PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Experimental Manipulation of Cerebral Cortical Areas in Primates

Pasko Rakic

Phil. Trans. R. Soc. Lond. B 1991 **331**, 291-294
doi: 10.1098/rstb.1991.0019

Experimental manipulation of cerebral cortical areas in primates

PASKO RAKIC

Yale University School of Medicine, New Haven, CT 06510 U.S.A.

SUMMARY

The developmental mechanisms underlying the subdivision of the neocortex into structurally and functionally distinct areas is central to our understanding of the development of human cognitive capacity and the pathogenesis of congenital disorders of higher brain functions. The protomap hypothesis suggests how the cytoarchitectonic pattern of the cerebral cortex may be generated by a combination of intrinsic and extrinsic influences during embryonic development. Although little is known about the genetic and molecular mechanisms underlying this individual and species-specific diversity of cellular and synaptic architecture, experimental manipulation of development in the primate embryo provides a glimpse into the cascade of cellular events involved in the control of cell numbers, specification of neuronal phenotypes, their apportions into cytoarchitectonic areas, and establishment of area-specific synaptic circuitry.

1. INTRODUCTION

2. MATERIALS AND METHODS

Advances in neurobiological techniques introduced during the past two decades have provided new insight into genetic and epigenetic regulation of cortical parcellation into cytoarchitectonic areas and formation of their synaptic region-specific circuitry. It is becoming clear that interactions between different classes of developing cells may regulate the number, pattern and chemistry of cortical connections. These complex interactions can be analysed by using histochemical and immunocytochemical labelling, *in vitro* binding and *in situ* hybridization using specific cellular markers and probes for cell types, and various neurotransmitters and their receptors. Many of the approaches have been initially tested in reduced preparations such as simple systems, isolated slice preparations and dissociated cell culture, but now can be applied to the developing mammalian cerebral cortex *in vivo*. As a result, considerable progress has been made particularly in the understanding of the development of somatosensory cortex of rodents and the visual cortex of carnivora. However, the present overview is based exclusively on the research programme carried out in my laboratory on the development of normal and experimentally altered neocortex in the rhesus monkey.

During the past two decades we studied four major groups of developmental cellular events in the cerebral neocortex of the rhesus monkey that have relevance to the theme of this meeting: (*a*) time, site of origin, kinetics of proliferation and regulation of cell number in the cortex; (*b*) mechanism of cell migration and acquisition of vertical (layer-specific) and horizontal (area-specific) positions of postmitotic cortical neurons; (*c*) determination of neuronal phenotype as recognized by their molecular (transmitter-receptor) and structural characteristics, and (*d*) epigenetic sequences and competitive interactions involved in the establishment of intrinsic and extrinsic synaptic circuitry of the selected areas of the cerebral neocortex. The basic concepts, critical developmental events, timing and sequences are discussed only as they pertain to the rhesus monkey and, therefore, numerous studies of this subject done on other species will not be reviewed here. However, I would like to emphasize that many of the approaches and ideas offered here probably have been already mentioned in one form or another in the vast literature. My intentions are merely to provide a broad overview based on my own experience in working on non-human primates. The description of material and methods, experimental details, factual data and additional relevant literature can be found in the papers listed in the bibliography.

3. RESULTS AND DISCUSSION

Our initial DNA labelling autoradiographic studies have shown that all cortical neurons in primates are generated near the lateral cerebral ventricle during the middle two months of the 165-day gestational period (Rakic 1974, 1982). Use of electron microscopic and immunocytochemical methods in a series of foetal telencephalons revealed the existence of separate neural and glial cell lines in the proliferative ventricular zone (Levitt *et al.* 1981). Furthermore, we observed that postmitotic neurons migrate from the site of their origin near the lateral ventricular surface to their final locations in the cortical plate situated below the pial surface, guided by surface-mediated interactions along radial glial fibres (Rakic 1972, 1990*a*; Rakic *et al.* 1974). In the cortical plate, successive generations of migrating neurons bypass each other and settle in the

Phil. Trans. R. Soc. Lond. B (1991) **331**, 291–294
Printed in Great Britain

inside-out pattern, as they do in all examined mammalian species so far.

Settling of postmitotic cells into specific layers and appropriate cytoarchitectonic areas of the cerebral cortex can be explained by the radial unit hypothesis (Rakic 1978, 1988*a*). According to this hypothesis, the horizontal position of cells into areas that subserves specific function depends on spatial parameters of neuron origin in the proliferative zone lining the ventricular surface (protomap) while the vertical organization of neurons displayed within each radial columnar unit (cell phenotype and their hierarchy) is affected by the time and sequence of their origin in the proliferative units. The competence for differentiation of neurons into neuronal phenotypes within each column are established before their entrance into the cortical plate, while their radial position depends on their time of arrival.

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 neighbouring cells is considered essential for all types of migration (see, for example, Rakic (1990*a*)). Disturbance of this cell–cell interaction may be important in several congenital or acquired brain abnormalities. I have proposed that one set of controlling genes provide general instructions for individual and species-specific changes in the protomap of the ventricular zone while another set of genes control cell production in the proliferative units (Rakic 1988*a*). These regulatory genes may arrest the cell cycle and trigger transcription of cell class-specific molecules as well as adhesion molecules that lead them to the cortical plate. Therefore, the surface area of the cortex as well as the general cytoarchitectonic pattern of the cerebral cortex in each species and individual may depend on the number of contributing proliferative units. In contrast, the thickness of the cortex in each region reflects on the number of cell divisions generated within the units. In support of this developmental model, experimental and neuropathological data indicate that each step (formation of proliferative units, formation of ontogenetic columns, and formation of cytoarchitectonic areas) can be separately affected by either genetic defects, extrinsic factors or a mixture of both (Rakic 1988*a*, *b*).

The radial unit hypothesis could also provide an insight into the evolution of the neocortical mantle. There is little doubt that the neocortical surface enlarges during phylogenetic development by unequal growth of existing cytoarchitectonic areas and by the addition of new ones. I have suggested that this is accomplished by the addition of ontogenetic columns, which in turn are produced by the proliferative units in the ventricular zone (Rakic 1978, 1988*a*, 1990*b*). The hypothesis consistent with our [^{3}H]thymidine autoradiographic findings is that cells of the proliferative units at the early stages arise mainly by symmetrical cell divisions, while later they produce cortical cells mainly by asymmetrical or stem divisions. The number of units in each individual, therefore, must be determined early, before the onset of neurogenesis by an exponential increase of progenitor cells in that ventricular zone (Rakic 1990*b*). A single additional round of symmetrical cell divisions at the stage of unit formation would, therefore, double the number of progenitors as well as the number of ontogenetic columns that they subsequently produce. Conversely, the number of neurons within the ontogenetic columns in the cortex depends on the rate of cell production by asymmetrical division in the proliferative units at the ventricular zone. An additional round of cell division at this stage would increase the number of neurons within a given ontogenetic column by only one row. Indeed, the number of cells in ontogenetic columns, reflected in the thickness of the cortex, changes relatively little during evolution. It may not be coincidental that the size of the columnated afferent terminal fields in the cortex is relatively constant, even in species with large differences in the cerebral surface (see, for example, Rakic (1991)). Although an increase in the number of ontogenetic columns explains the expansion of the cortical surface as a whole, it does not address the issue of a differential increase in the surface of various cytoarchitectonic areas.

The basic cellular mechanisms, or their combination, could account for differential expansion of cytoarchitectonic fields. According to the fate map hypothesis, the areal specificity of cortical neurons is rigidly determined within the ventricular zone (i.e. the number of proliferative units devoted to each area is fixed). According to this view, the differential increase in the number of units producing area-specific columns can be regulated at early embryonic stages by regulatory genes. Such master genes could, at later stages, control production of neuronal phenotypes within the proliferative units, thereby generating variations on the common neural pattern in ontogenetic columns subserving individual cytoarchitectonic areas. Therefore, regulatory genes expressed within the cortical progenitor cells are assigned to preserve an evolutionary component and provide instruction both for duplication and for changes in the mosaic of proliferative units at the ventricular surface.

An alternative possibility, known as the *tabula rasa* hypothesis, is that the areal positions and modal specificity of neurons are not determined in the proliferative units and that all cells of the cortical plate are pluripotential. According to this hypothesis, the phenotype of cortical neurons and their function are decided at later stages exclusively by the type of input they receive from the periphery via the thalamus. Although this hypothesis provides a logical and attractive explanation for the diversification of cytoarchitectonic areas, it is difficult to reconcile it with a variety of evidence, including the results from our studies of developing cortex in rhesus monkey. Indeed, the developmental sequence of visual pathways indicates that the information from the receptors of the periphery cannot be the sole determinant of cortical areas. For example, it is clear that the basic pattern of geniculocortical connections in the primate brain is present not only before the formation of contacts with

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the retinal receptors (Nishimura & Rakic 1987), but they develop appropriate topography and are maintained in the absence of both eyes from the early embryonic stages (Rakic 1988*a*; Kuljis & Rakic 1990; Rakic & Lidow 1991).

The third, and somewhat more complex mechanism has been formulated in the protomap hypothesis, which suggests that the embryonic telencephalic contains the basic species-specific cortical map, but that such a map can be fully developed only *via* interaction with appropriate input (Rakic 1988*a*). The results from studies of prenatal ablation of selected cortical and subcortical centres by foetal neurosurgery (Rakic 1988*b*; Kuljis & Rakic 1990; Rakic *et al.* 1991) or deletion of specific layers or cell classes by dilating their precursor by using X-irradiation (P. Rakic *et al.*, unpublished data) provides supporting evidence for the protomap hypothesis and indicates that the basic species-specific protomap in the ventricular proliferative zone may be determined during the early embryonic stages. However, it is also clear that the final size of each cytoarchitectonic area depends to a great extent on surface-mediated interactions with various afferents that invade the cortical plate mostly in the second half of gestation (Rakic 1988*a*). For example, downsizing experimentally specific thalamic afferents growing from the lateral geniculate nucleus to the primary visual cortex (area 17 or V1) during midgestation produces not only the diminished V1, but also generates a novel cytoarchitectonic areas within and/or at the periphery of otherwise normal-appearing V1 (Rakic *et al.* 1991). We termed this new area V_x because it differs cytoarchitectonically from the V1 and the adjacent prestriate cortex (area 18 or V2). The portion of developing cortical plate deprived of normal thalamic afferents may, therefore, develop as a new 'hybrid' area consisting of cells that are genetically destined for V1 but have acquired different input–output relations. These results, therefore, suggest that the final neural and synaptic organization of the neocortex depends on both intracellular genetic programmes and intercellular epigenetic regulation.

Ingrowth of the major cortical afferents proceeds by passing through the transient waiting compartment (subplate zone) where axonal terminals interact between themselves as well as with the local subplate cells before entering the developing cortical plate (Rakic 1977; Kostovic & Rakic 1990). Most of the connections in the immature cortex are initially more widespread and overlapping than in the adult monkeys (Rakic 1976, 1977, 1982). Our experiments indicate that sorting out and segregation of afferents into layers and columns depends on competitive interactions that involve selective elimination. Counts of neurons, axons and synapses in the cerebral cortex of developing monkeys show that they are initially overproduced and then selectively eliminated at a precise and predictable time schedule (Rakic *et al.* 1986; Williams *et al.* 1987; LaMantia & Rakic 1990). For example, asymmetric synapses on spines in the sensory, motor and association areas of the neocortex are more numerous during infancy and their number gradually declines towards puberty (Rakic *et al.* 1986; Zecevic & Rakic 1989). In contrast, symmetrical synapses remain relatively constant. We also found that, at least in the striate cortex, premature visual stimulation does not affect the rate of synaptic overproduction, but instead may act predominantly on modifying or eliminating synapses that have been formed (Bourgeois *et al.* 1989). In parallel with these structural changes we also observed overproduction and redistribution of neurotransmitters and their receptors (Lidow *et al.* 1991).

In conclusion, our studies of developing monkey telencephalon show how both intrinsic and extrinsic factors shape cerebral cytoarchitectonic areas. The results suggest that genetic alteration as well as mechanical lesions of distant but synaptically related structures that reduce specific input to the cortex could affect subsequent developmental events and provide the setting for new cell relations, the net outcome of which is a unique cytoarchitectonic map. Furthermore, we suggest the developmental models such as the radial unit hypothesis and the protomap hypotheses can serve as a framework for understanding genetic and cellular mechanisms of cerebral evolution as well as pathogenesis of certain congenital abnormalities of the neocortex. However, it seems to me that the most exciting times are still ahead of us as we begin to unravel genetic and molecular mechanisms involved in each step of cortical development and the process of activity-induced changes of the cerebral cyto- and chemoarchitecture.

This series of studies have been supported by the United States Public Health Service.

REFERENCES

Bourgeois, J.-P., Jastreboff, P. J. & Rakic, P. 1989 Synaptogenesis in visual cortex of normal and preterm monkeys: Evidence for intrinsic regulation of synaptic overproduction. *Proc. natl Acad. Sci. U.S.A.* **86**, 4297–4301.

Kostovic, I. & Rakic, P. 1990 Developmental history of transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *J. comp. Neurol.* **297**, 441–470.

Kuljis, R. O. & Rakic, P. 1990 Hypercolumns in primate visual cortex develop in the absence of cues from photoreceptors. *Proc. natl Acad. Sci. U.S.A.* **87**, 5303–5306.

LaMantia, A. S. & Rakic, P. 1990 Cytological and quantitative characteristics of four cerebral commissures in the rhesus monkey. *J. comp. Neurol.* **291**, 520–537.

Levitt, P., Cooper, M. L. & Rakic, P. 1981 Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: an ultrastructural immunoperoxidase analysis. *J. Neurosci.* **1**, 27–39.

Lidow, M. S., Goldman-Rakic, P. S. & Rakic, P. 1991 Synchronous development of major neurotransmitter receptors in diverse regions of the primate cerebral cortex. (Submitted.)

Nishimura, Y. & Rakic, P. 1987 Development of the rhesus monkey retina: II. A three-dimensional analysis of the sequences of synaptic combinations in the inner plexiform layer. *J. comp. Neurol.* **262**, 290–313.

Rakic, P. 1972 Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. comp. Neurol.* **145**, 61–83.

Rakic, P. 1974 Neurons in the monkey visual cortex: Systematic relation between time of origin and eventual disposition. *Science, Wash.* **183**, 425–427.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Rakic, P. 1976 Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. *Nature Lond.* **261**, 467–471.

Rakic, P. 1977 Prenatal development of the visual system in rhesus monkey. *Phil. Trans. R. Soc. Lond.* B **278**, 245–260.

Rakic, P. 1978 Neuronal migration and contact interaction in primate telencephalon. *Postgrad. Med. J.* **54**, 25–40.

Rakic, P. 1981 Development of visual centers in the primate brain depends on binocular competition before birth. *Science, Wash.* **214**, 928–931.

Rakic, P 1982 The role of neuronal-glial interaction during brain development. In *Neuronal-glial cell interrelationships*. (ed. T. A. Sears), pp. 25–38. Dahlem Konferenzen. Berlin: Springer–Verlag.

Rakic, P. 1985 Limits of neurogenesis in primates. *Science, Wash.* **227**, 154–156.

Rakic, P. 1988*a* Specification of cerebral cortical areas. *Science, Wash.* **241**, 170–176.

Rakic, P. 1988*b* Defects of neuronal migration and pathogenesis of cortical malformations. *Prog. Brain Res.* **73**, 15–37.

Rakic, P. 1990*a* Principles of neuronal cell migration. *Experientia.* **46**, 882–891.

Rakic, P. 1990*b* Critical cellular events during cortical evolution: Radial Unit Hypothesis. In *The neocortex: ontogeny and phylogeny* (ed. B. L. Finley & G. Innocenti). New York: Plenum Press.

Rakic, P. 1991 Radial unit hypothesis of cerebral cortical evolution. *Exp. Brain Res.* (Suppl.) (In the press.)

Rakic, P., Bourgeois, J.-P., Eckenhoff, M. E., Zecevic, N. & Goldman-Rakic, P. S. 1986 Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science, Wash.* **232**, 232–235.

Rakic, P. & Lidow, M. 1991 Distribution and density of neurotransmitter receptors in the absence of retinal input from early embryonic stages. *J. Neurosci.* (Submitted.)

Rakic, P., Stensaas, L. J., Sayre, E. P. & Sidman, R. L. 1974 Computer-aided three-dimensional reconstruction and quantitative analysis of cells from serial electronmicroscopic montages of fetal monkey brain. *Nature, Lond.* **250**, 31–34.

Rakic, P., Suñer, I. & Williams, R. 1991 Novel cytoarchitectonic field induced experimentally in primate striate cortex. *Proc. natl Acad. Sci. U.S.A.* (In the press.)

Williams, R. W., Ryder, K. & Rakic, P. 1987 Emergence of cytoarchitectonic differences between areas 17 and 18 in the developing rhesus monkey. *Abst. Soc. Neurosci.* **13**, 1044.

Zecevic, N. & Rakic, P. 1989 Changes in synaptic density in motor cortex of rhesus monkey during fetal and postnatal life. *Devl. Brain Res.* **50**, 11–32.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES