Multi-author Review

Commitment and migration of young neurons in the vertebrate brain

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Introduction

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The way in which the CNS is put together predisposes and constrains brain function. The genesis of neurons is pivotal to brain development; the temporal and spatial sequence in which neurons are laid down determines what types of neural circuits can form. Modifying the time and site of neurogenesis may be a common evolutionary practice to bring about changes in animal behavior during phylogeny.

Cells that divide to generate postmitotic young neurons are not distributed throughout the brain. Instead, neuronal precursors remain segregated in neurogenic regions usually within the ventricular zone (VZ), situated next to the brain ventricles. This could be a consequence of a not uncommon developmental paradigm: The commitment of neuroepithelial cells may happen before the three-dimensional structure of the brain has developed. As a consequence, neuronal precursors may end up segregated (e.g. in the VZ) far from the site of final neuronal residence. Migration is an obligatory step to move brain cells (and many other cells in the body, e.g. primordial germ cells, neural crest cells) from their site of birth to their site of work. Hence, commitment and migration may be causally interrelated.

The VZ holds clues to much of brain development; within this region are cell lineages from which most brain cells derive. Also it is here that migration begins and where some of the cell processes that guide this migration originate. The VZ can be traced during early development to a homogeneous, rapidly dividing population of elongated cells that form the palisade of the primitive neuroepithelium. During this early stage, neuroepithelial cells in interphase contact both the external limitans membrane and the inner ventricular wall. As development progresses the cell bodies from these stem cells remain compacted next to the ventricular cavity forming the VZ. This epithelium becomes separated from the surface of the brain by postmitotic cells that migrate away from the VZ and by incoming afferents.

Young neurons are masters of cell navigation; born within the VZ, they invade adjacent parenchyma and migrate away from the ventricle to find appropriate sites for differentiation. Depending on the size of the brain, young neurons may travel only 50 μm, or as far as several millimeters. But navigation does not end there: Axonal processes often engage in long journeys to find other target neurons and establish synaptic contacts, sometimes while the cell body is still migrating. Thus, the same cell that moves its cytoplasm and nucleus to the site of differentiation also extends long processes that semi-autonomously find the correct path to contact other cells.

This series of reviews collectively explores a number of basic questions: What guides the migration of young neurons and what mechanisms explain their movement? What initiates and guides neurite extensions and how do axons grow? How does neuronal diversity arise and when do young neurons become committed to a functional phenotype? How do the timing and pattern of neuronal birth and migration determine cytoarchitectonic features of the CNS?

In the first article of the series, Rakic [10] reviews the development of major concepts concerning neuronal migration. Two main orientation patterns of neuronal migration have emerged, radially, away from the VZ and tangentially parallel to the brain surface. The paths followed by migrating young neurons are processes of other cells. Radial glial processes guide radial migration, and axons from preexisting neurons guide tangential migration. Rakic's work suggests that selective molecular adhesion is used by neurons to cling onto the right path: 'gliophilic' along glia and 'neurophilic' along neurons.

Many radial fibers originating in the VZ of the mammalian cortex span the entire thickness of the brain. Consecutive generations of young neurons use these radial fibers as guides for migration into the cortical plate. Neurons in the deeper layers are born and migrate before neurons in more superficial layers. Rakic [10] proposes that postmitotic young neurons have some positional information for prospective brain structures that generates a protomap of the cortex at the level of the VZ. Neuronal migration enables distribution of neurons into appropriate lamina and/or area where they can interact with various inputs to produce the fine synaptic connectivity and architecture. Defects of neuronal migration may be the cause of many congenital brain disorders.

Two related issues emerge, one mechanistic on how neuronal migration happens, and the other, having to do

with how the patterns of neuronal migration influence brain histogenesis.

Mechanism of neuronal migration

The second and third articles by Chuong [2] and Liesi [7] (see also Hatten and Mason [5]) explore possible molecular mechanisms for neuronal migration. Both membrane-anchored cell adhesion molecules and extracellular matrix proteins have been implicated. However the picture that emerges is complex and controversial. Glial laminin [7] supports neurite extension and it might also have a role in neuronal migration, but this is not yet clear. Similarly, antibodies against certain cell adhesion proteins inhibit neuronal migration in some assays but not in others [2, 5]. Young neurons must adhere to the guiding path but not so strongly that they are unable to move. This is probably a multi-molecular process which includes adhesion molecules, adhesion inhibitors, matrix components and proteolytic enzymes. The mechanism remains a puzzle.

Much can be learned from the direct observation of neuronal migration under the microscope. The fourth review by Hatten and Mason [5] illustrates how isolated young neurons, confronted in vitro with glial processes, migrate along these fibers. The site of neuronal propulsion seems to be directly under the cell body where the migrating young neuron contacts the guiding fiber. This system affords direct experimental inquiry of neuronal migration 'in the dish'. Recombination experiments using young neurons from one region of the brain and glial cells (guides) from another, suggest that the guiding role of the glial fiber is non-specific and passive. Similarly, wild type neurons migrate normally along glia from *weaver* mice (a mutation affecting neuronal migration), suggesting that this mutation affects young neurons and not the guiding capacity of glia.

The mechanism that determines where young neurons stop their migration for final differentiation is of obvious relevance for all histogenetic processes described in this series. Young migrating neurons may recognize the site of differentiation by interpreting positional information and/or by interacting with other cells within the appropriate site. The interaction with other neurons (e.g. within a layer in the cortex [8]) at the site of differentiation, may signal the young migrating neuron, 'you have arrived'. This signal could be obligatory since neurons within the target region probably share functional and adhesive properties that could derail the young neuron from its guiding process. Axons and growth cones from other neurons may also play a role in terminating migration: Neurons migrating in vitro stop moving when contacted by neuronal processes [5]. Axonal afferents in vivo contact young migrating neurons and may induce them to stop [5]. Young neurons may use this information to end migration and start differentiation. However, in some cases young neurons are contacted by afferents before the end of their migration [5]. The mechanism that regulates the cessation of migration remains unclear and may involve a hierarchy of signals, contact with neurites being one of them.

It will be of interest to study in vitro the migration of neurons that use neuronal fibers as guides instead of glial cells; the pattern of movement and the response of these 'neurophilic' young migrating neurons to afferent axons may be very different from that of 'gliophilic' young neurons. Switching from 'gliophilic' to 'neurophilic' migration may be a common practice for many neurons [4, 10]; on this transition may hinge our understanding of the histogenesis of many brain nuclei (see below).

Perhaps we are most ignorant about the actual mechanism of neuronal propulsion. This is, however, only true for the actual displacement of the cell body. Thanks to in vitro model systems, we have learned much about the growth of neurites. In the fifth article Devoto [3] reviews how neurite extension starts and what cellular components mediate cytoplasm extrusion and membrane addition. We may think of neurite extension as a process of propulsion and pathfinding in which these two properties are closely interrelated. Observations on growth cone movement may inspire plausible mechanisms for neuronal propulsion; after all the growth cone is part of the neuron. However, the movement of the entire cell is significantly different from the extension of a slender cytoplasmic branch. While growth cones stride on the surface of properly coated tissue culture dishes, no simple artificial substrate is yet available for the migration of young neurons; young neurons prefer the surface of other cells, in particular thin cylindrical processes. The migration of neurons probably depends on complex interactions between the guiding cell process and the neuron.

Many of the in vitro experiments that revealed mechanisms of growth cone migration [3] can now be tried with young neurons. In vitro systems [5] will soon uncover the machinery inside and outside the young neuron that supports migration. This may hint at possible sites of regulation and clarify the role of some of the proteins that have been implicated in neuronal migration.

Neuronal migration and brain histogenesis

The patterns of neuronal migration are fundamental to our understanding of brain morphogenesis. Neocortex in mammals has been a favorite site for the study of neuronal migration and commitment. The orderly production of neurons in the different laminae has persuaded investigators to explore cortical histogenesis. How do neurons know which lamina they belong to and what path to follow? Heterochronic transplantation experiments reviewed by McConnell [8] in the sixth article, suggest that some neurons become committed to a certain layer while in the VZ, probably during their last cell cycle. Some of the grafted VZ cells that may undergo mitosis after transplantation retain considerable plastici-

ty and can generate neurons to fit the developmental timing of the host.

The radial unit hypothesis supports the notion that the VZ already contains a histogenetic plan for the neocortex[9,10]. The radial alignment imposed by glial fibers between the VZ and the cortical plate suggests that proliferative units in the VZ may be the anlage of presumptive cortical columns. In the strictest sense, this hypothesis predicts that progeny from single stem cells in the VZ should end up radially confined to one column in the cortical plate. The descendants of single proliferative cells in the VZ can now be followed with retroviral markers. The seventh article by Gray, Leber and Sanes[4], and the eighth by Walsh and Cepko[14], review lineage tracing experiments using retroviruses. This new technique allows the inquiry into basic concepts in neurobiology.

Some parts of the neocortex, optic tectum and retina seem to adhere to radial histogenesis; however some neurons derived from single clones in the cortex show considerable scattering[14]. The degree of dispersal of single clones varies between these radial structures[4,14] and this may point to interesting regional and species differences. Nevertheless, protomaps[10] in the VZ could partially explain some of the structural features of these CNS regions. In other non-radial brain structures, young neurons use radial migration for just part of the journey. These cells detach from the radial path and follow tangential cues[1,4]. For still other young neurons, radial fibers are alien paths and instead these cells use axons as their guides[10,12].

The histogenesis of different brain structures uses radial and/or tangential migration to different degrees. It is interesting that radial histogenesis is better represented in laminated regions of the brain. Radial fibers may play an important role in the specification of this type of brain tissue. Histogenesis of non-laminated brain parenchyma (e.g. basal ganglia, most of the bird telencephalon, spinal cord or pontine nuclei) may follow a different strategy; the initial radial migration that moves young neurons away from the VZ, is followed by tangential migration that brings the young neuron to its final site of residence[1,4,10]. In this case, radial alignment is lost and neurons that share similar functional specification may group together. These neuronal collectives or nuclei make up much of the volume of the brain, especially in vertebrates with less cortex. It is not known how neurons aggregate to form functional brain nuclei after losing their initial radial plan. This is of basic importance to the understanding of brain development.

Retroviruses used to trace the descendants of single VZ cells, have recently also proven invaluable in the unraveling of lineage relationships between the different cell types of the brain. This problem has engaged the minds of developmental neurobiologists for more than a century and is still unresolved and a matter of considerable debate. The belief that glial cells and neurons originate from separate pools of stem cells is deeply ingrained in the thinking of how the brain develops[6]. However, recent findings reviewed by Walsh and Cepko[14], and Gray, Leber and Sanes[4] suggest that neurons and glia may be closely related. Not only can a single VZ cell generate many different neuronal types (e.g. for the different optic tectum laminae[4]), but also glial cells. Thus, neurons and glia in some regions of the developing brain are cousins if not sisters.

The genesis of neurons is generally considered confined to development. In this case the modification of the functional output of the CNS after neurogenesis would depend on a prelaid framework of neurons. This temporal restriction on neurogenesis may impose limits on brain plasticity and repair. Birth, migration and differentiation of young neurons were thought to be typical of the developing brain and therefore to occur in a milieu specialized to support this function. As discussed above, the migration of young neurons often depends on a complex scaffolding typical of embryonic or fetal brain. However, a large group of vertebrates produce and replace neurons in adulthood.

Many birds and poikilotherms continue to produce new neurons into adulthood. A sizable region of the adult avian brain (the telencephalon) is serviced by new neurons. Article number nine of this series (Alvarez-Buylla[1]), reviews recent findings that suggest how birds do this. Adult birds retain discrete neurogenic centers on the walls of the lateral ventricle from where young neurons migrate into most of the telencephalon. Radial cells guide the initial dispersal but tangential migration scatters young neurons to regions far from the radial path. Given the tight schedule with which the different regions of the bird telencephalon are laid down during development[13], it is surprising that young neurons continue to be added in adulthood without much respect for cytoarchitectonic boundaries. Birth, migration and differentiation occur throughout much of the non-growing, functioning adult forebrain.

The liaison between commitment and migration is best illustrated in the tenth and last review of the series by Schwanzel-Fukuda and Pfaff. Despite the remarkable capacity of the CNS to generate a multitude of neuronal types, it imports some strategic cells from outside the brain. This is the case for luteinizing hormone-releasing hormone (LHRH) neurons that begin their differentiation in the olfactory placode, far from the site in the hypothalamus where their critical function is required. Following the route of the terminalis nerve, these cells invade the olfactory bulb to then migrate into the basal forebrain. Changes in the form of the head and brain over evolutionary time may have separated structures and cells that were originally neighbors and functionally linked. However neuronal birth sites may have remained fixed, so ontogeny had to solve the problem of how to bring together functionally related cells that were produced in segregated parts of the body. Neuronal migration was the solution. A recurrent theme in this series is

nature vs nurture: Is it the brain microenvironment or genetic predetermination that tells migrating young neurons where to go and what to become? A considerable degree of plasticity in the neuron's response to local cues explains some of the findings described in this multi-author review. However genetic restrictions are also suggested, and it is probably the unique proportion of the combination of both that shapes the different regions of the CNS.

As this century comes to a close – just as at the end of last century – developmental neurobiology is living a period of tremendous excitement. Ever since Ramón y Cajal showed that neurons were independent circuit elements, the genesis of these cells has remained the focus of much of our attention. Cajal recognized the relevance of this problem and described in extraordinary detail the transformation of the VZ postmitotic young neuron (Cajal's apolar neuroblast) into a migrating young neuron (bipolar neuroblast) and its differentiation into a mature neuron [11]. Today we can better explain where neurons come from and how committed they are. We know about some of the tracts that steer their migration and regulate their targeting. The understanding of these processes brings us closer to explaining CNS assembly and how brain function comes about.

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Principles of neural cell migration

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Summary. A basic property of immature neurons is their ability to change position from the place of their final mitotic division in proliferative centers of the developing brain to the specific positions they will occupy in a given structure of the adult nervous system. Proper acquisition of neuron position, attained through the process of active migration, ultimately affects a cell's morphology, synaptic connectivity and function. Although various classes of neurons may use different molecular cues to guide their migration to distant structures, a surface-mediated interaction between neighboring cells is considered essential for all types of migration. Disturbance of this cell-cell interaction may be important in several congenital and/or acquired brain abnormalities. The present article considers the basic mechanisms and principles of neuronal cell migration in the mammalian central nervous system.

Key words. Mammalian nervous system; neuron migration; neuron position; molecular cues; cell-cell interaction; radial migration; gliophilic cells; neurophilic cells; biphilic cells.

Immature nerve cells possess a remarkable capacity to move before they assume their final position and establish permanent synaptic relationships. In fact, the great majority of neurons in the developing vertebrate nervous system are generated in sites that are significantly different from those in which they reside in adult brain. The intervening process, termed migration, denotes the displacement of a neuronal cell body from its last cell division in the proliferative zone to its final destination in the mature brain [40, 41]. In many laminated structures of the mammalian brain, such as the cerebral or the cerebellar cortex, later generated neurons must pass by the early generated ones. Therefore, neuronal cell migration may be considered a 'biological necessity' in that it enables