Epilepsy as a neurodevelopmental disorder

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Epilepsy is characterized by spontaneous recurrent seizures and comprises a diverse group of syndromes with different etiologies. Epileptogenesis refers to the process whereby the brain becomes epileptic and can be related to several factors, such as acquired structural brain lesions, inborn brain malformations, alterations in neuronal signaling, and defects in maturation and plasticity of neuronal networks. In this review, we will focus on alterations of brain development that lead to an hyperexcitability phenotype in adulthood, providing examples from both animal and human studies. Malformations of cortical development (including focal cortical dysplasia, lissencephaly, heterotopia, and polymicrogyria) are frequently epileptogenic and result from defects in cell proliferation in the germinal zone and/or impaired neuronal migration and differentiation. Delayed or reduced arrival of inhibitory interneurons into the cortical plate is another possible cause of epileptogenesis. GABAergic neurons are generated during early development in the ganglionic eminences, and failure to pursue migration toward the cortex alters the excitatory/inhibitory balance resulting in aberrant network hyperexcitability. More subtle defects in the developmental assembly of excitatory and inhibitory synapses are also involved in epilepsy. For example, mutations in the presynaptic proteins synapsins and SNAP-25 cause derangements of synaptic transmission and plasticity which underlie appearance of an epileptic phenotype. Finally, there is evidence that defects in synapse elimination and remodeling during early "critical periods" can trigger hyperexcitability later in life. Further clarification of the developmental pathways to epilepsy has important implications for disease prevention and therapy.

Keywords: cortex, hippocampus, GABA, glutamate, critical period, sodium channels

INTRODUCTION

Epilepsy is one of the most common neurological disorders, characterized by the repeated occurrence of spontaneous bursts of neuronal overactivity, known as seizures. Seizures typically arise in restricted regions of the brain and may remain confined to these areas or spread to the whole cerebral hemispheres. The hippocampal formation and cerebral cortex are considered the most epileptogenic regions of the brain (Pitkanen and Sutula, 2002; Avanzini and Franceschetti, 2003). The behavioral manifestations of seizures, as well as the severity of the epileptic condition, strictly relate to the brain regions that are affected by overactivity. Epilepsy comprises a large group of syndromes with different etiologies. A large series of recent studies demonstrated that several developmental factors (including congenital brain malformations, altered neuronal signaling during embryonic life, and defects in postnatal maturation of neuronal networks) contribute to epileptogenesis, leading to the concept of epilepsy as a neurodevelopmental disorder.

In the first part of the review, we will focus on those alterations in embryonic development of the cerebral cortex that lead to an hyperexcitability phenotype in postnatal life. Taking examples from both animal and human studies, we will describe the role of a number of key developmental genes controlling the migration of projection (excitatory) and local circuit (inhibitory) neurons, and describe how their altered function may result in epileptogenesis.

In the second part of the review, we will describe the defects in the developmental assembly of excitatory and inhibitory synapses that are also involved in epileptogenesis. Specifically, we will focus on defects in synapse elimination and remodeling during early "critical periods" that may trigger hyperexcitability later in life. The deep understanding of the complex developmental processes involved in epileptogenesis may have important implications for disease prevention and therapy.

DEVELOPMENTAL DEFECTS OF THE CEREBRAL CORTEX LEAD TO EPILEPSY

The development of the mammalian cerebral cortex can be subdivided into three partially overlapping phases (Rubenstein, 2000, 2011; Lui et al., 2011). During the first phase, stem cells located in the ventricular and subventricular zones of the telencephalon proliferate and differentiate into neuronal precursors or glial cells. During the second phase, neurons migrate from their place of origin and reach their final destination in the cerebral cortex (Marin and Rubenstein, 2003; Kriegstein et al., 2006; Fishell, 2007; Fishell and Hanashima, 2008). The mature cerebral cortex is organized in six layers (Molyneaux et al., 2007) and contains two major types of projection neurons. The vast majority (~80%) are glutamatergic (excitatory) neurons extending their long axon into the ipsilateral or contralateral cortex (cortico-cortical neurons, located in layers 2/3) or toward subcortical regions (cortico-fugal neurons, located

in layers 5/6). The remainder (∼20%) are GABAergic local circuit neurons (inhibitory interneurons) that establish synaptic contacts with excitatory neurons located in their proximity.

Migration of glutamatergic and GABAergic cortical neurons occurs in two different ways. Glutamatergic projection neurons are generated from neuronal precursors located in the neocortical neuroepithelium via asymmetric cell divisions of cortical primary progenitors (radial glia) located in the ventricular and subventricular zones (Malatesta et al., 2003; Kriegstein et al., 2006; Hansen et al., 2010). Asymmetric divisions generate immature projection neurons, that migrate toward the cortical plate along radial glial processes (Rakic, 2007) and hence reach their final destination in specific cortical layers through the interaction with local molecular cues, such as Reelin (Frotscher, 2010). Cortical neurons are generated in an inside-out pattern, layer 6 neurons being the first to be born. The majority of GABAergic interneurons are instead generated outside of the cerebral cortex, in the ganglionic eminences of the basal forebrain (Wonders and Anderson, 2006). The immature interneurons initially migrate tangentially along the subventricular zone of the basal forebrain, and then change direction by following a radial or an oblique path to enter the cortical plate, from where they reach their final destination into the layers of the cerebral cortex (Kriegstein and Noctor, 2004).

After completion of neuronal migration, the third, last phase of cortical development involves a complex series of apoptotic and synaptogenic events aimed at finely regulating the number of mature neurons and their connections, ultimately leading to the shaping of cortical circuits.

The embryonic development of the cerebral cortex is a complex process, tightly controlled by a series of gene expression cascades (Guillemot et al., 2006). Alterations of these gene regulatory pathways during development may lead to cortical malformations, resulting in malfunction during postnatal life. Accordingly, several cortical abnormalities have been identified that are caused by mutations in key genes involved in the different phases of cortical development. Cortical malformations may result from abnormal neuronal proliferation, migration defects of both excitatory and inhibitory neurons, or altered synaptogenesis/circuit formation, and are usually epileptogenic (Barkovich et al., 2005; Guerrini and Parrini, 2010; Manzini and Walsh, 2011).

Defects of neuronal and glial proliferation during embryonic development result in epileptogenic cortical lesions. Tuberous sclerosis complex (TSC) is a genetic disorder characterized by the widespread development of benign tumors (hamartomas) in multiple organ systems including the brain. Cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas represent the typical lesions observed in TSC. These malformations result in early-onset seizures, that are often accompanied by intellectual disability and autism. TSC results from mutations of TSC1 (hamartin) or TSC2 (tuberin) genes that lead to hamartomatous growths of neuronal and glial cells (Holmes and Stafstrom, 2007).
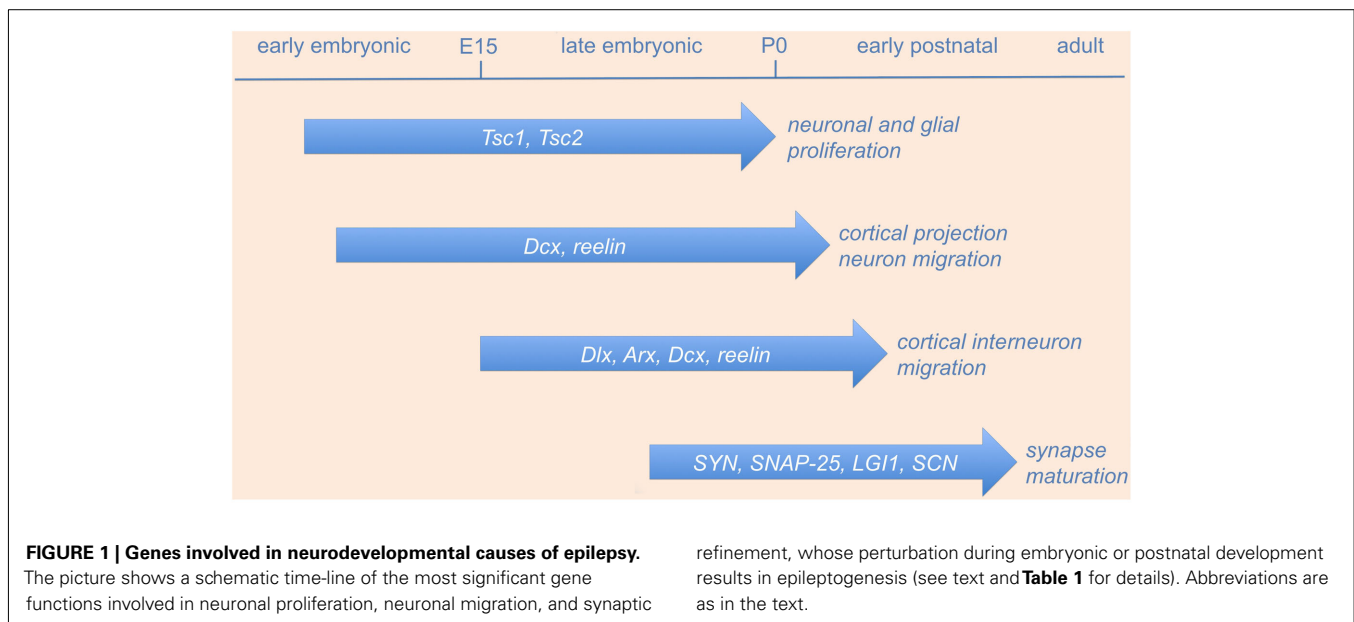
Neuronal migration disorders are a heterogeneous group of neurological conditions characterized by abnormal neuronal positioning in the cerebral cortex. Smooth cortex/lissencephaly (absent or reduced convolutions resulting in cortical thickening and smooth cerebral surface) and heterotopias (typically, ectopic nodules of gray matter located in a periventricular or subcortical position) have been associated to mutations in a number of genes regulating cortical neuron migration and are characterized by severe neurological impairment and epilepsy (Guerrini and Parrini, 2010). Migration defects of both excitatory and inhibitory neurons contribute to these conditions and lead to an altered excitation/inhibition balance and aberrant network hyperexcitability.

Following the initial generation and migration of excitatory and inhibitory neurons, immature neural networks are transformed into organized circuits through a process of refinement that is largely controlled by electrical activity (Katz and Shatz, 1996). During sensitive phases of the early postnatal life, called "critical periods," initially exuberant connections are eliminated and the remaining synapses undergo a functional maturation (Katz and Shatz, 1996; Berardi et al., 2000). Perturbations in this developmental refinement of neuronal circuitry during critical periods may trigger hyperexcitability and epilepsy later in life. In the following sections, we will detail some of the most significant molecular mechanisms involved in neuronal proliferation, neuronal migration, and synaptic refinement (schematically illustrated in **Figure 1** and summarized in **Table 1**), whose perturbation during embryonic or postnatal development results in epileptogenesis.

NEURONAL PROLIFERATION DEFECTS

Tuberous sclerosis complex is a neurocutaneous syndrome characterized by benign tumors, early-onset epilepsy, intellectual disability, and autism (Holmes and Stafstrom, 2007). TSC results from loss-of-function mutations of TSC1 or TSC2 genes, which are crucially involved in the control of neuronal and glial cell proliferation during embryonic development. TSC1 encodes a protein (hamartin) containing two coiled-coil domains, while TSC2 encodes a GTPase activating protein (tuberin) that inhibits small G-proteins belonging to the Ras-related super-family. Hamartin and tuberin are both expressed in neurons and astrocytes of specific central nervous system (CNS) regions such as forebrain, cerebellum, and brainstem, where they form a protein–protein complex that constitutively inhibits mammalian target of rapamycin (mTOR), a serine–threonine kinase positively regulating protein synthesis, cell proliferation, and survival. The function of the TSC1/2 complex is controlled by multiple intracellular signaling pathways converging on AKT, a pro-survival and pro-oncogenic kinase that directly phosphorylates TSC2 inhibiting its function. Loss of TSC1/2 function leads to activation of the mTOR cascade and results in increased cell proliferation; conversely, inhibition of mTOR function (e.g., by rapamycin) results in growth suppression and reduced cell size (Jozwiak, 2006; Holmes and Stafstrom, 2007). The occurrence of tubers in animal models of TSC has been a point of controversy in the field. In contrast with the main feature of the human disease, $Tsc1^{+/-}$ or $Tsc2^{+/-}$ mice do not develop tubers (Kobayashi et al., 2001; Uhlmann et al., 2002; Goorden et al., 2007; Ehninger et al., 2008; Bonnet et al., 2009). However, conditional inactivation of Tsc1 or Tsc2 in astrocytes leads to tuber-like lesions and severe seizures in mice (Zeng et al., 2008, 2011; Way et al., 2009). More recently, in utero electroporation was used to induce homozygous ($Tsc1^{-/-}$) cell clones on a TSC1 heterozygous ($Tsc1^{+/-}$) background. This strategy resulted in cortical tuber-like

FIGURE 1 | Genes involved in neurodevelopmental causes of epilepsy.
The picture shows a schematic time-line of the most significant gene functions involved in neuronal proliferation, neuronal migration, and synaptic refinement, whose perturbation during embryonic or postnatal development results in epileptogenesis (see text and **Table 1** for details). Abbreviations are as in the text.

lesions and a lower seizure threshold, suggesting that cells inside the tubers might have an additional somatic mutation that might contribute to the pathological phenotype (Feliciano et al., 2011). These studies clearly indicate that altering TSC1/2 signaling in specific CNS cell types is at the origin of TSC, but also point out the difficulty of modeling TSC in mice.

The histopathological features of cortical tubers in TSC reflect the antiproliferative role normally exerted by the TSC1/2 signaling complex. Cortical tubers in TSC patients are characterized by giant, dysplastic, and heterotopic neurons with aberrant dendrites and axons, as well as by proliferating astrocytes (Holmes and Stafstrom, 2007). Cortical hyperexcitability arises in the proximity of tubers, but its causes remain largely unknown (Major et al., 2009). Several hypotheses have been proposed to explain epileptogenesis in TSC. Increased expression of NMDA glutamate receptors and decreased expression of the GABA synthetic enzyme glutamic acid decarboxylase (GAD65), GABA vesicular transporter (vGAT), and GABA receptor subunits have been described in cortical tubers from human TSC patients (White et al., 2001), suggesting that excitation/inhibition imbalance in cortical circuits may contribute to epileptogenesis. Another intriguing hypothesis is that cortical tubers may alter thalamocortical connectivity during early brain development, thus resulting in hyperexcitable cortical circuits (Holmes and Stafstrom, 2007).

Seizure suppression in TSC remains a difficult task to be achieved. Infantile spasms in TSC often respond to vigabatrin (a GABA-transaminase inhibitor), but not to other antiepileptic drugs (AEDs), and the surgical removal of tubers remains in many cases the only therapeutic option (Holmes and Stafstrom, 2007). For these reasons, much emphasis has been put to the potential beneficial effects of the mTOR inhibitor rapamycin as a novel anticonvulsant and antiepileptogenic drug. Indeed, rapamycin is able to reduce seizures and prevent epileptogenesis in various animal models. For example, rapamycin treatment blocked epilepsy progression in conditional mutant mice lacking TSC1 or

the TSC-positive regulator PTEN (Zeng et al., 2008; Sunnen et al., 2011), and reversed learning deficits in a $Tsc2^{+/-}$ mice (Ehninger et al., 2008). Importantly, rapamycin administered to chronically epileptic rats following kainic acid (KA; Zeng et al., 2009) or pilocarpine (Huang et al., 2010) treatment has been shown to suppress acquired epilepsy, even though these results have not been replicated in mice (Buckmaster and Lew, 2011). However, some authors pointed out that rapamycin treatment in animal models is still far to be optimal, since seizures may reappear after treatment cessation, and continuous rapamycin exposure might severely affect animal growth and health (Sunnen et al., 2011). In this respect, valid alternative strategies might be represented by a high-dose pulse treatment (Raffo et al., 2011) or even prenatal exposure (to be applied in cases of familial TSC predisposition; Anderl et al., 2011), that have been successfully tested in rodents. According to these results obtained in animal models, preliminary findings in human patients are encouraging: rapamycin has been shown to induce the regression of astrocytomas in a small group ($n = 5$) of TSC cases (Franz et al., 2006), and to reduce seizure frequency in a single young TSC patient (Muncy et al., 2009). However, it is important to point out that while the effects of rapamycin on tumor growth in TSC patients are now well documented and reproduced in many cases, its efficacy on seizure control and other neurological deficits needs to be further investigated.

MIGRATION DEFECTS OF CORTICAL PROJECTION NEURONS
The classification and neuropathological features of genetic neuronal migration disorders have been described in other reviews (Guerrini and Marini, 2006; Guerrini and Parrini, 2010). Different forms of lissencephaly ("smooth brain") and heterotopias have been associated to mutations in genes involved in cortical neuron migration (LIS1, DCX, ARX, TUBA1A, RELN, FLNA, and ARFGEF2). Here we will focus on two of these genes, doublecortin (DCX) and reelin (RELN), whose function in neuronal migration and epilepsy has been investigated in more detail.

Table 1 | Genes involved in neurodevelopmental epileptic syndromes and their corresponding mouse models.

Syndrome (*phenotype*)	Affected genes	Animal models (*phenotype*)	Reference
TUBEROUS SCLEROSIS COMPLEX			
Benign tumors (hamartomas) in multiple organs (brain, skin, heart, kidneys, lung), renal and skin lesions, epilepsy, behavioral and learning disabilities, autism.	TSC1 (hamartin)	*Tsc1+/− mice. Renal and hepatic tumors. Increased number of astrocytes but no cerebral lesions. No spontaneous seizures, but impaired social behavior and impaired learning in hippocampal-dependent tasks. Tsc1−/− mice are lethal. Conditional mutant mice lacking Tsc1 in glia. Glial proliferation, enlarged brain size, progressive epilepsy, and premature death.*	Kobayashi et al. (2001), Uhlmann et al. (2002), Goorden et al. (2007), Zeng et al. (2008), Feliciano et al. (2011)
	TSC2 (tuberin)	*Tsc2+/− mice. Renal and hepatic defects. Cognitive deficits in the absence of neuropathology or seizures. Tsc2−/− mice are lethal. Conditional mutant mice lacking Tsc2 in glia. Cortical and hippocampal lamination defects, hippocampal heterotopias, enlarged dysplastic neurons and glia, abnormal myelination, astrocytosis, megalencephaly, epilepsy, and premature death.*	Uhlmann et al. (2002), Ehninger et al. (2008), Bonnet et al. (2009), Way et al. (2009), Zeng et al. (2011)
LISSENCEPHALY			
Different forms of the disease, due to different genetic mutations. Absent or decreased cerebral convolutions, resulting in cortical thickening and smooth cerebral surface. Developmental delay, myoclonic jerks, seizures.	LIS1	*Lis1 knockout mice. Lethal Lis1−/− mice (aberrant morphology of cortical neurons and radial glia, slower neuronal migration, cortical plate splitting, and abnormal thalamocortical innervation). Behavioral defects not reported.*	Cahana et al. (2001)
	TUBA1A	*N-ethyl-N-nitrosourea-induced mouse with S140G mutation in the TUBA1A gene. Abnormal neuronal migration in layers II/III and IV of the visual, auditory, and somatosensory cortices. Fractured pyramidal cell layer in the hippocampus. Impaired spatial working memory, reduced anxiety, abnormal nesting.*	Keays et al. (2007)
SUBCORTICAL BAND HETEROTOPIA (SBH)			
Bands of gray matter interposed between the cortex and lateral ventricles ("double cortex"). Developmental delay, myoclonic jerks, seizures. DCX mutations are also associated to lissencephaly.	DCX (doublecortin)	*Hemizygous Dcx−/y male mice. Hippocampal lamination defects, reduced number of hippocampal interneurons, no gross anatomical defects of the cerebral cortex. Learning deficits in hippocampus-dependent tasks. Spontaneous seizures with hippocampal onset, hyperexcitability. Dcx−/y Dclk1−/− mice. Perinatal lethality, disorganized neocortical, and hippocampal layering. Dcx in utero electroporation in rats. SBH-like migration defects of Dcx. seizures. Rescued by postnatal re-expression of Dcx.*	Corbo et al. (2002), Nosten-Bertrand et al. (2008) Deuel et al. (2006), Koizumi et al. (2006), Kerjan and Gleeson (2007), Bai et al. (2003), Ackman et al. (2009), Lapray et al. (2010), Manent et al. (2009), Kerjan and Gleeson (2009)
X-LINKED LISSENCEPHALY WITH ABNORMAL GENITALIA			
Intellectual disability, autism, and epilepsy without cortical malformations.	ARX	*Arx−/− mice. Perinatal lethality. Absent interneuron migration from the lateral and medial ganglionic eminences. Reduced number of CR, NPY, CB but not PV interneurons. Conditional deletion of Arx in Dlx5/6 expressing interneurons results in early-onset seizures in hemizygous (Arx−/y) male mice. Arx knock-in mice for human ARX mutations. P355R mutants die at P0, with anatomical defects similar to Arx−/− mice. 330ins(GCG)7 and P355L mutants die after birth, with cortical malformations and altered development of GABAergic and cholinergic neurons. More severe seizures and learning deficits in 330ins(GCG)7 than P355L mutants.*	Kitamura et al. (2002), Colombo et al. (2007), Friocourt et al. (2008), Marsh et al. (2009), Friocourt and Parnavelas (2010), Kitamura et al. (2009)

LISSENCEPHALY WITH CEREBELLAR HYPOPLASIA

Syndrome	Gene	Animal model	References
Moderate lissencephaly, severe cerebellar hypoplasia, dysmorphic facial features, developmental delay, and epilepsy.	RELN (reelin)	*Reeler mice. Impaired migration of cortical projection neurons, resulting in disorganized and dyslaminated cerebral cortex. Increased susceptibility to hippocampal and neocortical epileptiform activity.*	D'Arcangelo and Curran (1998), D'Arcangelo (2006), Patrylo et al. (2006)

PERIVENTRICULAR HETEROTOPIA (PH)

Periventricular nodules, seizures.	FLNA (filamin A)	*Flna-null mice. Die in utero due to cardiovascular defects (abnormal vessels, persistent truncus arteriosus, incomplete cardiac septation). No CNS analysis available*	Feng et al. (2006)

PH WITH MICROCEPHALY

Periventricular nodules, microcephaly, seizures, developmental delay.	ARFGEF2	*Not available.*	Guerrini and Parrini (2010)

AUTISM SPECTRUM DISORDER (ASD)

SNPs in DLX1 and DLX2 are associated to ASD, a neurodevelopmental disorder often associated to epilepsy.	DLX1, DLX2	*$Dlx1^{-/-}$ and $Dlx2^{-/-}$ mice. Selective apoptotic cell death of CR, SOM, and NPY cortical interneurons in $Dlx1/2^{-/-}$ mice. Noise- or handling-induced behavioral seizures and generalized electrographic activity in $Dlx1^{-/-}$ mice.*	Cobos et al. (2005b, 2007), Liu et al. (2009)

EPILEPTIC SYNDROMES

Autosomal dominant lateral temporal lobe epilepsy (ADLTE)	LGI1	*Overexpression of an Lgi1 gene with a truncating mutation. Arrested postnatal maturation of presynaptic and postsynaptic function in the hippocampus, lowered seizure threshold.*	Zhou et al. (2009)
		$Lgi1^{-/-}$ mice: Severe seizures during the second postnatal week and premature death. Seizure-induced cell loss and reorganization in the hippocampus. Altered excitatory synaptic transmission in the hippocampus; $Lgi1^{+/-}$ mice: Lowered seizure threshold.	Chabrol et al. (2010), Fukata et al. (2010), Yu et al. (2010)
GEFS+	SCN1A	*Mice homozygous for the R1648H mutation causing GEFS+. Spontaneous generalized seizures and premature death. Heterozygous mutants for the R1648H mutation. Infrequent spontaneous generalized seizures, reduced threshold for induced seizures. Impairments in inhibitory neuron function.*	Martin et al. (2010)
Dravet's syndrome	SCN1A	*$Scn1a^{+/-}$ and $Scn1a^{-/-}$ mice. Spontaneous seizures, reduced threshold for induced seizures, high mortality. Reduction of sodium currents in hippocampal interneurons. Knock-in mice carrying a truncation mutation in the Scn1a gene. Spontaneous seizures, alterations in parvalbumin interneuron firing.*	Yu et al. (2006), Oakley et al. (2009), Ogiwara et al. (2007)
Benign familial infantile seizures	SCN2A	*Transgenic mice (Q54) carrying a missense mutation of SCN2A. Spontaneous seizures and hippocampal sclerosis reminiscent of human MTLE.*	Kearney et al. (2001), Manno et al. (2011)
Epilepsy (X-linked, with variable learning disabilities and behavior disorders; associated to SYNI mutations)	SYNI	*Syn I and Syn II null mice. Spontaneous and sensory stimuli-evoked epileptic seizures of unspecified type. SynI null mice: the readily releasable pool is reduced at inhibitory synapses and increased at excitatory synapses.*	Rosahl et al. (1995), Garcia et al. (2004), Baldelli et al. (2007), Chiappalone et al. (2009)
Absence epilepsy	SNAP-25	*Coloboma mice. Cortical spike-wave discharges and increased thalamic T-type currents.*	Zhang et al. (2004)

The cited literature essentially refers to animal model studies; for the clinical features of the listed syndromes, the reader is referred to the "Online Mendelian Inheritance in Man" database (OMIM; www.omim.org). Abbreviations, if not otherwise specified, are as in the text.

DOUBLECORTIN

Dominant, X-linked DCX mutations cause lissencephaly or subcortical band heterotopia (SBH; "double cortex"), syndromes characterized by mental retardation and epilepsy in humans. Specifically, DCX mutations cause lissencephaly in hemizygous males, whereas heterozygous females show a mosaic phenotype with a normal cortex and a second band of heterotopic neurons beneath the cortex ("double cortex"; des Portes et al., 1998; Matsumoto et al., 2001). DCX encodes a 40-kDa microtubule-associated protein (doublecortin) expressed in migrating neuroblasts and containing two conserved tandem repeats required for tubulin binding and microtubule stabilization (des Portes et al., 1998; Gleeson et al., 1998; Gleeson and Walsh, 2000). How doublecortin influences microtubule dynamics during brain embryonic development remains largely unclear.

Mice lacking Dcx (hemizygous $Dcx^{-/Y}$ male and heterozygous $Dcx^{+/-}$ female mice) have been generated in the attempt to reproduce the human phenotype of DCX mutations (Corbo et al., 2002). $Dcx^{-/Y}$ male mice present hippocampal lamination defects and a reduced number of hippocampal interneurons, but have no gross anatomical defects at the level of the cerebral cortex (Corbo et al., 2002; Nosten-Bertrand et al., 2008). Similar defects are observed also in $Dcx^{+/-}$ female mice. Importantly, $Dcx^{-/Y}$ male mice have hippocampal-dependent learning deficits (Corbo et al., 2002), are hyperexcitable and show spontaneous seizures with hippocampal onset (Nosten-Bertrand et al., 2008).

Severe migration defects of cortical projection neurons and cortical malformation have been instead detected in mice lacking both doublecortin and doublecortin-like kinase 1 ($Dclk1$; Deuel et al., 2006; Koizumi et al., 2006; Kerjan and Gleeson, 2007). More importantly, Dcx RNA interference (RNAi) performed by *in utero* electroporation results in migration defects of pyramidal cortical neurons and spontaneous seizures in adult rats (Bai et al., 2003; Ackman et al., 2009; Lapray et al., 2010), similar to those observed in human SBH. Manent et al. (2009) tested whether the postnatal re-expression of Dcx could rescue these anatomical and neurological deficits. To this aim, the Dcx knockdown RNAi vector was *in utero* electroporated into cortical neurons together with a tamoxifen-inducible Dcx expression vector system. In this way, it was possible to knockdown Dcx in cortical neurons at embryonic day (E)14 and then re-activate it between postnatal day (P)0 and P10 by treating newborn pups with tamoxifen. Postnatal re-expression of Dcx induced in this way was able to reduce neuronal migration defects and seizures in this model of SBH (Kerjan and Gleeson, 2009; Manent et al., 2009).

Dcx and doublecortin-like kinase have also been implicated in the migration of cortical interneurons (Friocourt et al., 2007; see section below). Taken together, these data indicate that doublecortin is required for hippocampal lamination as well as migration of neocortical pyramidal neurons and interneurons; as a consequence of this important role, an altered function of doublecortin during embryonic forebrain development results in epilepsy in the postnatal life.

REELIN

Mutation of the RELN gene, coding for the extracellular glycoprotein Reelin, causes a neuronal migration disorder called lissencephaly with cerebellar hypoplasia (Zaki et al., 2007; Guerrini and Parrini, 2010). Reelin binds several receptors, including a complex composed by the apolipoprotein E receptor 2 (ApoER2) and the very low-density lipoprotein receptor (VLDLR) (Honda et al., 2011). Following receptor binding, Reelin activates the intracellular protein Disabled homolog 1 (Dab1), whose phosphorylation enhances signal transduction pathways leading to axonal growth, neuronal migration, and synaptic plasticity (Frotscher, 2010; Honda et al., 2011). Reelin-activated pathways include the PI3K/Akt/Gsk-3β and Notch cascades (Honda et al., 2011). During embryonic development, Reelin is mainly expressed by Cajal–Retzius cells in the marginal zone of the neocortex and outer molecular layer of the hippocampus, whereas in the adult brain it is expressed in GABAergic interneurons (Alcantara et al., 1998). Mice lacking Reelin (*reeler* mice) show a dramatic impairment of migration of cortical projection neurons, that results in a highly disorganized and dyslaminated cerebral cortex (D'Arcangelo and Curran, 1998; D'Arcangelo, 2006). The final outcome is an increased susceptibility to hippocampal and neocortical epileptiform activity (Patrylo et al., 2006). During embryogenesis, the firstly generated cortical neurons migrate from the germinal zone and form the preplate. At the arrival of subsequently generated neurons, the preplate splits into two layers: the Cajal–Retzius layer (expressing Reelin) and the subplate. Successive waves of neurons migrate past the subplate, but not past the Cajal–Retzius layer, that will form the most superficial layer of the mature cerebral cortex. In the absence of Reelin, neuronal migration does not occur properly and neocortical lamination is perturbed. The preplate does not split, and newly generated neurons pile up behind the previously generated ones, resulting in an inverted layering of the neocortex. Several hypotheses have been proposed to explain the role of Reelin in neuronal cortical migration: (i) Reelin might function as a chemoattractant for migrating neurons; (ii) it might control the preplate splitting that initiates the inside-out lamination; (iii) it might determine the detachment of neurons from radial glial processes and their subsequent differentiation (D'Arcangelo and Curran, 1998; Cooper, 2008; Gaiano, 2008). More recent studies suggest that Reelin and Notch signaling cooperate to regulate neuronal migration in the neocortex (Hashimoto-Torii et al., 2008).

Reelin is also implicated in the migration of excitatory neurons of the hippocampus; a marked dispersion of projection neurons is observed in the stratum pyramidale and dentate granule cell layer in *reeler* mice (D'Arcangelo and Curran, 1998). Granule cell dispersion (GCD) is often observed in human mesial temporal lobe epilepsy (MTLE), and recent studies investigated the role of Reelin in this form of epileptic condition. In the adult hippocampus, Reelin-positive interneurons (Cajal–Retzius cells) are normally found in the CA1 and CA3 regions, in the hilus, and along the hippocampal fissure, both in mouse (Heinrich et al., 2006) and human samples (Haas et al., 2002). Indeed, GCD correlates with hippocampal Reelin loss in MTLE patients, as shown by studies performed on hippocampal tissues resected from adult subjects who underwent temporal lobectomy for intractable MTLE (Haas et al., 2002). GCD and loss of Reelin was also observed in rodent models of MTLE (Heinrich et al., 2006; Gong et al., 2007; Antonucci et al., 2008, 2009; Duveau et al., 2010). The causative role of Reelin deficiency in GCD is confirmed by the observation

that GCD is induced by the infusion of Reeling-blocking anti-body, and prevented by the administration of exogenous Reelin (Haas and Frotscher, 2010). It remains still to be determined how Reelin loss and subsequent GCD contribute to epileptogenesis, and whether they are a cause or an effect of seizures (Haas and Frotscher, 2010).

MIGRATION DEFECTS OF CORTICAL INTERNEURONS

Cortical GABAergic interneurons are generated in ganglionic eminences of the basal forebrain, from where they migrate to reach their final location (Wonders and Anderson, 2006). Several genes have been implicated in interneuron differentiation and migration; the role of specific interneuron types in epilepsy is a developing field, which may help us to better understand the complex neurodevelopmental processes underlying seizure onset and control. Here we will focus on *Dlx, Arx, Dcx*, and Reelin genes, whose role in interneuron migration and possible implications in epileptogenesis has been extensively studied in mouse models.

Dlx

Migration and differentiation of cortical GABAergic interneurons has been shown to depend on the action of Dlx homeobox transcription factors, namely *Dlx1/2* and *Dlx5/6*. *Dlx* genes are expressed in GABAergic neuron progenitors of the developing forebrain and in subsets of mature GABAergic interneurons of the adult cerebral cortex (Cobos et al., 2005b, 2007; Wang et al., 2010). Loss-of-function mutations of pairs of mouse *Dlx* genes (*Dlx1/2* or *Dlx 5/6*) severely affect the migration and differentiation of distinct subpopulations of cortical interneurons. Specifically, *Dlx1/2* have a crucial role in the migration and survival of calretinin (CR), somatostatin (SOM), and neuropeptide Y (NPY) positive interneurons (Anderson et al., 1997; Cobos et al., 2005b, 2007) whereas Dlx5/6 preferentially control parvalbumin-positive interneurons (Wang et al., 2010).

Migration defects and subsequent reduced number of cortical interneurons result in epilepsy in Dlx mutant mice. Mice lacking *Dlx1* (*Dlx1$^{-/-}$* mice) show selective apoptotic cell death of CR, SOM, and NPY cortical interneurons, resulting in decreased synaptic inhibition at 1 month of age. Accordingly, adult *Dlx1$^{-/-}$* mice show noise- or handling-induced behavioral seizures and generalized electrographic activity (Cobos et al., 2005b). Mice homozygous for both *Dlx5* and *Dlx6* (*Dlx5/6$^{-/-}$* mice) show a normal layering of the cerebral cortex and a normal positioning of MGE-derived cortical interneurons, but die perinatally. A reduced number of PV cortical interneurons is observed in these mutants, and the surviving PV interneurons show an increased *dendritic* branching (Wang et al., 2010). Conversely, heterozygous *Dlx5/6$^{+/-}$* mice do not present interneuron loss nor other gross anatomical abnormalities, but develop epilepsy; this has been ascribed to functional deficit and aberrant connectivity of PV neurons (Wang et al., 2010).

Taken together, these studies clearly demonstrate that *Dlx* genes regulate the development and function of forebrain interneurons, which are crucial to maintain the inhibitory tone of the cerebral cortex and other brain structures. The reduced function of *Dlx* transcription factors (or of their transcriptional targets, such as GAD and vGAT; Stuhmer et al., 2002; Rubenstein, 2011)

might increase the excitation/inhibition ratio thus predisposing the cerebral cortex to hyperexcitability and epilepsy.

Arx

The aristaless-related homeobox gene (*Arx*) belongs to the family of paired-class homeobox transcription factors, and its expression is controlled by the Dlx genes in cells derived from basal ganglia progenitor domains (Cobos et al., 2005a). *Arx* plays a pivotal role in the development of the CNS. ARX large deletions, protein truncating mutations and missense mutations in the homeobox region lead to X-linked lissencephaly with abnormal genitalia, while other ARX mutations cause intellectual disability, autism, and epilepsy without cortical malformations (Kitamura et al., 2002). *Arx* expression has been detected in the developing lateral and medial ganglionic eminence and later in cortical progenitors and migrating interneurons (Colombo et al., 2004; Friocourt et al., 2006). Studies in animal models showed that *Arx* is critical for radial migration of cortical progenitors and is crucially involved in the development and migration of GABAergic interneurons (Colombo et al., 2007; Friocourt et al., 2008; Marsh et al., 2009). In *Arx*-null embryos, interneuron migration from the lateral and medial ganglionic eminences is nearly absent, whereas migration through the cortical layers is only partially impaired. As a consequence, CR, NPY, and calbindin (CB) interneurons are severely reduced (Kitamura et al., 2002; Colombo et al., 2007). No loss of PV interneurons was instead observed in hemizygous *Arx$^{-/-}$* mice suggesting the *Arx* loss results in specific interneuron subtype deficits (Friocourt and Parnavelas, 2010). These studies also revealed that abnormal expression of transcription factors potentially important for neuronal differentiation and migration occurs in *Arx$^{-/-}$* mice.

Dcx

Cortical interneuron migration is also controlled by both *Dcx* and *Dclk*. Dcx knockdown by RNAi slows the migration of interneurons from the ganglionic eminence to the cerebral cortex (Friocourt et al., 2007), and cortices of *Dcx, Dclk*, and *Dcx/Dclk* mutant mice contain less CB-positive interneurons as compared to control mice (Friocourt et al., 2007; Nosten-Bertrand et al., 2008). An increased number of CR-positive interneurons is detected in the dentate gyrus of *Dcx* mutant mice (Nosten-Bertrand et al., 2008), whereas no defects of PV interneurons are detected in *Dcx, Dclk*, and *Dcx/Dclk* mutant mice (Kerjan and Gleeson, 2009).

REELIN

A large subset of cortical and hippocampal GABAergic interneurons express Reelin. These interneurons derive from the caudal ganglionic eminence (CGE), and occupy superficial layer of the cerebral cortex (Miyoshi et al., 2010) and strata lacunosum moleculare and deep radiatum of the mature hippocampus (Tricoire et al., 2011). The role of Reelin in interneuron migration and positioning in forebrain areas has been debated. Some authors showed that in *reeler* mice, GABAergic interneurons migrate normally into the developing forebrain, but fail to acquire proper layer position in the mature cerebral cortex (Hammond et al., 2006; Yabut et al., 2007). Using transplants to analyze the mechanisms underlying the positioning of cortical interneurons, other authors instead

found that layer acquisition by these cells does not directly depend on Reelin signaling (Pla et al., 2006). Interestingly, a recent study showed that neuronal activity is essential for the migration and morphology of CGE-derived Reelin- and CR-positive interneurons (De Marco García et al., 2011). To suppress excitability in CGE-derived interneurons, the authors electroporated *in utero* the inward rectifying potassium channel Kir2.1 under the control of a *Dlx5/6* enhancer element at E15.5, and then analyzed the positioning and morphology of Reelin and CR interneurons. Results showed that neuronal activity is required before P3 for the correct migration of CGE-derived interneurons, and that after P3, glutamate-mediated activity controls the development of their connections (De Marco García et al., 2011). These findings indicate that the genetic program underlying the development of Reelin-expressing interneurons is strongly modulated by activity, and may have implications for the role of Reelin in neurodevelopmental epileptic syndromes.

GABA AND GLUTAMATE CONTROL NEURONAL MIGRATION IN THE FOREBRAIN

Studies performed on corticohippocampal organotypic co-cultures from EGFP-expressing mouse embryos demonstrated that GABA and glutamate may modulate neuronal migration in the developing forebrain. Specifically, GABA and glutamate modulate the migration of hippocampal pyramidal neurons respectively acting on $GABA_A$ and NMDA receptors (Manent et al., 2005), while glutamate controls the migration of GABAergic interneurons via AMPA receptor activation (Manent et al., 2006). These results led the authors to postulate that the migrations of glutamatergic and GABAergic interneurons are inter-dependent: glutamate released from pioneer glutamatergic neurons controls the migration of GABAergic interneurons, which in turn would facilitate glutamate neuron migration via GABA release (Manent and Represa, 2007). In keeping with these observation, the same authors showed that prenatal exposure to some AEDs acting on GABA signaling (such as vigabatrin, valproate, and lamotrigine) results in hippocampal and cortical dysplasias in the developing embryos (Manent et al., 2007, 2008), thus raising serious concerns about the possible consequences of AEDs use during pregnancy.

DEVELOPMENTAL REMODELING OF NEURONAL CIRCUITS

In the previous sections we have considered how defects in the early stages of brain development (i.e., neuronal proliferation and migration) can induce an epileptic phenotype. Following the initial assembly of excitatory and inhibitory neurons, immature neural networks are transformed into organized circuits that subserve adult brain function (Katz and Shatz, 1996). This process of network refinement is largely controlled by electrical activity. In particular, work in the sensory cortices has clearly established the existence of so-called "critical periods," during which patterns of activity generated by sensory experience play a critical role for maturation of cortical function (Berardi et al., 2000). During these sensitive phases of development, initially exuberant connections are eliminated and neuronal dendrites are correspondingly pruned and narrowed. The remaining synapses functionally mature (Katz and Shatz, 1996). In particular, in the visual system, synapse elimination and remodeling depends upon the amount and patterning of neural activity within the visual pathway (Fagiolini et al., 1994; Caleo et al., 2007).

Perturbations in this developmental refinement of neuronal circuitry during critical periods may trigger hyperexcitability and epilepsy later in life. One clear example comes from a study on blockade of hippocampal activity during early development (Galvan et al., 2000). In the rodent hippocampus, CA3 pyramidal neurons display an exuberant growth of axon collaterals during postnatal weeks 2 and 3; this developmental time period corresponds to a critical phase, when CA3 networks have a marked propensity to generate epileptic seizures (Swann and Brady, 1984). Exuberant CA3 axons are then remodeled with maturation, so that about half of the branches are eliminated (Gomez-Di Cesare et al., 1997). To prevent this axonal remodeling, Swann and colleagues infused tetrodotoxin (a blocker of voltage-gated sodium channels) into the rat hippocampus for about 10 days starting from P12 (Galvan et al., 2000). This transient blockade of neuronal activity resulted in the establishment of a chronic epileptic focus in adulthood, with prolonged electrographic seizures originating from the infused hippocampus (Galvan et al., 2000). These results are consistent with the idea that blockade of neuronal activity during early critical phases can enhance seizure susceptibility later in life by preventing developmental remodeling of neural circuits. Indeed, the immature brain is more prone to seizures than the adult brain (Ben-Ari and Holmes, 2006).

Recently, a persistent immaturity of glutamatergic circuitries in the hippocampus was found to underlie seizure susceptibility in autosomal dominant lateral temporal lobe epilepsy (ADLTE; Zhou et al., 2009). This study investigated the function of LGI1 (leucine-rich, glioma-inactivated 1), a gene that is implicated in about half the cases of ADLTE (Kalachikov et al., 2002; Striano et al., 2011). LGI1 encodes a protein that localizes to glutamatergic synapses, binds its receptors ADAM22 and ADAM23 (disintegrin and metalloproteinase domain 22 and 23), and copurifies with presynaptic and postsynaptic regulatory molecules (Fukata et al., 2006; Schulte et al., 2006). LGI1 expression increases in the rodent hippocampus exactly during the third postnatal week, when glutamatergic synapses are pruned and mature (Fukata et al., 2006; Zhou et al., 2009). In particular, excitatory neurons downregulate their presynaptic vesicular release probability and reduce their postsynaptic NMDA-receptor subunit NR2B; during this same period, dendritic arbors and spines are pruned and remodeled. Anderson and colleagues generated mice expressing a truncated form of the LGI1 gene, found in ADLTE patients, and showed that hippocampal glutamatergic synapses did not properly mature in these mice (Zhou et al., 2009). Specifically, (i) the normal, age-dependent decrease of probability of glutamate release was blocked in mutant mice; (ii) developmental changes in glutamate receptor composition were impaired by mutated LGI1; and (iii) dendrites of adult mutant mice remained immature, with a high density of branches and spine protrusions (Zhou et al., 2009). Importantly, mutant LGI1 mice displayed a lower threshold for the development of pharmacologically induced seizures. There was no significant effect of mutated LGI1 on GABAergic neurotransmission (Zhou et al., 2009; Yu et al., 2010). All together, these data suggest that mutated LGI1 blocks the normal functional maturation of both presynaptic and postsynaptic compartments and halts structural pruning,

thus maintaining a high density of excitatory synaptic inputs converging onto hippocampal neurons and leading to a hyperexcitability phenotype (Caleo, 2009; Zhou et al., 2009). Other reports are consistent with the idea that LGI1 regulates the development of glutamatergic synapses (Fukata et al., 2010; Yu et al., 2010). In keeping with this findings, recent data show that heterozygous mice lacking LGI1 (*LGI1*$^{+/-}$ mice) display a lower threshold to auditory stimuli induced seizures, and homozygous *LGI1*$^{-/-}$ mice develop spontaneous recurrent seizures followed hippocampal neuronal loss, mossy fiber sprouting, astrocyte reactivity, and GCD (Chabrol et al., 2010).

EPILEPTOGENIC ION CHANNEL MUTATIONS: FOCUS ON SCN2A

In the last years, a number of epileptogenic mutations have been identified in different genes (Noebels, 2003). The majority of these genes code for voltage- or ligand-gated ion channels. Mutations in different subunits of sodium, potassium, and calcium channels underlie different forms of genetic epilepsies ("channelopathies"; Kullmann, 2010; Mantegazza et al., 2010). Mutations of the nicotinic acetylcholine receptor subunits are associated with autosomal dominant nocturnal frontal lobe epilepsy (De Fusco et al., 2000). Mutations in the voltage-gated potassium channel subunit genes KCNQ2 and KCNQ3 are associated with benign familial neonatal seizures (Leppert and Singh, 1999). Mutations in the GABA$_A$ receptor, which is the primary mediator of synaptic inhibition, have also been found to contribute to several idiopathic epilepsies and febrile seizures (Galanopoulou, 2010). The functional consequences of these epileptogenic mutations have been amply studied, and the data have provided significant knowledge on the pathogenic mechanisms that lead to epilepsy. For more details on these findings, the reader is referred to a series of excellent reviews (Noebels, 2003; Kullmann, 2010; Mantegazza et al., 2010).

Mutations in voltage-gated sodium channel genes (Nav) are the most common genetic cause of familial epilepsy. Specifically, mutations in the Nav1.1 alpha subunit gene (SCN1A) are responsible for "generalized epilepsy with febrile seizures plus" (GEFS+; Scheffer and Berkovic, 1997) and Dravet's syndrome (Mantegazza et al., 2010; Meisler et al., 2010). Missense mutations in Nav1.2 alpha subunit (SCN2A) are found in patients with benign familial neonatal-infantile convulsions (Avanzini et al., 2007). Functional studies demonstrate that Nav1.2 mutations result in modifications of the gating properties of the channel, with a net amplification of the sodium current and resulting greater depolarization, consistent with the hyperexcitability phenotype (Scalmani et al., 2006).

It is important to point out that it is often difficult to predict the epileptic phenotype based solely from the change in the behavior of the mutated channel. First, it is crucial to determine cell-specific patterns of expression of the mutated subunit, as expression in excitatory vs. inhibitory neurons can lead to completely opposite effects on excitability status of the neuronal network (Yu et al., 2006). Second, the cellular background (i.e., the modifier effect of other channels expressed in the same neuron) has to be considered (Glasscock et al., 2007). "Humanized" mouse models (in which human mutated channel sequences are inserted in genetically engineered mice) offer an unique opportunity to study the modulatory role of the genetic background and of the interactions

between different genes. One example is represented by transgenic mice (Q54) carrying a human, epileptogenic missense mutation of the sodium channel SCN2A (Nav1.2; Kearney et al., 2001). Mice carrying the Q54 transgene on a SJL strain background exhibit severe spontaneous seizures originating in the hippocampus with onset at about 4 weeks, and progressive hippocampal sclerosis with extensive cell loss and gliosis in areas CA1, CA3, and hilus (Kearney et al., 2001; Manno et al., 2011). However, on a pure C57BL/6J background, onset of seizures is delayed and the epileptic phenotype is mild. Two modifier loci responsible for the difference in severity between strains C57BL/6J and SJL have been mapped, and the evidence points to the voltage-gated potassium channel gene Kcnv2 as one modifier (Bergren et al., 2005, 2009). These data indicate that severity of the epileptic condition may be significantly impacted by gene interactions.

Finally, in the study of channelopathies it is important to underlie the possible effects of the complex interaction between mutated genes and the environment. As we have described in the previous section, early environmental conditions shape neuronal connectivity, and the resulting changes in network organization can potently affect clinical expression of a mutated channel. In keeping with this notion, Q54 transgenic mice (carrying an epileptogenic mutated SCN2A; see above) exposed to environmental enrichment from birth show a dramatic reduction of spontaneous seizures and hippocampal cell loss (Manno et al., 2011). The data indicate that an enriched housing from birth may have profound antiepileptic and neuroprotective effects. Environmental enrichment may exert these actions by up-regulation of neurotrophic factors, plastic rearrangements in excitatory/inhibitory circuits, and stimulation of neurogenesis (Dhanushkodi and Shetty, 2008; Sale et al., 2009). Thus, changes in network organization due to environmental influences can halt epileptogenic changes and dampen hyperexcitability of neural networks.

EPILEPTOGENIC ALTERATIONS OF PRESYNAPTIC PROTEIN FUNCTION

In addition to channelopathies, defects in the control of neurotransmitter release account for a wide variety of epileptic syndromes. Presynaptic proteins that have been found to be involved in epilepsy include the synaptic vesicle (SV) protein SV2A (Crowder et al., 1999; Janz et al., 1999), the SV-anchoring phosphoproteins synapsins (Rosahl et al., 1995; Baldelli et al., 2007) and the plasma membrane fusion protein SNAP-25 (Zhang et al., 2004). Not all disruptions of the neurotransmitter machinery are equally epileptogenic, as shown by the lack of seizures in mice lacking other presynaptic proteins, such as synaptotagmin (Fernandez-Chacon et al., 2001) or synaptobrevin/VAMP (Schoch et al., 2001). Data obtained in mice mutant for synapsins and SNAP-25 are particularly interesting, since they suggest that hyperexcitability might result from developmental alterations causing an unbalance in the activity of excitatory and inhibitory neurons.

SYNAPSINS
Synapsins (Syns) comprise a family of neuron-specific proteins encoded by three distinct genes (SYNI, SYNII, and SYNIII) located in chromosome X, 3, and 22, respectively. The Syns are phosphoproteins implicated in the regulation of neurotransmitter release

and synapse formation. In particular, Syns are responsible for the formation and maintenance of SV pools within the nerve terminal (Benfenati et al., 1992) and their expression has been causally related to the formation and maturation of synaptic connectivity during neural development and synaptogenesis (Valtorta et al., 1995; Bonanomi et al., 2005). Despite the absence of gross defects in brain morphology, Syn I and Syn II (but not Syn III) knockout (KO) mice exhibit early-onset spontaneous and sensory stimuli-evoked epileptic seizures (Rosahl et al., 1995). Syn I mutations can also contribute to human epilepsy (Garcia et al., 2004). In Syn KO mice, spontaneous recurrent seizures develop after 2 months of age and the animals become more susceptible with age (Rosahl et al., 1995). This latent period of "epileptogenesis" suggests that alterations in the developmental assembly of neural networks may underlie hyperexcitability in these mice.

Several theories have been put forward to explain the nexus between the molecular function of Syns at the neuronal level and the onset of the epileptic phenotype. Since Syns are involved in crucial steps of presynaptic physiology and play a role in the formation of the synaptic connectivity during development, it is likely that mutations affecting their expression and/or phosphorylation-dependent functions will result in significant changes in synaptic transmission, plasticity and development which could be potentially related to the appearance of an epileptic phenotype. Thus, the epileptic phenotype of Syn KO animals might be due to a defect in the overall brain connectivity (Chin et al., 1995), or to an impairment in synaptogenesis within confined neuronal populations (Gitler et al., 2004; Bonanomi et al., 2005). There is also support for the idea that lack of Syns produces an imbalance in the activity of excitatory and inhibitory neurons. Indeed, Syn I KO reduces the readily releasable pool at inhibitory synapses, and increases it at excitatory synapses (Baldelli et al., 2007; Chiappalone et al., 2009).

SNAP-25

A further example of presynaptic protein involved in epilepsy is SNAP-25. SNAP-25, together with syntaxin and synaptobrevin/VAMP, is a member of the SNARE (soluble NSF attachment protein receptor) complex which contributes to the formation of the fusion complex required for SV exocytosis. Beside participating in SV fusion, SNAP-25 has been proposed to regulate exocytosis at additional steps. Through its interaction with other proteins, such as synaptotagmin or complexins (Jahn et al., 2003) or through putative calcium binding sites (Sorensen et al., 2002), SNAP-25 appears to participate in the calcium-dependent regulation of exocytosis. SNAP-25 has also been shown to interact with and modulate the activity of various voltage-activated calcium channels (Atlas, 2001; Pozzi et al., 2008; Condliffe et al., 2010). The possible involvement of SNAP-25 in neuronal hyperexcitability and

epilepsy comes from the neurological mouse mutant Coloboma (Cm/+), bearing an *autosomal dominant deletion* on chromosome 2, including the SNAP-25 gene (Hess et al., 1992, 1994). Cm/+ mice show 50% reduction in SNAP-25 mRNA and protein, and impaired evoked neurotransmitter release (Raber et al., 1997; Wilson, 2000). Interestingly, Cm/+ mice show robust cortical cortical spike-wave discharges and increased thalamic T-type currents (Zhang et al., 2004), two typical features of "absence" epilepsy (Tsakiridou et al., 1995; Coenen and Van Luijtelaar, 2003).

Similarly to what already described for Syns, the role of SNAP-25 in the etiopathology of epilepsy may involve alterations of synaptic formation/refinement during development. The epileptic phenotype of SNAP-25 mutant mice may be produced by a defect in the overall brain connectivity consequent to the reduced expression of the protein, which is known to play a crucial role in neurite extension (Osen-Sand et al., 1996). Alternatively, it may be the consequence of unbalances in neurotransmitter release due to deficits in the SNARE complex. Interestingly, SNAP-25 is expressed at much higher levels at excitatory vs. inhibitory synapses (Verderio et al., 2004; Bragina et al., 2007). Thus, hyperexcitability could result from perturbations of the processes that balance the developmental assembly of inhibitory and excitatory circuits. Furthermore, recent studies have shown a role of SNAP-25 in the regulation of calcium homeostasis in neurons. In particular, it has been shown that phosphorylated SNAP-25 negatively modulates calcium dynamics by inhibiting voltage-gated calcium channels (Verderio et al., 2004; Pozzi et al., 2008). Thus, reduced levels of SNAP-25 may lead to increased calcium currents (Condliffe et al., 2010) and consequently to the onset of epileptic discharges.

CONCLUSIONS AND FUTURE DIRECTIONS

In this review, we have highlighted some of the neurodevelopmental pathways that lead to a chronic epileptic condition in adulthood. Abnormal development of the cerebral cortex (due to perturbations of neuronal proliferation and/or migration) is a frequent cause of epilepsy. In addition, more subtle alterations in the assembly and fine-tuning of neuronal networks may also lead to an hyperexcitability phenotype later in life. A better understanding of the biology of these epileptogenic mechanisms has important implications for the development of novel therapeutic approaches.

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