CNS Neuroscience & Therapeutics

SPECIAL ISSUE ARTICLE

CNS Neuroscience & Therapeutics

Causes and Consequences of Gray Matter Heterotopia

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Keywords
Cortical development disorders; Epilepsy; Neuronal migration; Pathophysiology; Progenitors.

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Received 11 July 2014; revision 30 July 2014; accepted 6 August 2014

doi: 10.1111/cns.12322

SUMMARY

The objective of this article is to review the pathophysiological bases of gray matter heterotopia and to appreciate their involvement in brain cortical development and functional consequences, namely epilepsy. The development of the cerebral cortex results from complex sequential processes including cell proliferation, cell migration, cortical organization, and formation of neuronal networks. Disruption of these steps yields different types of cortical malformations including gray matter heterotopia, characterized by the ectopic position of neurons along the ventricular walls or in the deep white matter. Cortical malformations are major causes of epilepsy, being responsible for up to 40% of drug-resistant epilepsy, and the cognitive level of affected patients varies from normal to severely impaired. This review reports data from human patients and animal models highlighting the genetic causes for these disorders affecting not only neuronal migration but also the proliferation of cortical progenitors. Therefore, gray matter heterotopias should not be considered as solely due to an abnormal neuronal migration and classifying them as such may be too restrictive. The review will also summarize literature data indicating that besides ectopic neurons, neighbor cortical areas also play a consistent role in epileptogenesis, supporting the notion that plastic changes secondary to the initial malformation are instrumental in the pathophysiology of epilepsy in affected patients.

Gray matter heterotopia (GMH) is a group of neurological disorders characterized by the ectopic position of neurons. They present as ectopic clusters of neurons along the ventricular walls [mainly comprising periventricular nodular heterotopia (PNH)] or they form in the deep white matter a nodule (focal subcortical heterotopia) or a packaged band of neurons [subcortical band heterotopia or doublecortex (SBH)]. In the last update of the classification of malformations of cortical development [1], GMH were categorized as the result of an abnormal neuronal migration. Although the causes are not yet fully elucidated, a number of causative genes play also important roles on radial glia, proliferation, and differentiation of progenitors, supporting the notion that GMH may result from a diversity of alterations of developmental programs although the final phenotype can be read as a migration defect: the cells do not reach the appropriate destination layer. The first part of this review will provide an updated view of genetic causes and cellular and molecular mechanisms involved in the genesis of GMH.

GMH cause a variety of symptoms mainly including epilepsy, frequently resistant to medication. GMH often affects as well higher brain functions being responsible for mental delay, although symptoms range from absent to profound. Clinical investigations so far conducted failed to identify the epileptogenic focus in GMH patients, but it is proposed that reactive changes in peri-ectopic areas are instrumental. This precludes surgery and urges investigations of the pathophysiological changes leading to hyperexcitability in GMH. Data obtained in animal models will be presented in the second part of this review and compared with available data from human patients to propose a working model for future investigations.

Genetic Causes and Cellular and Molecular Mechanisms

Periventricular Nodular Heterotopia

Periventricular nodular heterotopia (PNH), the most common form of malformation of cortical development (MCD) in adulthood, is characterized by the presence of ectopic neuronal nodules lining the walls of the lateral ventricles. Theses nodules can readily be detected with MRI. There is a wide spectrum of anatomic and clinical presentations of PNH, ranging from asymptomatic small unilateral or bilateral nodules to extensive agglomerates of heterotopia lining the lateral ventricles in patients with intractable epilepsy and intellectual disabilities [2,3]. There is also a range of associated cerebral and systemic malformations. Mutations in the *FLNA* gene, on Xq28, were found in 100% of families with X-linked bilateral PNH and in 26% of sporadic patients with PNH [3,4] (Table 1). The *FLNA* gene encodes a very large (280 kD) cytoplasmic protein that binds to actin and a wide range of cytoplasmic signaling proteins involved in cell adhesion and migration [5]. In the brain, *FLNA* is expressed at high levels in prenatal and

Table 1 Genes and phenotypes associated with periventricular nodular heterotopia

Gene (Locus)	Protein	Etiology	Phenotype	References
FLNA (Xq28)	Filamin A	In females: *de novo* germline mutations (missense, nonsense, and frameshift mutations), intragenic deletions, and duplications In males: lethal in the majority of cases.	Bilateral PNH associated with coagulopathy and cardiovascular abnormalities in some patients	[3, 4]
ARFGEF2 (20q13.13)	BIG2	Inherited mutations (missense and frameshift), autosomal recessive	Bilateral PNH associated with microcephaly	[9]
C6orf70 (6q27)	ERMARD	*De novo* deletions and missense mutation (one)	Bilateral PNH	[14]
FAT4 (4q28.1)	FAT atypical cadherin 4	Inherited compound heterozygous (nonsense and missense) or homozygous (nonsense) mutations	Posterior PNH (partially penetrant)	[12]
DCHS1 (11p15.4)	Dachsous cadherin-related 1	Inherited homozygous (nonsense and missense) mutations	Posterior PNH (partially penetrant)	[12]

FLNA, filamin A, alpha; *ARFGEF2*, ADP-ribosylation factor guanine nucleotide exchange factor 2; BIG2, brefeldin A-inhibited guanine nucleotide exchange protein 2; C6orf70, chromosome 6 open reading frame 70; ERMARD, ER membrane-associated RNA degradation; *FAT4*, FAT atypical cadherin 4; *DCHS1*, dachsous cadherin-related 1.

neonatal stages and these levels diminish during adolescence to reach moderate expression in adulthood [6]. *FLNA* is also expressed in pyramidal neurons in the neocortex where it localizes in somatodendritic compartments [7]. Heterozygous females have normal to borderline intelligence and epilepsy [4]. A few living male patients with bilateral PNH due to *FLNA* mutations have been reported; however, most male fetuses are not viable [8]. Coagulopathy and cardiovascular abnormalities have been observed in some patients [4,8].

Other Genes than FLNA can Cause PNH

A rare recessive form caused by mutations in the *ARFGEF2* gene, on 20q13.1, has been reported in two consanguineous families [9]. *ARFGEF2* encodes a protein called BIG2 (or brefeldin A-inhibited guanine nucleotide exchange factor 2 protein) localized along the Golgi and recycling endosomes [10]. BIG2 is thought to carry out ARF-dependent vesicle trafficking along these subcellular compartments [11]. Recently, it has been reported that biallelic mutations in genes encoding the receptor-ligand cadherin pair DCHS1 and FAT4 lead to a multisystem disorder that includes PNH [12]. PNH has also been observed in patients with chromosomal rearrangements, such as deletions of the 5q14.3-15 [13] or 6q27 [14] regions. For the latter, a *de novo* missense mutation in the *C6orf70* gene, mapping the minimal critical deleted 6q27 region, was identified in a sporadic patient with developmental delay, epilepsy, and PNH [14]. To date, 13 distinct PNH disorders have been described but for the majority of them the etiology remains unknown [13].

The mechanism involved in the genesis of PNH remains elusive although it is widely accepted that it results from a defective migration of neurons which remain blocked in the ventricular (VZ)–subventricular zone (SVZ). Although two *Flna* knockout mice strains have been developed, progress has been hindered by the fact that none of them showed the presence of ectopic nodules [15,16]. In contrast, *in utero* knockdown of *Flna* expression has succeeded in reproducing a PNH phenotype in rat similar to the one observed in human patients and represents an appropriate model to investigate pathogenetic mechanisms underlying PNH associated to mutations in *FLNA* gene [17]. In this model, PNH is associated with an impairment of radial glial integrity in the VZ. Thus, the phenotype would associate a cell-autonomous migration defect as largely proposed and an alteration of RGCs and radial glial scaffold (Table 2). Interestingly, we demonstrated [17] a similar disruption of radial glial cells in human PH brains from a 35-week fetus and a 3-month-old child, harboring distinct FLNA mutations. Other studies have shown that mice mutant for *MEKK4*, a MAP kinase that regulates the CSBP2 and JNK-MAPK pathways, showed a PNH phenotype [18]. Interestingly, phosphorylation of FLNA at serine 2152 depends on MEKK4 signaling and phosphorylation at this site regulates FLNA localization at the cell membrane. Mice with mutations in the *Napa* gene, which encodes for the vesicle trafficking protein αSnap, also replicate the PNH phenotype [19]. The αSnap protein is involved in SNAP receptor (SNARE)-mediated vesicle fusion thus suggesting that it plays a role in vesicle trafficking in PNH formation. Finally, it has been shown that deletion of the RhoGTPase *Cdc42* gene in mouse disrupts the neuroependymal lining, local adherens junctions, and proliferation of basal progenitors, which may lead to neuronal heterotopia [20,21]. Overall, as the majority of PNH genes are required for some forms of vesicle trafficking, it has been proposed that an overriding defect in the vesicle trafficking machinery may contribute to PNH formation [22].

Experimental PNH can also be modeled in rodents using various nongenetic manipulations, including prenatal exposure to ionizing radiations, methylazoxymethanol (MAM), carmustine (1-3-bis-chloroethylnitrosourea or BCNU) in rats, or postnatal exposure to ibotenate in hamsters. These teratogens produce damages within the proliferative neuroepithelium, affecting both the genesis of newborn neurons and their migration along the radial glial scaffold [23–25]. As a consequence, animals generated with these

Table 2 Genetic animal models of periventricular nodular heterotopia

Gene	Animal model	Phenotype	Altered cellular process	Molecular function	References
Flna	Flna conditional knockout mouse	Small brain; severe vascular defects; high rate of early lethality in males	Unknown	Cytoplasmic protein; binds to actin and numerous signaling proteins; cell adhesion and migration	[15]
	Flna knockdown in rats	PNH; migration arrest in SVZ and IZ	Proliferation of NP; RGC scaffold; neuronal migration		[17]
	FLNA overexpression in mice	Migration arrest in SVZ and IZ			[132]
Fat4	*Fat4* knockdown in mice	Migration arrest in SVZ and IZ; white matter neuronal heteretopia	Neuronal migration	Member of the protocadherin superfamily	[12]
Dchs1	*Dchs1* knockdown in mice	Migration arrest in SVZ and IZ; white matter neuronal heteretopia	Neuronal migration	Member of the protocadherin superfamily; ligand for FAT4	
C6orf70	*C6orf70* knockdown in rats	Migration arrest in SVZ and IZ	Neuronal migration	Unknown (probably involved in vesicular trafficking)	[14]
Mekk4	*Mekk4* knockout mouse *Mekk4* knockdown in mice	Bilateral PNH; degenerated forebrain	Neuronal migration; VZ lining	MAPK kinase kinase; regulates CSBP2 and JNK-MAPK pathways	[18]
Napa	*Alpha Snap* (Napa) mouse; spontaneous genetic model, autosomal recessive	PNH	Neuronal migration; VZ lining	Involved in SNARE-mediated vesicle fusion	[19,133]

Flna, filamin a; *Fat4*, FAT atypical cadherin 4; *Dchs1*, dachsous cadherin related 1; C6orf70, chromosome 6 open reading frame 7; *Mekk4*, MEK kinase 4 (replaced with *Map3k4*, mitogen-activated protein kinase kinase kinase 4); *Napa*, N-ethylmaleimide-sensitive fusion protein attachment protein alpha.

treatments invariably have microcephaly and altered cortical structure and exhibit various types and combination of gray matter heterotopia, including periventricular nodular heterotopia, layer I ectopia, intracortical and subcortical heterotopia, and intrahippocampal heterotopia.

Subcortical Band Heterotopia

SBH or double cortex syndrome is a malformation of cortical development that represents the less severe form of the lissencephaly spectrum [26]. SBH refers to bilateral smooth bands of gray matter located in the subcortical white matter. It is generally associated with a normal or mildly simplified gyration pattern, broad circumvolutions, and an increased cortical thickness. SBH cortical malformations always have a genetic origin, and abnormalities in the *DCX* and *LIS1* genes account for the majority of the SBH cases. Although much less common, mutations in genes encoding microtubule subunits (TUBA1A; TUBG1) have also been identified in a few SBH patients [27–29], as well as in the microtubule-dependent motor protein *KIF2A* gene [29] (Table 3).

Most SBH patients are females because the most common genetic abnormalities are found in *DCX*, an X-linked gene, and whereas heterozygous females develop SBH, hemizygous males develop an isolated lissencephaly. The majority of female patients with *DCX* mutations are sporadic, but familial cases have been described and could represent up to one-third of the female patients [30]. *DCX* mutations are found in up to 88.5% and in 100% of female patients with sporadic SBH and familial SBH, respectively [30–32]. Although much less common than females, male SBH patients associated to *DCX* mutations or deletions have been described [33–35]. They may result from a rather mild mutation that allows some residual function of DCX or the mutation or deletion is mosaic, affecting a portion of the neurons only [30,33,36]. Somatic mosaicism in these male patients reproduces the female situation in which depending on the X inactivation pattern, a variable proportion of neurons are DCX deficient. Mosaic heterozygous point mutations in the *LIS1* gene account for a small number of SBH sporadic cases [37,38].

DCX encodes a microtubule-associated protein (MAP), which nucleates and binds to the 13-protofilament microtubules [39–41]. It is highly expressed in newly generated neurons as soon as they exit the cell cycle, all along their journey from VZ/SVZ to the cortical plate, and in their following differentiation steps, soon afterward it is downregulated. The DCX microtubule domain is made up of two microtubule-binding domains, an N-terminal (N-DC) and a C-terminal (C-DC) domain.

LIS1 encodes a highly conserved protein with an N-terminal homodimerization and coiled-coil domain, and seven C-terminal WD40 (tryptophan-aspartic acid-40) repeats [42]. LIS1 binds to the cytoplasmic dynein, a microtubule minus end-directed motor [43]. The LIS1/dynein complex has been shown to regulate the orientation of the spindle of dividing neuronal precursors at the VZ and decreased LIS1 levels lead to depletion of radial glial progenitor cells (RGCs) [44]. LIS1 also binds to several MAPs, including DCX [45] and genetic interactions between these two genes have been demonstrated *in vivo* in the mouse [46]. LIS1 is

Table 3 Genes and phenotypes associated with subcortical band heterotopia

Gene (Locus)	Protein	Etiology	Phenotype	References
DCX (Xq22.3-q23)	DCX	In females: *de novo* germline mutations (missense, nonsense, and frameshift mutations), deletions, and duplications	Anteriorly predominant SBH; *de novo* mutations generally associated with the most severe phenotype (thick band frequently associated with frontal pachygyria, shallow sulci, and ventricular enlargement)	[30,120,121]
		In females: inherited mutations (missense, nonsense, and frameshift)	Anteriorly predominant SBH; inherited mutations generally associated with a milder phenotype (thin band)	
		In males: *de novo* somatic mosaic mutations (missense, nonsense, and frameshift) and deletions	Anteriorly predominant SBH	[31,33,35,36,122–125]
		In males: inherited mutations (missense mutations only)	Anteriorly predominant SBH; milder phenotype	[33]
LIS1 or PAFAH1B1 (17p13.3)	LIS1	*De novo* somatic mosaic heterozygous (missense and nonsense) mutations	Posteriorly predominant SBH	[37,38]
KIF2A (5q12.1)	KIF2A	*De novo* germline heterozygous (missense) mutation, dominant negative effect	Frontal band heterotopia, posterior predominant pachygyria, and severe congenital microcephaly	[29]
TUBA1A (12q13.12)	α1-tubulin	*De novo* germline heterozygous (missense) mutation, dominant negative effect	Laminar heterotopia, partial agenesis of the corpus callosum, and hypoplasia of the cerebellar vermis	[27,48]
TUBG1 (17q21.2)	TUBG1 (γ-Tubulin)	*De novo* germline heterozygous (missense) mutation, dominant negative effect	Laminar heterotopia, posterior pachygyria, and a thick and dysmorphic corpus callosum	[29]
EML1 (14q32)	EML1	Inherited compound heterozygous (nonsense and missense) or homozygous (missense) mutations	Giant bilateral periventricular and ribbon-like subcortical heterotopia with polymicrogyria and agenesis of the corpus callosum	[50]

DCX, doublecortin; *LIS1*, lissencephaly-1; *PAFAH1B1*, platelet-activating factor acetylhydrolase 1b, regulatory subunit 1; *KIF2A*, kinesin heavy chain member 2A; *TUBA1A*, tubulin, alpha 1a; *TUBG1*, tubulin, gamma 1; *EML1*, echinoderm microtubule-associated protein-like 1.

required for nuclear movement during neuronal migration by coupling the nucleus to the centrosome [47].

In addition to *DCX* and *LIS1*, genes classically involved in tubulinopathies such as those encoding microtubule subunits and kinesins have been associated to SBH. A large number of *TUBA1* mutations have been identified in patients with lissencephaly but one mutation has been identified in a female patient with SBH [27,48]. *TUBA1* encodes the α-tubulin which heterodimerizes with the γ-tubulin. A mutation in *TUBG1* was also identified in a patient with laminar heterotopia associated with posterior pachygyria and a dysmorphic corpus callosum [29]. *TUBG1* encodes a γ-tubulin subunit, which is highly expressed in fetal brain. The γ-tubulin is a component of the centrosome and associates with other proteins to form the γ-tubulin ring complex implicated in microtubule nucleation [49]. A single *de novo* heterozygous missense (dominant negative) mutation in the *KIF2A* gene was identified in a female patient with frontal band heterotopia, posterior predominant pachygyria and severe congenital microcephaly [29]. The microtubule-dependent motor protein KIF2A is an M-kinesin and drives the ATP-dependent depolymerization of microtubules. The fact that mutations affecting all these genes are only missense heterozygous mutations suggests that they are dominant negative and that haplo-insufficiency is not the primary mechanism causing the SBH.

From Human to Animal Models of SBH

If human genetics studies have allowed the identification of mutant genes in SBH patients (such as *DCX* or *LIS1*), animal models in which expression of the corresponding genes have been inactivated are invaluable tools to identify the associated disrupted biological processes. In addition, spontaneous SBH animal models such as the tish rat, the HeCo, or *BXD29-Trl4lps 2J/J* mice have also led to a better understanding of potential SBH genesis mechanisms and, in the case of the HeCo mouse, to the identification of a new gene whose implication in human ribbon-like heterotopia has been subsequently confirmed [50]. Finally, knowledge of the molecular and cellular pathways in which the previously identified SBH genes are involved is an excellent starting point as implication of other genes participating into the same pathways can be tested in new animal models (Table 4).

SBH has been long envisaged as a cell-autonomous neuronal migration disorder; however, recent animal model studies show

Table 4 Genetic animal models of subcortical band heterotopia

Gene	Animal model	Phenotype	Altered cellular process	Molecular function	References
Dcx	*Dcx* knockdown in rats and mice	SBH and laminar displacement of neocortical neurons in rats; abnormal neocortical lamination in mice	Neuronal migration; neuronal differentiation	MAP; nucleation, assembly and stability of MTs; regulation of vesicle trafficking; regulation of the actin cytoskeleton	[61,62,66]
	Dcx knockout mouse	Abnormal hippocampal lamination	Neuronal migration; neuronal differentiation		[63,126–128]
Lis1	*Lis1* knockdown in rats	Migration arrest in SVZ and IZ	Proliferation of NP; neuronal migration; neuronal differentiation; neuronal apoptosis	Interacts with MTs, MT-based motors and MAPs	[129]
	Lis1 knockout mouse	Defects in neocortical and hippocampal neurogenesis and migration			[59,60]
Kif2a	*Kif2a* knockout mouse	Migratory defects; abnormal neocortical and hippocampal lamination; ventricle enlargement	Neuronal migration; neuronal differentiation	M-Kinesin; drives the ATP-dependent depolymerization of MTs	[130]
Tuba1	*Tuba1* heterozygous Jenna (Jna) mouse N-ethyl-N-nitrosourea (ENU) induced mutant	Abnormal neocortical and hippocampal lamination	Neuronal migration; neuronal differentiation	Component of the MT cytoskeleton	[48]
Tubg1	*Tubg1* knockdown in mice	Migration arrest in SVZ and IZ	Neuronal migration; neuronal polarization; neuronal differentiation	Component of the centrosome; nucleation of MTs	[29]
Eml1	HeCo mouse, spontaneous genetic model, autosomal recessive	Bilateral SBH	Proliferation of NP (ectopic NPs)	MAP; cell cycle-dependent localization	[50,51]
Unknown	Tish rat, spontaneous genetic model, autosomal recessive	Bilateral SBH; ventricle enlargement	Proliferation of NP (ectopic NPs)	Unknown	[55–57]
RhoA	*Rhoa* conditional knockout	Bilateral SBH; cobblestone lissencephaly	Proliferation of NP (ectopic NPs); RGC scaffold; neuronal migration	GTPase; stabilization of the actin and MTs cytoskeleton	[52]
Wnt3a	*Wnt3a* transgenic mouse	Cortical dysplasia; large neuronal heterotopia	Proliferation of RGCs; differentiation of IPs	Wnt-β-catenin signaling pathway	[58]
Unknown	*BXD29-Trl4lps-2J/J* mouse; spontaneous genetic model, two-loci autosomal	Bilateral SBH; partial callosal agenesis	Neuronal migration	Unknown	[131]
Rapgef2	*RA-GEF-1* conditional knockout mouse	Bilateral SBH; commissural and callosal agenesis; ventricle enlargement	Neuronal migration	Guanine nucleotide exchange factor (GEF) specific for the small GTPases Rap1 and Rap2; Rap1-mediated signaling pathway	[111]

Dcx, doublecortin; *Lis-1*, lissencephaly-1; *Kif2a*, kinesin family member 2A; *Tuba1a*, tubulin, alpha 1A; *Tubg1*, tubulin, gamma 1; *Eml1*, echinoderm microtubule-associated protein-like 1; *RhoA*, ras homolog gene family, member A; *Wnt3a*, wingless-type MMTV integration site family, member 3A; *Rapgef2*, Rap guanine nucleotide exchange factor (GEF) 2; NP, neural progenitor; RGC, radial glial cell; MAP, microtubule-associated protein; MT, microtubule.

that SBH can result from dysregulation of cellular events involving the neuroprogenitors such as abnormal proliferation, mispositioning and/or differentiation, which will eventually lead to an abnormal neuronal migration or a mispositioning of neurons in the cortical wall. On the other hand, single molecules can be involved in multiple cellular processes involving both the neuroprogenitors and neurons, as it is the case for Dcx and Lis1.

SBH, A Neuroprogenitor Defect?

Two rodent models, the spontaneous HeCo and conditional *RhoA*$^{-/-}$ cKO (*Emx1::Cre/RhoAfl/fl*) mice, display a SBH which clearly results from abnormalities in neuronal progenitor cells [50–52]. In the HeCo mouse, dividing neuronal progenitors are found from early to late stages of corticogenesis in ectopic places throughout the cortical wall, such as the IZ and CP. Although many progenitors are mislocalized, the adherens junctions

between the RGCs lining the ventricular wall are normal. However, dividing RGCs in the VZ display abnormal spindle orientations which might explain the appearance of ectopic progenitors. These ectopic progenitors display an abnormal proliferation pattern (higher labeling index/slowed cell cycle exit). On the other hand, video microscopy of electroporated eGFP+ neurons shows that HeCo neurons migrate normally either in a HeCo or wild-type context. *Eml1* was recently identified as the mutant gene in the HeCo mouse: it is a microtubule-associated protein belonging to the EMAP family of proteins whose members have been shown to play a role in microtubule dynamics and cell division [53,54]. In the mouse embryonic brain, Eml1 is expressed in neuroprogenitors of the VZ and neurons of the CP. *In utero Eml1* knockdown in the WT mouse mimics the HeCo phenotype whereas *Eml1* reexpression in HeCo mouse RGCs rescues it. The HeCo mouse mutation is autosomal recessive, and human genetic studies have further confirmed the implication of EML1 in SBH genesis as compound or homozygous mutations were found in two families with ribbon-like heterotopia [50].

The conditional $RhoA^{-/-}$ cKO mouse model shares similarities with the HeCo mouse in that dividing neuronal progenitors are found from early stages of corticogenesis in ectopic places throughout the cortical wall and display an increased proliferation (PH3+ cells) [52]. Later on, neuronal progenitors [RGCs and intermediate progenitors (IPs)] tend to form a broad band located in the middle of the cortical wall, from which the neurons separate in two bands either toward the cortical plate or the VZ. The lower band, near the VZ, gives rise to the SBH composed mostly of late born neurons. From early stages of corticogenesis, most RGCs lose their apical anchoring, and RG processes are highly disorganized. Furthermore, transplanted GFP+ WT neurons into E14 $RhoA^{-/-}$ cKO brains distributed as $RhoA^{-/-}$ neurons either in the cortical plate or in the SBH at P2. Both microtubule and actin cytoskeletons are destabilized in $RhoA^{-/-}$ cKO brains, mainly in RGCs and less so in neurons. Altogether, these observations demonstrate that the SBH genesis is not due to an intrinsic neuronal defect but clearly results from RGC defects: absence of apical anchoring and adherens junctions, defects in RGC scaffold. $RhoA^{-/-}$ cKO mice also display in addition a protrusion of neurons beyond layer I at the pial surface of the brain, mimicking cobblestone lissencephaly. RhoA belongs to the family of small Rho GTPases. As Cdc42 and Rac1, two other members of this family, it is expressed in the VZ/SVZ of the developing brain. Cdc42 has been shown to regulate neural progenitor fate at the apical ventricular surface [20]. Up to now, mutations or deletions in the *RhoA* gene have not been characterized in human patients with SBH.

A third rodent model, the spontaneous tish rat displays a SBH which also results from abnormalities in neuronal progenitor cells and shares strong similarities with the HeCo mouse model [55–57]. From early corticogenesis stages, RGCs and IPs, most likely generated from the VZ/SVZ, are scattered throughout the cortical wall, but an intact VZ is maintained in which RGCs display normal adherens junctions. Ectopic $tish^{-/-}$ progenitors display an abnormal proliferation (shortened cell cycle) which has been proposed to be a consequence of their mislocalization rather than a cell-autonomous defect [57]. *In utero* electroporation experiments show that neurons generated from the VZ/SVZ contribute to both the heterotopia and cortical plate, and although the radial fibers are somewhat disorganized, neurons can still migrate throughout the heterotopia to reach the cortical plate [57]. The gene associated to the tish phenotype has not yet been identified.

An additional mechanism involving specifically the IPs has been uncovered with an *in utero* electroporation mouse model [58] in which upregulation of the Wnt-β-catenin signaling pathway, by overexpressing Wnt3a, induces two distinct phenotypes: an increased proliferation of RGCs combined with a premature differentiation of IPs into neurons. The accumulation of these newly born neurons at the SVZ/IZ border leads eventually to the formation of large neuronal heterotopia.

These rodent models strongly demonstrate that SBH can result from an alteration of neuronal progenitors. It is even possible that part of the effects linked to *Lis1* and *Dcx* mutations results from a similar mechanism as it has been shown that Lis1 affects the generation and survival of neuroprogenitors [59,60] and that $Dcx^{-/y}$ RGCs display spindle orientation abnormalities affecting their proliferation [46].

SBH, An Intrinsic Neuronal Migration Defect?

Radial migration of newborn neurons, from the VZ toward the pial surface, is a critical step in the development of the cerebral cortex. Early generated pyramidal neurons migrate by soma translocation, independently from radial glia scaffold. As development goes on, newly generated neurons generated from asymmetric division of RGCs or symmetric division of IPs become multipolar in the SVZ and migrate as multipolar cells through the SVZ and lower IZ. Neurons become bipolar as they leave the IZ and switch their migration mode to travel radially through the cortical plate. They migrate along the radial glia scaffold by locomotion, with a three step migratory mode: extension of a leading process, translocation of the nucleus in the leading process (nuclear kinesis), and retraction of the trailing process. All these migration steps and associated neuronal morphological changes are critically regulated and involve precise microtubule and actin cytoskeleton remodeling. Numerous genes, among which *Dcx* and *Lis1*, have been shown to be involved in the transition from multipolar to bipolar migration modes. *In utero* short hairpin RNA (shRNA) mediated knocking down of *Dcx* (*Dcx* KD) expression in the rat embryo leads to a massive accumulation of multipolar neurons in the IZ, which form a SBH after birth [61]. A minority of these *Dcx* KD neurons can still reach the cortical plate and be identified at ectopic places in the normotopic cortex. This mosaic model mimics the case of heterozygous female patients in whom the absence of *DCX* expression occurs in a subpopulation of cells only, those having inactivated the X chromosome bearing the *DCX* WT allele. Strikingly in the mouse, downregulation of *Dcx* expression by *in utero* shRNA interference leads to a cortical lamination defect, but no SBH [62] and germ-line inactivation of the *Dcx* gene has no major consequence on cortex development but only on the hippocampus lamination [63]. Discrepancies between the rat and mouse *Dcx* KD phenotypes have been proposed to arise from species differences in the expression of the doublecortin-like kinase 1 gene (*Dclk1*), a *Dcx*-related gene encoding a microtubule-associated protein, and those between the mouse *Dcx* KD and KO phenotypes to arise from an acute (KD) rather than chronic inactivation (KO) which might allow compensatory mechanisms to take place. In support this hypothesis are the observations that double

Dcx/Dclk1 KO mice ($Dcx^{-/y}$;$Dclk1^{-/-}$) display a clear cortical migration defect, whereas single *Dcx* ($Dcx^{-/y}$) or *Dclk1* ($Dclk1^{-/-}$) KO mice do not [63,64] and that whereas the chronic germ-line *Dclk1* gene inactivation does not lead to a cortical migration defect, acute *Dclk1* inactivation in *Dclk1* KD does [64]. An off-target effect of *Dcx* shRNAs encoding the *Dcx* interfering RNAs (RNAis) has recently been proposed to explain the migration phenotype obtained in the mouse (heterotopic cortical neurons) as similar phenotypes could not be reproduced with identical *Dcx* RNAis produced from artificial microRNAs (shmiRNAs) [65]. Moreover, although no cortical migration defect was detected in the mouse germ-line *Dcx* KO, it was detected after *in utero* electroporation of *Dcx* shRNAs in *Dcx* KO mouse embryos which do not express *Dcx* anymore [65]. Similar observations were made after electroporation of *Dclk1* shRNAs in the $Dclk1^{-/-}$ KO mouse embryos. Finally, although single *Dcx* or *Dclk1* KD with RNAis produced from shmiRNAs did not lead to a cortical migration phenotype, double *Dcx/Dclk1* KD with RNAis produced from shmiRNAs did. The cortical migration phenotype obtained with the *Dcx* shRNAs has been proposed to arise from a dysregulation of specific endogenous miRNAS. However, these results are difficult to reconcile with the human genetic studies which clearly implicate *DCX* mutations in SBH and with results obtained in the rat shRNA *Dcx* KD model as *Dcx* overexpression in these rat embryos or neonates can rescue the migration phenotype [61,66]. Further, it was recently demonstrated that a fine tuning of *Dcx* expression levels in migrating neurons via a miRNA-mediated regulation of CoREST/REST is required for properly regulating neuron polarization and migration in the neocortex [67]. It also remains to understand why cortical migration defects do not generate an SBH in the double *Dcx/Dclk1* KO or shmiRNAs *Dcx/Dclk1* KD mouse models. Additional studies will be needed to clarify these issues.

Are Rodent Good Models of Human SBH?

It is remarkable that so few rodent animal models display a SBH even for genes which have been shown to lead to SBH in human patients such as *DCX* or *LIS1*. From the currently available SBH rodent models, it seems that the mutations impairing the RGCs are more likely to induce a SBH than those affecting neuronal migration at least in the mouse. Although most of the developmental mechanisms of brain development are shared between rodents and humans, development of a gyrated cortex in human involves far more complex developmental processes than those required for the development of a rodent lissencephalic cortex. Recent studies have shown that the increased neocortical volume and surface area of the human brain (and gyrencephalic brains from other species) are related to the expansion of progenitor cells (radial glial-like cells and IPs) localized in an additional SVZ, the outer SVZ (OSVZ). OSVZ radial glial-like cells undergo both symmetric and self-renewing asymmetric divisions that allow the generation of additional neurons presumed to occupy the outer cortical layers [68,69]. Neurons also have to migrate a much longer way. Taking into account these developmental differences, a common genetic alteration might generate very divergent phenotypes in rodents and human patients thus raising concerns about the use of lissencephalic rodent models for studying the mechanisms involved in SBH genesis.

Pathophysiology of GMH

PNH Patients

Studies using intracranial EEG recordings in patients with PNH suggested that epileptic discharges may originate from a large epileptogenic network that includes heterotopic nodules and other cortical areas. Intracerebral exploration with deep electrodes revealed two situations, either no ictal discharges from the explored nodule [70,71], or, most frequently, involvement of at least one nodule in ictal discharges [70,72–78]. Seizures were found to start simultaneously from heterotopic nodules and cortical regions [70,73–77], from heterotopic nodules [70,75,77,79], or from several regions including the temporal cortex and mesial structures [70,76,78,80]. Similar observations were made using EEG–fMRI and also revealed concomitant involvement of sites distant to the malformation [81–83], reinforcing the notion that a large epileptogenic network including heterotopic nodules and other cortical areas may be involved.

Spontaneous and Induced Seizures in PNH Models

Prenatally irradiated rats were found to exhibit spontaneous seizures arising from the frontal cortex (75% of seizures) or from the hippocampus (25% of seizures) [84], or recorded simultaneously from the hippocampus and the frontal cortex in some cases [85], whereas rats prenatally exposed to MAM were rarely observed to exhibit spontaneous seizures (less than 20% of rats) [86]. Although no spontaneous seizures were reported in the other models of experimental PNH so far, increased susceptibility levels to induced seizures were found in all models, regardless of the mode of seizure induction: sedating agents [87], flurothyl [88], Kainic acid [89–91], pentylenetetrazole [17,90], hippocampal kindling [92,93], or hyperthermia [94].

Origin and Propagation of Epileptiform Activity in PNH Models

Experimentally induced PNH in MAM rats were never observed to initiate bicuculline- or pentylenetetrazole-induced seizures, neither *in vivo* [95] or *in vitro* [95,96], and epileptiform activity in PNH, most commonly initiated in the dysplastic hippocampus, was generally synchronized with that of the surrounding brain tissue. Accordingly, isolated intrahippocampal heterotopias were observed to generate spontaneous bicuculline- and 4-aminopyridine-induced epileptiform activity, independently of other hippocampal synaptic inputs [97]. Tracing experiments in MAM rats revealed the presence of reciprocal connections between both PNH and intrahippocampal heterotopia and ipsilateral and contralateral cortices, and abnormal cortico-hippocampal and cortico-cortical connections [98]. Ectopic hippocampal neurons composing intrahippocampal heterotopia were characterized as displaced neurons normally fated to upper cortical layers that secondarily invaded the hippocampus [90,99,100] and formed a functional bridge between the hippocampus and neocortex [90,101]. In the presence of bicuculline, this aberrant bridge was found to allow propagation of hippocampal epileptiform activity evoked by elec-

trical stimulation of the dentate gyrus to the neocortex via the intrahippocampal heterotopia [101]. Heterotopic neurons and those located in the dysplastic cortex in irradiated rats were found to develop long-distance subcortical projections [102,103]. Altered organization of thalamic fibers and abnormally projecting callosal fibers were described in BCNU-treated rats [104,105]. Although propagation of epileptiform discharges along these fiber pathways has not been investigated in irradiated and BCNU-treated rats, this pathological circuitry may contribute to the epileptogenic network.

SBH Patients

Depth recordings are rarely carried out in patients with SBH who are not considered as good candidates for epilepsy surgery given its poor outcome [106]. In the few reported cases, epileptiform activities were recorded from both the heterotopic and normotopic cortices, independently or not, and they sometime propagated to other brain structures [106,107]. Electrical discharges starting elsewhere and subsequently propagating to both the heterotopic and normotopic cortices were also reported [108], as well as an absence of any epileptiform activities recorded from the heterotopic band [106]. Studies using EEG-fMRI [81,82] revealed that both the heterotopic band and the normotopic cortex showed fMRI signal changes during interictal and ictal epileptiform events. Signal changes can be restricted to a portion of the heterotopic band or involve a large activation of the entire double cortex [82].

Spontaneous and Induced Seizures in SBH Models

Frequent spontaneous seizures were recorded in only two models: tish mutant rats with seizures arising from both the heterotopic and normotopic cortices [109], and *Dcx* KD rats, showing spontaneous seizures in adulthood [110]. Other models were only reported to exhibit increased susceptibility to convulsant-evoked seizures: pilocarpine-induced seizures in Heco mice [51] and *RAGEF-1* conditional KO [111]. Surprisingly, *BXD29-Trl4lps 2J/J* mice were found more resistant to PTZ-induced seizures than wild-type controls [112]. Seizure susceptibility was not investigated in *RhoA* conditional KO.

Origin and Propagation of Epileptiform Activity in SBH Models

Seizure activity in tish rats was investigated using depth electrode recordings *in vivo* and revealed an almost synchronous onset in the normotopic cortex and the heterotopic band, although lower thresholds for penicillin- and 4-aminopyridine-induced interictal spikes were found in the normotopic cortex of acute slices. Interestingly, focal injection of TTX in the white matter separating the normotopic cortex and the band heterotopia resulted in decreased amplitudes of epileptiform spikes recorded from the band, suggesting that the normotopic cortex may initiate epileptiform activity [109]. Tracing experiments revealed that neurons located within the band heterotopia display typical subcortical projections [55,113], and staining for cytochrome oxidase showed that some of the individual vibrissae have dual representations in both the normotopic primary somatosensory cortex and the band heterotopia suggesting altered functional connectivity [114]. Dynamic calcium imaging in slices from *Dcx* KD rats demonstrated that neurons in both the normotopic cortex and SBH were more frequently coactive in coherent synchronized oscillations than neurons from control slices, and both areas were found to display network-driven oscillations during evoked epileptiform bursts [115]. Extracellular recordings from 60-channels microelectrode arrays on slices from *Dcx* KD rats revealed that most interictal-like discharges originating in the overlying cortex secondarily propagates to the band heterotopia [116]. Interestingly, *in vivo* suppression of neuronal excitability in SBH does not alter the higher propensity of Dcx KD rats to display seizures, suggesting a major role of the normotopic cortex for generating seizures in brain with SBH [116]. At the morphological level, SBH neurons were found to send axonal collaterals to deep layers of the normally migrated cortex, as well as long run axons reaching the contralateral cortex, or the striatum or thalamus, that may contribute to the epileptogenic network [115].

Collectively, clinical and experimental observations support the notion that apparently anatomically unaltered cortical regions surrounding both PNH and SBH are included in a large epileptogenic network prone to generate epileptiform discharges. Further, these observations suggest that cortical areas overlying malformations may play a major role for generating epileptiform discharges and that plastic changes within these areas, together with circuit-level defects, may be instrumental in both epileptogenesis and seizure generation. Accordingly, abnormal intrinsic features were described in experimental heterotopia, not only in the malformation, but in the overlying cortex as well. In experimental SBH, the overlying cortex of *Dcx* KD rats was found to exhibit a massive increase of ongoing glutamatergic synaptic currents [115]. Similar observations were made in experimental PVNH, with neurons in the dysplastic cortex overlying nodules showing increased glutamatergic synaptic currents and decreased GABAergic synaptic currents in irradiated rats [117], and a decreased sensitivity to GABA inhibition in BCNU-treated rats [118]. Pyramidal neurons with repetitive burst firing patterns were also described in the dysplastic cortex of MAM-treated rats [119]. These observations in animal models may support the hypothesis that increased neuronal excitability and abnormal circuitry both contribute to favor the emergence of seizures from the overlying cortex.

Conflict of Interest

The authors declare no conflict of interest.

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