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TOTAL NUMBER OF CELLS IN THE HUMAN NEWBORN TELENCEPHALIC WALL

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Abstract—The total cell numbers were estimated in the neocortical part of the human telencephalon in 10 normal brains of newborn babies within four major developmental zones: the cortical plate/marginal zone, the subplate, the intermediate zone and the ventricular/subventricular zone. Furthermore, the total number of neuron and glial cells was estimated in the cortical plate. The gestational ages ranged from 38+0–42+5 weeks+days of gestation. The mean total cell number was 32.6×10^9 (coefficient of error=0.04) and the total number of neurons in the cortical plate 19.8×10^9 (coefficient of error=0.06). This indicates that the total number of neocortical neurons equals the total number in the adults, which, however, is not the case for the glial cells. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: brain cell growth, cerebral cortex, newborn, human, neurons, stereology.

The establishment of the human fetal neocortex is critically important because disturbances of cellular formation can cause congenital brain disorders and result in adverse effects on morphology of the brain (abnormal proliferation, differentiation and/or migration of cells), e.g. quantitative changes such as decreases in number of neurons at the location of their final destination. Excessive neuron loss in the developing brain has negative consequences for the mental and physical abilities of the adult individual, becoming even more pronounced during senescence (Herschkowitz, 1988). However, such developmental morphological changes may go unrecognized by qualitative microscopical investigation and it is hypothesized in this study that quantitative neuropathology endpoints may be essential to identify early effects on the developing brain. Evidently knowledge of the normal fetal human brain can serve as a normative reference in the analysis of developmental neuroanatomy.

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Abbreviations: asf, area sampling fraction; CE, coefficient of error; CP, cortical plate; CV, coefficient of variance; IZ, intermediate zone; MZ, marginal zone; SP, subplate; SURS, systematic uniform random sampling; SVZ, subventricular zone; VZ, ventricular zone.

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During early gestation the process of differential cell proliferation causes the anterior part of the embryonic neural tube to expand outwards, forming a pair of telencephalic vesicles that becomes the cerebral hemispheres. The neocortex is formed from within the dorsal walls of the vesicles. The neurons are generated within the cerebral ventricles by an epithelial sheet of dividing progenitor cells (Herschkowitz, 1988). Before the onset of neurogenesis, the neuron progenitor cells will divide symmetrically in order to establish a pool of progenitor cells. During neurogenesis an asymmetric cell division takes place where one progenitor cell and one postmitotic neuron are generated and from where the pool of cells will grow. The postmitotic neuron will leave the ventricular zone (VZ) and settle just outside, forming the preplate. The subsequent postmitotic neurons migrate from the VZ along radial glial cell fibers positioning themselves in the preplate forming the cortical plate. As the cortical plate grows it is divided into an inner subplate (SP) and an outer marginal zone that will become layer I of the future cerebral cortex (McConnell, 1995). The first generated postmitotic neurons settle themselves in the deep layers of the cortex, whereas neurons generated later will settle in the superficial layer of the cortex creating the concentric layers designated layers VI–II. As the neurogenesis ceases around embryonic day 125, symmetric cell division replaces the progenitor cell pool with postmitotic neurons (Kornack, 2000). At one point the VZ stops generating neurons and eventually is replaced with ependymal cells (Kornack, 2000). The growth in cell number in the human fetal forebrain appears to be two-phased: one rapid, exponential phase from 13–20 weeks of gestation, and a second and slower phase which increases linearly from approximately 22 weeks of gestation to term (Samuelsen et al., 2003; Dobbing and Sands, 1973). DNA [^{3}H]thymidine studies have shown that primates acquire the majority of their neocortical neurons during the first half of gestation (Rakic, 1978, 1988).

The aim of our study was to estimate the total number of cells in the neocortical part of the telencephalic wall within four major fetal zones: the cortical plate/marginal zone (CP/MZ) of the prospective neocortex and three zones underneath the neocortical anlage: the SP, the intermediate zone (IZ) and the VZ/subventricular zone (VZ/SVZ) in the normal human newborn neocortex to determine if the neocortical neuron number is established already prenatally.

EXPERIMENTAL PROCEDURES

The material comprises 10 brains from newborn humans, five male and five females, with gestational ages ranging from 38+0–

Table 1. Clinical data

Gestational age (weeks+days)	Sex	Body weight (g)	Fetal growth ratio	Brain weight (g)	Fetal condition	Placental/maternal conditions	Age of mother (years)
38+0	Female	2900	0.93	144 (One hemisphere)	Pneumoni	Normal	24
38+6	Female	3647	1.10	402	Acute asphyxia	Normal	34
40+0	Female	3330	0.85	420	Acute asphyxia	Small artifacts in placenta	35
40+4	Female	3770	1.04	407	Acute asphyxia	Normal	35
41+0	Female	3859	1.04	558	Acute asphyxia	Normal	28
39+2	Male	3667	1.04	442	Acute asphyxia	Normal	28
40+5	Male	3926	1.03	482	Acute asphyxia	Tightening of true cord knot	30
41+0	Male	4657	1.20	471	Subcapsular Liver hematoma Hemoperitoneum Acute asphyxia	Chorioamnionitis	33
41+3	Male	3855	0.97	441	Isolated lung hypoplasia	Normal	29
42+5	Male	4138	0.99	540	Acute asphyxia	Chorioamnionitis	33

41+5 weeks+days, all Caucasian. A complete brain autopsy was performed in each case, including histological diagnosis and thorough neuropathological examination on one hemisphere. The contralateral hemisphere was prepared for stereological analysis. Seven subjects died of acute asphyxia, one from bronchopneumonia, one from lung hypoplasia, and one died for unknown causes. The age of the mothers varied from 24 years to 35 years. All fetuses were without malformations (except the case of lung hypoplasia), no apparent chromosomal abnormality, hydrops, or systemic infections, and all had normal birth weights with fetal growth indices (observed birth weight/expected mean birth weight) between 0.80–1.2 (Larsen et al., 1990), see Table 1. Such a ratio-based classification was preferred to more traditional ones based on percentiles or standard deviations because it conveys important clinical and statistical information, as it gives the percentage of weight relative to the mean. Multiple pregnancies were not included. At the time of delivery one mother was primiparous, seven were secundiparous, and one multiparous while this information was not available for the last subject. If divided into quarters of the year at time of death: one died in the first quarter, two in the second quarter, two in the third quarter and five in the fourth quarter. Nine out of 10 subjects died during birth while the last subject died 60 h after birth. The autopsies were performed within one or two days after death but the time until fixation of the brains was not consistently recorded. Sometimes, due to weekends and holidays, the autopsy was delayed and thus performed up to three and a half days after death. From death until autopsy the fetuses were refrigerated at 5 °C in order to minimize cellular degeneration. The brains were collected in the period 1991–1999 and obtained from necropsies after parental consent and the material was studied with the approval of the local ethical committee for Copenhagen and Frederiksberg (Jr. nr. (KF) 01–247/95).

Tissue processing

The brains were fixed in 10% formalin for four weeks. Soft and vulnerable brains were further fixed in 25% picric acid and 20% formalin for two to four weeks to harden the tissue prior to cutting. The left or right hemisphere was chosen systematically at random. The hemispheres were cut into 2–3 cm thick blocks before embedding in paraffin, and then sectioned coronally on a sledge microtome with a setting of 40 μm. The blocks were cut serially and throughout the hemisphere.

The sections were mounted on double silane-coated glass slides and instantly dried at 40 °C for 24 h. The sections were then heated to 60 °C for 30 min, deparaffinated in xylene for 45 min, followed by 15 min in 99% alcohol, 10 min in 96% alcohol, 5 min in 70% alcohol, and 5 min in distilled water. The sections were stained by a modified Giemsa staining containing 25 ml Giemsa stock solution (Merck, Darmstadt, Germany), 250 ml potassium-hydrogen-phosphate, pH 4.5, being filtered before use. Finally the sections were dehydrated through 96% ethanol for 1–5 min, 99% ethanol for 5–10 min, and xylene for 15 min.

Delineations of developmental zones

In order to estimate the total number of cells in the neocortical part of the cerebral wall a clear definition of the boundaries of the four major developmental zones, the VZ/SVZ, IZ, SP and CP/MZ, was necessary.

In the newborn brain the VZ/SVZ is a proliferative zone characterized by its densely packed immature neuroepithelial cells, which makes it easy to establish the delineation toward the IZ. Eventually the VZ differentiates into the future ependyma. The IZ is the future white matter, consisting of migrating cells and ingrowing axons. It is less cell-packed than the VZ/SVZ and is separated from the SP by the presence of horizontal axons at the border to the SP. The SP is histologically recognized by its large polymorphic neurons scattered in abundant neuropil. The neurons are characterized by their cytoplasmic maturation compared with the cells of the CP. This maturational feature is important in distinguishing the SP from the other zones. The SP disappears by the 6th postnatal month (Kostovic and Rakic, 1990). The outer border of the SP is easy to differentiate from the cell-dense and heavily stained CP. The CP/MZ represents the future neocortex and was analyzed as one, as the cell-sparse MZ is included in the future neocortex. Uncus, hippocampus, parahippocampal gyrus, gyrus fornicatus, and the subcallosal area was considered archicortex and not included in neocortex.

Stereology

Stereology is a technique that enables data to be obtained by sampling a number of identifiable objects in a three-dimensional space. Thus, it provides a method for counting objects on a section from the structure such as a histological specimen viewed under the microscope. It has become evident that it is neither desirable nor necessary to count all the cells in a structure to obtain data that are useful for descriptive and comparative studies. Estimates of the total number are sufficient, as long as they have an appropriate amount of precision and are estimates of the true total number, i.e. are based on unbiased counting and sampling techniques. Thus, the stereological methods allow reli-