

# Normal neuronal migration

María Guadalupe Flores Cruz,<sup>1</sup> Alfonso Escobar<sup>1</sup>

Artículo original

## SUMMARY

Ontogenesis of both central and peripheral nervous systems depends on basic, molecular and cellular mechanisms of the normal neuronal migration. Any deviation leads to neural malformations. All neural cells and structures derive from the neural ectoderm, which under the influence of the notochord and the molecules Noggin and Chordin, is transformed consecutively into neural plate, neural groove, neural tube and primary vesicles. Of the latter, the most rostral, the prosencephalon, two vesicles are bilaterally generated, the telencephalon and in the middle, the unpaired diencephalons. The telencephalic vesicles generate the cerebral hemispheres and the lateral ventricles; the latter constitutes the main source of progenitor neuroepithelial cells (NEC) in the subventricular zone. The NEC massively migrates to constitute the cerebral cortex and other hemispheric structures in the telencephalon and diencephalon. The NEC expresses a broad repertory of markers: BLBP, GLAST, vimentin, tenascin, S100 $\beta$  and, in primates GFAP; in a sequential order the NEC form the first cortical layer formed by the marginal zone and the subplate. The marginal zone harbors the Cajal-Retzius reelin positive neurons and reelin negative neurons.

Reelin, besides signaling stop to migrating neurons, also participates in ordering the cortical layers; it is known that in mutant mice lacking reelin cortical layers are disrupted. Genetic studies indicate that ApoER2, Vldr (both reelin receptors) and Dab1, reelin signaling adaptor protein, enter into a common pathway leading Dab1 to phosphorylation in migrating neurons.

Cortical pyramidal neurons generate at germinal zone; interneurons generate both in Vz and SVZ in medial ganglionic eminence and caudal GE.

Two types of neuronal migration coexist, radial and tangential. In radial migration, the neurons move perpendicular to marginal zone and radial glia serves as a scaffold to migrating cells; in the tangential way, neurons migrate in parallel to brain surface guided by semaphorins, neuropilins, cell adhesion molecules, neuregulins, chemokines and the slit and robo families of attractant and repellent molecules.

The migratory cycle of neurons involves leading process dynamics and somal translocation, which involves the movement of perinuclear material, organelles and nucleus.

Leading process stability depends on the microtubular array that links the leading edge of the cell with the soma. The centrosome is a microtubule center to control microtubule polymerization. In radially migrating neurons, the centrosome establishes a link between centrioles and nuclear membrane. The effective neuronal migration is only completed by translocation of the cell soma, which occurs

with cytoplasmic dilatation, and then the centrosome and Golgi apparatus approach it, finally nucleus advances to the cytoplasmic dilatation. Movement of centrosome and nucleus depends on integrity of a microtubule network. Most of the microtubules surrounding the nucleus are tyrosinated, making them dynamic; microtubules at the anterior pole of the nucleus, near the centrosome, are acetylated.

Once neurons reach their final destination, they need to cancel the migratory program and differentiate. The mechanisms are unknown; possibly early patterns of activity in the target region could influence. Ca<sup>2+</sup> influx is a proposed mechanism for halting migration.

**Key words:** Neuronal migration, cerebral cortex, molecular mechanisms.

## RESUMEN

La ontogenia de los Sistemas Nervioso Central y Nervioso Periférico depende de procesos como la proliferación, diferenciación y migración neuronal, entre otros. Cualquier desviación resulta en malformaciones. Las estructuras y células nerviosas derivan del ectodermo, la notocorda induce la formación de la placa neural mediante la secreción de las moléculas Noggin y Chordin; posteriormente la placa neural se convierte en surco y tubo neurales.

Una vez que el tubo neural está formado, las células neuroepiteliales (CNE), futuras neuronas y glía, en la zona subventricular migran masivamente para constituir la corteza cerebral y otras estructuras. Las CNE, al ser células gliales, expresan múltiples marcadores: BLBP, GLAST, vimentin, tenascin, S100 $\beta$  y en primates GFAP.

Las CNE forman la primera capa cortical, también llamada preplato. Las siguientes divisiones celulares darán origen a la zona marginal y al subplato. Las subsecuentes neuronas que arriban al subplato desplazan a las anteriores de modo que en las capas superficiales se encuentran las últimas neuronas que llegaron. La capa marginal o capa I contiene células de Cajal-Retzius inmunorreactivas a reelin y neuronas reelin-negativas situadas más profundamente.

La proteína reelin, además de servir como señal de alto a las neuronas migratorias, también interviene en el orden de la laminación cortical, la cual es desordenada en los ratones que carecen de reelin. No se conoce en su totalidad el mecanismo molecular mediante el cual reelin regula los procesos antes mencionados. Hasta el momento se conoce que ApoER2, Vldr (ambos receptores de reelin) y Dab1, proteína adaptadora en la señalización por reelin, participan en una vía común que lleva a la fosforilación de Dab1 en las neuronas en migración.

<sup>1</sup> Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

Corresponding: María Guadalupe Flores Cruz, Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, México, D.F. Tel. & Fax. +52 55 5622-3850. E-mail: mgfcruz@gmail.com

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Las neuronas piramidales corticales se generan en el telencéfalo dorsal, mientras que las interneuronas se generan en la zona y subzona ventriculares del telencéfalo ventral, en las bien definidas subdivisiones de la eminencia gangliónica (EG): lateral, medial y caudal.

La migración neuronal puede ser radial o tangencial; la migración radial emplea a la glía radial mientras que en la tangencial las neuronas migran paralelamente a la superficie cortical. En los dos tipos hay formación de neuritas, translocación somática y núcleocinesis. Varios factores participan en la migración tangencial: semaforinas, neuropilinas, moléculas de adhesión celular, neuregulinas, quimiocinas y moléculas atrayentes y repelentes de las familias slit y robo.

El ciclo migratorio de las neuronas incluye la translocación del soma con movilización de material perinuclear, organelos y del núcleo. Así mismo, dicho ciclo aparece con morfología bien definida en una variedad de neuronas lo que refleja adaptación a ambientes específicos. De tal modo que las claves guías influyen en la frecuencia y orientación de la emergencia dendrítica, que a su vez permite a las neuronas migrantes cambiar de dirección sin reorientar las dendritas preexistentes. La estabilidad del mecanismo depende de la organización microtubular que asocia el borde celular con el soma; ya que el sistema de microtúbulos apoya dicho mecanismo y también permite el flujo de vesículas.

En las células animales el centrosoma es el centro que organiza el citoesqueleto, la polimerización, el arreglo de los microtúbulos perinucleares y establece el contacto de los centriolos con la membrana nuclear. En la migración radial el movimiento hacia

delante de los centriolos deforma el conjunto perinuclear de microtúbulos. Se debe a la elasticidad de ese conjunto microtubular y sus proteínas motoras asociadas al desplazamiento del núcleo.

La nucleocinesis o movimiento del núcleo determina la dirección del movimiento nuclear, la migración neuronal efectiva sólo se completa por la translocación subsecuente del soma, lo cual ocurre por la dilatación del citoplasma y el movimiento del centrosoma y del aparato de Golgi hacia el mecanismo; finalmente el núcleo avanza e invade la dilatación del citoplasma.

El movimiento del centrosoma y del núcleo depende de la integridad de la red microtubular y de las modificaciones post-transcripción. La mayoría de los microtúbulos perinucleares están tiosinados, lo cual los hace extremadamente dinámicos; en cambio, los microtúbulos del polo anterior del núcleo, vecinos del centrosoma, están acetilados y por ende más estables. Se ha dicho que los microtúbulos perinucleares se hallan conectados con el centrosoma que en sí es el centro que los organiza. Además, se han descrito otras proteínas asociadas con la polaridad celular que desempeñan un papel esencial en la coordinación del movimiento del centrosoma y del núcleo en cada ciclo migratorio.

Finalmente, una vez que las neuronas alcanzan su posición definitiva, requieren cancelar el programa migratorio y continuar su diferenciación hasta alcanzar las características morfológicas y funcionales que les corresponden.

**Palabras clave:** Migración neuronal, corteza cerebral, mecanismos moleculares.

## INTRODUCTION

The concept of neuronal migration refers to the essential mechanisms, molecular and cellular aspects that, during embryonic development, constitute the structures of both the Central and the Peripheral Nervous Systems. Early neuroscience studies back in the 19<sup>th</sup> century, contributed to firmly establish knowledge in the precise sequential stages of the embryonic development in vertebrates, and also recognized that any failure in the stage sequence would lead to malformations of the Nervous System.

All the Nervous System, structures and cells, without exception, are derived from the ectoderm –the neuroectoderm–, the outer layer of the primitive embryo, which under the influence of the underlying notochord, generated by the mesoderm, gives the signal, the neural induction coupled with the molecules Noggin and Chordin, to transform that zone of the ectoderm into the neural plate, actually the skin of the embryo that has thickened. By the third week of gestation in the human embryonic development, due to significant stem cell proliferation, the neural plate grooves in to form the neural groove followed in sequence to form the neural tube. The neural tube closes its open ends –the neuropores– by the fourth week. The rostral end of the neural tube expands to form three swellings, called the primary vesicles. The most rostral vesicle is called the prosencephalon, also called the

forebrain. Shortly afterwards the prosencephalon laterally generates two optic vesicles and two telencephalic vesicles plus the unpaired structure in the middle, the diencephalon. The two telencephalic vesicles constitute the primitive cerebral hemispheres; inside the telencephalon two fluid-filled spaces constitute the lateral ventricles. The wall of the ventricles constitutes the main source of neuroepithelial progenitor cells, the future neurons that will migrate to form the cerebral cortex and other structures in the developing brain.<sup>1</sup>

## THE EMBRYONIC EMERGENCE OF THE CEREBRAL CORTEX

### Delimitation of the zones

At early stages of embryonic development, most neuroepithelial progenitor cells in the ventricular zone undergo symmetric division, this mechanism allows the expansion of the population; meanwhile, at the onset of neurogenesis, neuroepithelial cells take the features of glial cells. They express a broad repertory of markers: BLBP, GLAST, vimentin, tenascin, S100 $\beta$  and, in primates, GFAP.<sup>2</sup>

Radial glial cells are one of the key components of the developing cerebral cortex; radial glia undergo asymmetric division, generating with each division another glial cell and

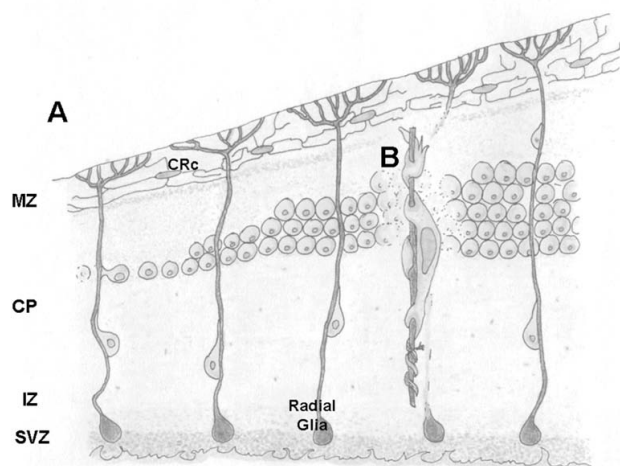
one daughter cell that becomes a neuron. The aforementioned divisions take place in the ventricular zone.<sup>3</sup>

The first postmitotic cells, that underwent asymmetric division, migrate in a radial way out of the neuroepithelium to form the first cortical layer or preplate. The subsequent developing cortical plate is formed within the preplate and divides this layer and its neuronal population into a superficial zone, named the marginal zone, and a deep, lower zone called the subplate.<sup>4</sup> As additional waves migrating neurons arrive in the cortical plate, they bypass previously generated neurons to form the cortical layers; hence the deeper layers are the first to form, while the superficial layers are the last, excepting the marginal zone or layer I.<sup>5</sup>

At this time-point, the marginal zone contains at least two types of neurons, named subpial or pioneer cells: large reelin-positive Cajal-Retzius neurons that extend their axons in the marginal zone, and other large, reelin-negative neurons located deeper in the marginal zone.<sup>6</sup>

The extracellular matrix protein reelin serves as a stop signal for migrating neurons, it also controls the formation of cortical layers. Lack of reelin, as in the reeler mutants, causes disorders in cortical lamination.<sup>4,7</sup> Besides the importance of the molecule for the lamination of the cortical structures, the molecular mechanisms underlying its action remain unclear.

Several genetic studies have positioned Reelin, ApoER2, Vldlr (both of them reelin receptors) and Dab1 (an adaptor protein in reelin signaling) into a common signaling pathway that leads to the phosphorylation of Dab1 in migrating neurons.<sup>8</sup>



**Figure 1.** Schematic representation of the subdivisions of the developing cortex. In A, developing cortex showing the principal subdivisions; in MZ is possible to appreciate the terminal process of the radial glia attached to the subpial surface and the most abundant pioneer cells, the CRc. In B, amplified view of a migrating neuron attached to radial glia.

Abbreviations: MZ= marginal zone, CP= cortical plate, IZ= intermediate zone, SVZ= subventricular zone, CRc= Cajal-Retzius cell.

## DIVERSE ORIGIN OF CORTICAL NEURONS

Cortical pyramidal neurons are generated at the germinal zones of the dorsal telencephalon.<sup>9</sup>

Interneurons are generated in the VZ and SVZ of the ventral telencephalon, or subpallium, in the distinct subdivisions of the ganglionic eminence –GE–.<sup>10</sup> The GE is subdivided into three principal progenitor domains: the lateral ganglionic eminence –LGE–, the medial ganglionic eminence –MGE–, and the caudal ganglionic eminence –CGE–.<sup>5</sup>

## TYPES OF MOVEMENT: RADIAL AND TANGENTIAL MIGRATION

Across the embryonic stages, two types of neuronal migration predominate, radial and tangential movement. The first one consists in movement in perpendicular direction to the marginal zone, and a scaffold of radial glial cells guides migrating neurons. The second movement, the tangential type, describes neurons that migrate in parallel to the brain surface, or marginal zone; tangential migration does not depend on radial glia, unlike radial migration.<sup>11</sup>

Both types of movement involve processes such as the extension of a leading neurite, somal translocation, nucleokinesis.<sup>12</sup>

### Radial migration

Radial migration of neurons along radial glial fibers is also termed gliophilic migration. The neurons generated in the dorsal cortex proliferative zones pass through a series of distinct migrational stages characterized by changes in cell shape, direction of movement and speed of migration. The stages of radial migration are four: first, neurons generated at the ventricular zone move radially to the subventricular zone; in the second stage, neurons pause in the intermediate zone-SVZ for as long as 24h and become multipolar. A fraction of neurons pass through a third stage in which they extend a process toward the ventricle (and sometimes they also translocate the cell body toward the ventricle). Once the neurons reach the ventricle, they enter in stage four of migration. Neurons reverse multipolar to bipolar morphology and extend a pia-directed leading process and begin radial migration to the cortical plate.<sup>5</sup>

Correct migration in the cortical plate requires coordination of adhesive and cytoskeletal dynamics between neurons and radial glia and changes in the disposability of extracellular matrix molecules interacting with them.

### Tangential migration

Tangential migration has been shown to be guided by a variety of factors. These factors include semaphorins,

neuropilins, cell adhesion molecules, neuregulins, chemokines and the slit and robo families of attractant and repellent molecules.<sup>5,9</sup>

The polysialylated form of the neural adhesion molecule (PSA-N-CAM), a member of the immunoglobulin superfamily that mediates homo-and heterophilic cell-cell interactions, is important in the migratory process. Mutation of N-CAM in mice results in a small olfactory bulb and the accumulation of olfactory interneuron precursors in the SVZ.<sup>13</sup>

Homeodomain transcription factors are essential in specification and differentiation of proliferating GE progenitors, and in later phases, orchestrate neuronal migration away from the GE.

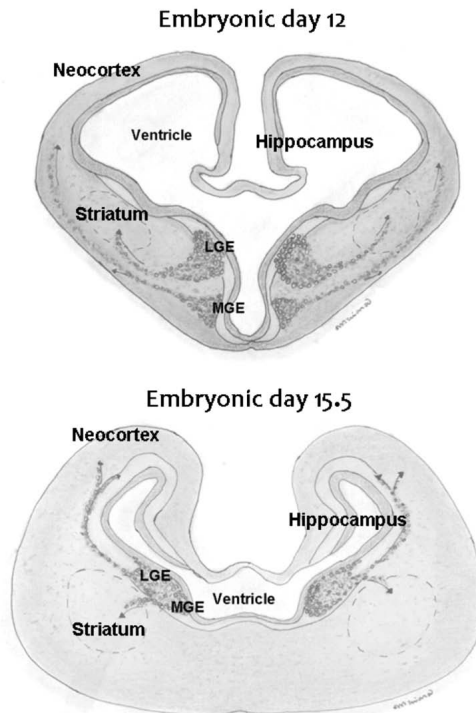
Dlx genes promote GABAergic phenotype and interneuron migration. In Dlx null brains, GE tangentially migrating neurons are blocked in the SVZ. A primary target of DLX genes is another transcription factor, Arx (X-linked aristaless-related homeobox gene); Arx is located on X chromosome and is involved in human neurological disorders including epilepsy, lissencephaly and mental retardation. A Dlx protein binds and activates an Arx enhancer element. Male Arx ko mouse embryos have a smaller olfactory bulb, neocortex and hippocampus due to

a delayed migration and cannot invade the cortical plate. Those results suggest that the Dlx-dependent switch from tangential to radial migration might be mediated by Arx.<sup>14</sup>

The migratory failure in Dlx mutants has been attributed to premature differentiation, associated with the extension of longer and highly branched processes; this feature is shared with Arx mutants. Homeodomain transcription factors appear to control interneuron migration by influencing the expression of cytoskeletal and cell dynamics related genes, like PAK3 (p21-activated serine/threonine kinases), MAP2 (microtubule associated protein2), Tau and GAP43.<sup>9</sup>

Many downstream genes appear to be selectively controlled by either Arx or Dlx proteins, suggesting that these transcription factors may also display distinct activities in subpallial neurons. The LIM (Lin-11, Isl-1, Mec-3) homeodomain transcription factor Lhx6 downregulation results in MGE-derived neurons accumulation at the cortico-striatal boundary; in Dlx and Arx ko, Lhx6 is still expressed by interneurons, suggesting that this transcription factor is not sufficient to trigger a normal migration program. Nkx2.1 is another transcription factor essential to the migration of cortical interneurons. Nkx2.1 is expressed in MGE progenitor domains and is maintained in neurons migration to the striatum but it is downregulated in interneurons targeting the neocortex.<sup>9</sup>

Another influence for both neurogenesis and tangential migration is dopaminergic signaling. Activation of the dopamine D1-like receptors promotes GABA interneuron migration from the basal forebrain to the cerebral cortex, whereas the activation of D2-like receptors decreases it. The activation of D1-like receptors mobilizes cytoplasmic dynein heavy chain and tubulin to cellular process, whereas activation of D2-like receptors produces a condensation of these proteins around the nucleus.<sup>15</sup>



**Figure 2.** Diverse origin of interneuron population. Interneurons are generated in the distinct subdivisions of the ganglionic eminence, the lateral ganglionic eminence –LGE- the birthplace of the interneurons that will populate the neocortex; the medial ganglionic eminence –MGE-, giving rise to interneurons that migrate to the striatum, and the caudal ganglionic eminence –CGE-. The GE also generates neurons that populate other structures such as the septum, olfactory bulb and amygdala.

## MIGRATION MECHANISMS

The migratory cycle of neurons involves leading process dynamics and somal translocation, which involves the movement of perinuclear material, organelles and nucleus.

The leading process of migrating neurons has relatively distinct morphologies in different classes of neurons, probably reflecting their adaptation to specific micro-environments. Thus guidance cues influence the frequency and orientation at which new leading process branches emerge, which allows migrating neurons to rapidly change direction without having to reorient pre-existing branches.<sup>8</sup>

Leading process stability depends on the microtubular array that links the leading edge of the cell with the soma; the microtubule system supports the leading process and also allows the flow of vesicles required for intracellular communication. P600 is a microtubule associated protein



that interacts with the endoplasmic reticulum; knockdown of p600 in cortical pyramidal cells leads to an important reduction in acetylated tubulin and an almost complete loss of the endoplasmic reticulum in the leading process, conferring a wavy appearance and disrupting migration.<sup>8</sup>

In animal cells, the centrosome is a microtubule-organizing center that controls microtubule polymerization, organizing the cytoskeleton. In radially migrating neurons, the centrosome controls the formation of a microtubule array that surrounds the nucleus as a cage and establishes a link between centrioles and nuclear membrane. During radial migration, the forward movement of centrioles leads to deformation of the perinuclear microtubule cage. The elasticity of the cage together with the microtubule associated motor proteins cooperates in pulling the nucleus.<sup>13</sup>

### Nucleokinesis

While the leading process determines the direction in which the neuron moves, the effective neuronal migration is only completed by the subsequent translocation of the cell soma. Soma translocation occurs in the following way: first cytoplasmic dilatation appears in the proximal part of the leading process, then the centrosome and the Golgi apparatus moves toward it; finally the nucleus advances forward and invades the cytoplasmic dilatation.<sup>8</sup>

The movement of both the centrosome and the nucleus is dependent on the integrity of a microtubules network with different post-transcriptional modifications. Most of the microtubules surrounding the nucleus are tyrosinated, which make them extremely dynamic; by contrast, microtubules at the anterior pole of the nucleus, near the centrosome, are acetylated and therefore more stable. It has been hypothesized that the microtubule array surrounding the nucleus is connected with the centrosome, which is the main microtubule-organizing center. In addition, several proteins involved in cell polarity have been described to play central roles in coordinating the movement of the centrosome and the nucleus in each migratory cycle.<sup>8</sup>

Another type of factors that need to be revised here are the core-cell cycle regulators. The cell cycle coordination requires both positive and negative regulators. Cip/Kip and INK4 CKIs are negative regulators. In addition to their roles as CKIs, Cip/Kip proteins regulate cell motility and migration by facilitating actin cytoskeleton rearrangement. Cip/Kip proteins promote cell motility and migration by inhibiting the Rho signaling pathway. Another core-cell cycle regulators that regulate neuronal migration are Rb and E2F transcription factors; Rb's major function in the cell cycle is to sequester and inhibit E2F transcription factors in order to control the timing of the DNA replication. Rb loss of function elicits radial and tangential migration defects.<sup>16</sup>

Finally, neurons reach their final destination, so they need to cancel their migratory program and continue their

differentiation. The mechanisms through which neurons detect that they have reached their target position remain elusive. It has been suggested that early patterns of activity generated in the target region could be influencing this process. Ca<sup>2+</sup> influx is one proposed mechanism for halting migration.<sup>8</sup>

## COROLLARY

The central nervous development could be compared with a symphony, a complex molecular symphony, in which the precise participation in time and space of each marker is crucial for the harmonic evolution of the movements. For example, core cell cycle proteins provide the *tempo* and establish the duration of each movement; and the tonal features are given by the extracellular markers.

The neuronal migration is one of the most beautiful and dynamic process in the Nervous System, and because of its complexity, it is vulnerable to a broad range of disruptive events such as mutations, infections and chemical insults.

Learning about the molecular interactions that regulate embryonic neuronal migration could help us to understand more than the genesis of severe cortical malformations; it is a promissory way to aboard stem cell investigation in neurodegeneration and neuronal plasticity.

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