Cortical development and focal cortical dysplasia

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ABSTRACT – A brief survey of cortical development is presented, focusing on neuronal migration and its alterations. Corticogenesis is achieved through ordered temporospatial steps, via the formation of transient structures, and successive waves of cell proliferation and migration (followed by cell differentiation and maturation), and apoptotic cell death. The appearance of the proliferative ventricular zone and marginal zone, and of the superficial primordial plexiform layer, is followed by the formation of the prospective layer I, of the subplate, whose neurons are destined to die, and of the cortical plate that will give rise to layers II-VI. Cells arising in the ventricular zone migrate radially using radial glia as a scaffold, and are destined to form pyramidal cells. Cortical interneurons are mainly generated in the ganglionic eminence and migrate along axonal substrates following tangential routes. Disorders of this complex process lead to a wide range of alterations, and focal derangements of cortical organization have been grouped under the term focal cortical dysplasia (FCD). As the result of a neuropathological revision of FCD cases with intractable epilepsy, a novel classification comprising three subgroups of FCD has been introduced, and is supported by electroclinical and neuroimaging data, as well as by the postsurgical outcome of patients: i) architectural dysplasia, characterized by altered cortical lamination; ii) cytoarchitectural dysplasia, with the occurrence of giant neurons besides cortical dyslamination; iii) Taylor-type cortical dysplasia, in which altered cortical lamination is consistently associated with the occurrence of giant, dysmorphic and ectopic neurons, and frequently with the so-called balloon cells.

KEY WORDS: ontogenesis, cerebral cortex, epilepsy, neuronal migration, malformations

Normal cortical development

Foundations of the concept of neuronal migration and radial glia

Studies and debates on the developmental events that shape the nervous system date back to the pioneers of neuroscience. In 1885, Camillo Golgi [1], using the silver impregnation technique that he had introduced in 1873 began investigating, the developing nervous system ‘convinced that the key for the solution of many questions is enclosed in the embryogenesis of the nervous central organs’. He published his observations in the chick embryo, describing glial fibers ‘radiating’ from the central canal towards the periphery of the spinal cord [1].

In a series of fundamental contributions, His [2-4] stimulated numerous embryological studies at the turn of the 19th century, leading to the con-
cluision that the pallium consisted exclusively of epithelial elements (i.e., deriving from the ectoderm), extending from the ventricular cavities to the brain surface. In addition, Hirsch demonstrated that the internal zone was made by rapidly dividing germinal cells capable of mitosis.

Giuseppe Magini, an investigator working in Rome, using the Golgi impregnation method on the fetal cerebral cortex of different mammals including humans [5, 6], described ‘cylindrical epithelial cells’ situated around the ventricular cavity and proceeding ‘like rays’ towards the brain surface. These ‘radiating neuroglial cells’ exhibited a high number of ‘varicosities’, and Magini hypothesized that they could represent developing nerve cells. Santiago Ramón y Cajal, deeply influenced by Hirsch’s works, and also aware of the works of Golgi and Magini, published his first report on the developing cerebral cortex in 1889 [7]. He stated that cell division occurred preferentially in the ‘vicinity of the epithelium’, that neuroblasts migrated beneath the outer part of the developing cortex and that radial glia served as scaffolding for embryonic development. Cajal severely criticized Magini’s opinion that neuronal cells were threaded in radial glial cells, and stated that the varicosities observed by this Italian scientist represented instead ‘protoplasmic accumulations’ [8].

The mechanism of outward displacement of neurons in the developing cortex remained unknown for decades and became obvious only in the 1960s, when autoradiographic studies showed that cortical neurons were generated deep in the cortex and were then taking up a distant, superficial position forming the cortical layers. However, at that time it was believed that neurons did not undertake an active migration but were instead translocating their nuclei within cylinders of cytoplasm extending from the ventricular zone to the cortical plate. It was only in the early 1970s that Rakic [9] clarified the intercellular neuron-glial relationship, demonstrating that ‘late-generated cells find their way to the cortex by assuming a bipolar shape and moving outward in direct and constant apposition to radial glial processes that span almost the entire width of the telencephalic wall’.

At the same time as active radial migration was finally recognized as the basic mechanism for building up the cerebral cortex, studies based on the Golgi impregnation method or electron microscopy reported the existence of cells with a tangential orientation (i.e., perpendicular to radial glia) in the intermediate zone of the developing neocortex [10, 11]. Interest in the latter observations has been recently revived by a wealth of findings that have provided evidence for distinct, non-radial routes of migration of some cell cohorts that migrate parallel to the pial surface ([12, 13]; see also further).

Interest in perikaryal or somal translocation has also been recently revived, on the basis of data indicating that neurons destined to migrate radially adopt two different strategies, and that somal translocation occurs during the early stages of corticogenesis, when the cerebral wall is relatively thin, whereas a radial glia-guided migration becomes the main mechanism during the subsequent stages of cortical formation (reviewed in ref. [14]).

Last but not least, the history of radial glia has been recently enriched by novel, exciting data indicating that these cells are capable, by asymmetric division, of giving rise to neurons and glial cells, and that radial glial cells are, therefore, neurogenic [15, 16]. On this basis, it has been suggested that the historical term of radial glia be modified, renaming these elements ‘radial cells’ [15].

The gypsy cells in the developing cortex

At the beginning of cortical development, a homogeneous population of undifferentiated cells is present. Cell proliferation, migration, differentiation, maturation, as well as programmed cell death represent the fundamental events involved in the developmental shaping of the cerebral cortex. From the undifferentiated cell population, the neocortex is generated through successive and partially irreversible steps implying, for a single cell, decisions that progressively restrict its choices and fate. The matrix cells, organized as a pseudostratified columnar epithelium, will generate part of the neuronal and glial elements of the neocortex and the final laminated cortex of mammals, including humans, is generated through the formation of transient structures (figure 1).

After the appearance of the ventricular zone, composed of the undifferentiated matrix cells, a new area, called the marginal zone, is formed just below the pial surface. This area is supposed to be the first functionally active zone of the developing cortex, being the site of formation of the early synapses from brainstem incoming afferents. The marginal zone also promotes the maturation of early generated neurons in the subpial region, presumably triggering the subsequent migratory events [17]. Thus, the appearance of the marginal zone marks the beginning of cortical neurogenesis and precedes the formation of all the other cortical layers. The superficial lamina containing the first differentiated neuronal elements is called the primordial plexiform layer (figure 1).

Subsequently, after a mitotic cycle, the neuroblasts from the ventricular zone migrate upward, and the arrival of the first migratory neurons splits the primordial plexiform layer in two regions (figure 1). The most dorsal one, close to the pial surface, becomes the prospective layer I; the second one, called the subplate (SP), lies below the newly arrived cohort of neurons forming the cortical plate (CP). The SP is considered the area recipient of fibers incoming from subcortical structures, which establish synapses with the neurons of SP, and represents the waiting compartment for these afferents during CP development. SP neurons are destined to die later on through a process of programmed cell death, and the fibers will then be free to reach their appropriate and final cortical target.
Figure 1. Summary of the main steps of the formation of the human cerebral cortex. Abbreviations: CP, cortical plate; IZ, intermediate zone; L, layer; PPL, primordial plexiform layer; SP, subplate; SVZ, subventricular zone; VZ, ventricular zone; W, week; WM, white matter.

Figure 2. Schematic representation of radial and tangential mechanisms of neuronal migration in cortical development.
During further developmental stages, the thickness of the CP progressively increases due to the arrival of subsequent waves of migrating neuroblasts. This process follows an inside-out sequence, so that the early generated and migrated neurons will form the deep layers of the future neocortex, displaced downward by the neurons generated later and forming the most superficial layers [9, 17]. By these mechanisms, layers II through VI are formed (figure 1).

As mentioned above, two main trajectories, a radial and a tangential route, have been identified for neuronal migration (figure 1). The process of radial migration implies that newly generated neuroblasts from the proliferative zone migrate to the CP, climbing along radially oriented fibers that extend from the ventricle to the pial surface [9, 14-16].

Non-radially migrating neurons are generated in the ganglionic eminence (figure 2), the primordium of the future basal ganglia, located in the ventral forebrain. Radially migrating neurons give rise primarily to pyramidal neurons (i.e., projection neurons), which are excitatory and utilize glutamate as neurotransmitter. The cells generated in the ganglionic eminence follow a long tangential migratory route to reach the CP and form instead the majority of nonpyramidal neurons (i.e., cortical interneurons), which are GABAergic [12-14]. While neocortical cells arising in the ventricular zone utilize radial glia as scaffolding to migrate to the developing CP, tangentially migrating neurons move in close association with axons of the corticofugal fiber system [12, 14].

This brief summary shows that during cortical development a spatial and ordered sequence of migratory events is necessary to build up the cerebral cortex. The complexity of these mechanisms increases in the large cortex of primates. Both intrinsic (genetic) and environmental mechanisms are involved in corticogenesis, although the intimate processes leading to the mature cortical structure are not yet completely understood. Disturbances of these processes can lead to a wide range of alterations, from severe brain malformations to local disruption of cortical structure.

**Focal cortical dysplasia**

Malformations of cortical development (MCD) have been recognized pathologically since the end of the 19th century; most are attributed to a defect of neuronal migration during a crucial period of cortical development [18-20]. MCD are a heterogeneous group of focal or diffuse anatomical derangement whose pathological features depend largely on the timing of the defect in the developmental process and to a lesser extent on its cause [21].

The advent of high-resolution imaging techniques, particularly magnetic resonance imaging (MRI) has made it possible to diagnose MCD *in vivo* and provide correlations between imaging and electroclinical findings since most of these malformations are epileptogenic.

As a consequence, several types of partial epilepsies, previously defined as cryptogenic, are now recognized as secondary to cortical lesions. In particular, changes due to abnormal cortical development are frequently associated with intractable epilepsy, resulting in increasing numbers of patient candidates for surgical treatment [22].

Neuronal migration disorders (NMDs) are generally considered a subgroup of MCD, and their definition may imply that disruption of migration is the only mechanism upon which the malformation is based. However, not all the so-called NMDs have been shown to be due directly to impaired neuronal migration, and other mechanisms are therefore involved.

The cortical malformations previously grouped under the general term of NMDs are currently diversified to reflect the improved knowledge of the pathological substrate, the possible aetiological factors and the relationship between altered structural features of the malformation and type of epilepsy [21]. Nevertheless, despite many efforts, it is widely recognized that the classification of these disorders is far from satisfactory and there is no general consensus on these complex structural abnormalities [21-23]. Furthermore, their aetiology is often uncertain and the mechanisms by which they generate epilepsy is unclear [24-29].

Special attention has been devoted in recent years to focal derangements of cortical organization. Taylor et al. [30] were the first to describe distinctive focal anomalies of cortical structure, to which they gave the name of focal cortical dysplasia (FCD). Subsequently this term has been used extensively in the literature to refer to a wide range of derangements of cortical anatomy. Alterations originally observed in surgical specimens and subsequently detected by MRI have been referred to as mild cortical dysplasia, or microdysgenesis, as well as FCD. This latter terminology was originally adopted by Meencke [31] to describe histological alterations of layer I, in patients with generalized epilepsy. During the following years, this term was used in a broader meaning to indicate small, microscopic or very localized forms of FCD affecting layers other than layer I. Thus, the term microdysgenesis has now become seriously misleading.

FCDs are among the cortical malformations most frequently detected with MRI [22, 27-29]. In order to contribute to a terminological clarification of these disorders, some of the authors of the present overview recently contributed to a simplified classification system based on easily recognized neuropathological characteristics [32]. This work was based on the retrospective re-evaluation of the neurohistological data of selected patients, undergoing surgery for intractable epilepsy at the ‘Claudio Munari’ Epilepsy Surgery Center, and characterized by the pres-
ence of FCD not associated with other cortical abnormalities or lesions.

Based on the neuropathological revision of permanent slides processed by routine histological methods (haematoxylin/eosin, thionin, luxol fast blue and Bielschowski), the presence of cortical laminar disruption and cytological abnormalities were considered [32]. According to these criteria, the patients were grouped into three main categories:

1. **Architectural dysplasia (AD):** characterized by an altered cortical lamination but with no major cytological abnormalities. In specimens from this group, heterotopic neurons in the white matter can be observed, exceeding the number of cells found in normal material [33, 34]. Within the cortical mantle, scattered or clustered cells of small diameter with a large nucleus and a thin rim of cytoplasm were found; these elements were identified as immature neurons.

Figure 3. Schematic drawings showing normal cortex with regularly distributed layers and related cells and fibers (A), compared with a dysplastic, unlayered cortex with haphazardly distributed neurons (B).
2. Cytoarchitectural dysplasia (CD): this group is defined by the presence, in addition to cortical dyslamination, of giant neurons scattered in different cortical layers; these cells are larger than the large-sized neurons normally present and are restricted to layer V, their neurofilament content is greater than normal, but their morphology is otherwise normal.

3. Taylor-type cortical dysplasia (TFCD): characterized by abnormal cortical lamination always associated with giant, dysmorphic neurons, and large ectopic neurons (figure 3). In most of the cases balloon cells are also present (figure 4A, B); the main elements are huge cells with an ill-defined membrane, pale eosinophilic cytoplasm and one or more eccentric nuclei.

Figure 4 A, B. Photomicrographs showing large dysmorphic neurons labeled by neurofilament immunostaining (A), and balloon cells immunostained with anti-vimentin serum (B) in TFCD. C: Schematic drawings showing the morphology of pyramidal neurons and axon terminals (the latter represented by dots outlining the soma, dendrites and proximal axons) in the normal cortex, architectural dysplasia and TFCD. Note the dysmorphic aspect of neurons, impinged upon by neurofilaments in TFCD.
It was then assessed whether these groups of alterations, defined on the basis of their main neuropathological features, corresponded to clinically homogeneous groups. To this end, the electroclinical findings, MRI data and postsurgical outcome in cases of each subgroup were then independently re-evaluated and correlated with immunocytochemical findings obtained in surgical specimens [32, 35].

Although the number of patients belonging to the CD group (11% of the total studied population) was too limited to make a definitive and representative group [32], a significant correlation was found between AD and TFCFD with respect to the electroclinical, MRI, surgical outcome and immunocytochemical findings. In particular, seizure frequency was much higher in patients with TFCFD than in AD; the location of this latter dysplasia predominated in the temporal lobe, while in TFCFD they were mainly extratemporal and affecting particularly the frontal lobe. In patients submitted to stereo-EEG invasive presurgical evaluation, a distinctive interictal electrical pattern, never observed in patients with either AD or CD, was observed in TFCFD [35]. Although MRI was unrevealing in almost one-third of the population studied, focal hypoplasia was generally found in AD, while distinctive signal alterations were characteristic of TFCFD.

Despite the severe neuropathological aspect of TFCFD, patients with this type of dysplasia had a better surgical outcome compared to those presenting AD [32]. This was presumably due to the fact that lesions in TFCFD are more localized than in AD and therefore their surgical resection, particularly when guided by stereo-EEG that permits a more accurate definition of the epileptogenic zone, can be more radical than in AD.

Immunocytochemical studies also revealed that TFCFD and AD are characterized by different and selective morpho-functional alterations particularly related to the GABAergic system (figure 4C). A reduction of neurons expressing glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, and some calcium binding proteins (such as calretinin, parvalbumin and calbindin), was evident in all the subtypes of dysplasia. However, peculiar features were revealed by immunocytochemistry in TFCFD, but not in the other forms of dysplasia. In particular, a sprouting of parvalbuminergic, GAD-positive terminals, presumably deriving from a subset of GABAergic interneurons, is present only in TFCFD [36–38]. This reorganization of microcircuitry (figure 4C) could be responsible in TFCFD for the peculiar interictal pattern observed during stereo-EEG recordings, which is never observed in other forms of alterations of cortical development.

In conclusion, AD, CD and TFCFD represent three subgroups of dysplasia defined on the basis of easily recognizable, histopathological characteristics, as well as three different clinical entities. The proposed classification [32, 35] can therefore be useful to clinicians and radiologists for a better definition of the diagnosis and prognosis prior to surgical treatment.

References