Fondation Fyssen
1993 International Prize
Pasko Rakic

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Mr. Minister of Higher Education and of Research
Mrs. United States Ambassador to Paris,
Madame President,
Members of the Administrative Council and Scientific Council,
My Good Friends and Colleagues,
Ladies and Gentlemen,

Introduction

I am both honored and happy to be chosen by the selecting committee to receive 1993 International Prize of the Fyssen Fondation. It is, of course, rewarding for any scientist when the years of effort are recognized by their colleagues or even more if they prove to be useful to society.

There are, however, several reasons that make me especially happy to receive the Fyssen Prize:

(1) First, as I understand it, the main mission of this Fondation is to foster research on the evolutionary most advanced part of the brain, the neocortex, that underlies the highest cognitive functions and makes us different from any other species. To be recognized in this field seems to have a particular significance because it is the most difficult subject to study.

(2) To the best of my knowledge, this is the first Fyssen Prize given for studies on the development of the cerebral cortex. Thus, I feel that this occasion also recognizes the contributions of my co-workers in this field, without whom progress would not be possible.

(3) The third reason why I appreciate the Fyssen Prize, is that it is given in this wonderful city where I have many friends, and where a stay of even a few days is a reward in itself.

I have focused my research mostly on the development of the central nervous system in mammals, with particular emphasis on the primate cerebral cortex. I selected to concentrate on the monkey brain because the structural similarity of the cerebral cortex in this species and humans suggests the involvement of similar cellular and molecular mechanisms in its development. When I entered the world of research many scientists were convinced that the mechanisms of development, that is, how the brain is put together by genes, starting from individual molecules to orderly neuronal assemblies, will be figured out well before we understand how it works. Although I was not particularly looking for something easy to do, in the early 70's I, indeed, believed that the mysteries of brain development would be solved in my lifetime. However, the more I learned about this subject, the more humble I became. Haldane said that "the universe is not only queerer than we suppose, but queerer than we can suppose" .To paraphrase Haldane, I eventually began to recognize that the development of the nervous system is not only more complicated than we have imagined, but even more complicated than we can imagine.

The fact that many secrets of development are still illusive is not disappointing. The discovery of each step, and unraveling the miraculous world of increasing complexity, even without fully understanding it, has proved to be great pleasure. Indeed, we have witnessed a fascinating array of cellular and molecular events. I will share with you some of the principles of cortical development that have emerged from this work and that may have relevance for the emergence of higher brain functions.(which make us human).

I wished very much to give my acceptance talk in French. However it proved to be rather difficult for me to learn a third foreign language at my age, so I will force you to listen to the remainder of my talk in English.

Scientific Presentation

The topic of the Fyssen Prize was Evolution and Cognition, and I am glad that the Scientific Committee thought that my research on the development of the monkey cerebral cortex has relevance to the understanding of the evolutionary ascent of human intellectual and cognitive abilities. Indeed, since the common ancestors of humans and other primates are extinct, ontogenetic development in living primates may be the only way to approach the evolutionary process at the genetic, molecular or cellular levels. Any permanent feature that is introduced during evolution must be hereditary, and, therefore, the secret of what and how this occurred millions of years ago is still preserved and may be deduced from the cellular events in ontogenetic development. So, when I study the embryonic brain I feel like an archeologist who digs through the layers of the long ago buried past. Every small element, every minor detail, may give us some important and unexpected clue. Therefore, I will give you a short account of selected findings from my laboratory and some of my thoughts on the development of the primate neocortex that hopefully have some relevance to the evolutionary process behind our cognitive and intellectual abilities.

Cortical Neurons are Generated Prenatally

Now it seems to be generally accepted that all cortical neurons in primates are generated prenatally, but it was not always so because there were no adequate methods to address this issue. In the early 70's we used the method of labeling DNA replication to show that, in monkey, and, by implication, also in human, the genesis of all cortical neurons occurs during the middle period of gestation and that no neocortical neuron is produced during the remainder of gestation or at any time during the life span of these primates. Although the same experimental approach cannot be applied to human postmortem material, we have used various cytological criteria, including supravital DNA synthesis in living slices of fetal cerebrum, to estimate that the production of cortical

neurons in human lasts between 6th and 17th gestational week. Therefore, the upper limit in the number of cortical neurons is set up in the wombs of our mothers.

Neurons Originate Near Cerebral Ventricle and Migrate to the Cortex

We learned that cortical neurons do not originate in the cortex itself. Use of the same technique of DNA labeling revealed that neurons destined for the neocortex are produced exclusively in the proliferative zone lining the cerebral ventricle. After dividing for the last time, immature neurons begin to migrate to their prespecified areal and laminar positions in the cortex. In 1972 we proposed that a majority of postmitotic neurons find their way to the proper position in the cortex by following elongated shafts of the fetal radial glial cells. These non-neuronal cells stop transiently to divide and stretch their elongated processes across the developing cerebral wall during the stage of neuronal migration, and eventually transform to other glial cell types, or disappear, after birth.

Molecular Mechanisms of Migration are Now Being Unraveled

With the advent of new methods in molecular biology over the past decade, several candidates for recognition and adhesion molecules situated on the neuronal or glial surface have been discovered and are currently being characterized and tested in our, as well as in several other, laboratories. We now recognize that several types of complimentary molecules may be involved, one type situated on glial and the other on neuronal surface. Interaction of such molecules allows recognition and selection of the migratory pathway. We also found that membrane bound ion channels regulate the influx of calcium ions that are essential for cell motility and its morphogenetic transformation. The proper concentration of these ions is required by cytoskeletal and contractile molecules to propel cells across their journey to the distant, final destinations in the primate cerebral cortex. Understanding of the molecular mechanisms of neuronal movement in the fetal brain have helped to explain the pathogenesis of several genetic or

environmental factors involved in previously inexplicable conditions, such as childhood epilepsy, mental retardation or developmental dyslexia.

Development of the Transient "Waiting Compartment"

The developing cerebral wall consists of several cellular layers, also called embryonic zones, that do not exist in the mature cerebrum. Although most of these zones were described by Wilhelm His at the turn of century, the subplate zone has been recognized only recently. This zone consists of early-generated neurons situated below the developing cortical plate in the prospective white matter. It is, perhaps, not accidental that this zone has been discovered in primates, since it has expanded during evolution of the cerebral cortex, ostensibly to subserve the increasing number of its associative connections in higher primates. We suggested that the subplate zone may serve as a waiting compartment where various afferents generated ahead of their cortical targets interact and compete before invading the cortex. After completing their developmental function, most subplate cells degenerate and only a vestige remains after birth.

Determination of Neuronal Phenotypes

Initially all neuronal progenitors divide symmetrically, that is. they produce new sets of equal progenitors during each division, increasing their number exponentially. However, eventually the progenitors begin to generate dissimilar daughters, one remaining as a stem cell and the other becoming postmitotic neuron. Use of a variety of methods revealed that the proliferative ventricular zone produced multiple cell phenotypes (i.e., glial cell, pyramidal, or stellate neurons). Recent use of RNA retrovirus-mediated gene transfer and transgenic animals enables marking permanently the progeny of dividing cells. Use of this approach confirmed that a majority of clonally-related neurons in the cortex become deployed in a form of radial columns and gradually become restricted in the repertoire of possible fates. Recent analysis using retroviral gene transfer indicates that basic neuronal classes, e.g., local circuit neurons or projection neurons, may

be clonally related. These studies collectively indicate that the range of morphologies and patterns of synaptic contacts that cortical neurons eventually form may be specified in large measure before reaching their final positions.

Cortical Neurons are Arranged in an Inside-out Order

In general, earlier-generated neurons destined for the cerebral cortex occupy deeper positions, and those arriving later have to pass by them to settle more superficially. This spatial relation among neurons with different birthdays, called the "inside-out" gradient of neurogenesis, was suspected already by classical anatomists, but has been proven only by the use of the labeling of DNA replication. We found that the inside-out gradient of neurogenesis is particularly prominent in primates where each injection of [^{3}H]thymidine labels a highly selective sample of cortical neurons. It is not clear why this inside-out order of genesis is essential for cortical development, except that it perhaps enables later-generated neurons to communicate with the earlier generated at the time they pass by each other. This, actually, makes sense since it is generally agreed that the deeper laying neurons and their connections are phylogenetically older, while the more superficial neurons, which form a bulk of the corticocortical connections, are evolutionary younger.

Radial Unit Hypothesis of Cortical Development and Evolution

Eventually, postmitotic cells form a morphologically identifiable stack of radially-deployed neurons in the cortex termed "ontogenetic" or "embryonic" columns. Prominence of these columns in primate embryos led us to propose the radial unit hypothesis of cortical development and evolution. This hypothesis predicts that the ventricular zone produces postmitotic neurons that are constrained in their migratory behavior, or otherwise prevented from randomly mixing, while on their way to the cortex. This radial organization has recently been confirmed by the use of transgenic animals in which clonally-related neurons are labeled by neutral, but histochemically detectable

markers. We reasoned that, if the ventricular proliferative mosaic is translated via the radial glial scaffolding to the expanding cortical plate, then the variability in the number of radial units among species and individuals is determined in the ventricular zone et early embryonic stages. An array of interrelated columns, or radially-organized modules of neurons in the adult neocortex, may be a reflection of their developmental history. It was originally shown by Mountcastle, one of the earlier recipients of the Fyssen Prize, that neurons situated within a single column in the somatosensory cortex are responsive to a specific modality and receptive field of stimulation. A similar anatomical and functional columnar organization was found in association cortices by Goldman-Rakic, who received this Prize in 1990. The relatively constant size of columnar afferent terminal fields among species with vastly different sizes of cerebral surface area supports the idea that the cortex expands during evolution by the addition of radial units rather than by enlargement of modules themselves.

Determinants of Overall Cortical Size

The expansion of the cortical surface during evolution is not associated with a corresponding increase in cortical thickness. Since proliferation in the ventricular zone initially proceeds by symmetrical division, an additional round of mitotic divisions at that time doubles the number of proliferative units and, consequently, doubles the number of radial ontogenetic columns in the cortex. Therefore, even a short prolongation of this phase produces a large increase in the cortical surface by an exponential increase in the number of proliferative units. In contrast, after corticogenesis begins, an extra round of asymmetrical divisions adds only a single cell per column and thus has a relatively small effect on the overall thickness of the cortex . Our working hypothesis is that one set of regulatory genes that operates at early developmental stages could determine the number of mitotic divisions at the ventricular surface, and thereby the size of the cortical surface. Therefore, only one extra round of symmetrical cell divisions occurring at the early stage can double the number of radial units and, therefore, double the surface of the cortex.

Another set of genes that becomes activated at later stages could control the identity of various neuronal phenotypes produced within the proliferative units, generating variations in the composition of ontogenetic columns. It can be expected that regulatory genes similar to the those found in the fruit fly operate throughout vertebrate evolution, including the mammalian telencephalon; and several candidates are currently being tested.

The Protomap Hypothesis of Cytoarchitectonic Diversity

The prominent feature of the cerebral cortex is its parcellation into cytoarchitectonic areas that underlie motor coordination or specific sensory modalities, and even complex cognitive functions such as language or facial recognition. At present, it is not known how individual and species-specific cytoarchitectonic areas have emerged. Our working model, formulated in a *protomap hypothesis*, suggests that cortical areas emerge through synergistic, interdependent interactions between developmental programs intrinsic to cortical neurons and extrinsic signals supplied by specific input from subcortical structures. According to this hypothesis, the neurons of the embryonic cortical plate—indeed even the proliferative ventricular zone where they originate—set up a primordial species-specific cortical map of areas that preferentially attract appropriate afferents.

According to this model, the *protomap* in the cerebrum provides only a potential and set of individual and species-specific biological constraints. The position of interareal borders, the size of the cytoarchitectonic areas, and detailed molecular, cellular, and synaptic characteristics of the adult cerebral cortex are achieved through a cascade of reciprocal interactions between cortical cells and afferents arriving from a variety of extracortical sources. For example, we found that the size of the visual cortex is regulated by afferents originating from a single thalamic nucleus. The concept of *protomap*, therefore, involves both intrinsic and extrinsic regulation of cortical parcellation. This

model also assumes the initial presence of a larger number of participating neurons and their connections from which the final pattern of the cytoarchitectonic map has to be carved.

Overproduction of Cortical Neurons and Their Connections

Studies in the rhesus monkey show that both neurons and their axons are overproduced in the cerebral cortex during well-delineated stages of development. For example, there are about 40% more neurons in the monkey visual cortex during the second half of pregnancy than in the adult. Furthermore, the newborn monkey has almost 200 million callosal axons compared to less than 50 million in adult. The axons are lost at the rate of about 8 million per day or 50 per second during the first three weeks after birth. Thereafter, they are lost at an estimated rate of half a million per day or 5 per second until the adult value is reached . Other interhemispheric connections, including anterior and hippocampal commissures ,display a similar period of axonal loss. The functional significance of loss of axons is not fully understood, but the prevailing hypothesis has been that activity-dependent stabilization plays a critical role.

Overproduction of Cortical Synapses

In the rhesus monkey, during the first two to three months of postnatal life synaptic density increases rapidly and reaches a peak which is about two times higher than in adult. The synaptic density well above those found in the adult lasts throughout infancy and adolescence. Dr. Jean-Pierre Bourgeois, from Institut Pasteur, who was visiting my laboratory at Yale, has calculated that the enormous number of about 1.8×10^{11} synapses are lost in the visual cortex of a single cerebral hemisphere during sexual maturation of the macaque monkey. The magnitude of this decline is stunning when expressed as a loss of about 2500 synapses per second during this period. Since other areas, including association cortices, undergo simultaneously comparable synaptic loss, over 30,000 synapses per second are deleted from the entire cortical mantle during monkey

adolescence. In human, in which this period of life lasts about three times longer, but the cortex is ten times larger, the synapses may be lost at an even higher rate.

The decline in synaptic density is due primarily to elimination of excitatory junctions located on dendritic spines, while inhibitory synapses on dendritic shafts remain relatively constant. Our recent results show that the density of major neurotransmitter receptors in the cortex also reaches a maximum level between two and four months of age and then declines to the adult level during the period of sexual maturation. These findings reveal unusual coordination between biochemical and structural differentiation and indicate that these events may be related to maturation of function.

Elimination of Synapses is Regulated by Competitive Interactions

The prolonged phase of postnatal development in primates provides unparalleled opportunity for competitive, activity-driven stabilization among initially supernumerary inter- and intracortical connections. The formation of the final pattern of cortical connections is achieved through dynamic interactions that involve at least two well-defined steps. In the first step, one set of axons project to the target structure guided by prespecified, membrane-bound, or short-lived molecular markers. This initial step occurs without regard to specific location on the individual neurons or their parts. In the second step, which is activity-dependent, connections within a given structure are sorted out and remain on only a selective sets of neurons or their dendrites. Our experimental studies of the formation of binocular vision in fetal primates provides a dramatic example of this biphasic development and support the hypothesis that competitive interactions between two or more populations of neurons play a significant role in the elimination of the axons and segregation of their synapses. These interactions in primates are initiated prenatally and, therefore, in the absence of visual experience.

Relevance to Cognitive Functions

In my research I was motivated by the hope that our findings may have relevance for normal development of the human mind. Some implications are obvious. For example, educational systems which postpone disciplined intellectual learning until later stages in life may not be biologically optimal, as the first 15 years are the most important formative phase as far as synaptic stabilization is concerned. This is the critical age when exceptional musicians or athletes are created, and there is no reason why cognitive skills essential for human intellectual abilities are not set up also at this period. Our data provide a strong biological argument that intensive, targeted intellectual training should start early in childhood.

The studies on developing cerebral cortex have equally compelling implications for understanding hereditary cognitive disorders that occur due to defective cell migration, mismatching of neuronal connections, or an excessive or insufficient rate of synaptic elimination. Finally, the competitive synapse elimination that occurs in childhood enables the sculpting of an extraordinary variety of connectional combinations, thus providing the biological basis of individual diversity and differences in mental abilities and talents. The challenge in the decade to come is to unravel the cellular and molecular mechanisms involved in selective gene transcription and to use the theoretical approaches to study the pattern of synaptic formation. I believe that neural development offers crucial insight into the evolution of the human mind and behavior, and may be central to the formulation of the basic concepts that underlie uniquely human intellectual abilities.