The Changing Number of Cells in the Human Fetal Forebrain and its Subdivisions: A Stereological Analysis

The total number of cells – including both neurons and glial cells – was estimated in the neocortical part of the human fetal telencephalon in 22 normal brains within four major developmental zones: the cortical plate/marginal zone, the subplate, the intermediate zone and the ventricular/subventricular zone. The fetal ages ranged from 13 to 41 weeks of gestation. The cellular growth in the human fetal forebrain appears to be two-phased: one rapid, exponential phase from 13 to 20 weeks of gestation and a second and slower phase, which increases linearly, from approximately 22 weeks of gestation to term. From 13 to 20 weeks of gestation the total number of cells increases by a factor of 4.3 from \(3 \times 10^9\) cells to \(13 \times 10^9\) cells at 20 weeks of gestation. From mid-gestation to term, the total cell number increases by a factor of 2.9 to \(38 \times 10^9\) cells in the newborn infant. Studying cellular growth in the normal human fetal brain is important since it may serve as a useful parameter for the assessment of cortical growth in non-invasive and histological studies, and thus improve the analysis of fetal brain disturbances.

Introduction

In spite of the impressive amount of data that has been provided over the past three decades to evaluate the mechanisms regulating cortical histogenesis, only a few studies have quantified the development of the human fetal brain in terms of cellular growth (Rabinowicz et al., 1996). Previous quantitative studies have been based on non-uniform sampling and various correctional factors, which has made interpretation difficult, especially because fetal brain tissue undergoes considerable and unpredictable changes during fixation and preparation. Dobbing and Sands avoided these technical problems by using chemical analysis of DNA contents, and reported cellular growth in the human forebrain to be two-phased (Dobbing and Sands, 1975). They found the first rapid phase at 10–18 weeks of gestation to be exponential, while the second and slower phase occurred from approximately 20 weeks of gestation until term. In the latter period the increase in DNA amount was almost linear. DNA \(^{3H}\)thymidine studies suggested that humans acquire the majority of their neocortical neurons during the first half of gestation (Rakic and Sidman, 1968; Sidman and Rakic, 1973; Rakic, 1974, 1978, 1988). Thus, it is most likely that this period corresponds to the time of neurogenesis with exception of the presence of radial glial cells which are needed during corticogenesis for the neurons to reach the cortical plate (Rakic, 1972; Rakic, 1975; Antanitus et al., 1976; Choi and Lapham, 1978; Cameron and Rakic, 1991). The coexistence of neurons and glial cells in the ventricular/subventricular zone is compatible with several studies that have described a heterogeneous population in this zone with separate progenitors for neurons and glial cells at the beginning of corticogenesis (Levitt et al., 1981; Luskin et al., 1988, 1993).

In the present study, cell numbers were estimated in distinct fetal compartments in the neocortical part of the cerebral wall; these fetal zones will be briefly described in the following.

Most of the neurons destined for the prospective cortex are produced deep within the brain in the ventricular zone (VZ). Initially, the superficial processes of the ventricular cells together with processes of the first postmitotic neurons forms a pale superficial layer – the marginal zone (MZ). The following postmitotic neurons generate a new intermediate zone (IZ) – the prospective white matter – situated between the VZ and the MZ. Later, neurons arise indirectly from the VZ by means of secondary proliferative cells that congregate in the subventricular zone (SVZ), a zone situated between the VZ and the IZ (Rakic, 1972; Levitt et al., 1981). A distinct part of the SVZ situated within the basolateral part of the cerebral wall, the ganglionic eminence (Kostovic and Judas, 1995), gives rise to the cells of the basal ganglia. However, recent studies suggest that this area also gives rise to the inhibitory GABAergic interneurons in the neocortex by tangential migration (Anderson et al., 1997; Tamamaki et al., 1997).

From the VZ and the SVZ the majority of young neurons will migrate along the transient radial glial cells toward the pial surface of the cerebral wall, where they form the cortical plate (CP). In the cerebral cortex, the cells that underwent their mitosis early in development end up in the deep layers of the cortex, whereas neurons produced later migrate to the superficial layers (Angevine and Sidman, 1961; Berry and Rogers, 1965; Rakic, 1972). When migration is completed, neurons are locked in position by the formation of specific axon-target interaction and subsequently, radial glial cells most probably transform into astrocytes (Hatten, 1993, 1999).

In the beginning of the second trimester, another zone will appear at the intersection between the IZ and the CP. This zone, the subplate zone (SP), is a transient zone characteristic for the development of the cerebral cortex in humans and higher mammals. Together with the CP and the MZ, it also contributes to the prospective neocortex (Kostovic and Rakic, 1990; Kostovic and Judas, 1995). As it is a transient developmental zone, the SP undergoes intensive reorganization from the time of its formation until its gradual dissolution after birth. It reaches its maximum thickness by the 22nd week of gestation, and after the 35th week of gestation the SP gradually dissolves and is considered to disappear around the sixth postnatal month in humans (Kostovic et al., 1989; Kostovic and Rakic, 1990).

The aim of this study was to estimate the total number of cells in the neocortical part of the telencephalic wall within the following major fetal zones: the CP/MZ of the prospective neocortex and three zones underneath the neocortical anlage – the SP, the IZ and the VZ/SVZ in the normal human brain from 13 weeks of gestation until term. This study gives the first normative, numerical data in the human fetal forebrain at various prenatal stages related to already known qualitative events in this period (Kostovic et al., 2002). Our data may provide important
parameters that can be used as guidelines in both histological and non-invasive studies of fetal brain development.

Materials and Methods

The study included 22 human fetal brains, 12 males and 10 females, with gestational ages ranging from 13 to 41 weeks; all fetuses were Caucasian. Twenty brains were collected from a consecutive series of ~1200 fetal autopsies from Rigshospitalet, Copenhagen and Herlev University Hospital after parental consent and approval of the study by the local ethical committee. In addition, two hemispheres from autopsies performed according to the same principles at other Danish hospitals were also included. A complete autopsy, including a neuropathological examination, was performed on one of the brain hemispheres while the other hemisphere was prepared for quantitative analysis.

Sometimes, due to weekends or holidays, an autopsy was delayed up to 3.5 days after death, but most autopsies were performed within one or two days postmortem. The exact time from death until fixation of the brain, the hemispheres were cut into two or three blocks or right hemisphere was chosen systematically at random. Depending on the size of the brain, the hemispheres were cut into two or three blocks before further treatment or directly embedded in paraffin and sectioned coronally on a sledge microtome with a setting of 40 μm. The sections were mounted on double-silane-coated glass slides and dried immediately at 40°C for 24 h. Before staining, the sections were heated to 60°C for 15 min. Samples were fixed in 4% buffered formaldehyde (Lillie), eight were fixed in 4% formalin (Larsen et al. 1990). Some brains were immersed in a 10% solution for 4 weeks prior to cutting in order to harden the tissue. The left brain hemisphere was processed according to the same principles at other Danish hospitals, including two hemispheres from autopsies from Rigshospitalet, Copenhagen and Herlev University Hospital after parental consent and approval of the study by the local ethical committee. In addition, two hemispheres from autopsies performed according to the same principles at other Danish hospitals were also included. A complete autopsy, including a neuropathological examination, was performed on one of the brain hemispheres while the other hemisphere was prepared for quantitative analysis.

The brains were divided into two parts according to gestational age: one early period relating to the time traditionally known as the period of neurogenesis from 13 to 20 weeks of gestation, and a second period covering the interval from 22 weeks of gestation to term (for further details, see Table 1).

In fetuses younger than 20 weeks, the gestational age was estimated from the menstrual history of the mother, foot length and in two cases an ultrasound examination had also been performed. The measurement of the gestational age was expressed in commenced gestational weeks, e.g. 13 weeks of gestation ranged from 12 completed weeks plus one day to the termination of week 13. The selection of the fetuses older than 20 weeks was based on their body weights measured at autopsy corrected for gestational age to include only fetuses with normal birth weights within ±10% of the expected birth weight as given by a Danish ultrasonic intrauterine growth curve, which is adjusted for sex, males being 4% heavier than females (Larsen et al., 1990). 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.

### Table 1

<table>
<thead>
<tr>
<th>Gestational age (days/weeks)</th>
<th>Sex</th>
<th>Body weight (g)</th>
<th>Fetal growth ratio</th>
<th>Brain weight (g)</th>
<th>Fetal condition</th>
<th>Placental/maternal conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>male</td>
<td>53</td>
<td>–</td>
<td>–</td>
<td>social abortion</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
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<td>63</td>
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<td>9.7</td>
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<td>normal</td>
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<td>15</td>
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<td>74</td>
<td>–</td>
<td>9.0</td>
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<td>normal</td>
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<td>16</td>
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<td>80</td>
<td>–</td>
<td>–</td>
<td>social abortion</td>
<td>normal</td>
</tr>
<tr>
<td>17</td>
<td>female</td>
<td>129</td>
<td>–</td>
<td>20</td>
<td>spontaneous abortion</td>
<td>chorionamnionitis</td>
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<tr>
<td>17</td>
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<td>133</td>
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<td>167</td>
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<td>293</td>
<td>–</td>
<td>39</td>
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<td>cervix insufficiency</td>
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<td>chorionamnionitis</td>
</tr>
<tr>
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<td>114</td>
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<td>chorionamnionitis</td>
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<tr>
<td>Early 24</td>
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<td>619</td>
<td>1.07</td>
<td>82</td>
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<td>Early 25</td>
<td>male</td>
<td>679</td>
<td>0.98</td>
<td>–</td>
<td>liveborn immaturity</td>
<td>chorionamnionitis</td>
</tr>
<tr>
<td>Early 25</td>
<td>male</td>
<td>670</td>
<td>0.92</td>
<td>86</td>
<td>immaturity</td>
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<tr>
<td>Early 26</td>
<td>female</td>
<td>845</td>
<td>1.08</td>
<td>99</td>
<td>spontaneous abortion</td>
<td>chorionamnionitis</td>
</tr>
<tr>
<td>Early 27</td>
<td>female</td>
<td>945</td>
<td>1.04</td>
<td>116</td>
<td>–</td>
<td>normal</td>
</tr>
<tr>
<td>Early 31</td>
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<td>1700</td>
<td>1.08</td>
<td>–</td>
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<td>normal</td>
</tr>
<tr>
<td>Early 31</td>
<td>male</td>
<td>1690</td>
<td>1.02</td>
<td>–</td>
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<td>acute fetomaternal haemorrhage</td>
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<td>Early 36</td>
<td>female</td>
<td>2567</td>
<td>1.02</td>
<td>302</td>
<td>–</td>
<td>normal</td>
</tr>
<tr>
<td>Late 39</td>
<td>female</td>
<td>3647</td>
<td>1.1</td>
<td>402</td>
<td>–</td>
<td>normal</td>
</tr>
<tr>
<td>Early 40</td>
<td>male</td>
<td>3667</td>
<td>1.04</td>
<td>442</td>
<td>–</td>
<td>short umbilical cord</td>
</tr>
<tr>
<td>Late 41</td>
<td>male</td>
<td>3926</td>
<td>1.03</td>
<td>482</td>
<td>–</td>
<td>tightening of true cord knot</td>
</tr>
</tbody>
</table>

Delineation of the Developmental Zones

The neocortex develops a prominent CP and SP while the archicortex develops an exceptionally wide MZ and a thin convoluted CP without a CP. The paleocortex never develops a true CP (Kostovic and Judas, 1995). Since we were quantifying cell numbers only in the neocortical part of the cerebral wall, a clear definition of the boundaries was necessary. The cerebral wall is arranged from inside-out: the VZ, the SVZ, the IZ, the SP, the CP and the MZ. The capsule externa was considered as a part of the IZ minus the claustrum, which was excluded. The ganglionic eminence was only included from 20 weeks to term because recent literature reports a tangential migration from this area to the neocortex (Anderson et al., 1997; Tamamaki et al., 1997). The ganglionic eminence was not included in the first period. Since the ganglionic eminence is part of the VZ/SVZ, the total cell numbers in the VZ/SVZ are not shown in any figures since they are not directly comparable. The CP and the MZ were analysed as one fetal zone since the cell-sparse MZ is included in the prospective cortex as layer 1. The VZ and the SVZ were chosen to be analysed as one

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zone, since the borders are not clearly distinguishable. Both zones are proliferative zones and recognized by their densely packed immature, neuroepithelial cells, (Fig. 1).

**Counting Method**
A combination of two stereological principles, the optical dissector and the fractionator sampling design – the so-called optical fractionator method – was applied (West et al., 1991). This technique involves counting particles with optical dissectors in a systematic, uniform, random sample that constitutes a known fraction of the structure to be analysed. The only caveats are that the entire structure must be available and generally intact, and, in this design, that the structure be sectioned exhaustively. All handling and counting was performed on coded brains.

The optical fractionator method was ideal for this study because it is unaffected by the considerable and unpredictable shrinkage during fixation of the human fetal brain. The optical fractionator method was applied in three stages:

1. One fully intact brain hemisphere was embedded in paraffin and sectioned exhaustively into 40 µm thick sections. A known sampling fraction,ssf, of all sections was sampled systematically with a random start. Depending on the size of the brain, thessfwas chosen to be between 1/80th and 1/200th in order to achieve 10-16 sections per brain.

2. After random placement of a two-dimensional counting frame in the first predetermined x,y interval, the counting frame was placed at subsequent x,y intervals using a motorized stepping motor. The area of the counting frame, a(frame), of the dissector was known relative to the area associated with each step in the x,y direction, a(x,y step). The area sampling fraction,ssf, was calculated as a(frame)/a(x,y step). The size of the counting frame and the x,y distance were adjusted in each brain to count ~100–200 cells per region. The size of the counting frame varied from 4^2 to 650 µm^2 overall, while the x,y interval varied from 1000 to 2107 µm in the VZ/SVZ, 1250 to 3500 µm in the IZ, 1000 to 3000 µm in the SP, and 1000 to 3000 µm in the CP/MZ.

3. The mean thickness, t, was estimated from measurements made at every fifth dissector. The fixed height, h, of the dissector was chosen to be 10 µm throughout. The height sampling fraction is calculated as:

\[
hsf = \frac{h}{Q} \quad \text{for} \quad i = \frac{\sum q_i}{Q} - \frac{\sum t_iq_i}{Q} \sum q_i
\]

where t_i is the local section thickness centrally in the ith counting frame with a dissector cell count of q_i (Dorph-Petersen et al., 2001). A Heidenhain microcator measured movements in the z-axis with a precision of 0.5 µm.

The optical dissector equipment consisted of an Olympus microscope with a high numerical aperture (NA = 1.4) and a 100× oil objective. A videocamera transmitted the image to a video screen where a counting frame was superimposed using a commercial software package (CAST-GRID, Olympus, Denmark).

For each sampled section, the different fetal zones were delineated by the same neuroanatomist (N.B.). This was accomplished by making an indication in Indian ink corresponding to the histologically verified borders on a section under a stereo microscope. The borders of the fetal zones were transferred to CAST-GRID and each fetal zone counted separately. The inclusion line for the CP/MZ was drawn according to the outer pial surface, whereas the exclusion line for the CP/MZ was made at the interface between the CP/MZ and the SP. The cells were then counted directly in optical dissectors in the CP/MZ. Subsequently, the exclusion line of the CP/MZ was used as an inclusion line for the underlying SP, whereas a new exclusion line was drawn at the interface between the SP and the IZ. Afterwards, the same procedure was used for the IZ and the VZ/SVZ zones, thereby guaranteeing that no area, and thus no cells, were omitted or counted twice due to inconsistent delineation.

For one brain hemisphere, the sum of cells counted in all the dissectors was denoted as \( \Sigma Q' \). The total number of cells in the particular fetal zone was now calculated as:

\[
N = 2 \times \sum Q' \times \frac{1}{ssf} \times \frac{1}{asf} \times \frac{1}{hsf}
\]

The multiplication by two provides a bilateral estimate and this is allowed because each hemisphere was selected systematically randomly assuming no side differences. The total number of cells throughout the cerebral wall was provided by adding \( N \) cells from each fetal zone.

**Statistics**
The cellular growth in the human fetal forebrain appeared to be two-phased, and therefore the data were allocated into two different groups: one from 13 to 20 weeks of gestation and another from 22 weeks of gestation until term. The unit of gestational age is weeks.

From 13 to 20 weeks of gestation all of the total cell numbers in the four fetal zones develop in exponential patterns. Because of the exponential growth the total cell numbers are expressed as logarithms. A linear regression analysis of the total cell number was made from:

\[
\log(N) = \alpha + (\beta \times \text{gestational age})
\]

where \( N \) is the total number of cells in billions (10^9). The total cell number is thus calculated from:

\[
N = \exp(\alpha + (\beta \times \text{gestational age}))
\]

The coefficient of determination, \( R^2 \), is the proportion of the variation explained by the regression model. This coefficient was used to describe how well the regression model fits the dependent variable. A value of \( R^2 \) close to 1 implies that most of the variability in the dependent variable is explained by the regression model. If the linear regression has only one dependent variable, \( y = \alpha + \beta x \), the square root of the coefficient of determination, \( \sqrt{R^2} \), is equal to the correlation coefficient, \( r \).

As the total cell numbers appeared to develop in linear patterns from 22 weeks of gestation until term, a multiple linear regression analysis was applied in this period to describe the total cell number in the four developmental zones using gestational age as the independent regression variable:

\[
N = \alpha + (\beta \times \text{gestational age})
\]

It could not be determined which of the two mathematical approaches – showing either exponential or linear growth – fitted best for the period between 20 and 22 weeks of gestation, partly due to the limited number of cases in the entire study, and partly due to the lack of cases in this specific period.

Subsequently, multiple linear regression analysis was applied to describe total cell number using head circumference as an independent regression variable.

A second-order polynomial was applied to describe the age-dependent development of the total cell number in the SP.

All tests are two-sided and performed at the 5% significance level.

**Precision of the Estimate**
A mean of 1009 cells in an average of 447 dissectors was counted per brain (261 cells in an average of 121 dissectors in the CP/MZ, 316 cells in an average of 158 dissectors in the SP, 247 cells in an average of 104 dissectors in the IZ and 184 cells in an average of 63 dissectors in the VZ/SVZ). The coefficient of error, CE = SEM/mean, indicates the precision of the estimate of a single brain. The CE was estimated from

\[
\text{CE}(N) = \sqrt{\frac{\text{VarSURS} + \text{Noise}}{Q'}}
\]

where Noise is the sum of counted particles and VarSURS is the estimator variance under Systematic Uniform Random Sampling (SURS). The VarSURS(N) is obtained from the formula

\[
\text{VarSURS}(N) = \frac{3(A - \text{Noise}) - 4B + C}{240}
\]
where the systematic section series of particle count are denoted $f_1$, $f_2$, ..., $f_n$ and

$$A = \sum_{i=1}^{n} f_i^2 \quad B = \sum_{i=1}^{n-1} f_i f_{i+1} \quad C = \sum_{i=1}^{n-2} f_i f_{i+2}$$

(for further details see equations 20–22, Gundersen et al., 1999). Because of high variation of brain size, the sampling scheme was designed on an individual basis. However, the CEs were all <10%. The final estimate of the sum of the total number of cells in the human fetal forebrain had a mean CE of 4.3%. The mean CEs for the CP/MZ, SP, IZ, VZ/SVZ were 7.1, 6.6, 7.4 and 8.6%, respectively. One brain was counted twice with a repeatability of <5.0%.

Results

First Period: 13–20 Weeks of Gestation

The total number of cells in the neocortical part of the human fetal telencephalon, $N_{\text{total}}$, is increased by a factor of 4.3 from $3.01 \times 10^9$ cells at 13 weeks of gestation to $12.8 \times 10^9$ cells at 20 weeks of gestation, given by the formula:

$$\log(N_{\text{total}}) = -1.591 + (0.207 \times \text{gestational age})$$

($R^2 = 0.96$) (Fig. 2). The prospective neocortex, CP/MZ, comprises 0.96 $\times 10^9$ cells at 13 weeks of gestation, increasing by a factor of 6.1 to 5.87 $\times 10^9$ cells at 20 weeks of gestation given by the formula:

$$\log(N_{\text{CP/MZ}}) = -3.411 + (0.259 \times \text{gestational age})$$

($R^2 = 0.96$) (Fig. 2). Underneath the cortical plate, the total number of cells of the subplate also increases in this particular period by a factor of 3.6 from $0.66 \times 10^9$ cells at 13 weeks of gestation to $2.40 \times 10^9$ cells at 20 weeks of gestation given by the formula:

$$\log(N_{\text{SP}}) = -2.826 + (0.185 \times \text{gestational age})$$

($R^2 = 0.88$) (Fig. 2). The prospective white matter, the IZ, contains $0.80 \times 10^9$ cells at 13 weeks of gestation and increases by a factor of 3.8 to $3.06 \times 10^9$ cells at 20 weeks of gestation given by the formula:

$$\log(N_{\text{IZ}}) = -2.702 + (0.191 \times \text{gestational age})$$

($R^2 = 0.94$) (Fig. 2). According to the growth formula for the VZ/SVZ made in this study, the VZ/SVZ comprises $0.57 \times 10^9$ cells at 13 weeks of gestation, which increases by a factor of 2.5 to $1.44 \times 10^9$ cells at 20 weeks of gestation ($R^2 = 0.51$) given by the formula:

$$\log(N_{\text{VZ/SVZ}}) = -2.275 + (0.132 \times \text{gestational age})$$

Second Period: 22 Weeks of Gestation until Term

Of the four developmental zones, all but the SP develop in linear patterns in this period. Consequently, the CP/MZ, IZ and VZ/SVZ
are described by a first-order polynomial, whereas the SP is described by a second-order polynomial.

In the prospective neocortex, the total cell population, \( N_{CP/MZ} \), is increased by a factor of 4.2 during the last half of gestation, from \( 7.05 \times 10^9 \) cells at 22 weeks of gestation to \( 29.4 \times 10^9 \) cells at term given by the formula:

\[
N_{CP/MZ} = -20.21 + (1.239 \times \text{gestational age})
\]  
\( (R^2 = 0.93) \) (Fig. 2). As illustrated in Figure 2, the development of the total cell number in the SP, \( N_{SP} \), follows a second-order polynomial \( (R^2 = 0.72) \), confirming that this zone is transient. The SP comprises \( \sim 1.90 \times 10^9 \) cells at 22 weeks of gestation, reaching a maximum of \( \sim 3.59 \times 10^9 \) cells at 35 weeks of gestation given by the formula:

\[
N_{SP} = -12.51 + (0.985 \times \text{gestational age}) - (0.015 \times \text{gestational age})^2
\]  

Hereafter the number of cells slowly declines as the zone dissolves.

The total cell number in the feeding zone, \( N_{VZ/SVZ} \), decreases linearly from mid-gestation to term. From 22 weeks of gestation to term the total cell number in this zone is reduced by a factor of almost 4 from \( 4.58 \times 10^9 \) cells at 22 weeks of gestation to \( 0.79 \times 10^9 \) cells at term \( (R^2 = 0.92) \) given by the formula:

\[
N_{VZ/SVZ} = 9.197 - (0.210 \times \text{gestational age})
\]

A sum of the results from the four developmental zones was made to provide an estimate of the total number of cells throughout the entire neocortical cerebral wall. The sum of total cell numbers in all four zones, \( N_{Total} \), also increases linearly and parallels the development of the total cell number in the CP/MZ. A linear model of the total cell number is given by the formula:

\[
N_{Total} = -9.629 + (1.198 \times \text{gestational age})
\]  
\( (R^2 = 0.88) \) (Fig. 2). The total number of cells in the human fetal forebrain increases by a factor of \( \sim 2 \) from \( 16.7 \times 10^9 \) cells at 22 weeks of gestation to \( 38.3 \times 10^9 \) cells at term. The weekly accession of brain cells in the entire neocortical cerebral wall is \( 1.20 \times 10^9 \) cells, equal to \( \sim 171 \) million \( (10^6) \) cells per day.

**Head Circumference**

An important parameter is head circumference, which is considered to be an indirect measurement of brain size (Winick and Rosso, 1969). Head circumference was therefore analysed in relation to the total number of brain cells in both the prospective

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**Figure 2.** The total number of cells in billions \( (10^9) \) in three major developmental zones of the human fetal forebrain (CP/MZ, SP, IZ) and all four zones added (CP/MZ, SP, IZ, VZ/SVZ), representing the total number of cells in the entire human fetal forebrain. The material has been allocated into two study periods: one from 13 to 20 weeks of gestation and another from 22 weeks of gestation to term. Due to the limited number of cases during the period from 18 to 22 weeks of gestation, it could not be determined which of the two mathematical approaches that fitted best between 20 and 22 weeks of gestation.
neocortex and all four zones together from 20 weeks of gestation to term. There is a high correlation between the total cell number in both the CP/MZ, \( N_{\text{CP/MZ}} \), and throughout the cerebral wall, \( N_{\text{Total}} \), and head circumference \( (R^2 = 0.88 \text{ and } R^2 = 0.86, \text{ respectively}) \). The latter is shown in Figure 3.

**Discussion**

**First Period: 13–20 Weeks of Gestation**

In this period the CP/MZ is characterized by a rapid exponential growth in cell numbers, reaching \( 5.87 \times 10^9 \) cells at 20 weeks of gestation. This is in agreement with the qualitative observation of the secondary consolidation of the cortical plate during at 16–18 weeks of gestation (Kostovic and Judas, 1995).

From 13 to 20 weeks, the subplate has three different stages: subplate formation stage at 13–15 weeks of gestation, the subplate stage in the expansive phase at 16–18 weeks of gestation, and the subplate stage in the beginning of the stationary phase at 19–20 weeks of gestation. Immature neurons wait in the subplate to mature and form connections with ingrowing afferents from the brainstem, the basal forebrain, thalamus, and ipsi- and contralateral cortex, before reaching the cortical plate (Kostovic and Judas, 1998). In this context, it is in full agreement that the content of cells in the subplate increases by a factor of 3.6 during the period from 13 to 20 weeks of gestation.

The prospective white matter, the IZ, increases its content of cells by a factor of 3.8 during this period of gestation. Inward growing axons occupy a large area in the IZ, which explains the relatively low density of cells in this zone.

The low coefficient of determination \( (R^2 = 0.51) \) in the VZ/SVZ indicates that the development of cellular numbers is not explained satisfactorily by an exponential growth model from 13 to 20 weeks of gestation. This may be a consequence of a predominantly asymmetric cell division at the beginning of the period. The asymmetric cell division is a stem cell mode of division. One cell remains in the VZ/SVZ zone while the other migrates to reach the CP (McConnell, 1995), resulting in a steady number of cells in spite of a high cell production. Thus, the total number of cells in the VZ/SVZ zone does not change, even though a great number of cells are generated. At 13–15 weeks of gestation the unchanging total number of cells in the VZ/SVZ zone could be a consequence of asymmetric cell division predominating as one of the generated cells is sent away to populate the other zones. At the beginning at 16 weeks of gestation the total number of cells in the VZ/SVZ zone increases once again. This increment may be due to the symmetric terminating cell division, which appears to continue to \( \sim 22 \) weeks of gestation. Both cells wait in the VZ/SVZ zone before migrating to the CP (Kornack, 2000). Generally, a low value of \( R^2 \) might be due to imprecise data or to large biological variation. The CE for the VZ/SVZ was 8.6\%, which is close to CE in the other regions.

**Second Period: From Mid-gestation to Term**

In the prospective neocortex, the total cell number further increases almost by a factor of five from \( \sim 6 \times 10^9 \) cells at 20 weeks of gestation to \( \sim 30 \times 10^9 \) cells at term. Since neurogenesis in humans is generally accepted as being completed at mid-gestation (Rakic and Sidman, 1968; Sidman and Rakic, 1973; Rakic, 1974, 1978, 1988), we expected to find a total cell number that would approach the number of neocortical neurons found in adult human brains. In the present study, however, we estimated a total of \( \sim 6 \times 10^9 \) cells at 20 weeks of gestation in the CP/MZ. Even when the total cell number of all four developmental zones were summed — and assuming that most of these cells were neurons that eventually will settle down in the neocortex — a total of \( \sim 15 \times 10^9 \) cells is still far from the \( 19–23 \times 10^9 \) neurons found in the adult human brain (Pakkenberg and Gundersen, 1997). This could indicate that neurogenesis is not yet completed at 20 weeks of gestation and might continue a few weeks longer. Furthermore, a recent study suggests that a subpopulation of GABAergic neurons migrates from the ganglionic eminence of the ventral forebrain to the dorsal forebrain, and these clones of GABAergic neurons have been shown to appear in the human fetal cerebral cortex at \( \sim 20 \) weeks of gestation (Letinic et al., 2002).

The development of total cell numbers in the SP during the second period is best described using a second-order polynomial. The SP comprises \( \sim 2 \times 10^9 \) cells at 22 weeks of gestation, reaching a maximum of \( \sim 3.6 \times 10^9 \) cells at 35 weeks of gestation. Thus, in terms of total cell number, the SP is not in a stationary phase from 22 to 35 weeks of gestation. The present results demonstrate an increase in total cell number from 20 to 35 weeks of gestation, after which the total cell number declines. If we disregard a two-phased growth model in the SP, the development in total cell numbers could also be described as a linear increment from 13 to 35 weeks of gestation, after which the total cell number decreases. Irrespective of mathematical approach, the results confirm that the SP is a transient zone and begins to dissolve after 35 weeks of gestation. However, at term the SP still contains a little less than \( 3 \times 10^9 \) cells, suggesting that the cellular interactions taking place in this zone between the immature neurons and incoming fibers also could be influenced by events in early postnatal life.

Corresponding to the linear increases found in both the CP/MZ and the IZ from mid-gestation to term, the proliferative ventricular and subventricular zones, VZ/SVZ, are demonstrated to decrease linearly in this period, by a factor of approximately four. The most plausible explanation for the reduction in total cell number in the proliferative regions is the vast migration of cells away from the VZ/SVZ in order to ‘feed’ the concurrently thickening cerebral wall with cells.

**Head Circumference and Total Cell Number**

Head circumference is an often-used measurement to follow growth during intrauterine life, infancy and childhood. Using the already-mentioned DNA quantification method, Winick found head circumference to be an indirect measurement of brain size and that DNA content correlated linearly with head circumference (Winick and Rosso, 1969). In the present study a similar correlation is found from mid-gestation to term. Head circumference is linearly correlated with the total cell number in both the prospective neocortex as well as the entire neocortical cerebral wall (the latter is shown in Fig. 2). Thus, the present
study supports the hypothesis that head circumference may reflect brain size, i.e. total cell number.

Conclusion
We report an exponential growth in total cell numbers in the human fetal forebrain from 13 to 20 weeks of gestation. This is in contrast to the study of Winick (Winick, 1968) who described a linear growth in total cell number from 13 weeks of gestation to term, but in agreement with the study of Dobbing and Sands (Dobbing and Sands, 1973) who, in a larger investigation, described a rapid, exponential growth from 10 to 18 weeks of gestation.

From mid-gestation to term there is a shift in the developmental pattern of the total cell numbers — from an exponential to a linear increase. A linear increase in the total cell number throughout the entire cerebral wall during the last half of gestation is in accordance with the studies of both Winick (Winick, 1968) and Dobbing and Sands (Dobbing and Sands, 1973). In contrast, a morphometric study by Rabinowicz et al. (Rabinowicz et al., 1996) reported a maximum in total neuron number in the neocortex at 28–32 weeks of gestation, after which the total neuron number declined by 70% to term. The total neuron number reported in that study was, however, based on a non-uniform sampling scheme, various correction factors and many assumptions, making the results difficult to evaluate.

It should be noted that, due to technical difficulties, a distinction between neuronal and non-neuronal cell types was not possible in the present study, and therefore the total cell numbers pertain only to the sum of neuron and glial cells. A specific staining for apoptotic cells was not performed, and thus the ratio of cell production versus cell death was not estimated. We only report the total number of cells at any given time. Furthermore, the number of cases was rather limited, which was primarily due to strict inclusion criteria and the prerequisite that one fully intact hemisphere had to be available. However, the distribution of the cases did cover most of the spectrum.

This study provides the first quantitative evaluation of total cell numbers in the human fetal forebrain from 13 weeks of gestation to term estimated by methods based on unbiased principles.

Notes
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