Int. J. Dev. Biol. 35: 215-230 (1991)

Zagreb research collection of human brains for developmental neurobiologists and clinical neuroscientists

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ABSTRACT The aim of this paper was to offer for the first time a selective and systematic description of the «Zagreb Neuroembryological Collection» of human brains and to illustrate the major results of our research team. Throughout these 16 years of continuous and systematic research, we have applied different techniques for demonstrating the cytoarchitectonics (Nissl staining), neuronal morphology (Golgi impregnation), synaptogenesis (EM analysis), growing pathways (acetylcholinesterase histochemistry) and transmitter-related properties of developing neuronal populations (immunocytochemistry and acetylcholinesterase histochemistry) on several hundred human brains ranging in age from the 5th week post-conception to 90 years. The combination of classical and modern research techniques applied to the constantly growing developmental collection, as well as the continuous evaluation of our data in the light of experimental work in non-human primates, has led to the discovery of an early synaptogenesis within the human cortical anlage and hitherto undescribed transient subplate zone; our results also provided the first comprehensive evidence concerning the timing and pattern of development of afferent fiber systems in the human cortex. All this enabled us to offer a well-documented and coherent reconstruction of major histogenetic events in the human brain. We concluded that structural remodeling and reorganization of the brain, from the transient patterns of the fetal organization through the postnatal phase of transient overproduction of circuitry elements to the final maturation, is the crucial principle of development. Fetal neuronal elements (afferents, synapses and postsynaptic neurons) display transient patterns of laminar, vertical and modular organization and transient cellular interactions and competition in the subplate zone are crucial for the formation of cortical connections. The elucidation of the nature and timing of these histogenetic reorganizational events in the human brain represents the first step towards determining the neurobiological basis of the emergence of behavior, neural functions and cognition in human fetuses, infants and children, which takes place during perinatal and early postnatal life.

KEY WORDS: *human brain, development, collection, cerebral cortex, transient*

Introduction

Many substantial facts about the development of the human brain have been discovered through the study of valuable material from different neuroembryological collections. For example, the careful analysis, by different research groups, of brains from the Yakovlev Collection has resulted in a corpus of data on the development of early as well as later stages of human brain development (see Kretschmann *et al.*, 1982).

The senior author decided to establish the Zagreb Neuroembryological Collection of human brains during his involvement in intensive research carried out in collaboration with Drs. Mark Molliver and Hendrik Van der Loos at The Johns Hopkins University in Baltimore from 1972 to 1974. The combination of classical morphological and descriptive-topographical data on human brain development (His, 1904; Hochstetter, 1919; Economo and Koskinas, 1925) with modern Golgi (Poliakov, 1979) and electron microscope analysis (Molliver *et al.*, 1973; Kostovic and Molliver, 1974) has led to the discovery of an early synaptogenesis within the human cortical anlage as well as to the delineation of the hitherto undescribed and essential compartment of the telencephalic wall and cortical anlage – the subplate zone (Kostovic and Molliver, 1974; Kostovic and Rakic, 1980, 1990). The development of a new experimental approach to the analysis of primate brain development (Rakic and Goldman-Rakic, 1985) resulted in new ideas and concepts about histogenetic events (especially proliferation and

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0214-6282/91/$03.00
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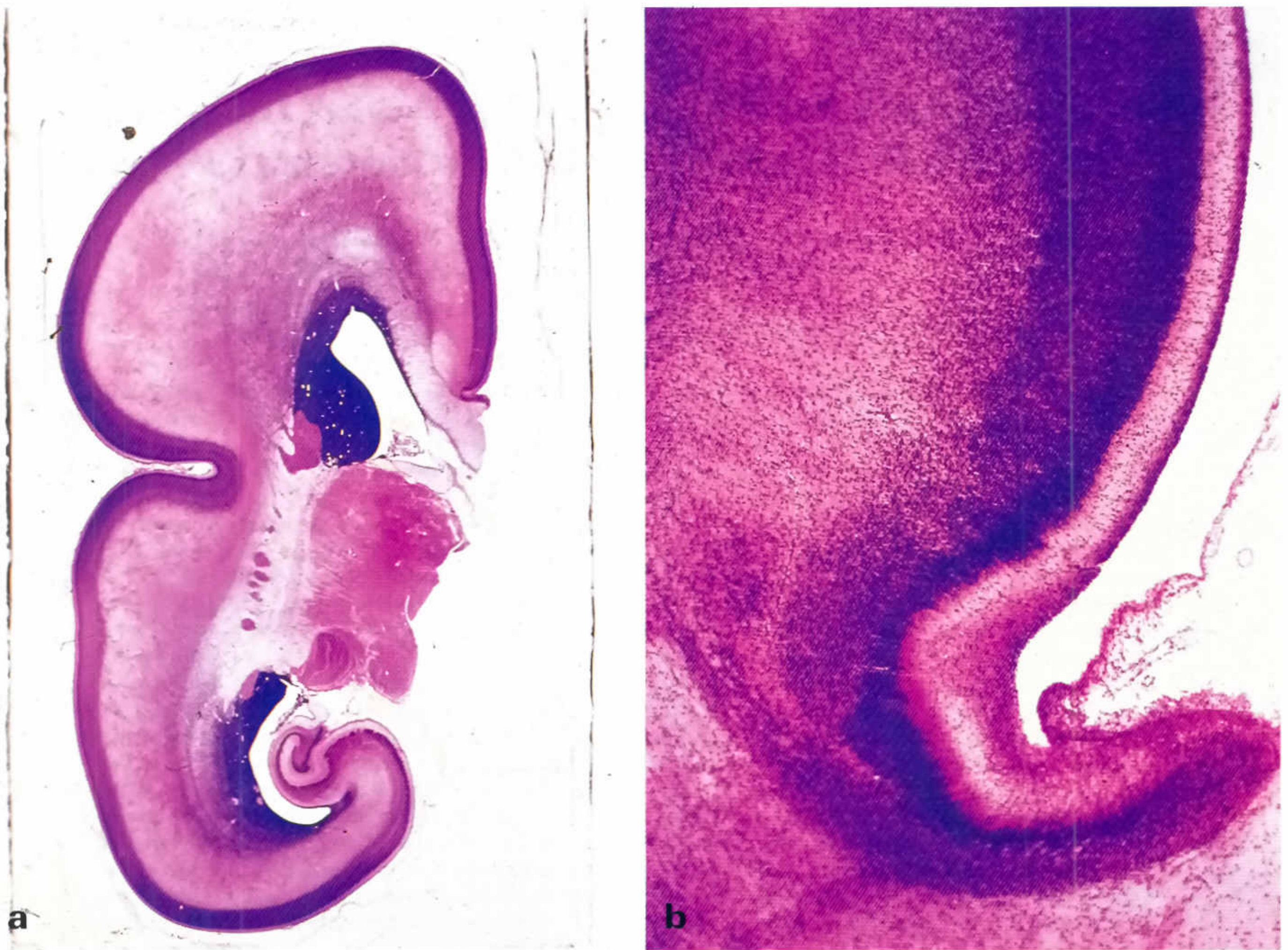

Fig. 1. (A) *Coronal section through the one hemisphere of the developing human telencephalon. Fetus at 24 postovulatory weeks, Nissl staining. Note the cell-dense cortical plate and below it the pale subplate zone. The ventral hemispheric limbus contains the developing hippocampal formation. Magnification x10.* **(B)** *The early differentiation of the dorsal allocortex in a 16-week-old human fetus. Note the differences between archi-, meso- and neocortex. Magnification x40.*

migration) involved in the shaping of the primate brain. This systematic, thorough and imaginative approach of Drs. Pasko Rakic and Patricia S. Goldman-Rakic stimulated the collaboration and collection of comparative data in the human brain (Kostovic and Rakic, 1980, 1984, 1989, 1990; Kostovic and Goldman-Rakic, 1983), realized through the three consecutive US-Yugoslav Joint Board grants sponsored by the National Institutes of Health of the USA. This collaboration enabled us to interpret data on human development in the light of current experimental work in primates, as well as to obtain a permanent experimental evaluation of our results. However, the most important factors for evaluation of our results and conceptions were the access to the constantly growing developmental collection and the discovery of a thick (3-5 mm!) synaptic compartment (subplate zone) in the human fetal cortex.

Throughout these 16 years of continuous and systematic research, we have applied different techniques for demonstrating the cytoarchitectonics (Nissl staining), neuronal morphology (Golgi impregnation), synaptogenesis (E.M. analysis), growing pathways (AChE histochemistry) and transmitter-related properties of developing neuronal populations (immunocytochemistry and AChE histochemistry) on numerous human brains. In this way we have demonstrated that the careful analysis of all spatial and temporal developmental parameters in the closely spaced developmental series can yield a well-documented and coherent reconstruction of major histogenetic events in the human brain (Kostovic, 1975, 1979, 1980, 1981, 1982, 1983a,b,c, 1984a,b, 1985, 1986a,b, 1987, 1989, 1990a,b; Kostovic and Fucic, 1985; Kostovic and Goldman-Rakic, 1983; Kostovic and Kostovic-Knezevic, 1984; Kostovic and Krmpotic-Nemanic, 1975, 1976; Kostovic and Molliver, 1974; Kostovic and Rakic, 1980, 1984, 1989, 1990; Kostovic *et al.*, 1975, 1976, 1978, 1980a,b, 1981, 1985, 1986, 1987a,b, 1988a,b, 1989a,b, 1990, 1991; Kostovic-Knezevic and Kostovic, 1986; Kostovic-Knezevic *et al.*, 1978, 1979, 1987, 1988; Kracun *et al.*, 1983; Krmpotic-Nemanic *et al.*, 1979, 1980a,b, 1981,

1983a,b, 1984, 1987, 1988; Molliver *et al.*, 1973; Mrzljak *et al.*, 1988, 1990; Nikolic and Kostovic, 1986; Seress and Mrzljak, 1987; Stampalija and Kostovic, 1981; Uylings *et al.*, 1987, 1988).

The analysis of the midfetal and late fetal period resulted in our pinpointing the exact timing of synaptogenesis, development of neuronal morphology and the ingrowth of thalamocortical and basal forebrain afferents in the human fetal telencephalon.

The conductance of these long-term projects was greatly facilitated by successful and long-lasting collaboration with different clinical and university hospitals in Zagreb, but also in other parts of the Republic of Croatia or Yugoslavia (see Acknowledgments). This collaboration has led to the establishment of the most versatile collection of neuroembryological material, as judged by several experts and international granting institutions. This collection has been increasing steadily from 1974 (when the senior investigator returned to Zagreb from the USA) and now consists of more than six hundred normal and pathological human brains covering the whole life span (from the fifth week post-conception to 83 years). However, throughout all this time, it has served as a part of long-term and quite specifically aimed research projects, and is therefore incomplete with respect to some brain regions (especially the brain stem and spinal cord). Therefore, it is more a developmental-neuroanatomical research collection than a true neuroembryological collection (like, *e.g.*, the Yakovlev or Carnegie collections). However, in terms of the development of the telencephalon (*i.e.*, cerebral cortex, basal forebrain and basal ganglia) and diencephalon, it is very rich and well-suited to extensive scientific analysis. Therefore it has enabled us to collect much new and valuable data, part of which has already been published in various strictly refereed international journals, and to establish a successful network of international collaboration.

The purpose of this paper is to offer for the first time a systematic (but concise and selective) description of our collection of human neurodevelopmental material to a general developmental-biological community. It is also our intention to illustrate major results of 16 years of research within the field of human developmental neurobiology, performed at the Section of Neuroanatomy in Zagreb, and to point out the hidden and still insufficiently explored potential of the research on human brain development. Our Collection is now becoming a part of the Croatian Institute for Brain Research. This Institute has been recently founded and its building and development represents a priority project of the School of Medicine in Zagreb under the sponsorship of the Government of the Republic of Croatia. The Institute has been conceived as a center for coordinating multidisciplinary neuroscience research in the Republic of Croatia, and will act openly and cooperatively towards the wide community of neuroscientists and developmental neurobiologists throughout the world.

Results

There is only one solution for comprehensive study of human cortex development: morphologic, histochemical and biochemical parameters of cortical neurogenesis must be defined with reference to the common spatial parameter in a series of closely spaced stages of development (time is fourth dimension). Due to the size and slow development (months!) of the human brain, the data on development of the human cortex and subcortical structures give a good resolution in time and space for the morphologic, histochemical and biochemical parameters of axon ingrowth, synaptogenesis and cellular interactions within the given cytoarchitectonic compartment (layer or area). The most important spatial parameter is the cytoarchitectonic layer; the second spatial parameter is the area/region; the third is the vertical (modular, columnar) organization. Moreover, we interpreted fetal cortical layers as changing cellular compartments and not as a simple forerunners of adult layers.

One of the first goals of our long-term project was to analyze the prenatal development (histogenesis and histochemical differentiation) of the human cerebral cortex with special emphasis on the development of afferent (subcortical) systems. We tried to obtain spatial (regional and areal) and temporal (developmental stages and critical periods) parameters of cortical differentiation by analyzing histochemical and connectional properties of: a) developing cortical layers (as defined by cytoarchitectonic, Golgi and synaptogenesis analysis) in each pallial division (neocortex: prefrontal, premotor-motor, auditory, somatosensory and visual cortex; mesocortex: limbic and entorhinal cortex; archicortex: hippocampus; and paleocortex: prepyriform and periamygdaloid region); b) approaching fiber systems (thalamocortical and basal forebrain afferents); and c) related projection nuclei (dorsomedial nucleus, lateral and medial geniculate body and pulvinar of thalamus; basal forebrain and medial septal nuclei).

The primary aim was to correlate the development of cortical lamination with the ingrowth of thalamic and other afferent fibers in several selected areas of the human cortex. Special attention was paid to the transient fetal synaptic subplate zone (waiting compartment) in developing human and monkey cortex. To approach this question, it was necessary to develop a suitable method for identification of thalamic afferents in the human brain. We have found that relatively simple AChE histochemistry may serve as an excellent label for non-cholinergic thalamic afferents (see Fig. 2A). The method is selective since some thalamic nuclei and fibers are intensely stained while neighboring structures are devoid of stain (*e.g.*, primary visual pathway). To our knowledge, our results provided the first comprehensive evidence concerning the timing and pattern of development of afferent fiber systems in the human cortex (Kelovic *et al.*, 1979; Kelovic and Kostovic, 1981; Kostovic, 1983c, 1986a,b, 1989; Kostovic *et al.*, 1978, 1979, 1980a,b; Kostovic-Knezevic *et al.*, 1979; Krmpotic-Nemanic *et al.*, 1979, 1980a,b, 1981, 1983a,b). In collaboration with Dr. P. Rakic and Dr. P.S. Goldman-Rakic at Yale University, we determined comparative stages for ingrowth of thalamocortical fibers in both human and rhesus monkey forebrain (Kostovic and Goldman-Rakic, 1983; Kostovic and Rakic, 1980, 1984, 1990). These results may briefly be summarized as follows: in stage I, strongly AChE reactive fibers are directed towards the frontal (from mediodorsal nucleus) and occipital lobe (from pulvinar) through the internal capsule. In stage II, AChE reactive fibers can be traced to the subplate zone beneath the developing cortical plate where they wait («waiting compartment») before invasion of the cortex. In stage III fibers penetrate the cortical plate in a laminar fashion and this stage coincides in time with the intensive cortical synaptogenesis. In stage IV AChE reactivity gradually disappears and the cortical dense staining pattern begins to lighten.

The comprehensive Nissl, Golgi, and EM studies of the subplate zone in the human (Fig. 1A) and monkey brain (Kostovic and Rakic, 1980) have shown that this zone is a transient fetal layer of paramount importance for the differentiation of the primate cortex especially as a «waiting compartment» for thalamic and other cortical afferents and as a transient fetal circuitry compartment for potential interactions between «waiting» afferents and transient

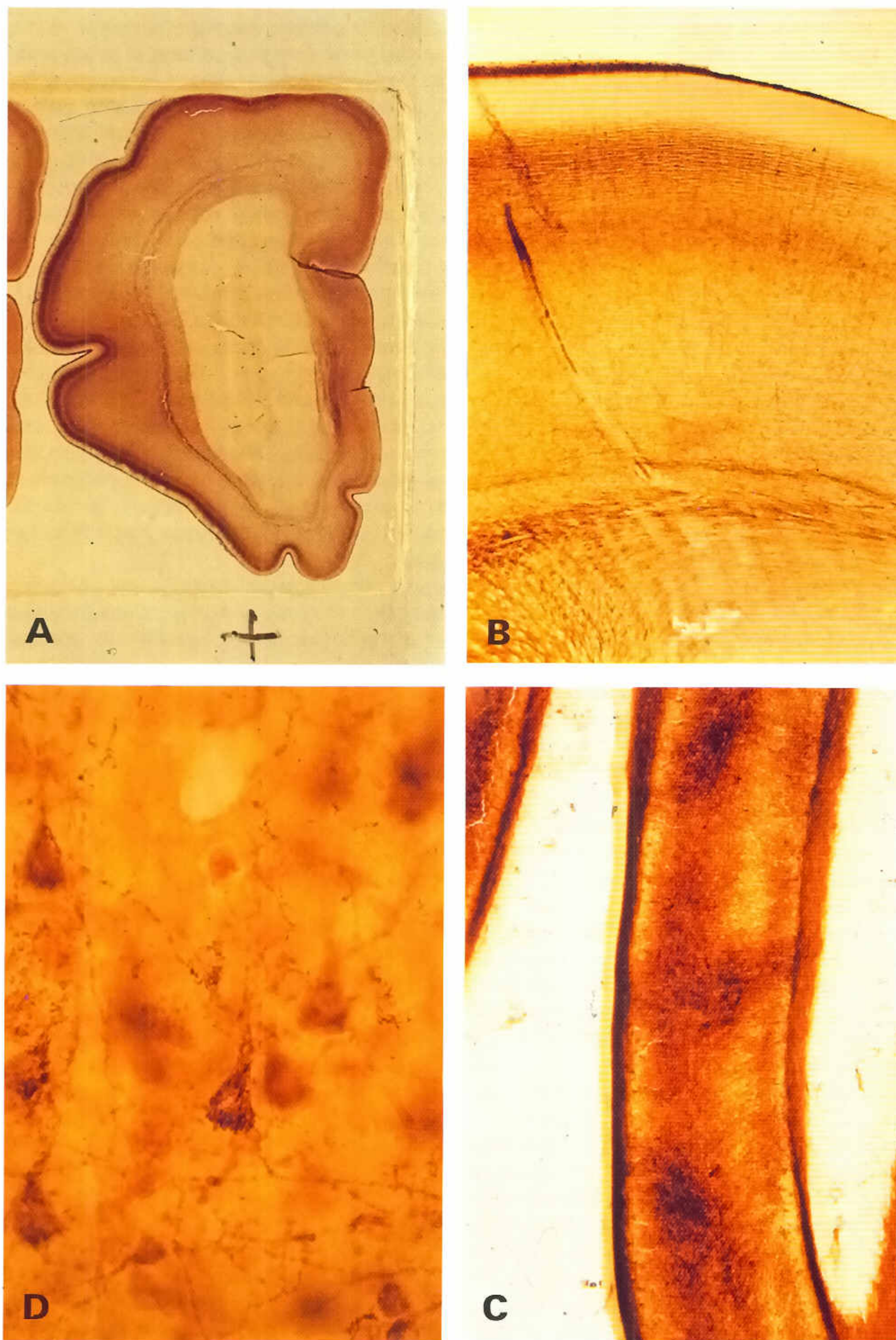

Fig. 2. (A) *Acetylcholinesterase staining of the frontal cortex in a 28-week-old preterm infant. Note the transient, dark staining of the cortical plate and the disperse staining of the underlying subplate zone. Magnification x10.* **(B)** *Acetylcholinesterase-reactive fiber systems (internal and external capsule) in the frontal cortex of the preterm infant (24th postovulatory week). Magnification x40.* **(C)** *Acetylcholinesterase-reactive longitudinal bands (a transient phenomenon) in the human fetal cerebellum. Magnification x50.* **(D)** *Acetylcholinesterase staining of the pyramidal neuron (layer III) in a 23-year-old adult. This reactivity develops late in postnatal life. Magnification x100.*

fetal populations of neurons. The study of the developmental history of the transient subplate zone in the frontal, somatosensory and visual cortex of the monkey and human brain turned out to be a long and demanding project; but, this is so far the only complete comparative study of transient fetal compartments in the human and monkey cortex (Kostovic and Rakic, 1990). Moreover, our results indicate that (contrary to prevailing notions) the subplate zone is a phylogenetically new transient structure that serves the expansion of the neocortex and establishment of complexity of cortical connections (Kostovic and Rakic, 1990). The dissolution of the transient subplate zone begins during the last third of gestation. The subplate zone of the frontal cortex shows protracted development and may serve as a structural basis of developmental plasticity throughout perinatal life (Kostovic, 1981, 1982, 1986a, 1987, 1989; Kostovic *et al.*, 1989a).

Qualitative and quantitative Golgi studies of the human prefrontal

cortex (Mrzljak *et al.*, 1987, 1988, 1990; Uylings *et al.*, 1987) showed that the acceleration of differentiation of neurons around 15 and 24 weeks of gestation was correlated with the ingrowth of afferents (as revealed by AChE histochemistry). Two additional significant findings were: a) there is continuing differentiation of fetal subplate neurons during the perinatal period and b) nonpyramidal double bouquet cells of supragranular layers show precocious development and do not follow the pattern of deep- to superficial («inside-out») differentiation.

It was also shown that almost all Cajal-Retzius cells show strong AChE reactivity and that these cells do not undergo degenerative changes during the early postnatal period but become significantly «diluted» due to the growth of the cerebral cortex (Kostovic *et al.*, 1985, 1989a; Krmpotic-Nemanic *et al.*, 1987). In this respect, the maturation of Cajal-Retzius cells resembles that of subplate and interstitial neurons (Kostovic, 1984b; Kostovic and Rakic, 1980; Kostovic *et al.*, 1981). Thus, these two cell types may serve as special fetal neurons for transient connectivity during prenatal and perinatal development (Kostovic and Rakic, 1980, 1990).

We defined new parameters for developmental cytoarchitectonics (Judas, 1987; Kostovic *et al.*, 1987a,b) and described transient discrete cytoarchitectonic modules within several telencephalic structures during the midgestational period (Delalle and Kostovic, 1989; Judas, 1989; Kelovic *et al.*, 1986; Kostovic, 1980, 1983a,b, 1984a, 1985; Kostovic and Kostovic-Knezevic, 1984; Krmpotic-Nemanic *et al.*, 1984; Nikolic and Kostovic, 1986; Nikolic *et al.*, 1982). These transient architectonic features are present during the period of the prospective growth of efferent cortical pathways and the ingrowth of afferent fiber systems.

Perinatal development of cholinergic markers was analyzed by histochemical (AChE) and immunocytochemical methods (ChAT) and two important features of the developing cholinesterasic system were demonstrated (Kostovic, 1983c, 1986b; Kostovic *et al.*, 1980b, 1986, 1988a,b): a) transient innervation of the subplate zone and the spatial overlap with the thalamocortical system, and b) trends for the prolonged maturation and laminar shifts. Immunocytochemical analysis has revealed an early maturation of cholineacetyltransferase in the nucleus basalis (Meynert) and the presence of nuclear groups characteristic for the human basal forebrain.

Recently we have reviewed histogenetic events and transmitter-related maturational changes in the human cerebral cortex (Kostovic, 1990a,b) concluding that structural remodeling and reorganization of the brain, from transient patterns of the fetal organization through the postnatal phase of transient overproduction of circuitry elements to the final maturation, is the crucial principle of development. Fetal neuronal elements (afferents, synapses and postsynaptic neurons) display transient patterns of laminar, vertical and modular organization and transient cellular interactions and competition in the subplate zone are crucial for the formation of cortical connections. These transient patterns of neuronal organization in preterm infants represent a substrate for transient functional and behavioral phenomena. This conclusion is based upon data on: 1) the reorganization of transient layers, transient populations of cells and other transient patterns of cortical organization; 2) the developmental redistribution of major afferent cortical systems (subcortical afferents, commissural and associational pathways); 3) the overproduction and subsequent elimination of dendritic spines and synapses and 4) laminar shifts and developmental overexpression of transmitters and synaptic modulators. Naturally occurring cell death (Cowan *et al.*, 1985) is also a normal developmental process which leads to the reorganization of the cerebrum rather than being a simple «regressive event». The elucidation of the nature and timing of these histogenetic reorganizational events in the human brain represents the first step towards determining the neurobiological basis of emergence of behavior, neural functions and cognition in human fetuses, infants and children, which takes place during perinatal and early postnatal life.

Here we summarize our current views on the perinatal and postnatal developmental reorganization of the human cerebral cortex, within the context of modern developmental neurobiology.

Developmental reorganization of transient layers, cells and other transient patterns of cortical organization

Transient laminar and cellular patterns of the cortical organization are most pronounced during the late fetal period (Kostovic, 1990a,b). The most prominent transient fetal layer is the subplate zone (Kostovic and Molliver, 1974; Kostovic and Rakic, 1980, 1990). This zone serves as a transient «waiting» compartment for cellular interactions and as a substrate for competition and segregation of afferents from various subcortical centers and from the ipsi- and contralateral cerebral hemisphere (Kostovic and Rakic, 1990). In addition, the subplate zone contains perikarya and dendrites of the oldest postmigratory neurons (Kostovic and Molliver, 1974; Kostovic and Rakic, 1980, 1990) which express an early peptidergic activity (Kostovic and Fucic, 1985; Kostovic *et al.*, 1991). The subplate neurons participate in the earliest synaptic circuits in the human fetal cortex (Molliver *et al.*, 1973; Kostovic *et al.*, 1974). The cellular organization of the subplate zone shows transient, fetal characteristics: loose arrangement of growing axons, transitory synapses, growth cones and abundance of extracellular space (Kostovic and Rakic, 1990). Although the cortical plate as well as the overlying marginal zone are forerunners of permanent cortical layers I-VI, they also display various transient patterns of organization. The vertical alignment of densely-packed embryonic columns is the most characteristic feature of the fetal cortical plate (Kostovic-Knezevic *et al.*, 1978; Krmpotic-Nemanic *et al.*, 1979, 1984; Rakic, 1988a,b). Another transient feature of the cortical plate is the transient columnar distribution of AChE reactivity (Kostovic, 1979, 1982, 1986a; Kostovic and Goldman-Rakic, 1983; Kostovic *et al.*, 1979, 1980a). Developmental reorganization and the gradual disappearance of the subplate zone begins in the visual cortex at the end of gestation (Kostovic and Rakic, 1990). However, in the prefrontal cortex the subplate zone is present in the 6th postnatal month (Kostovic *et al.*, 1989a). The prolonged existence of the subplate zone in the prefrontal cortex is probably related to the prolonged growth of frontal cortico-cortical pathways through the subplate compartment and its role in postnatal shaping of tertiary gyri (Kostovic and Rakic, 1990). The developmental reorganization of the subplate zone involves also the disappearance of subplate neurons. The most attractive mechanism of disappearance of subplate neurons is cell death (Kostovic and Rakic, 1980). However, a significant portion of subplate neurons become incorporated in the hemispheric white matter where they undergo significant changes in morphology during later postnatal life (Kostovic and Rakic, 1980, 1990).

Developmental redistribution of major afferent systems

Experimental studies on the monkey have shown that thalamocortical and cortico-cortical afferents are transiently distrib-

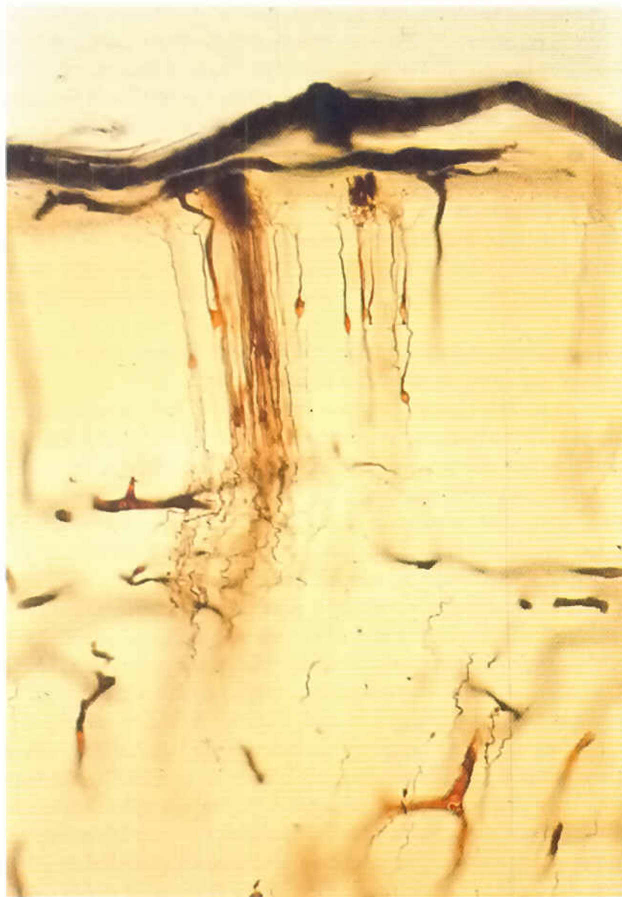

Fig. 3. Golgi impregnated immature neuron in the cortical plate at 10 weeks of postovulatory age. *Stensaas impregnation. Magnification x100.*

uted within the «waiting» compartment of the subplate zone before they penetrate their final target - cortical plate (Rakic, 1976, 1977). In man, the transient «waiting» period of thalamocortical afferents has been indicated by histochemical markers (Kostovic and Goldman-Rakic, 1983; Krmpotic-Nemanic *et al.*, 1983; Kostovic and Rakic, 1984). After the penetration of the cortical plate, thalamocortical fibers show transient random distribution and a characteristic mature columnar pattern emerges during the next phase of development (Rakic, 1977). Very little is known about transient projection of cortico-cortical associational pathways. Since a critical period seems to exist for structural plasticity of cortico-cortical pathways in monkeys (Goldman-Rakic, 1987), one can predict a similar «waiting» period in the transient human subplate compartment (Kostovic and Rakic, 1990).

Laminar shifts and developmental overexpression of transmitters and prolonged chemical maturation of pyramidal neurons in the primate cortex

Laminar, columnar and areal shifts of acetylcholinesterase (AChE) are not necessarily related to the maturation of the cholinergic system (Kostovic and Goldman-Rakic, 1983). The most prominent histochemical change in the developing human frontal and prestriate cortex is the decrease of the AChE reactivity in layer IV during the perinatal period. During this period AChE reactivity in the mediodorsal nucleus and pulvinar of the thalamus gradually disappears (Kostovic and Goldman-Rakic, 1983; Kostovic and Rakic, 1984). The loss of AChE reactivity is an obvious sign of the change in chemical properties of thalamocortical axons. According to the hypothesis of the developmental role of AChE (Kostovic and Goldman-Rakic, 1983), postnatal disappearance of the thalamic AChE simply indicates the end of active growth and differentiation in the particular thalamocortical system. On the other hand, the histochemical differentiation of the cholinergic fibers originating from the basal forebrain continues during postnatal development. After birth the AChE network, which is composed of well-stained individual axons, gradually develops in a «deep to superficial» fashion (Kostovic *et al.*, 1988b). The AChE reactivity of layer III pyramidal cell bodies and surrounding fibrillar network begins to develop after the first postnatal year, and reaches its peak intensity in young adults (Kostovic *et al.*, 1988b – see Fig. 2C). Peptidergic activity of the human fetal cortex (Figs. 4A and 4B) appears in the transient subplate zone after 15 weeks of gestation (Kostovic and Fucic, 1985; Kostovic *et al.*, 1991).

In a recent study on the histochemical maturation of the human frontal associative cortex we focused attention on the associative-commissural pyramidal neurons of layer III (Kostovic *et al.*, 1988b). These neurons are the major source of associative and commissural projections and are important neuronal elements underlying cognitive processing in the cerebral cortex (Goldman-Rakic, 1987).

We demonstrated the late development of AChE reactivity in layer III pyramidal cell bodies and surrounding fibrillar networks (Kostovic, 1987; Kostovic *et al.*, 1988b). The AChE reactivity of layer III pyramidal neurons begins to develop after the first postnatal year, increases gradually, and reaches its peak intensity in young adults (Kostovic *et al.*, 1988b; Mesulam and Geula, 1988). These findings led to the hypothesis that AChE-rich elements play a role in the innervation of cortical associative neurons during cognitive development in man. The AChE-rich innervation of layer III pyramidal neurons has so far been documented only in the human cortex and this late developmental process seems to be characteristic of the primate brain (Kostovic *et al.*, 1988b).

Areal differentiation

The question of areal specialization and differentiation is crucial for our understanding of the organization of the human cerebral cortex since many cortical areas such as speech and associational areas are specific for the human brain and do not exist in experimental primates. In spite of current interest for this problem (Rakic, 1988a,b; O'Leary, 1989), very little is known about the ontogenetic processes underlying areal differentiation. In the human cerebral cortex, regional and areal cytoarchitectonic specialization (see Fig. 1B) begins during prenatal life (Brodmann ,1909). Topography of thalamocortical projection parallels or even precedes this process (Kostovic and Rakic, 1984, 1990). The process of cytoarchitectonic differentiation of cortical areas lasts for a long period (Kostovic *et al.*, 1987a,b) and in the Broca area is not finished until 3 postnatal years (Judas, 1987, 1989, 1990). It is not known whether there is developmental reorganization of areal borders during development, but this question is interesting in the light of experimental evidence about plasticity and changing patterns of cortical maps in both the adult and developing brain.

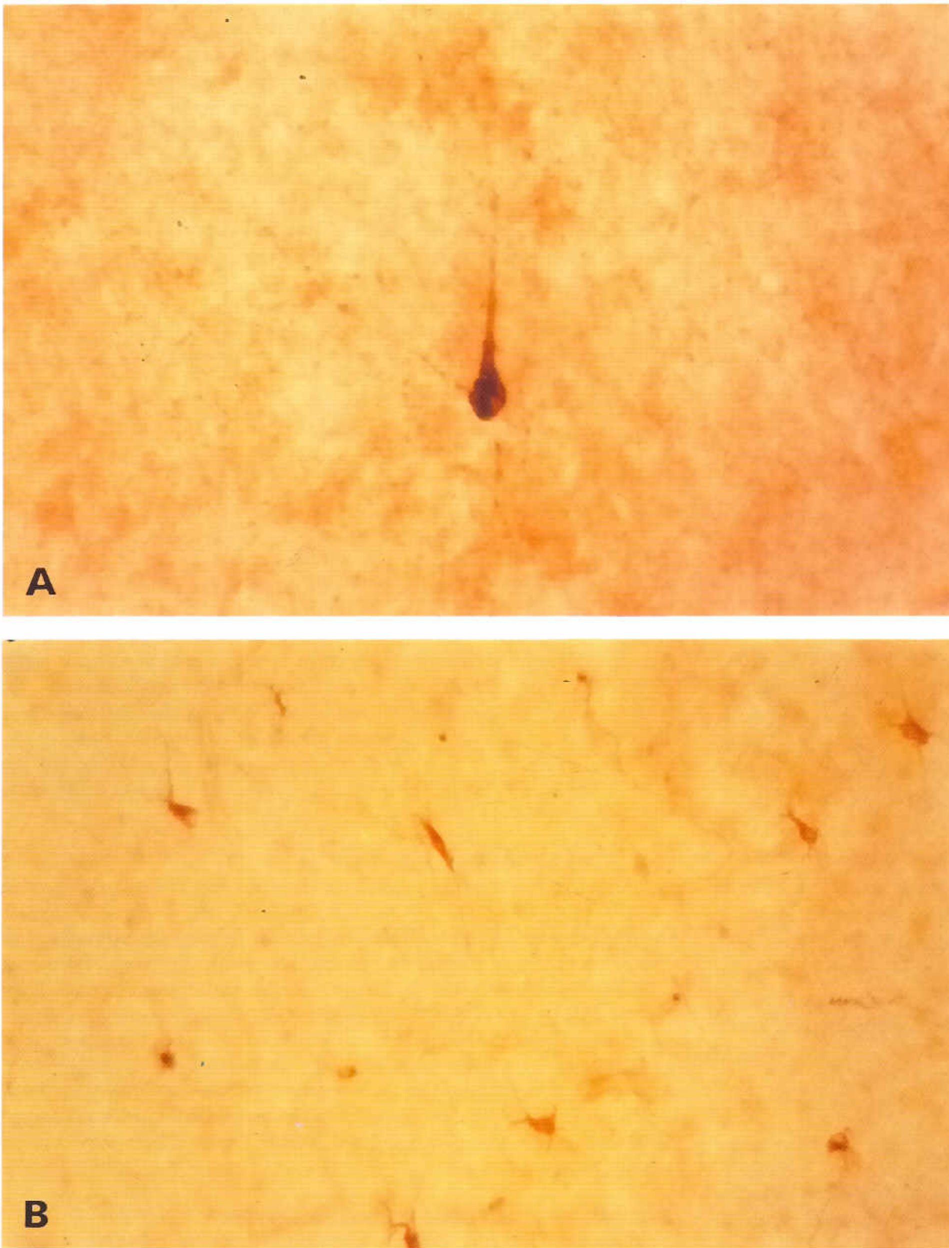

Fig. 4. (A) *Somatostatin-immunoreactive neuron in the subplate zone of the frontal cortex of a 23-week-old human fetus. Magnification x100.* **(B)** *NPY-immunoreactive neurons in the subplate zone of the newborn human frontal cortex. Magnification x100.*

Discussion

The systematic exposure of our collection demonstrated its great potential for hitherto unexplored morphogenetic and histogenetic events and chemical maturation of the brain during the midfetal and late fetal period. The most interesting finding was the presence of different transient patterns of organization as revealed by Nissl, Golgi, AChE histochemistry and immunocytochemistry (see Figs. 1A, 2B, 2D, 4A and 4B). The organization of the late fetal period and perinatal period shows that the premature brain is substantially different from that in the child or adult in terms of the presence of transient fetal zones (Fig. 1A), transient populations of fetal cells (Figs. 4A and 4B), transient arrangement of pathways (Fig. 2B) and transient expression of transmitter properties.

The aim of our long-term research was to determine changes in the laminar, columnar, cellular, and transmitter organization of the human frontal lobe which underlie these transient, neurophysiological, neurological, behavioral and cognitive patterns observed in human infants during perinatal and early postnatal life. We proposed that the cerebral cortex passes through two phases of the reorganization characterized by different levels or rearrangements of cortical elements: 1) First, the perinatal phase involves disappearance of the fetal subplate zone and laminar relocation of afferent pathways. The duration in this phase and the size of the transient subplate zone are proportional to the complexity of cortico-cortical pathways in a given area of the cortex. 2)

Second, fine reorganization occurs during infancy and early childhood and is related to the dendritic and synaptic rearrangements.

At present, we are engaged in an intensive study of postnatal development of the human brain. Admittedly, this will be the most difficult developmental period to study. One important thing is, however, already clear: substantial and measurable changes occur during this period and these changes are somehow related to the appearance and further development of cognitive and other higher nervous functions of infants and children. If one accepts that there is a structural (neurobiological) basis for these functions, an obvious task for the developmental neurobiologist is to apply all available methods and to establish a new system of developmental parameters and criteria for staging. We have already moved in this direction (Kostovic *et al.*, 1987a,b; Judas, 1987; Kostovic, 1990a,b; Kostovic and Rakic, 1990; Kostovic *et al.*, 1988a, 1989a, 1991; Mrzljak *et al.*, 1990) but much work still has to be done. The difficulties in obtaining excellent specimens from this developmental period, together with the size of the brain, require the collaboration of different neurodevelopmental laboratories and better communication among different researchers. The computer-based international data bank and exchange of information will be needed in order to define developmental periods during this crucial period for the development of the human intellect and personality. The establishment of the international data bank and the incorporation of the Zagreb Collection into the European Neuroembryological Network is one of the major goals of our team in the near future. Our collection is obviously a major resource for the future studies of brain developmental disorders and abnormalities. We expect that systematic elaboration of normative parameters on human brain development will undoubtedly help reveal the neurobiological basis of different developmental disorders such as Down's syndrome, mental retardation and autism, developmental language disturbances, schizophrenia or disorders of proliferation and migration leading to micropolygiria, lissencephalia or pachygyria. The first step towards this important issue was undertaken by analyzing the development of basal forebrain-frontal cortex system in Down's syndrome (Kostovic *et al.*, 1988a) – but most of this promising work is still in progress.

Materials and Methods

Description of sample

We have analyzed a large series of human brains at different developmental ages ranging from an embryonic period to senescence. Through collaboration with different Departments of Gynecology and Obstetrics, Pediatric Departments and Departments of Pathology and Forensic Medicine, we collected more than 600 normal and pathological brains. This extensive material includes almost 200 specimens with developmental pathological changes of various nature: periventricular-intraventricular hemorrhage, congenital malformations (*e.g.*, micropolygyria, holoprosencephalia and some other disorders of migration; hydrocephalus, anencephaly and spina bifida), brains of fetuses and infants with trisomy syndromes (Down's syndrome, Syndroma Patau and Syndroma Edwards). Adult specimens also include pathological brains (*e.g.*, intraoperative bioptic material or brains with carbon monoxide poisoning). Prenatal and perinatal «normal» (*i.e.*, normative control accessions) brains were obtained from spontaneous abortions or prematurely born and newborn infants who died of non-neurological diseases. Older «normal» specimens were obtained from infants, adolescents and adults who died due to respiratory failure, non-neurological diseases or accidents. All our studies were conducted on the postmortem brain tissue obtained from regular autopsies at the Departments of Forensic Medicine and Pathology.

Our primary goal in this paper was to list a representative sample of normative control accessions covering all developmental ages (see Table 1) and to present selective lists of specimens of excellent histological quality which have served as the basis for obtaining our major results: perinatal and postnatal development of neuronal morphology in the human cerebral cortex (Table 3), cytoarchitectonic and histochemical development of the cholinergic basal forebrain system in normal and Down's syndrome brains (Table 4), development of peptidergic reactivity for various neuropeptides in human cerebral cortex (Table 5), and histochemical development of thalamocortical and basal forebrain-cortex afferent systems as well as the histochemical development of cortical associative layer III neurons (Table 6). Extensive material representing the basis for our thorough studies of cytoarchitectonic and ultrastructural development of human telencephalon is represented in Table 1; in Table 2 we list the brains from patients with Down's syndrome to illustrate the potential of our collection for developmental neuropathological studies as well.

From each specimen obtained within 6-8 hours (or 1-3 hours for young fetuses!) after death, the coronal slabs from the right hemisphere (or the whole telencephalon in the youngest specimens) were used for acetylcholinesterase histochemistry, immunocytochemistry and Golgi analysis; horizontal or coronal slabs from the left hemisphere were used for celloidin embedding and Nissl staining. According to our experience, three well-fixed specimens for each age displaying successful staining or impregnation give satisfactory parameters for the delineation of the developmental stage, but due to prominent individual variations the analysis of associative cortical areas may require at least five specimens. Cytoarchitectonic (Nissl staining) data were compared with «normal» specimens from our neuroembryological collection (for each developmental age, several «normal» specimens are available). Acetylcholinesterase histochemistry, immunocytochemical, electron-microscopic and Golgi material was analyzed both qualitatively and quantitatively using common laminar (layers and fetal zones), regional (areas, regions) and topographic landmarks (main sulci and gyri).

Golgi analysis of fetal material

Frontal slices were immersed in the Rio del Hortega fixative as modified by Stensaas (Stensaas, 1967). For older fetuses, fixation proceeded according to Marín-Padilla (Marín-Padilla, 1971). After impregnation, selected sections were prepared with a razor-blade and the remaining blocks were embedded in celloidin. It is essential to make some sections by hand in case of later «fading» caused by the embedding procedure. Celloidin blocks were cut on a sliding microtome in sections of 200 micrometers in thickness, and adjacent 25 micrometer sections were Nissl-stained to obtain the precise juxtaposition of impregnated neurons with cytoarchitectonic landmarks. In our hands, the Rio del Hortega method (Stensaas modification) gives very constant results and good general impregnation of all cortical layers (see Fig. 3).

Golgi analysis of premature and postnatal brains

Tissue blocks were obtained at regular autopsies, with a 2-8 hour interval between death and the first fixation. Consecutive adjacent blocks were impregnated with either the rapid Golgi (chrome osmium fixative) or Golgi-Cox method according to Van der Loos (1959). Both Golgi techniques are applied on adjacent blocks since the Golgi-rapid technique (the most commonly applied Golgi method in neuropathology) is very sensitive to postmortem delays greater than 6 hours, while our experience indicates that the Golgi-Cox technique is much less susceptible to a postmortem delay and gives satisfactorily impregnated neurons even 18 hours after death in brains of children older than 2 years. The detailed procedure is described in our previous papers (Kostovic and Rakic, 1980; Mrzljak *et al.*, 1988, 1990). The collected Golgi material has been analyzed both qualitatively and quantitatively and representative neurons are documented by camera lucida drawings or photomicrographs.

Fine cytological analysis was performed on 1 micrometer thick plastic sections, and for the *electron microscope analysis* slices of the frontal lobe were fixed by immersion in the buffered mixture of 1% paraformaldehyde and 1.25% distilled glutaraldehyde (EM fixative). The buffer was 0.1M PBS

(phosphate buffer saline, pH = 7.35; 220 M osm.). The tissue was further processed and analyzed as described in detail previously (Molliver *et al.*, 1973; Kostovic and Rakic, 1980, 1990).

Acetylcholinesterase histochemistry

One hemisphere (usually the right) was routinely used for AChE histochemistry. The hemisphere is removed from the cranial cavity and fixed for 24-48 hours by immersion in 0.1 M PBS containing 1% glutaraldehyde plus 2% paraformaldehyde at 4-6°C. After fixation, the brains were cut into 8-10 mm (for fetuses) or 1-2 cm (for older brains) thick slabs and frozen sectioned at 70 micrometers. Free-floating sections were then stained for AChE with Lewis's modification of Koelle's method (Krnjevic and Silver, 1965, 1966) and with the Karnovsky-Roots method as described by Broderson, Westrum and Sutton (Broderson *et al.*, 1974). The control tests were performed as described in previous papers (Kostovic and Goldman-Rakic, 1983; Kostovic, 1986b; Kostovic *et al.*, 1988b).

Immunocytochemistry

The brains obtained at autopsy are fixed by immersion for 1-2 hours in the solution of 4% paraformaldehyde in 0.1 M phosphate buffer and then cut in 1 cm thick coronal slabs which are left overnight in freshly prepared fixative. After the cryoprotection and sectioning (80 μm sections), free-floating sections for immunohistochemistry are processed according to the avidin-biotin method (Hsu *et al.*, 1981) using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Adjacent Nissl-stained sections serve for the cytoarchitectonic delineation of layers. We use commercially available antisera to the following antigens, located in primate cortical neurons: a) peptides: neuropeptide Y (NPY), somatostatin (SOM) and cholecystokinin (CCK); b) calcium binding protein parvalbumin; c) cytoskeletal neuronal proteins – microtubule associated protein 2 - MAP 2 and neurofilament SMI-32; d) cholinergic markers: choline acetyltransferase - ChAT and acetylcholinesterase - AChE. So far, we have obtained good results in peptide immunocytochemistry on human postmortem tissue even after the long fixation period.

Human subjects

All research was approved by the Institutional Review Board (School of Medicine, Zagreb). We analyzed post-mortem brain tissue removed as a part of a routine diagnostic procedure that is regulated by law in our country. All autopsies were performed at the Department of Forensic Medicine and Department of Pathology. All data are recorded in such a way that identification of a person is not possible.

Acknowledgments

This work has been supported by the US-Yugoslav Joint Board grant No. JF 855 and the Scientific Foundation of the Republic of Croatia. We appreciate the excellent and long-lasting technical assistance of Zdenka Cmuk, Danica Budinscak and Bozica Popovic. We also use this opportunity to express our deep gratitude to numerous colleagues (listed in alphabetical order) who through the years have helped us to establish the valuable collection of human neuroembryological material: S. Anlelinovic, V. Bosnjak, D. Brezovacki, A. Bunarevic, V. Burja, S. Ciglar, A. Cviko, D. Cadez, V. Gec, S. Gusic, S. Jukic, F. Knezevic, B. Kruslin, M. Kubat, N. Lukinovic, P. Miklic, M. Mosunjac, D. Strinovic, J. Skavic, A. Stampalija, D. Zecevic, and N. Zecevic.

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APPENDIX

TABLE 1

NORMATIVE CONTROL ACCESSIONS

1. EMBRYONIC PERIOD (embryos to 31 mm CRL, Carnegie stages I-XXIII):

Acc. No.	Sex	CRL/mm	BW/gr	BL/cm	GA/w.g.	PN Srv.	N	G	H	I	EM
CF 76	-	2	-	-	-	-					+
CF 112	-	5	-	-	-	-					+
CF 110	-	8	-	-	-	-	+				
CF 65	-	9	-	1.7	-	-					+
CF 69	-	11	-	-	9	-	+				+
CF 121	-	14	-	-	-	-					+
CF 109	-	15	-	-	-	-	+				
CF 83	-	16	-	-	-	-	+				
CF 113	-	16	-	-	-	-					+
CF 117	-	16	-	-	-	-	+	+			+
CF 84	-	17	-	-	-	-	+				
CF 89	-	17	-	-	-	-					+
CF 111	-	18	-	-	-	-	+				+
CF 107	-	19	-	-	-	-	+				
CF 77	-	20	-	-	-	-	+				
CF 93	-	20	-	-	-	-	+				
CF 120	-	20	-	-	-	-					+
CF 108	-	23	-	-	-	-	+				
CF 78	-	23	-	-	-	-	+				
CF 71	-	24	-	-	-	-	+				+
CF 81	-	24	-	-	-	-	+				
CF 41	-	25	-	-	-	-	+				
CF 128	-	27	-	-	9	-			+		
CF 80	-	28	-	-	-	-	+				
CF 94	-	29	-	-	-	-	+				
CF 57	-	30	-	-	-	-	+				
CF 116	-	40	-	-	-	-	+				
CF 96	-	43	-	-	-	-	+				
CF 49	-	45	-	-	-	-	+				
CF 67	-	45	-	-	9	-	+				

2. EARLY AND MIDDLE FETAL PERIOD (non-viable fetuses to 200 mm CRL *i.e.*, up to 22 weeks post-ovulation):

Acc. No	Sex	CRL/mm	BW/gr	BL/cm	GA/w.g.	PN Srv.	N	G	H	I	EM
CF 123	-	46	-	-	-	-	+				
CF 95	-	55	-	-	-	-	+				
CF 47	-	60	-	-	-	-	+				
CF 59	-	60	-	-	-	-		+			
CF 118	-	60	-	-	-	-	+				
CF 79	-	67	-	-	-	-	+				
CF 43	-	70	-	-	-	-	+				
CF 127	-	75	-	-	-	-	+		+		
CF 91	-	80	-	-	12	-	+				
CF 66	-	83	-	-	-	-	+				
CF 61	-	85	-	-	-	-	+				
CF 10	-	85	-	-	14	-	+	+			
CF 170	M	90	-	-	14	-			+		
CF 102	-	97	-	-	16	-	+				
CF 17	-	100	-	-	-	-		+			
CF 27	-	110	-	-	-	-	+				
CF 48	-	110	-	-	-	-	+				
CF 60	-	110	-	-	-	-	+				
CF 90	-	110	-	-	-	-	+				
CF 125	-	120	-	-	17	-	+				
CF 134	-	120	-	-	-	-	+		+		
CF 281	-	120	-	-	15	-	+				
CF 322	-	128	149	-	18	-	+				
CF 174	-	130	-	-	18	-	+				
CF 199	-	130	-	-	20	-			+		
CF 320	M	130	180	19	19	-	+				
CF 12	-	135	-	-	-	-		+			
CF 168	M	135	-	-	19	-	+				
CF 28	-	137	-	-	-	-	+				
CF 8	-	145	-	-	24	-	+				
CF 16	-	145	-	-	-	-	+	+			
CF 130	-	145	-	-	-	-	+				
CF 216	-	150	-	-	21	-		+			
CF 290	-	153	-	-	-	-		+			
CF 166	-	-	250	19	18	-	+				
CF 36	-	155	270	24	-	-	+				
CF 129	-	155	-	-	22	-	+				
CF 198	-	160	-	-	17	-			+		
CF 45	-	175	300	-	-	-	+				
CF 62	-	175	-	-	20	-	+				
CF 183	-	178	-	-	-	-	+				
CF 307	-	180	-	-	-	-		+			
CF 288	-	180	-	-	-	-		+			
CF 21	-	185	400	-	-	-	+	+			
CF 63	-	185?	-	-	-	-	+				
CF 55	-	190	-	-	-	-	+				
CF 150	-	190	-	-	-	-	+				
CF 197	F	190	-	-	23	-			+		
CF 44	-	193	440	-	-	-	+				
CF 101	M	195	-	31	-	-	+				
CF 32	-	200	400	-	-	-	+				
CF 38	-	200	500	-	-	-	+	+			
CF 160	-	200	-	-	-	-	+				
CF 164	-	200	-	-	-	-	+				
CF 181	M	200	750	-	24	-	+	+	+		
CF 135	-	-	800	35	26	-	+				

3. PREMATURELY BORN (LOW-BIRTH-WEIGHT) INFANTS AND FULL TERM NEWBORNS FROM 201 mm CRL (23 weeks post-ovulation) UP TO 30 DAYS OF POSTNATAL SURVIVAL:

Acc. No	Sex	CRL/mm	BW/gr	BL/cm	GA/w.g.	PN Srv.	N	G	H	I	EM
CF 25	-	203	500	-	-	-	+				
CF 19	-	205	-	-	-	-	+				
CF 54	-	210	-	-	28	-	+				
CF 250	F	210	540	-	26	-	+				
CF 31	-	220	780	-	-	-	+				
CF 177	M	220	-	31	25	-	+	+	+		
CF 245	M	220	800	35	-	3 d.	+				
CF 294	-	225	800	-	-	-	+				
CF 9	-	230	650	-	24	-	+				
CF 24	-	230	560	-	24	-	+				
CF 100	-	230	-	34	26	-	+				
CF 35	-	235	800	-	-	-	+				
CF 23	-	240	820	-	-	-	+				
CF 180	M	245	-	26	-	-	+	+			
CF 182	F	270	1450	38	28	11 h.	+	+			
CF 324	M	-	720	33	25	-	+	+			
CD 171	-	-	800	33	26	1 d.	+		+		
CF 352	F	-	800	-	26	11 d.	+				
CF 70	-	-	850	36	-	3 d.	+				
CF 208	M	-	900	35	-	2 d.	+				
CF 308	M	-	1010	36	-	1 h.		+			
CF 305	M	-	1070	37	29	1 d.		+		+	
CF 52	-	-	1100	37	-	-	+				
CF 209	-	-	1100	38	32	5 h.	+		+		
CF 205	F	-	1200	38	30	4 h.	+		+		
CF 327	-	-	1230	25	31	2 d.	+				
CF 246	-	-	1250	32	32	-	+				
CF 206	F	-	1350	40	28	5 h.	+		+		
CF 194	M	-	1420	38	-	-	+				
CD 164	F	-	1430	40	31	7 d.				+	
CF 260	M	260	1430	45	-	5 h.	+				
CF 257	F	290	1450	42	27	6 h.	+				
CF 201	-	-	1450	39	35	8 d.	+				
CF 207	F	-	1500	46	-	18 h.	+		+		
CF 225	M	300	1620	42	-	1 d.	+		+		
CF 224	M	320	1650	-	-	2 d.	+				
CD 44	F	-	1750	41	37	6 d.	+				
CF 204	-	-	1750	43	-	3 d.	+		+		
CF 72	-	-	1800	44	-	20 d.	+				
CF 251	M	-	1800	45	35	10 min.	+		+		
CD 41	M	-	1820	42	-	-	+				
CF 51	-	-	1850	40	-	-	+				
CF 210	M	-	1850	43	31	2 h.	+		+		
CF 265	F	-	1900	43	32	16 h.	+				
CD 20	F	-	2010	47	-	5 d.	+				
CF 255	M	230	2300	45	36	6 h.	+				
CD 48	M	320	2450	47	36	17 h.	+				
CF 350	-	-	2450	48	-	4 d.		+			
CD 76	M	-	2480	49	37	11 d.	+				
CD 162	F	-	2980	48	41	-	+				
CD 4	F	-	3000	54	-	-	+				

Acc. No	Sex	CRL/mm	BW/gr	BL/cm	GA/w.g.	PN Srv.	N	G	H	I	EM
CD 40	M	-	3200	51	-	6 d.	+				
CD 29	F	-	3500	50	-	-			+		
CD 28	M	-	3500	52	-	38 h.	+		+		
CD 27	M	-	3600	49	-	4 d.	+				
CD 45	M	-	3650	52	-	42 h.	+				
CD 178	-	-	3750	53	39	10 d.				+	
CD 24	M	-	4000	52	-	3 d.	+				
CD 77	M	-	4100	51	-	1 d.	+		+		
CD 121	-	-	newborn		-	1 d.	+				
CD 110	M	-	newborn		-	5 d.	+		+		
CD 96	-	-	newborn		-	6 d.	+	+	+	+	
CD 99	F	-	newborn		-	15 d.	+			+	
CD 132	F	-	newborn		-	22 d.	+		+		
CD 116	M	-	newborn		-	25 d.	+				
CD 140	M	-	newborn		36	28 d.	+	+			
CD 82	-	-	-	-	37	-	+				
CD 87	M	-	-	50	-	-	+				
CD 39	F	-	-	-	-	37 h.	+				
CD 36	M	-	-	-	-	4 d.	+		+		

4. INFANTS UP TO 12 MONTHS OF POSTNATAL SURVIVAL:

Acc. No.	Sex	PN Srv.	N	G	H	I
CD 35	M	1 m.	+		+	
CD 37	F	1 m.	+			
CD 147	M	1 m.		+	+	
CD 115	F	51 d.	+			
CD 155	M	1.5 m.	+		+	
CD 188	M	1.5 m.				+
CD 172	-	55 d.				+
CD 18	M	2 m.	+			
CD 25	F	2 m.	+			
CD 85	F	2 m.	+	+		
CD 105	M	2.5 m.		+		
CD 88	-	3 m.	+	+		
CD 22	F	3 m.	+	+	+	
CD 32	M	3 m.	+			
CD 47	M	3 m.	+			
CD 50	M	3 m.	+			
CD 122	F	3 m.	+		+	
CD 124	-	3 m.	+		+	
CD 148A	-	3 m.	+			
CD 103	M	3.5 m.	+			
CD 78	M	4 m.	+			
CD 120	F	4 m.	+	+		
CD 173	-	4 m.			+	+
CD 106	-	4 m.	+			
CD 17	F	4.5 m.	+			
CD 2	-	4m 18d	+			
CD 7	-	5 m.	+			
CD 100	-	5 m.	+		+	
CD 131	F	5 m.	+			
CD 26	M	5m 20d	+			
CD 51	M	6 m.	+			
CD 118	-	6 m.	+		+	
CD 119	-	6.5 m.	+		+	
CD 102	F	7 m.	+			
CD 123	M	7 m.	+	+	+	
CD 101	M	7.5 m.	+	+	+	
CD 133	F	8 m.?	+			
CD 23	M	8 m.	+	+	+	
CD 38	F	9 m.	+	+	+	
CD 124	M	9 m.	+		+	
CD 174	-	9 m.			+	+
CD 113	M	9.5 m.	+		+	
CD 8	M	10 m.	+			
CD 57	M	10 m.	+			
CD 138	M	10 m.	+			
CD 9	F	10.5 m.	+			
CD 107	M	1 y.	+		+	+

5. CHILDREN FROM 13 MONTHS OF AGE THROUGH THE FIRST DECADE:

Acc. No.	Sex	PN Srv.	N	G	H	I
CD 181	-	14 m.	+			+
CD 143	-	15 m.	+	+		
CD 159	F	16 m.	+	+	+	+
CD 180	-	16 m.			+	+
CD 167	-	16.5 m.				+
CD 33	M	18 m.	+		+	
CD 111	F	2 y.	+		+	
CD 175	-	2y 5m		+	+	+
CD 182	-	2y 10m				+
CD 80	M	3 y.	+	+		
CD 112	F	3 y.	+		+	
CD 134	M	3 y.	+			
CD 148	M	3.8 y.	+			
CD 31	F	4 y.			+	
CD 149	M	4y 2m	+			
CD 98	M	5y 5m	+	+	+	
CD 125	F	5 y 10m	+	+		
CD 21	F	6 y.	+			
CD 156	M	6 y.	+	+	+	
CD 161	F	6.5 y.	+			
CD 129	F	7 y.	+			
CD 160	M	7 y.			+	
CD 93	M	8 y.	+			
CD 126	M	9 y.	+	+	+	
CD 185	-	9 y.		+		+
CD 157	M	9y 3m	+	+	+	
CD 136	F	10 y.	+			
CD 150	F	10 y.	+			

6. CHILDREN AND YOUNG ADULTS THROUGH THE SECOND DECADE:

Acc. No.:	Sex	PN Srv.	N	G	H	I
CD 90	M	11 y.	+	+		
CD 151	M	11 y.			+	
CD 49	F	12 y.?	+			

Acc. No.:	Sex	PN Srv.	N	G	H	I
CD 137	M	12 y.	+			
CD 187	M	12 y.				+
CD 89	M	14 y.	+			
CD 74	M	15 y.	+			
CO 160	M	16 y.			+	
CO 170	-	16 y.	+	+		
CO 185	F	16 y.		+		+
CO 159	-	17 y.		+		
CO 175	F	19 y.	+			
CO 167	M	19 y.	+	+		
7. ADULTS IN THIRD DECADE:						
CO 122	-	21 y.		+		
CO 194	F	23 y.		+		+
CO 184	M	24 y.				+
CO 34	F	26 y.	+	+		
CO 161	-	27 y.		+		
CO 192	M	28 y.		+		+
CO 180	M	30 y.		+	+	
8. ADULTS IN FOURTH DECADE:						
CO 157	M	31 y.	+			
CO 87	M	33 y.	+			
CO 190	F	34 y.	+			+
CO 135	-	35 y.		+		
CO 127	M	36 y.			+	
CO 140	M	36 y.	+		+	
CO 145	F	36 y.	+			
CO 129	M	39 y.		+	+	
CO 38	M	40 y.	+			
CO 189	M	40 y.		+		
9. ADULTS IN FIFTH DECADE:						
CO 37	F	43 y.	+			
CO 150	F	46 y.		+		
CO 133	M	47 y.	+			
CO 162	M	48 y.	+			
10. ADULTS IN SIXTH TO NINTH DECADE:						
CO 51	F	52 y.	+			
CO 134	M	55 y.		+		
CO 182	M	56 y.	+			+
CO 35	M	59 y.	+			
CO 25	M	70 y.	+			
CO 171	M	86 y.		+		
CO 82	F	88 y.	+			

(271 age-matched cerebra from embryos from first trimester to adults of eighth postnatal decade). For each accession with serial protocol number (Acc. No.) the following data are listed: sex, crown-rump length expressed in millimeters (CRL/mm), weight at birth (BW/gr) in grams, and length at birth (BL/cm) in centimeters; gestational age in weeks (GA/w.g.) as recorded by clinician according to last menstrual period; postnatal survival (PN Srv.) expressed in hours (h.), days (d.), months (m.) or years (y.). Pluses in last five columns indicate successfully applied method, as follows: Nissl staining (N), Golgi impregnation (G), acetylcholinesterase histochemistry (H), immunocytochemistry (I) and electron microscopy (EM).

TABLE 2

BRAINS OF FETUSES AND INFANTS WITH DOWN SYNDROME

Acc. No.	Sex	CRL/mm	BW/gr	BL/cm	GA/w.g.	PN Srv.	N	G	H	I
CF 318	-	-	300	-	-	-	+	+		
CF 317	M	-	600	-	-	-	+			
CF 333	F	180	-	-	20	-		+		
CF 343	-	190	-	-	-	-	+			
CF 335	-	-	-	-	24	-	+		+	+
CF 390	M	-	800	35	29	-	+			
CF 336	F	-	-	-	30	-	+		+	
CF 382	M	-	1300	30	26	1 d.	+		+	+
CF 303	M	-	1944	44	-	6 d.	+	+	+	+
CF 372	M	-	2390	46	36	24 d.	+			
CD 146	M	-	2800	47	-	77 d.	+	+		
CD 73	M	-	3000	44	39	-	+	+	+	
CD 144	-	-	newborn		-	-	+		+	
CD 84	-	-	newborn		-	7 d.	+			
CD 141	F	-	-	-	-	6 d.	+			
CD 135	M	-	-	-	-	7 d.	+		+	+
CD 179	-	-	-	-	-	11 d.	+		+	
CD 127	-	-	-	-	-	20 d.	+	+	+	
CD 106	-	-	-	-	-	4 m.	+			
CD 170	-	-	-	-	-	4m 20d	+	+	+	+
CD 142	-	-	-	-	-	8 m.	+			
CD 191	-	-	-	-	-	15 m.	+	+	+	+
CD 117	M	-	-	-	-	2,5 y.	+		+	
CD 14	F	-	-	-	-	2y 10m	+	+		
CD 156	F	-	-	-	-	38 y.	+			

Abbreviations as in table 1.

TABLE 3

POSTNATAL DEVELOPMENT OF NEURONS OF THE HUMAN CEREBRAL CORTEX AS REVEALED BY GOLGI IMPREGNATION (RAPID GOLGI AND GOLGI-COX).

		CORTICALREGION							
ACC.. No.	AGE	PFC	MOT.	PREMOT.	BROCA	HIPPOC.	TEMP.	SEPT.	ANG.
CD 140	newb.		+	+	+	+			
CD 96	newb.	+	+	+		+			
CD 147	1m				+	+			
CD 105	2.5m	+		+		+			
CD 88	3m	+	+			+			
CD 123	7m	+	+			+			
CD 101	7.5m	+							
CD 143	15m	+	+		+	+			
CD 159	16m	+							
CD 175	2y5m	+		+	+				
CD 98	5y	+							
CD 125	6y	+	+			+			
CD 156	6y	+							
CD 126	9y	+	+			+			
CD 157	9y	+							

ACC.. No.	AGE	CORTICALREGION PFC	MOT.	PREMOT.	BROCA	HIPPOC.	TEMP.	SEPT.	ANG.
CD 185	9y	+							
CD 90	11y	+							
CO 170	16y	+							
CO 185	16y	+	+				+		
CO 159	17y	+							
CO 167	17y	+							
CO 194	24y	+						+	
CO 34	26y	+							
CO 161	27y	+							
CO 192	28y	+			+				
CO 180	30y	+							+
CO 135	35y	+							
CO 189	40y	+							
CO 150	46y	+							
CO 134	55y	+							
CO 171	86y					+			

Abbreviations: PFC = prefrontal cortex; MOT = motor cortex; PREMOT = premotor cortex; BROCA = cortex of opercular and triangular part of the left third frontal convolution; HIPPOC = hippocampus; TEMP = superior temporal cortex; ANG = gyrus angularis cortex; SEPT = septal region

TABLE 4

DEVELOPMENT OF THE CHOLINERGIC BASAL FOREBRAIN IN NORMAL BRAIN AND IN DOWN SYNDROME (NISSL STAINING AND AChE HISTOCHEMISTRY)

ACC. No:	AGE:	NORMAL BASAL FOREBRAIN	BASAL FOREBRAIN IN DOWN SYNDROME	NORMAL SEPTAL REGION
CF 171	9 w.g.	+		+
CF 95	11 w.g.	+		+
CF 169	11 w.g.	+		+
CF 10	13 w.g.	+		+
CF 43	13 w.g.	+		+
CF 127	13 w.g.	+		+
CF 134	15 w.g.	+		+
CF 9	24 w.g.	+		+
CF 356	26 w.g.	+		+
CF 182	28 w.g.	+		+
CF 207	31 w.g.	+		
CF 372	36 w.g.		+	
CD 4	newborn	+		
CD 135	6 d.		+	
CD 127	20 d.		+	
CD 132	22 d.	+		
CD 155	1.5 m.	+		+
CD 154	2 m.		+	
CD 85	2 m.	+		
CD 103	3.5 m.	+		
CD 78	4 m.	+		
CD 106	4 m.		+	
CD 170	4m 20d		+	
CD 131	5 m.	+		+
CF 353	6 m.	+		+
CD 124	6 m.			+
CD 101	7.5 m.	+		+
CD 142	8 m.		+	
CD 107	1 y.			+
CD 191	1y 3m.		+	
CD 111	2 y.	+		+
CD 117	2.5 y.		+	
CD 80	3 y.			+
CD 148	3.8 y.			+
CD 161	6.5 y.			+

TABLE 5

DEVELOPMENT OF PEPTIDERGIC ACTIVITY IN HUMAN CEREBRAL CORTEX AS JUDGED BY IMMUNOCYTOCHEMISTRY FOR SOMATOSTATIN (SOM), NEURO- PEPTIDE Y (NPY), PARVALBUMINE (PARV), SMI-32, CALBINDIN (CALB), FIBRONECTINE (FN) AND CHOLECYSTOKININ (CCK).

ACC.. No.	AGE	NPY	SOM	PARV	SMI-32	CCK	CALB	FN
CF 387	16 w.g.	+						+
CF 371	18 w.g.	+				+		
CF 373B	18 w.g.	+						
CF 306	22 w.g.		+					
CF 366A	23 w.g.	+						
CF 362	26 w.g.	+						
CF 376	26 w.g.	+				+		+
CF 379	26 w.g.	+				+		
CF 357	27 w.g.			+	+			
CF 384	28 w.g.	+						+
CF 305	29 w.g.		+					
CF 370	30 w.g.	+						
CD 164	31 w.g.	+						
CF 301	32 w.g.				+			
CF 380	35 w.g.	+		+		+		
CF 369	36 w.g.	+						
CD 96	newborn		+					
CD 178	newborn	+						
CD 176	38 d.	+						
CD 172	3 m.	+			+			
CD 173	4 m.	+			+			
CD 174	9 m.	+						
CD 181	14 m.	+						
CD 180	16 m.	+						
CD 167	16.5 m.			+	+		+	
CD 175	2 y.	+						
CD 182	2y 10m	+						
CD 159	5 y.		+					
CD 187	12 y.	+		+		+		
CO 185	16 y.	+		+	+			
CO 184	24 y.			+	+			
CO 192	28 y.	+		+				
CO 190	34 y	+						
CO 183	46 y.			+	+		+	
CO 193	53 y.	+		+		+		
CO 182	57 y.			+	+		+	

TABLE 6

HISTOCHEMICAL (AChE) DEVELOPMENT OF HUMAN CEREBRAL (FRONTAL, VISUAL, AUDITORY AND PARIETAL) CORTEX, HIPPOCAMPUS (HIPPOC.) AND BASAL FOREBRAIN (BF).

ACC. No.	AGE	BF	FRONTAL	VISUAL	AUDITORY	HIPPOC.	PARIETAL
CD 162	newb.		+	+	+		
CD 140	newb.		+				
CD 190	newb.			+		+	
CD 183	newb.		+	+			
CD 179	11 d.		+				
CD 127	20 d.	+					
CD 116	25 d.		+	+			
CD 139	1 m.		+				
CD 147	1 m.		+	+			+
CD 35	1 m.						
CD 166	1 m.			+			
CD 176	38 d.			+		+	
CD 115	51 d.		+	+			
CD 155	51 d.	+	+	+			
CD 188	1.5 m.						
CD 85	2 m.						
CD 114	2.5 m.	+	+	+			
CD 153	2.5 m.		+				
CD 172	2.5 m.		+				
CD 122	3 m.	+	+	+			
CD 22	3 m.						
CD 158	3 m.		+				
CD 173	4 m.		+	+			
CD 78	4 m.	+	+				
CD 120	4 m.		+	+			
CD 100	5 m.						
CD 131	5 m.	+	+				
CD 118	6 m.		+	+			
CD 119	6.5 m.		+	+			
CD 123	7 m.	+	+				
CD 101	7.5 m.			+			
CD 38	9 m.						
CD 124	9 m.		+	+			
CD 174	9 m.		+	+			
CD 113	9.5 m.	+	+	+			
CD 138	10 m.	+					
CD 107	1 y.		+	+			+
CD 133							
CD 181	14 m.		+	+		+	
CD 143	15 m.	+	+	+			
CD 159	16 m.		+	+			
CD 180	16 m.			+		+	
CD 167	16.5m.			+			
CD 33	18 m.						
CD 111	2 y.	+	+	+			
CD 175	2y 5m				+	+	
CD 182	2y 10m		+				
CD 112	3 y.	+	+	+			
CD 134	3 y.	+	+	+			
CD 149	4y 2m		+				
CD 31	4 y.						
CD 125	5 y.		+	+			
CD 98	5y 5m		+	+			
CD 156	6 y.	+	+	+	+		
CD 161	6.5 y.		+	+			
CD 129	7 y.	+	+				
CD 160	7 y.		+	+			
CD 93	8 y.	+	+	+	+		
CD 126	9 y.	+	+	+			
CD 185	9 y.		+	+			
CD 157	9y 3m	+	+	+	+		+
CD 136	10 y.			+	+		
CD 150	10 y.	+	+	+	+	+	+
CD 90	11 y.		+				
CD 151	11 y.		+	+	+		
CD 187	12 y.	+	+	+			
CD 89	14 y.	+	+				
CD 186	15 y		+				