

Regional Differences in Synaptogenesis in Human Cerebral Cortex

PETER R. HUTTENLOCHER* AND ARUN S. DABHOLKAR

Departments of Pediatrics and Neurology and Committee on Neurobiology,
University of Chicago, Chicago, Illinois 60637

ABSTRACT

The formation of synaptic contacts in human cerebral cortex was compared in two cortical regions: auditory cortex (Heschl's gyrus) and prefrontal cortex (middle frontal gyrus). Synapse formation in both cortical regions begins in the fetus, before conceptual age 27 weeks. Synaptic density increases more rapidly in auditory cortex, where the maximum is reached near postnatal age 3 months. Maximum synaptic density in middle frontal gyrus is not reached until after age 15 months. Synaptogenesis occurs concurrently with dendritic and axonal growth and with myelination of the subcortical white matter. A phase of net synapse elimination occurs late in childhood, earlier in auditory cortex, where it has ended by age 12 years, than in prefrontal cortex, where it extends to midadolescence. Synaptogenesis and synapse elimination in humans appear to be heterochronous in different cortical regions and, in that respect, appears to differ from the rhesus monkey, where they are concurrent. In other respects, including overproduction of synaptic contacts in infancy, persistence of high levels of synaptic density to late childhood or adolescence, the absolute values of maximum and adult synaptic density, and layer specific differences, findings in the human resemble those in rhesus monkeys. *J. Comp. Neurol.* 387:167–178, 1997. © 1997 Wiley-Liss, Inc.

Indexing terms: auditory cortex; prefrontal cortex; neural development

Synaptogenesis in human neocortex occurs during the third trimester of gestation and during the first 2 postnatal years. It is followed by a period of synapse elimination during which synaptic density and number decrease to about 60% of the maximum. Data are most complete for calcarine cortex (area 17 of Brodmann), including synaptic density values in all cortical layers and estimates of total number of synapses at various ages (Huttenlocher et al., 1982; Huttenlocher and de Courten, 1987). More limited data, for layer 3 only, are available for prefrontal cortex (middle frontal gyrus) (Huttenlocher, 1979). Comparison of these two data sets suggests that synapse elimination occurs earlier in visual cortex than in precentral gyrus. However, the data from the two studies are not strictly comparable, because synapse quantitation in prefrontal cortex was carried out in selected regions of cortex, free of cell nuclei or blood vessels (essentially neuropil), whereas in visual cortex, no such selection was made.

More recently, Rakic et al. (1986, 1994) have reported that in neocortex of the rhesus monkey, synaptogenesis and synapse elimination occur simultaneously and at an equal rate in all cortical regions. In the human, this would be an unexpected finding, because there are known regional differences in development, including timing of

maximum brain growth (Kretschmann et al., 1986), dendritic arborizations (Conel, 1939–1963; Schade and Van Groenigen, 1961; Becker et al., 1984; Mrzljak et al., 1990), and myelination of cortical afferents and efferents (Barkovich, 1990; Salamon, 1990; Wolpar and Barnes, 1992). These events occur earliest in primary motor and sensory areas, and latest in prefrontal cortex. Concurrent synaptogenesis in all cortical regions, therefore, would imply lack of synchrony between synapse formation and other developmental events such as dendritic growth.

The present study was undertaken to determine whether synaptogenesis in human cerebral cortex is concurrent in different neocortical regions or whether there are regional differences. Prefrontal cortex was chosen for analysis because both dendritic growth and myelination of the subcortical white matter in this region lag behind other

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*Correspondence to: Dr. Peter R. Huttenlocher, University of Chicago, Department of Pediatrics - MC/3055, Chicago, IL 60637.
E-mail: phutten@peds.bsd.uchicago.edu

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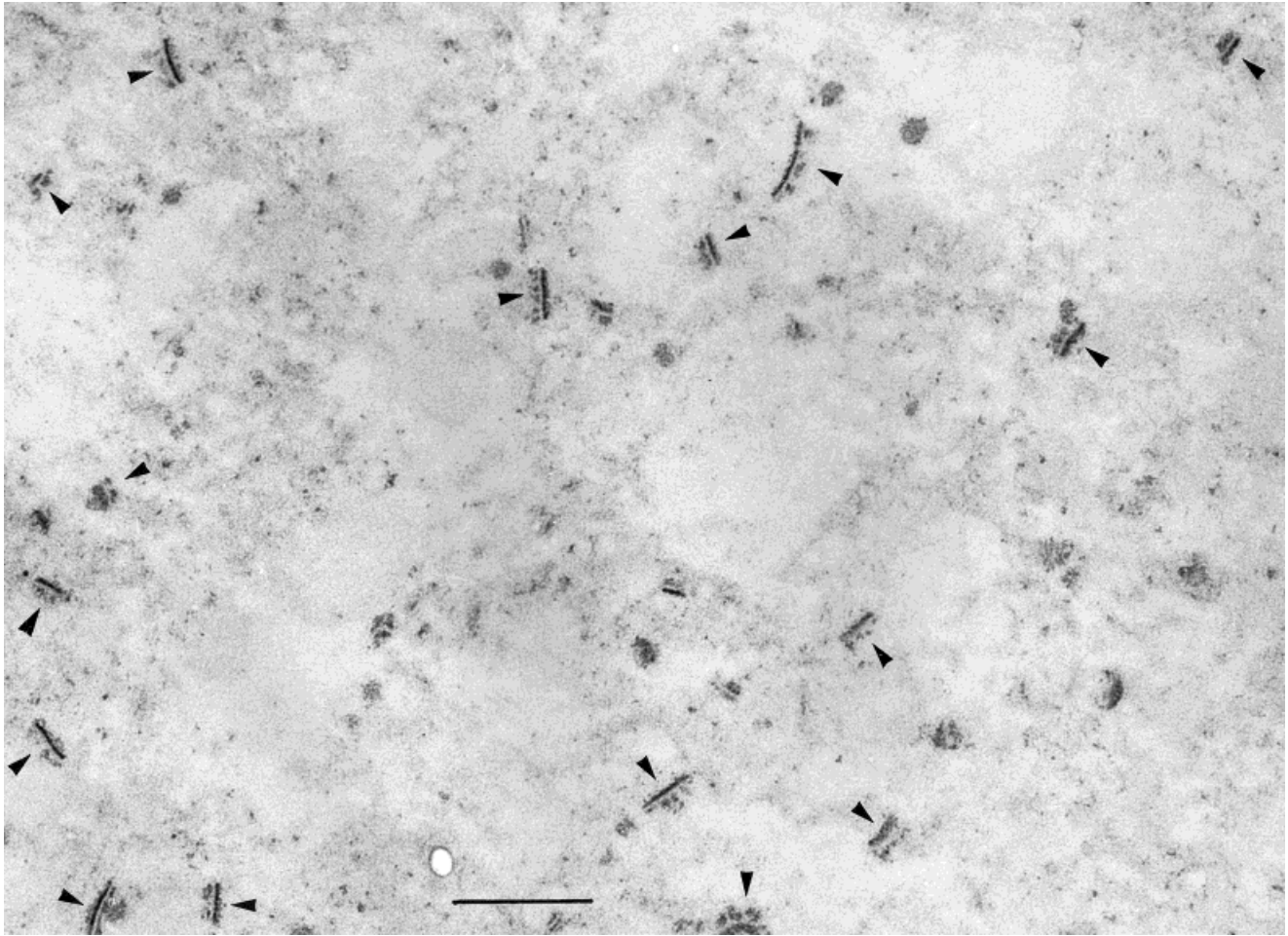


Fig. 1. Synaptic profiles stained by the phosphotungstic acid method, from middle frontal gyrus, male, conceptual age 1,620 days. The figure contains 16 clearly identifiable synaptic profiles (arrowheads). Scale bar = 1 μ m.

cortical areas. Auditory cortex was chosen for comparison as a primary sensory area, that might have early development.

MATERIALS AND METHODS

Small pieces of unfixed cerebral cortex extending from pia to white matter were removed from the anterior third of the left middle frontal gyrus (MFG) and from the left Heschl's gyrus at the time of autopsy. The location of these gyri in human cerebral cortex is provided by Crosby et al. (1962) in Figures 233 and 316. The tissue was fixed overnight in 2.5% glutaraldehyde in 0.1 M Sorensen's buffer, pH 7.4. It was washed in Sorensen's buffer and dehydrated in graded ethanol (35–100%). The tissue was then stained in 1% phosphotungstic acid in 100% ethanol, with a trace of added water (Bloom and Aghajanian, 1968). After processing in propylene oxide, the tissue was embedded in epoxy resin (Spurr, Polysciences, Inc., Warrington, PA). Sections, 2- μ m-thick, were cut and stained with Richardson stain for measurement of the cortical layers.

Silver interference (0.075 μ m), thin sections were cut, extending from pia to white matter, and were placed on

Formvar-coated, single-slot grids for electron microscopy. Serial photographs were taken at $\times 3,000$ magnification, extending from pia to white matter. Prints of $\times 2.5$ enlargement were made, and synaptic profiles were counted on the prints. Profiles were included in the counts when at least two presynaptic projections and a postsynaptic band or a thin continuous presynaptic line plus a thicker, parallel postsynaptic band could be identified (Fig. 1). The person performing the synapse counts was blinded as to the age and brain region of the tissue from which counts were obtained.

Synaptic density values obtained by the phosphotungstic acid (PTA) method have been similar to those obtained by conventional electron microscopy in the cerebral cortex of the mature rat. Aghajanian and Bloom (1967) reported a value of about 14×10^{11} synapses/cm³ in the molecular layer of rat parietal cortex with the PTA method, versus 12.6×10^{11} /cm³ obtained by Armstrong-James and Johnson (1970) in the rat motor cortex by conventional electron microscopy. In early infancy, the results obtained by the PTA method were lower than those obtained by lead/uranyl acetate staining, suggesting that the PTA method may underestimate immature synapses.

TABLE 1. Data on Source of Brain Tissue Used

Case	Conceptual age (days)	Sex	Cause of death	Postmortem interval (hours)	Ethnicity ¹	Brain weight (g)
1	192	Female	Immaturity of lungs, respiratory distress syndrome	23	AA	150
2	210	Male	Sudden infant death	16	C	140
3	280	Male	Intrapartum asphyxia	27	AA	445
4	320	Male	Sudden infant death	NA	C	592
5	360	Female	Sudden infant death	26	AA	560
6	363	Male	Sudden infant death	25	AA	NA
7	700	Female	Cyanotic congenital heart disease, operative death	10	AA	759
8	1620	Male	Drowning	22	AA	1260
9	4700	Male	Drowning	15	C	1600
10	5000	Female	Leukemia, pulmonary embolus	7	C	1285
11	5700	Male	Suicide by firearm	7	C	1640
12	7300	Male	Motorcycle accident	12	C	1465
13	11700	Male	Metastatic carcinoma of liver	12	C	1470
14	21500	Female	Anaphylactoid reaction	22	C	1295
15	22300	Female	Lymphoma	25	C	1310

¹AA, African American; C, Caucasian.TABLE 2. Mean Synaptic Density in Whole Cortex at Various Ages¹

Case	C.A. (days)	Age (years)	Synaptic Profiles/100 μm^2			Synapses/100 μm^3		
			Auditory cortex	MFG	P value	Auditory cortex	MFG	P value
1	192	—	2.00 (0.19)	.46 (0.08)	<0.001	12.2 (1.2)	3.2 (0.5)	0.001
2	210	—	1.22 (0.09)	.37 (0.08)	<0.0001	7.5 (0.6)	2.2 (0.5)	<0.0001
3	280	N.B.	4.77 (0.44)	3.11 (0.34)	0.03	29.4 (2.7)	19.5 (2.2)	0.03
4	320	$\frac{1}{12}$	3.36 (0.23)	1.80 (0.18)	0.0001	21.0 (2.4)	11.2 (1.1)	0.0001
5	360	$\frac{3}{12}$	8.20 (1.11)	5.86 (0.16)	0.04	40.7 (5.4)	28.3 (0.9)	0.03
6	363	$\frac{3}{12}$	10.17 (0.57)	6.44 (0.88)	0.002	54.3 (3.0)	34.3 (4.7)	0.002
7	700	$\frac{1}{12}$	9.40 (0.95)	6.72 (0.87)	0.05	53.0 (5.4)	37.9 (4.9)	0.05
8	1,620	$\frac{3}{12}$	11.28 (1.10)	10.62 (1.21)	N.S.	55.7 (5.4)	52.4 (6.0)	N.S.
9	4,700	12	5.02 (0.79)	9.50 (0.64)	<0.0001	24.7 (3.9)	46.9 (3.1)	<0.0001
10	5,000	13	5.27 (0.88)	8.05 (1.23)	0.02	26.0 (4.3)	39.7 (6.1)	0.02
11	5,700	15	8.02 (0.79)	8.10 (0.80)	N.S.	38.9 (3.5)	40.0 (3.9)	N.S.
12	7,300	19	4.85 (0.43)	5.50 (0.51)	N.S.	24.0 (2.1)	27.1 (2.5)	N.S.
13	11,700	32	7.08 (0.57)	8.21 (0.75)	N.S.	35.0 (2.8)	40.2 (3.7)	N.S.
14	21,500	59	7.02 (0.55)	5.80 (0.40)	N.S.	34.7 (2.7)	28.7 (2.0)	N.S.

¹The data are expressed both in terms of synaptic profiles/100 μm^2 in sections about 0.75 μm in thickness (i.e., as counted), and after correction to synapses/100 μm^3 of tissue. CA, conceptual age; MFG, middle frontal gyrus. The numbers in parentheses represent the standard error of the mean (SEM).

The PTA method is especially well-suited to the study of human postmortem brain tissue, because the perisynaptic proteins that are demonstrated, persist for at least 24 hours after death (Huttenlocher, 1979). That synaptic profiles stand out against a largely unstained background makes it possible to perform synapse counts rapidly on relatively low-magnification prints.

Synapse counts were expressed in terms of synaptic profiles/100 μm^2 in sections approximately 0.075 μm in thickness. These values do not directly translate into synaptic density, because a synaptic profile may extend over more than one section. A second error may arise because synapses sectioned parallel or nearly parallel to the synaptic cleft would not be recognizable by the PTA method. Synaptic density (synapses/100 μm^3) was calculated from the synaptic profile counts, by using an assumption-based correction first proposed by Bloom and Aghajanian (1968). The method represents a modification of the Abercrombie correction (Abercrombie, 1946), which includes the assumption that only about half of the synapses present are recognizable by the PTA method. The correction factor is $2A/(M+L)$, where A represents the density of synaptic profiles as counted per 100 μm^2 , M the thickness of the section, and L the mean length of synaptic profiles. The length of synaptic profiles was measured at each age on photographic prints. It was found to be constant from age 2 months to the adult at a mean of 0.33 μm . Synaptic length was smaller in the fetal and neonatal brains with a mean of 0.24 μm . We have previously com-

pared results obtained in PTA stained sections with the modified Abercrombie correction to those obtained by the stereological method proposed by De Hoff (1966) and have found little difference (Huttenlocher and de Courten, 1987).

Assumption-based correction methods for calculation of neuronal and synaptic density are subject to a number of biases, related to the often false assumption that the structures that are counted are spherical, and to the inability to accurately classify small cut fragments. These considerations have led to the recent recommendation that they be discarded in favor of modern, assumption-free stereological methods (Coggeshall and Lekan, 1996). However, neither of the two proposed stereological methods are suitable for synapse quantitation in large areas of tissue. The optical disector method, which requires thick sections, is limited to light microscopy. The physical disector method has been successfully applied to synapse counts from electron micrographs (Kleim et al., 1996). It requires matching of the same profile on contiguous sections, which is not possible in large sections, spanning the entire depth of cerebral cortex. Furthermore, the stereological method would not be assumption-free when applied to synapse counts. It contains the assumption that every synapse in a given section is identifiable as a synaptic profile. This assumption would lead to underestimation of synapse number, because synapses may not be identifiable when they are cut parallel to the synaptic cleft. Bloom and Aghajanian used the assumption that only about half of the synapses in a given section are identifiable with the

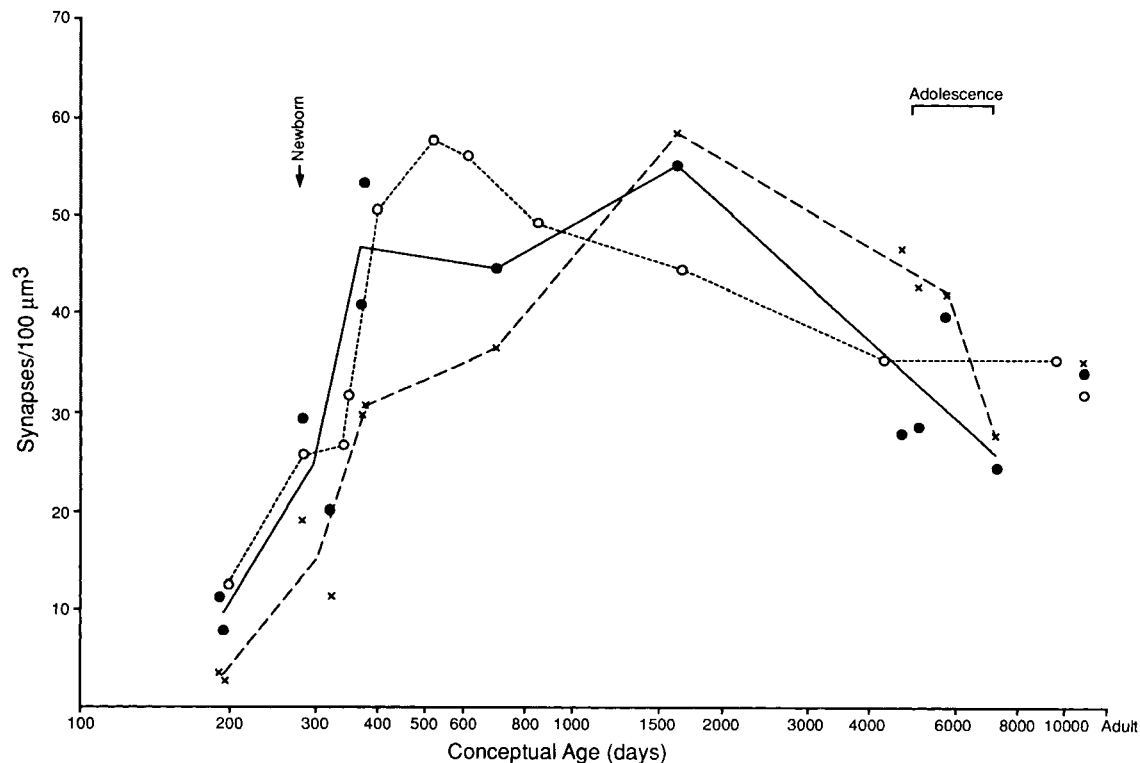


Fig. 2. Mean synaptic density in synapses/100 μm^3 in auditory, calcarine, and prefrontal cortex at various ages. Open circles, visual cortex (area 17); filled circles, auditory cortex; x, prefrontal cortex (middle frontal gyrus).

PTA method. A similar assumption would have to be made if stereological methods were used for synapse counts in PTA-stained sections.

Errors related to the use of an assumption-based correction method will affect the absolute synaptic density values reported in this study, but will not change the major findings of the study, which are based not on absolute values but on comparisons of synapse counts in different cortical regions in the same brain. These results are not affected by the correction factor used, and are as evident on analysis of the uncorrected synaptic profile counts as on analysis based on corrected synaptic density values.

In each cortical area, synapse counts were carried out in four side by side strips of tissue from the same section, 7.3 μm in width and extending from pia to white matter. These four determinations were used to calculate the mean and standard error of the mean for synaptic density for each cortical layer. The results for auditory and prefrontal cortex in each cortical layer from the same brain were tested for statistically significant differences, by using the *t* test. Only differences that were $P < 0.01$ were accepted as 'significant' to avoid errors related to chance in the use of repeated *t* tests. Mean synaptic density, standard error of the mean and significance of the difference between means for auditory and prefrontal cortex were also calculated for cortex as a whole. For these comparisons, of which there were only a total of 14, values of $P < 0.05$ were accepted as showing statistically significant differences.

The synaptic density data were not corrected for shrinkage of the tissue during fixation and dehydration. Presentation of uncorrected values facilitates comparisons with prior data that have not been corrected for shrinkage

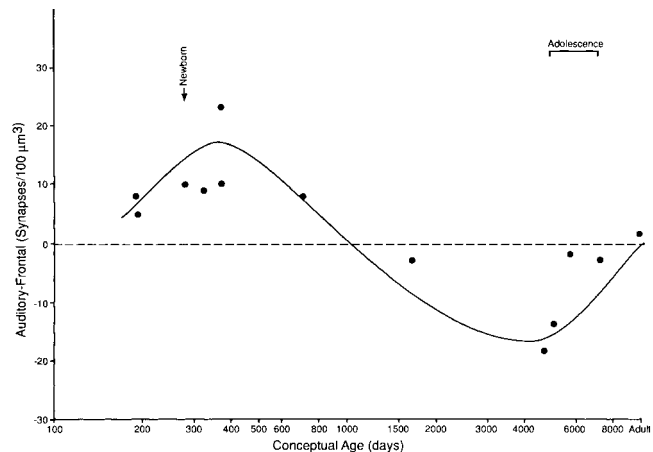


Fig. 3. Difference scores (synaptic density in auditory cortex - synaptic density in middle frontal gyrus) plotted against conceptual age. The dotted line represents the line of zero difference in time course of synapse formation and elimination between the two cortical regions. The solid line represents a curve of best fit for the data. The data points are derived from the data in Table 2.

(Rakic et al., 1986; Huttenlocher and de Courten, 1987). Estimates of shrinkage of human cerebral cortex during fixation and dehydration show approximately the following volume shrinkage: 50% of initial volume at conceptual age (CA) 28 weeks, 45% at postnatal age 2 months, 42% at 4 months, 37% at 7 months, and 35% thereafter (Hutten-

TABLE 3. Mean Synaptic Density in Synapses/100 μm^3 by Cortical Layer¹

CA (days)	Cortical layer					
	1	2	3	4	5	6
192 Aud.	8.1 (1.1)	14.4 (3.1)	14.1 (1.8)	15.3 (2.7)	11.7 (3.2)	9.4 (0.8)
192 MFG	5.0 (0.2)	2.5 (0.8)	3.7 (0.3)	1.5 (0.5)	3.2 (0.7)	3.1 (1.3)
210 Aud.	7.5 (0.5)	5.3 (1.1)	7.1 (0.3)	9.0 (0.9)	9.0 (1.2)	7.2 (1.0)
210 MFG	2.6 (0.5)	0.4 (0.4)	3.7 (1.0)	3.0 (0.3)	2.0 (0.2)	1.6 (0.5)
280 Aud.	28.4 (1.5)	30.6 (3.6)	37.3 (2.5)	35.8 (2.6)	24.0 (2.0)	20.5 (1.5)
280 MFG	26.0 (2.0)	20.3 (1.1)	18.4 (1.5)	10.0 (2.2)	20.6 (2.2)	21.7 (0.6)
320 Aud.	21.9 (6.1)	23.4 (3.7)	24.1 (5.1)	23.3 (2.3)	17.4 (3.1)	15.8 (1.8)
320 MFG	8.2 (2.3)	15.0 (3.9)	13.3 (4.0)	12.4 (1.8)	9.1 (1.6)	9.4 (4.2)
360 Aud.	37.0 (4.3)	46.3 (1.8)	58.0 (1.5)	49.0 (0.4)	34.0 (1.3)	20.0 (1.7)
360 MFG	24.0 (1.1)	29.0 (0.9)	30.0 (1.1)	29.0 (1.1)	28.0 (0.9)	30.0 (0.6)
363 Aud.	52.1 (2.3)	56.6 (2.0)	65.4 (3.6)	50.2 (1.4)	57.6 (1.8)	43.8 (1.4)
363 MFG	30.8 (2.6)	42.8 (2.7)	46.9 (1.6)	43.1 (2.8)	20.8 (2.4)	21.3 (1.9)
700 Aud.	59.1 (2.4)	75.1 (4.0)	55.5 (1.5)	47.5 (1.1)	40.8 (0.7)	40.2 (2.1)
700 MFG	21.8 (2.5)	35.8 (1.4)	46.3 (2.4)	50.5 (0.4)	46.9 (2.5)	25.9 (0.9)
1620 Aud.	52.8 (2.0)	68.6 (5.2)	72.7 (2.0)	53.2 (3.2)	50.8 (2.2)	36.1 (2.2)
1620 MFG	33.0 (1.6)	63.0 (4.8)	72.8 (2.0)	57.1 (1.6)	45.6 (2.5)	42.9 (1.0)
4700 Aud.	25.8 (4.4)	35.5 (4.2)	35.9 (1.7)	21.1 (1.0)	18.1 (2.5)	12.0 (3.3)
4700 MFG	44.4 (4.6)	53.1 (4.9)	55.1 (7.9)	50.9 (3.1)	43.2 (3.8)	34.5 (2.3)
5000 Aud.	20.9 (3.3)	36.7 (3.0)	39.5 (2.4)	24.2 (2.3)	24.1 (2.3)	10.6 (2.1)
5000 MFG	19.1 (3.3)	34.1 (1.3)	61.1 (4.9)	49.0 (3.6)	45.2 (4.7)	29.6 (5.4)
5700 Aud.	38.8 (4.4)	49.7 (1.6)	48.5 (2.8)	34.0 (2.5)	29.9 (2.6)	32.4 (3.8)
5700 MFG	36.0 (4.6)	45.9 (3.2)	50.5 (2.4)	46.9 (5.6)	35.7 (5.3)	24.7 (4.0)
7300 Aud.	21.1 (1.8)	34.1 (0.1)	24.8 (4.9)	22.7 (1.5)	20.9 (1.7)	20.2 (2.6)
7300 MFG	22.9 (2.8)	34.4 (3.3)	30.6 (4.5)	26.8 (2.1)	30.7 (1.2)	17.3 (2.0)
11700 Aud.	36.0 (2.5)	42.3 (1.1)	40.1 (1.0)	38.1 (1.6)	28.8 (0.4)	24.4 (0.6)
11700 MFG	32.6 (1.1)	47.1 (1.9)	54.1 (1.0)	40.5 (1.3)	37.9 (3.0)	29.4 (0.7)
21500 Aud.	30.7 (1.1)	40.8 (1.2)	41.9 (1.2)	38.3 (1.0)	31.9 (0.4)	24.8 (2.7)
21500 MFG	21.7 (2.2)	28.5 (3.3)	29.7 (1.5)	26.8 (0.5)	36.8 (1.2)	28.7 (0.3)
22300 MFG	27.6 (1.5)	27.2 (2.1)	42.0 (2.7)	38.9 (5.5)	27.4 (1.2)	25.6 (0.9)

¹The numbers in parentheses represent 1 SEM of four counts in the same cortical area. Aud., auditory cortex; MFG, middle frontal gyrus; CA, conceptual age.

locher and deCourten, 1987). This corresponds fairly well to a linear shrinkage of 18% reported in human postmortem cerebral cortex during fixation and histologic processing (Rockel et al., 1980). The shrinkage data suggest that the uncorrected synaptic density data represent a relative overestimation of synaptic density in the infant brains up to about age 7 months as compared with adult brains, and that comparisons made at later ages should be unaffected by tissue shrinkage.

Tissue from both MFG and auditory cortex (Heschl's gyrus) was obtained from eight brains ranging in age from CA 28 weeks to age 3.5 years, i.e., during the age range of synaptogenesis in human cerebral cortex. In addition, tissue from four adolescent brains ranging in age from 12 to 19 years was analyzed, for determination of the approximate end of the synapse elimination phase in the two cortical regions. Three adult brains were included for comparison. The number of brains studied had to be limited because of the considerable time required for synapse quantitation in serial EM prints extending through all layers of cortex. In addition, availability of fresh brain tissue required for these studies was limited, especially during childhood. The problem of availability of only single cases at most ages was addressed by making four separate synapse counts in each cortical area, and by using these data from single brains for calculation of the significance of the difference between auditory and frontal cortex.

Age at time of death, causes of death, sex, and postmortem interval are listed in Table 1. The brain tissue was obtained from several sources: University of Chicago Hospitals, Chicago, IL (cases 1–3, 7, 10, and 13–15, in Table 1); Cook County Morgue, Chicago, IL (cases 5, 6, and 8); Brain and Tissue Banks for Developmental Disorders, University of Maryland, Baltimore, MD (cases 9, 11, and 12); and University of Miami, Miami, FL (case 4).

RESULTS

Mean synaptic density in cerebral cortex

Mean synaptic density values for whole cortex are provided in Table 2. The data are presented in terms of synaptic profiles as counted on photographic prints from electron microscope images, and after transformation to synapses/100 μm^3 of brain tissue. Differences between cortical regions are not affected by this transformation. In the fetal and infancy brains, synaptic density was consistently higher in auditory cortex than in prefrontal cortex (MFG). In auditory cortex, synaptic density at age 3 months approached the highest values found in this study, which were observed at age 3.5 years in MFG. At age 3 months, synaptic density in MFG was only about half the maximum. Comparison of paired data from auditory cortex and MFG shows that in all seven brains in the age group from CA 27 weeks to postnatal age 15 months, mean synaptic density was significantly higher in auditory cortex than in MFG. By age 3.5 years synaptic density in the two cortical regions was approximately equal. The data suggest that synaptogenesis in prefrontal cortex lags behind auditory cortex by several months throughout infancy, but that prefrontal cortex has caught up to auditory cortex by age 3.5 years.

The data in two late childhood-early adolescent brains showed higher mean synaptic density in MFG than in auditory cortex. Synapse elimination appears to be complete by age 12 years in auditory cortex, but appears to continue in MFG until midadolescence. This conclusion is tentative, because only four brains were available in adolescence and because there is considerable variability of synaptic density values in this age group.

Figure 2 compares data from primary visual cortex, obtained in a previous study (Huttenlocher and de Courten, 1987), with our present findings in auditory cortex and

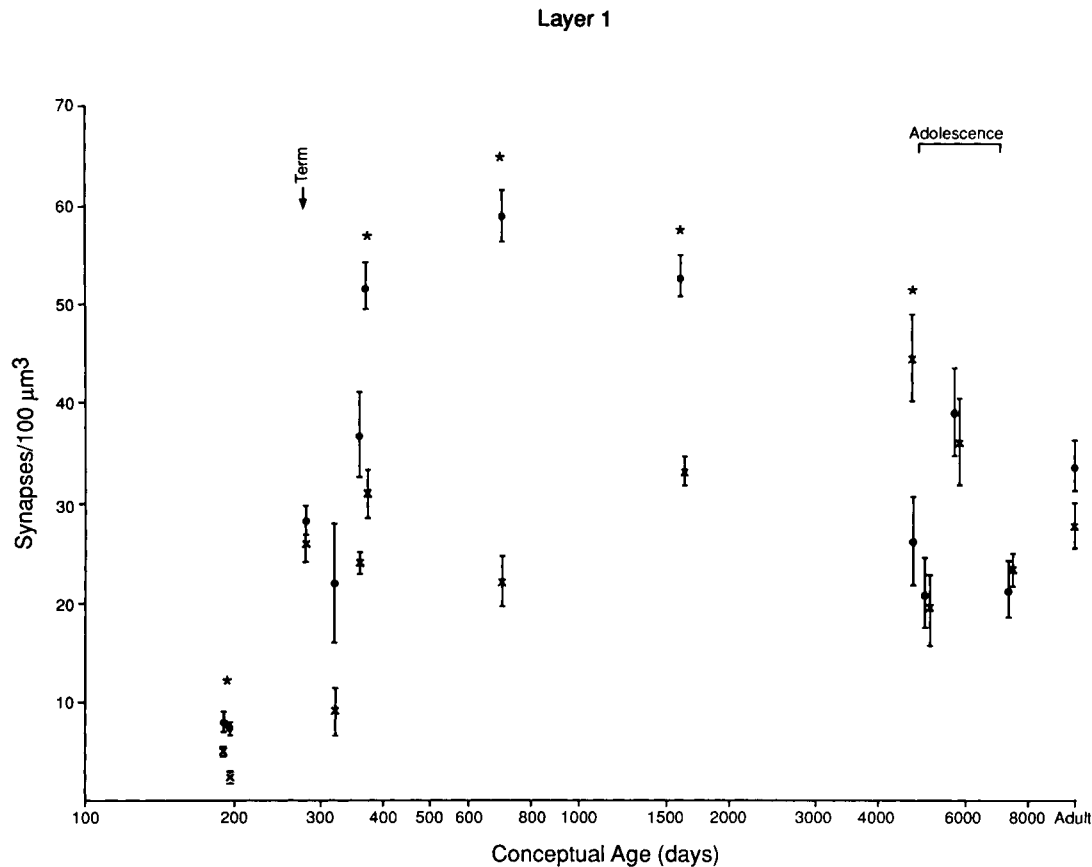


Fig. 4. Mean \pm SEM of synaptic density in layer 1 at various conceptual ages. Filled circles, auditory cortex; x, prefrontal cortex (middle frontal gyrus). *, significant difference between auditory cortex and middle frontal gyrus at $P < 0.01$.

in MFG. The time course of synaptogenesis in visual cortex resembles that in auditory cortex, except that synapse elimination appears to begin earlier in visual cortex. However, more data points for auditory cortex would be needed for confirmation of this point.

The maximum and adult synaptic density values for all three cortical regions are similar, suggesting that synapse elimination occurs to an equal extent in different regions of human cerebral cortex. The data also suggest that there is a normal range of values for synaptic density that is identical in cortical regions with markedly different functions.

The availability of synaptic density values for different regions of the same brain makes it possible to calculate difference scores (synaptic density in area A - synaptic density in area B) and to construct curves of the difference scores against age. Such curves should show random fluctuation of difference scores around a straight line (the line for zero difference between synaptic density values), if synapse formation and synapse elimination are concurrent in two brain regions. In the case where synaptogenesis and synapse elimination both occur earlier in area A than in area B, a curve is generated which initially shows positive values, followed by an inflection point and by negative values. The difference scores derived from our data show a distribution that suggests earlier synaptogenesis and earlier synapse elimination in auditory than in prefrontal cortex (Fig. 3).

Synaptic density for individual cortical layers

Synaptic density values for individual cortical layers are provided in Table 3 and in Figures 4–8. Statistically significant differences between synaptic density in auditory cortex and in MFG were found during infancy in all cortical layers, with later formation of synaptic profiles in MFG. In layers 2 and 3, synaptic density values were lower in MFG from CA 27 weeks, the earliest age examined, to age 14 months. In layers 4 and 5, values differed from CA 27 weeks to age 3 months, values at 14 months being approximately equal for the two cortical areas. Results in layer 6 may have been influenced by difficulties in the identification of the border between gray and white matter, which is indistinct, especially in the infant brain. This problem may account for higher variability of results in layer 6. Synaptic density in layer 1 was lower in prefrontal than in auditory cortex in all eight immature brains (under CA 2,000 days) examined (Fig. 4). Whereas evidence of overproduction of synapses in layer 1 of auditory cortex was seen between CA 360 to 1,620 days, no such period could be identified in layer 1 of MFG.

The synaptic density values for layer 1 and for the lower cortical layers tend to be lower than those for layers 2 and 3 (Fig. 9). This difference is present already in the full-term neonate in auditory cortex and appears by age 3

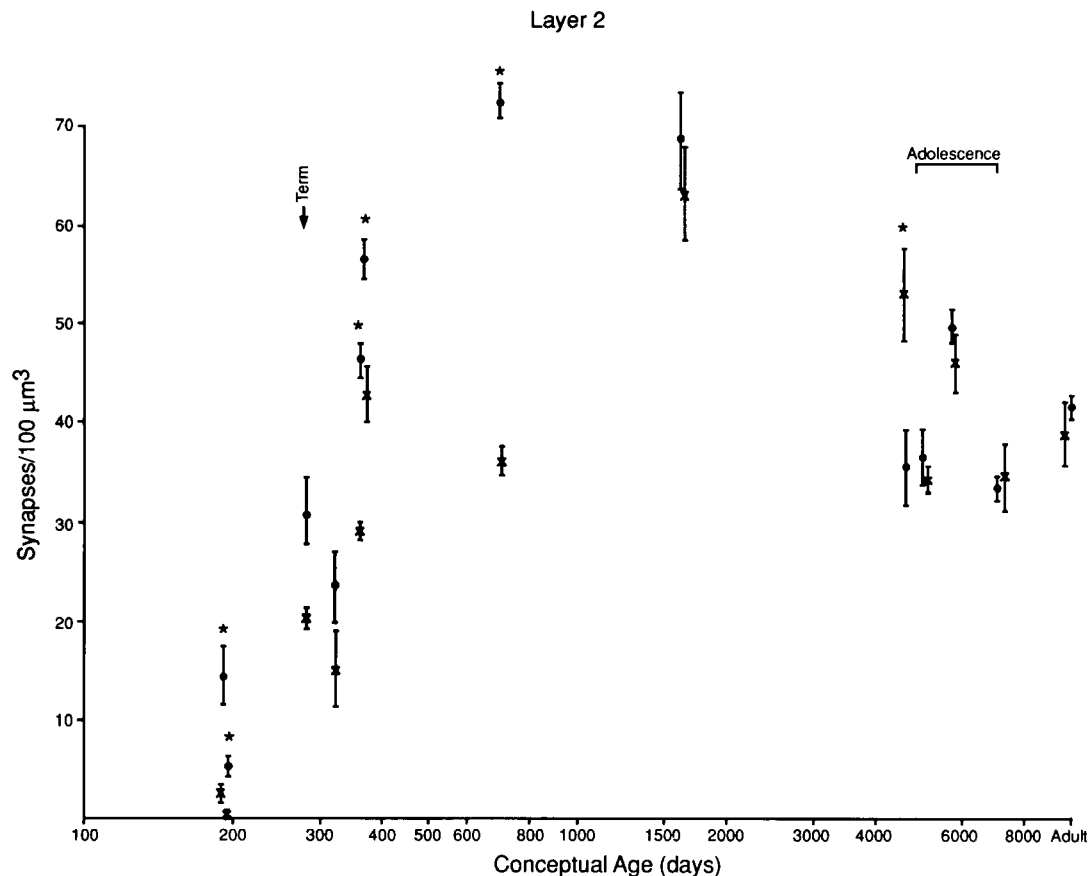


Fig. 5. Mean \pm SEM of synaptic density in layer 2. Symbols same as in Figure 4.

months in MFG. It persists in the adult brain, but is most marked during the period of exuberant synaptic density, in the present study at age 3.5 years. In the neonate, the two cortical regions differ in this respect, with MFG maintaining the 'fetal' pattern of approximately equal synaptic density in all cortical layers, whereas auditory cortex already shows the 'mature' pattern of increased synaptic density in layer 3 (Fig. 9).

DISCUSSION

Synaptogenesis in human neocortex appears to be heterochronous

The present data show regional differences in synaptogenesis in human neocortex both in fetal brains and postnatally up to at least age 15 months (CA 700 days) in cortical layers 2 to 3, and up to at least age 3 months (CA 363 days) in the lower cortical layers. A rapid burst in synaptogenesis occurs postnatally in visual and auditory cortex. Prefrontal cortex appears to acquire synaptic junctions more slowly. By age 3.5 years, prefrontal cortex has caught up to auditory cortex. In primary visual cortex net synapse elimination appears to have started already at that age.

Comparison of human data with those in rhesus monkeys

The present data show heterochronous synaptogenesis in neocortex of humans, whereas in rhesus monkeys

concurrent synaptogenesis was found in all neocortical areas (Rakic et al., 1986, 1994). Another difference between human and subhuman primate cortical development is the much shorter total period of synaptogenesis in the latter. In the monkeys, synapse formation occurs during the last 2 months of pregnancy and for the first 2 postnatal months, a total of about 4 months. In the human prefrontal cortex, it extends from the sixth month of gestation to at least age 15 months, a period of about 20 months. It is shorter in visual and auditory cortex, where synaptic density reaches a maximum at age 3 to 4 months, but even in these cortical regions it extends over about twice the time period in monkeys. Maturation differences in different cortical regions in monkeys would therefore be expected to be of shorter duration than in humans, if they occurred at all, and might be more difficult to detect.

A possibility that needs to be considered is that apparent differences between the rhesus monkey and the human data are an artifact of differences in the methods used for synapse quantitation. The rhesus monkey data (Rakic et al., 1986, 1994; Bourgeois and Rakic, 1993; Bourgeois et al., 1994) are expressed in terms of synapses per unit of neuropil, whereas our data are expressed as synapses per unit of whole cortex. Neuropil makes up a smaller percentage of total cortex in immature brain in which cell bodies are tightly packed with relatively few intervening axons and dendrites, than in mature brain in which cell bodies are widely separated. In immature brain, a small total number of synapses may be crowded into a small volume of

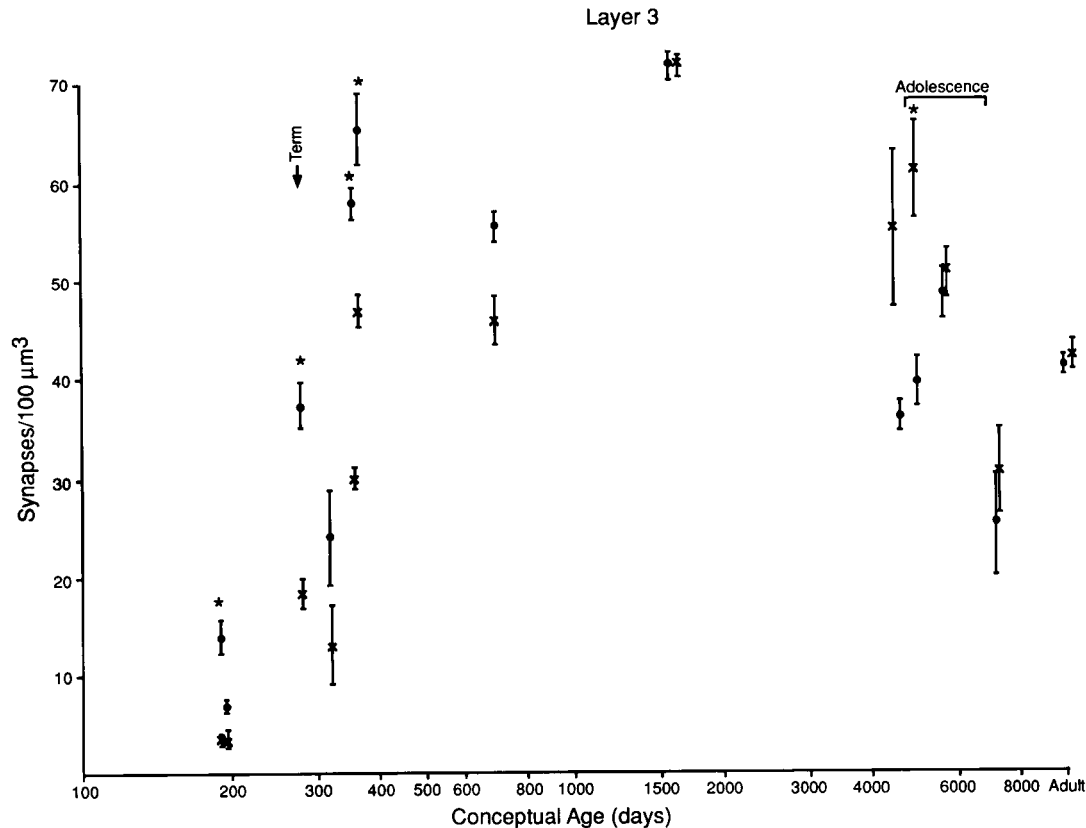


Fig. 6. Mean \pm SEM of synaptic density in layer 3. Symbols same as in Figure 4.

neuropil, giving a high density value for neuropil, but a low value for cortex as a whole. The difference in synaptic density values between the two methods is illustrated by comparison of two data sets on synaptogenesis in human MFG, one reported in Huttenlocher (1979), the other from the present study. The 1979 study reported synaptic density in selected cortical regions, free of nuclei, blood vessels and empty spaces, resembling neuropil as best as this can be identified in PTA stained sections (see below). The present data set is expressed as synapses/volume of whole cortex. The data, normalized for percentage maximum synaptic density, are summarized in Figure 10. After about age 15 months, data points from both studies fall on approximately the same curve. In infancy, however, maximum synaptic density is reached earlier in 'neuropil' than in whole cortex. The data are consistent with early saturation of neuropil with synapses, and with existence of a later phase of synaptogenesis that is largely due to expansion of neuropil in relation to other components of cerebral cortex. This later phase of synaptogenesis, related to elongation and/or branching of axons and dendrites, is missed when synaptic density is expressed per volume of neuropil. This may lead to an erroneously early estimate of the age at which maximum synapse numbers are reached in cerebral cortex, and may minimize differences in time course of synaptogenesis in different cortical areas.

Two other factors were taken into account in the decision to express synaptic density per volume of cortex. Expression of synaptic density per unit whole cortex facilitates comparison with other parameters having bearing on

synaptic function, such as cerebral metabolic rate for glucose and cerebral oxygen consumption, which are expressed in terms of cortex as a whole (Chugani and Phelps, 1986, 1990). Finally, accurate definition of the limits of neuropil would not be possible on tissue sections prepared by the PTA method, because structures other than synaptic profiles are not well defined by this method (Fig. 1).

Comparison of cortical synaptogenesis in humans and rhesus monkeys shows striking similarities as well as the above noted differences. In both species, synaptic contacts appear near the cortical plate soon after neuronal migration. They are first seen above and below the cortical plate (Molliver et al., 1973; Kostovic and Rakic, 1990; Bourgeois and Rakic, 1993), by CA 15 weeks in the human, followed within a few weeks by intracortical synapses, present by CA 23 weeks in human fetuses (Molliver et al., 1973). A period of rapid synaptogenesis pre- and postnatally is followed by a plateau during childhood during which synaptic density is significantly above adult levels. This plateau is better defined in the subhuman primate data, because few brains were available for study in the human during the likely age range of this plateau (ages, 1–10 years). However, the combination of data from MFG in the present study with those reported in 1979 (Huttenlocher, 1979) shows a plateau extending over several years (Fig. 10).

Synapse elimination occurs late in childhood and in adolescence in both humans and rhesus monkeys. It is followed by a much slower decline in synaptic density during the adult years, which in the human data occurs

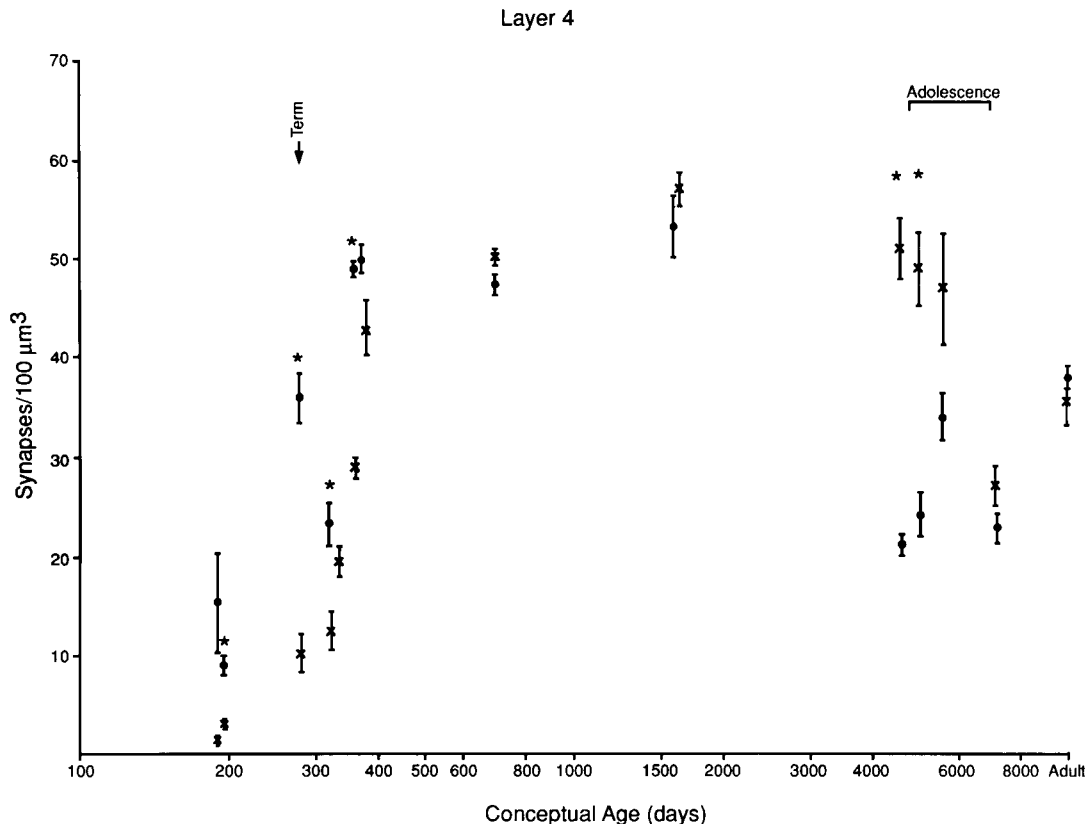


Fig. 7. Mean \pm SEM of synaptic density in layer 4. Symbols same as in Figure 4.

primarily in old age (Huttenlocher, 1979). The synapse elimination that occurs in late childhood and early adolescence is clearly distinct from the much later and smaller magnitude aging changes.

The maximum synaptic density is about the same for both species, 55 synapses/100 μm^3 whole cortex including somata and blood vessels (Fig. 2 and Rakic et al., 1986). The values are higher when density is measured in neuropil excluding cell bodies, blood vessels, and extracellular space, with maximum synaptic density of about 75/100 μm^3 .

Human and monkey cerebral cortex show remarkable resemblance in the extent of synapse elimination during development. In humans, the adult value for synaptic density is about 60% of the maximum (Huttenlocher, 1979; Huttenlocher and de Courten, 1987), similar to what has been found in rhesus monkeys (Rakic et al., 1986, 1994). There are also layer specific differences in synaptic density in both species, with synaptic density somewhat lower in layers 1, 5, and 6 than in layers 2–4 (Bourgeois et al., 1994; and the present data). These differences appear during fetal and early postnatal development in both species.

Time course of synaptogenesis resembles that of dendritic development, myelination, and cerebral energy metabolism

Regional differences in development of cerebral cortex are supported by data obtained with the Golgi method. Drawings from Golgi preparations showing dendritic growth in various cortical regions during human postnatal

brain development are provided by Conel (1939–1963). Dendritic trees appear less developed in prefrontal cortex than in primary sensory regions at birth and at postnatal age 3 months. Quantitative data from Golgi impregnated sections are available for MFG and for primary visual cortex. They show late development of cortical dendrites in MFG, especially in layer 3. In a study by Schade and Van Groenigen (1961), estimated mean total length of dendrites in layer 3 pyramidal neurons of MFG was 3% of the adult value at birth, 35% at 6 months, and about 50% at age 24 months. In a study by Becker et al. (1984) in visual cortex, the oldest age available for comparison was 7 years. Mean dendritic length of layer 3 pyramidal neurons was about 25% of the 7-year value at birth, 40% at 6 months, and 100% at 1 year. Dendritic development in layer 5 pyramidal neurons appears to occur more rapidly: Estimated mean dendritic length at age 6 months equalled 56% of adult value in MFG and about 90% of the 7-year level in visual cortex. A developmental difference between layers 3 and 5 also has been reported by Poliakov (1961) and by Marin-Padilla (1970). It is reflected in our synaptogenesis data, in which maturational differences persist for a shorter period in lower than in upper cortical layers. It appears that cortical layers that receive primary afferent input (layer 4) and that contain the neurons that give rise to efferent fibers (layers 5 and 6) develop more rapidly than those concerned with information processing (layers 2 and 3).

A recent Golgi study of dendritic development in human prefrontal cortex further supports late development of this

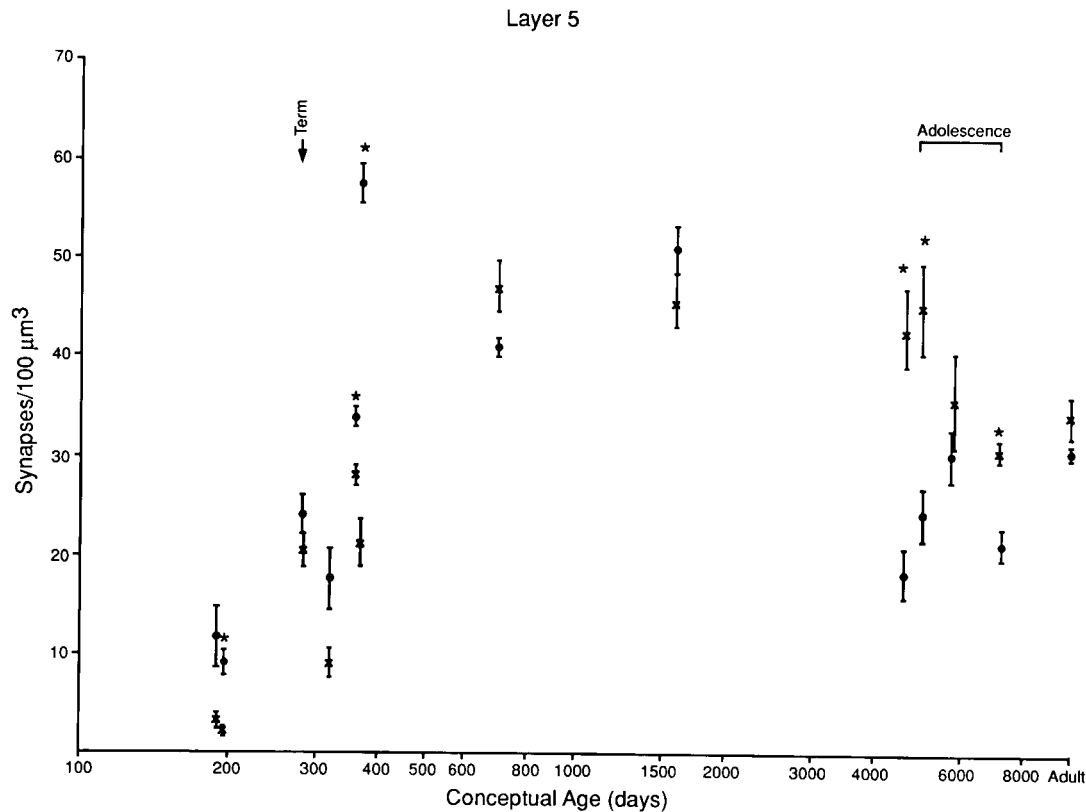


Fig. 8. Mean \pm SEM of synaptic density in layer 5. Symbols same as in Figure 4.

cortical area (Mrzljak et al., 1990). These investigators found late persistence of subplate and layer 1 neurons in prefrontal cortex, with demonstration of Cajal-Retzius cells in layer 1 as late as age 19 years. This unusual developmental time course may account for the absence of overproduction and elimination of synapses during the age range studied by us in layer 1 of MFG.

Myelination of subcortical white matter follows a regional pattern that resembles that of synaptogenesis and of dendritic development, with late myelination of prefrontal regions. Myelination in the prefrontal cortex is as yet incomplete at age 12 months, as determined by magnetic resonance imaging, a time when adult patterns have been reached in other cortical areas (Barkovich, 1990; Salamon, 1990; Wolpar and Barnes, 1992).

Differences between cortical regions have also been described in the development of regional metabolism of brain as determined by positron emission tomography. Cerebral energy metabolism as measured by 18 fluorodeoxyglucose utilization is low in cerebral cortex at birth, except for sensorimotor regions. It increases in parietal, temporal, and occipital cortex by age 3 months, and does not reach similar levels in prefrontal cortex until about age 8 months (Chugani and Phelps, 1986, 1990). The data suggest a 5- to 8-month lag in development of cerebral energy metabolism in prefrontal cortex compared with more posterior cortical regions. 'The last region to undergo a maturational rise in local cerebral metabolic rate for glucose is the frontal cortex, where maturation of the lateral portions precedes that of the phylogenetically newer dorsal prefrontal regions' (Chugani and Phelps,

1990, pp 23–24). Cerebral glucose metabolism in turn is thought to be closely linked to synaptic activity.

The data on cerebral energy metabolism show an interesting parallel to the data on synaptogenesis and synapse elimination. The cerebral metabolic rate for glucose rises rapidly during infancy, remains high throughout childhood, and decreases during adolescence, at about the age when synaptic density in prefrontal cortex falls to the adult range (Chugani et al., 1987). The positron emission tomography data do not show earlier decline of metabolic rate in visual or auditory cortex than in prefrontal cortex. However, primary visual and auditory cortex are quite small in comparison to association cortex and are highly infolded, which may make it difficult to detect differences from surrounding cortical regions.

Theories of synaptogenesis and its relation to cortical function

Events that lead to the formation of synapses have been defined in recent years. A recent view has been summarized by Haydon and Drapeau (1995). In many neurons, synaptic components are synthesized through intrinsic programs before cell-cell interactions. Synapse formation is triggered by contact of two neurites and may occur within minutes of such contact. Most synaptic contacts appear to be made randomly at first, whereas others are made selectively, through retrograde and anterograde signaling. Neurotransmitter release before synapse formation appears to be an important signaling mechanism. Stabilization of randomly made synapses appears to be

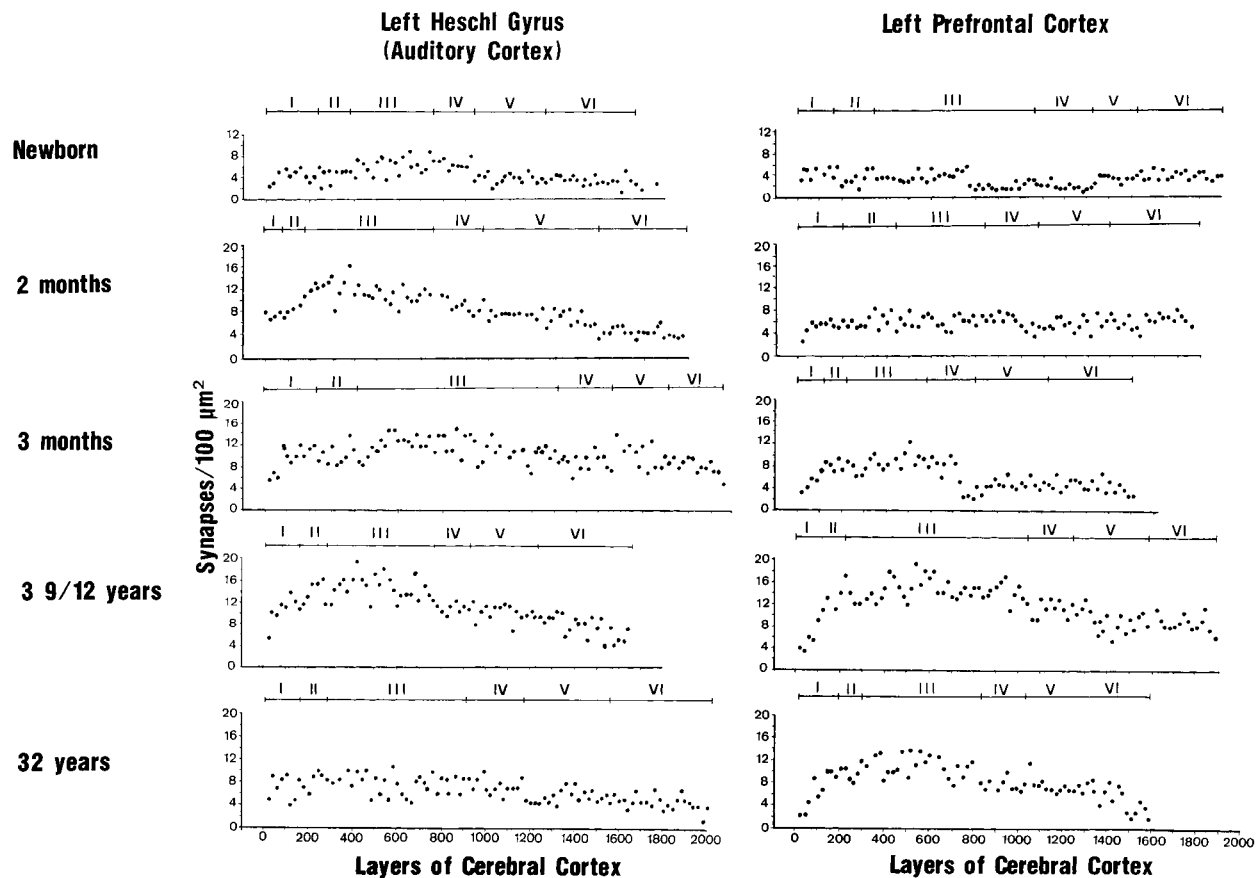


Fig. 9. Synaptic profile counts in profiles/100 μm^2 of electron microscope sections related to cortical layers. The abscissa represents distance from the pial surface in micrometers. Each data point represents the number of synaptic profiles per 100 μm^2 of cortex in a 20- μm interval.

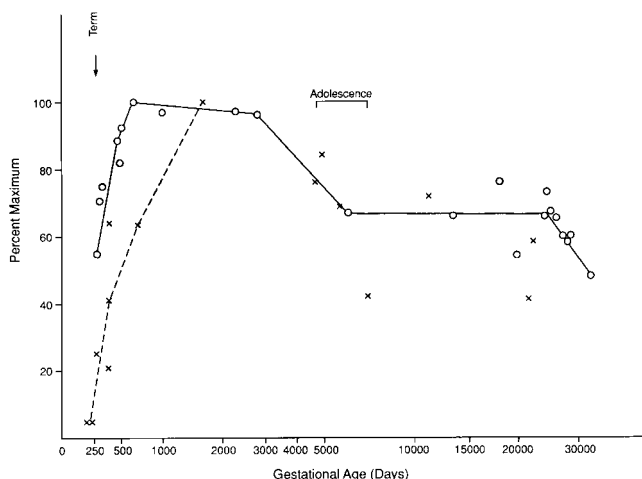


Fig. 10. Synaptic density in percent of maximum observed value in layer 3 of human MFG. Open circles, data based on counts in 'neuropil' with exclusion of cell nuclei, blood vessels and empty spaces; x, data based on counts in whole cortex (see text).

activity-dependent. Synaptic contacts that are not included in neuronal circuits are gradually eliminated (Changeux and Danchin, 1976; Changeux et al., 1984). According to this scheme of synaptogenesis, early formation of syn-

apses depends on axonal and dendritic growth, leading to neurite contact which in turn promotes synapse formation. A region of late formation of axonal and dendritic branches, such as the prefrontal cortex would therefore be expected to have late synaptogenesis, as observed in the present data. The scheme also predicts that early synaptogenesis is intrinsically regulated, and not under environmental control. Intrinsic control of early synaptogenesis is confirmed by the absence of effects of age of first exposure to light on synaptogenesis in visual cortex (Bourgeois et al., 1989). This is in contrast to the formation of new synapses later in life, which has been shown to occur in relation to learning and memory (Kleim et al., 1996).

Onset of function in cerebral cortex seems to occur during the late phase of rapid synaptogenesis. Wilson (1988) points out that visual cortex functions, as for example stereopsis, emerge when synaptic density in visual cortex approaches the maximum.

Synapse elimination, in contrast to synaptogenesis, seems to be at least to some extent environmentally regulated. In visual cortex, unilateral deprivation of formed visual input during the period of synapse elimination results in asymmetric synaptic input from the two eyes (Hubel et al., 1977; Goodman and Shatz, 1993), and in permanent visual deficits (VonNoorden and Crawford, 1979; Assaf, 1982).

Onset of cortical function in the human appears to occur at different ages in different cortical regions. Functional

development of prefrontal cortex appears to be more gradual and at a later age than visual cortex. A simple task that is thought to be mediated by prefrontal cortex and that has been studied extensively is Piaget's A not B task (Diamond, 1985; Diamond and Goldman-Rakic, 1989). Competence on this task in human infants is reached at about age 12 months, about 8 months after the appearance of basic visual cortex functions, and at a time when synaptogenesis in prefrontal cortex is at about the stage observed in visual cortex at 2–4 months postnatally. More complex 'executive' functions of prefrontal cortex such as reasoning, motivation, and judgment appear to develop gradually during childhood and adolescence, perhaps continuing during the adult years (Sternberg and Powell, 1983). These uniquely human functions appear late during development, and their emergence may be aided by late persistence of exuberant synapses in prefrontal cortex.

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