al.* 1996; Lazaro *et al.* 2004; Maity *et al.* 2005; Traiffort *et al.* 2004; Wallis & Muenke 2000).

Alterations in dorsally produced signals, such as BMPs, or their respective molecular partners, frequently generate errors in the dorsal midline fusion of the CNS: spina bifida and/or myelomeningocele (caudally) as well as HPE (rostrally; Anderson *et al.* 2002; Rice 2005; Warren *et al.* 2003).

AP patterning

AP patterning is the process that leads to the generation of distinct transverse domains at different axial positions in the CNS. There is evidence that AP patterning begins during early gastrulation (Cobos *et al.* 2001; Crossley *et al.* 2001; Foley *et al.* 2000).

Several experiments suggest that vertical signals diffusing from the underlying tissues (mesoderm and endoderm) to the overlying dorsal ectoderm, and perhaps also planar signals spreading from the primary organizer (Hensen's node) through the plane of the ectodermal epithelium, contribute to the specification of AP regional differences (Echevarria *et al.* 2003).

Two homeodomain transcription factors, *Lim1* and *Otx2*, are essential in the development of anterior CNS structures. These genes are expressed in the tissues underlying the anterior neural plate. Loss-of-function mutants of these genes result in mouse embryos lacking both forebrain and midbrain, suggesting that *Lim1* and *Otx2* play a role in early AP patterning (Kinder *et al.* 2001). *Lim1* is expressed in the primitive streak and prechordal mesoderm. Because expression is not detected in the neural plate, the lack of forebrain and midbrain in *Lim1* mouse mutants represents evidence for an essential role of the prechordal mesoderm in anterior CNS development (Shawlot & Behringer 1995). Understanding the mechanisms underlying the *Otx2* phenotype is more difficult because of the complexity of its expression pattern (Kinder *et al.* 2001).

There are experimental data suggesting that in some cases, distinct gene expression programs at different AP positions within the neural plate and tube are because of intrinsic differences in the competence to respond to a common signal. These local differences are controlled by essentially still unknown mechanisms of positional information. Fgf8 is an example of an inductive diffusible signal that generates distinct molecular responses at different axial levels. When Fgf8 is applied to prosencephalic and mesencephalic domains of neural plate explants, it induces distinct genes: anteriorly, *Foxg1 (Bf1)*, whereas posteriorly, *En2* (Shimamura & Rubenstein 1997).

Regionalization of the rostral brain involves secondary organizing centers

Regionalization of the prosencephalic neural plate appears to result from the superposition of multiple distinct patterning mechanisms. AP patterning creates transverse zones with differential competence, while patterning along the ML axis generates longitudinally aligned domains. The combination of ML and AP patterning then generates a grid-like organization of distinct histogenic forebrain primordia, consistent with the dual longitudinal and transverse subdivisions contemplated in the prosomeric model.

We have described previously how elaborate cellular interactions regulate the establishment of the common complex structural pattern of the developing brain in vertebrates. Distinct neural and glial identities are acquired by neuroepithelial cells according to their relative positions, through progressive restriction of histogenetic potential, under the influence of local environmental signals. Evidence for controlling morphogenetic processes at specific locations of the developing neural primordium has suggested the concept of secondary organizers. Such centers regulate by their own signaling the choices of identity and regional polarity of neighboring neuroepithelial regions, usually differentially in rostral or in caudal directions, because of differential potential of the adjoining territories (for review, see Echevarria *et al.* 2003; Martinez 2001).

Thus, these organizers operate secondarily after those that operate throughout the embryo during gastrulation. They usually develop within the previously broadly regionalized neuroectoderm at given molecular boundaries (frequently where cells expressing different transcription factors are juxtaposed). Their subsequent activity refines local neural identities along the AP or DV axes, giving rise to differentially specified subregions in the forebrain, midbrain and hindbrain vesicles.

Three regions in the neural plate and tube have been identified as putative secondary organizers (Fig. 2). Two of these organizers control prosencephalic regionalization: the anterior neural ridge (ANR) at the anterior end of the neural plate and the zona limitans intrathalamica (ZLI) in the middle of the diencephalon. In addition, the isthmic organizer, at the mid-hindbrain boundary, controls mesencephalic and rostral rhombencephalic (including cerebellar) regionalization. In order to focus the present review on telencephalic regionalization, we will concentrate on the function of ANR.

Anterior neural ridge

The most anterior secondary organizer, the ANR, is a morphologically indistinct median sector at the junction between the neural plate and the nonneural ectoderm. The ANR controls

prosencephalic development, regionalization and proliferation (Houart *et al.* 1998), and it was shown that some genes expressed in this region control others necessary for telencephalic regionalization (Shimamura & Rubenstein 1997; Storm *et al.* 2006; Ye *et al.* 1998). In particular, the *Fgf8* gene is expressed very early in ANR cells and has been shown to be crucial for the specification of the anterior areas of the forebrain and telencephalon. *Fgf8* hypomorphic mutations in both mouse and zebra fish result in a small telencephalon (Shanmugalingam *et al.* 2000; Storm *et al.* 2003, 2006).

The ANR is necessary for the induction and/or maintenance of Foxg1 (*Bf1*) expression, essential for telencephalic regionalization and proliferation (Shimamura & Rubenstein 1997). In addition, implantation of Fgf8 protein into the prospective area of the telencephalon of chick embryos generates changes in the patterns of gene expression in this region and leads consequently to a redistribution of telencephalic and optic derivatives (Cobos *et al.* 2001; Crossley *et al.* 2001). Ectopic expression of *Fgf8* in the caudal telencephalon of mouse embryos produces duplication of functional areas of the cortex (Fukuchi-Shimogori & Grove 2001). Prosencephalic regionalization by Fgf8 is regulated at least in part through inhibition of *Otx2* and *Emx2* expression, in co-operation with *Bmp4*, *Wnt* and *Shh* (Crossley *et al.* 2001; Garel *et al.* 2003; Grove & Fukuchi-Shimogori 2003; Sansom *et al.* 2005; Storm *et al.* 2006). Modifications in this interactive molecular network produce important anomalies in the brain and skull development. For instance, decreasing Fgf activity produces brain alterations and different forms of craniosynostosis (including Apert syndrome) in humans and mouse (revised in Chi *et al.* 2003; Rice 2005; Storm *et al.* 2003, 2006).

Another secreted signaling protein produced slightly later in the subpallium near the ANR is Shh. Abundant data suggest that Shh is both necessary and sufficient for the specification of ventral pattern throughout the nervous system, reportedly including the telencephalon (Chiang *et al.* 1996), although the topologic meaning of the term 'ventral' as used in the telencephalon and spinal cord is not comparable because the ANR and the entire prospective telencephalic area are by definition 'dorsal' ('lateral') neural plate loci and lack any history of axial mesoendodermal causal effects. Recent authors, nevertheless, widely prefer to study the evaginated telencephalon as having its own length axis (from olfactory pole to amygdaloid pole), independently of the neural tube length axis. It is only with regard to this extra axis that the source of telencephalic Fgf8 and Shh signals may be conceived to be ventral. According to such a common viewpoint, normal patterning in the telencephalon depends on the ventral repression of *Gli3* function by *Shh* and conversely on the dorsal repression of *Shh* signaling by *Gli3* (Rallu *et al.* 2002). *Gli3* mutation in mouse and humans produces craniofacial and brain anomalies (Rice *et al.* 2005). *Shh* has also been shown to be involved in the regulatory activity of *Nkx2.1*, a homeodomain gene required for the development of the telencephalic subpallium as well as the hypothalamus, the latter being a properly ventral part of the forebrain (Fig. 2; Ericson *et al.* 1995). All these recent findings support therefore a model for neocortical formation and areal regionalization, in which neocortical progenitor cells become patterned by extracellular signals to generate a protomap of progenitor cell areas that in turn generate area-specific neurons (Miyashita-Lin *et al.* 1999; Rakic 2002). The protomap is thought to be underpinned by spatial differences in progenitor cell identity that is reflected by regional-specific genes at the transcriptional level (Grove & Fukuchi-Shimogori 2003; Storm *et al.* 2006).

Growth, regional specification and morphogenesis of the telencephalon show a profound sensitivity to the dosage of *Fgf8*. Furthermore, cross-regulation between the rostral (Fgf), dorsal (Bmp; Wnt) and ventral (Shh) secondary patterning centers plays an essential role in patterning the early telencephalon (Fig. 3). Modulation of this cross-regulation has the potential to control the relative size of structures whose morphogenesis is controlled by a given patterning center. For instance, a reduction in Fgf8 signaling reduces the ratio of the frontal motor to sensory regions of the neocortex (Fukuchi-Shimogori & Grove 2001; Garel *et al.* 2003; Storm *et al.* 2006). Therefore, controlling the relative strength and the range of a given patterning signal may provide a fundamental mechanism to modify the relative sizes of brain subdivisions during evolution and in disease states.

A mechanistic model can be proposed in which morphogenetic signals from different organizer regions interact in regulative networks to establish initial territories of specified progenitor cells. Then, different, or the same, molecular signals acting at different temporal scales may stabilize the final specific molecular code in these territories and activate the production of specific cell types, allowing the establishment of particular neuronal networks of the neural system (Fig. 3). Eventually, ambient influences, acting through post-transcriptional mechanisms, can modify the structural and functional properties of such neural systems, both in positive (adaptative) and in negative (toxic) directions.

It has been postulated that prosencephalic regionalization defects may be the cause of cortical malformations, such as HPE (*Shh* functional mutations, described previously), and parietal foramina defects (e.g. in *ALX4* and *MSX2* mutations; Mavrogiannis *et al.* 2006; revised by Rice 2005; Table 1). However, most of the mechanisms brought forward by experimental data in mouse, showing modifications in the extent or character of functional cortical regions, have not been well defined in humans.

Neural migration in the cortex

Developmental defects in neuronal positioning and synaptic connectivity are commonly found in neurological and psychiatric diseases, and they are believed to underlie many cognitive and affective disorders (Barkovich *et al.* 2005; Harrison & Weinberger 2005; Tabares-Seisdedos *et al.* 2006). Several mouse mutants are currently available that model at least some aspects of human developmental brain disorders that might be related to similar structural alterations (Table 1; Molnar *et al.* 2006). With the identification of the genes mutated in these animals and the study of the cellular basis of their phenotypes, we have taken significant strides toward an understanding of the mechanisms controlling proper brain development and the consequences of their dysfunction. In particular, mouse mutants deficient in the *Reelin* and *Lis1* gene have provided valuable insights into the

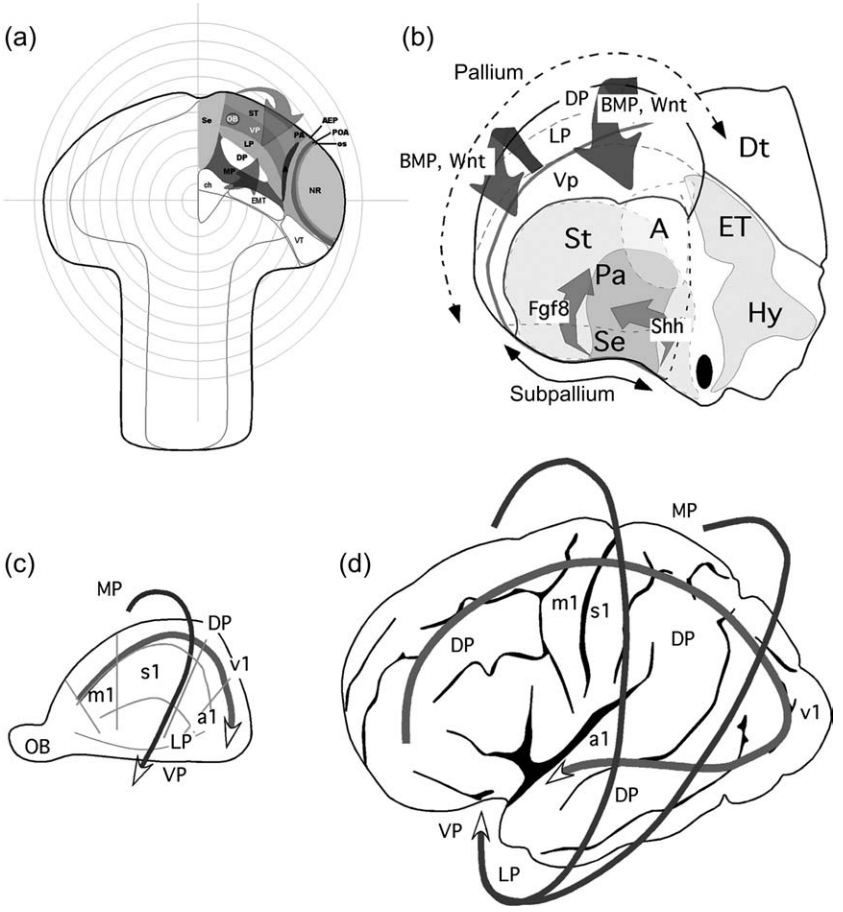

Figure 3: Telencephalic regionalization. (a) Dorsal view of the chick embryo neural tube. The presumptive regions of the pallial and subpallial regions have been mapped in the dorsal part of the prosencephalic vesicle. Arrows indicate the direction of morphogenetic signals from the anterior and dorsal organizers. (b) Lateral view of mouse anterior neural tube (E11.5) showing the localization of main pallial and subpallial regions in relation to the activity of signaling molecules: BMP and Wnt from the dorsal midline; Fgf8 from the ANR and Shh from the ventral telencephalon. (c, d) Lateral view of an adult mouse (c) and an adult human (d) pallium where AP and DV topological axes have been labeled by arrows. Pallial domains and primary cortical regions have been localized. a1, primary auditory cortex; ch, choroidal plexus; DP, dorsal pallium; DT, dorsal thalamus; EMT, eminentia thalami; ET, emimentia thalami; LP, lateral pallium; m1, motor cortex; MP, medial pallium; NR, nasal retina; OB, olfactory bulb; PA, pallidum; POA, preoptic area; s1, primary sensorial cortex; Se, septum; St, striatum; v1, primary visual cortex; VP, ventral pallium; VT, ventral thalamus.

mechanisms of cortical development (Assadi *et al.* 2003). Absence of reelin expression in the spontaneous mutant mouse reeler leads to extensive defects in neuronal position and dendrite development. In humans, loss of reelin results in a type of lissencephaly with severe cortical and cerebellar malformation (Barkovich *et al.* 2005; revised by D'Arcangelo 2006). Genetic and biochemical studies using mouse mutants suggest that the Lis1 protein may participate in reelin signaling pathway controlling cortical development by its interaction with the dynein system and, therefore, the neuronal motility (Assadi *et al.* 2003; D'Arcangelo 2006; Reiner *et al.* 2002).

Table 1: Related phenotypes and genes in human and mice

Phenotype	Human gene	Mouse gene
HPE	*SHH (HPE3)*	*Shh*
HPE-cephalopolysyndactily	*GLI3*	*Gli3*
Craniosynostosis	*FGFR*	*Fgf8/FgfR*
Parietal foramina deffects	*MSX2*	*Msx2*
	ALX4	*Alx4*
Spina bifida/myelomeningocele	*BMPs*	*BMP4*
Lissencephaly	*LIS1*	*Lis1*
	RELN	*Reelin*
	DCX	*Dcx*
	ARX	*Arx*
Psychosis predisposition	*DSC1*	*Dsc1*
	SP53	*sP53*

A recent classification proposed for developmental cortical malformations in humans by Barkovich *et al.* has listed the human genetic mutations identified as malformations because of abnormal neuronal migration (Barkovich *et al.* 2005). Most of the genes identified in humans have been also proved as functionally relevant to neuronal migration in mice; this suggests that in these situations, the mouse models are of great value for analyzing the physiopathological mechanisms of these human diseases. Indeed, it is of special relevance that alterations in the reelin-dependent genetic cascade activate complex molecular interactions regulating cell migration and microtubular transport (D'Arcangelo 2005). Mutation in some of the molecules involved in this interactive cascade produced important alterations in cell migration and, subsequently, in cortical structure, characterized phenotypically by different degrees of cortical dysplasia, such as the double-cortex syndrome within the lissencephaly spectrum (Cardoso *et al.* 2003), while mutations in some other molecules (like DSC1) or moderate genetic alteration in

lissencephaly-critical-region genes produce functional alterations, without important structural malformation, that may manifest schizophrenic or bipolar disease symptoms (Harrison & Weinberger 2005; Tabares-Seisdedos *et al.* 2006).

Summary and future perspective

We can conclude that the analysis of animal models has been most important to partially understand the physiopathology of an important number of human genetic diseases, at least at the level of basic molecular and cellular mechanisms. But, nevertheless, the mouse brain, like most of the common experimental models in neuroscience laboratories, does not fully reflex the complexity of human brains, neither in the structure nor in function. Therefore, it is necessary to develop more accurate studies in animal models, especially identifying the particular biological systems that appear more adequate as models of human pathologies, instead of looking for a model of an entire process. Furthermore, the understanding of particular basic aspects of a given disease will permit to articulate these singular mechanisms and, likely, to establish the physiopathological pathways of primary and emergent symptoms of human diseases.

Developmental neuroscience will describe soon the molecular mechanisms underlying brain regionalization and development in mammals; therefore, new genes and their function are going to be involved in regulating brain development. The cellular processes controlled by these genetic interactions have to be described in order to establish adequate correlation between genes and functions in the brain and, finally, to extend our knowledge on the molecular basis of brain developmental diseases. Thus, adequate diagnostic methods and better genetic and prognostic advice will become available.

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