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Lifespan Alterations of Basal Dendritic Trees of Pyramidal Neurons in the Human Prefrontal Cortex: A Layer-Specific Pattern

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The postnatal development and lifespan alterations in basal dendrites of large layer IIIC and layer V pyramidal neurons were quantitatively studied. Both classes of neurons were characterized by rapid dendritic growth during the first postnatal months. At birth, layer V pyramidal neurons had larger and more complex dendritic trees than those of layer IIIC; however, at 1 postnatal month both classes of neurons displayed a similar extent of dendritic outgrowth. In addition, after a more than year-long "dormant" period of only fine dendritic rearrangement, layer IIIC pyramidal neurons displayed a second period of dendritic growth, starting at the end of the second year and continuing in the third year. During that period, the dendritic tree of layer IIIC pyramidal neurons became more extensive than that of layer V pyramidal neurons. Thus, layer IIIC pyramidal neurons appear to show a biphasic pattern of postnatal dendritic development. Furthermore, the childhood period was characterized by transient increase in size of pyramidal cell somata, which was more pronounced for neurons in layer IIIC. These structural changes occurred during both the period of rapid cognitive development in preschool children and the period of protracted cognitive maturation during the childhood, puberty, and adolescence.

Keywords: associative cortex, cortico-cortical connections neurons, dendritic spines, working memory

Introduction

The development of dendrites, which form a major neuronal receptive field, is an essential process in the maturation of the neuronal circuitry (Uylings et al. 1994; Uylings 2001; van Pelt and Uylings 2002). Dendritic growth occurs in parallel with rapid synaptogenesis (Rakic et al. 1994) and the period of rapid dendritic maturation coincides with rapid changes in functional properties of neurons and cortical circuitry (Khazipov et al. 2001).

Large scale changes in brain functional activity continue throughout the childhood, up to early adulthood (Fischer and Rose 1994; Chugani 1998; Casey et al. 2000; Johnson 2001; Segalowitz and Davies 2004). However, no significant changes in length and complexity of dendritic arbor were found to correlate with these functional changes (Takashima et al. 1980; Becker et al. 1984; Mrzljak et al. 1990; Koenderink et al. 1994; Koenderink and Uylings 1995; Uylings et al. 2002). To further examine the postnatal development of dendritic arborizations we focused on the dorsolateral prefrontal cortex (magnopyramidal Brodmann's area 9) because it is characterized by prolonged structural and functional development and involvement in higher cognitive functions (Kostović et al. 1992; Huttenlocher and Dabholkar 1997; Woo et al. 1997; Casey et al. 2000; Diamond 2002; Fuster 2002; Dubois and Levy 2004; Gogtay et al. 2004; Segalowitz and Davies 2004). Large pyramidal neurons of layers IIIC and V represent key elements of dorsolateral prefrontal cortical circuitry (Petanjek et al. 1998, 2000; Petrides 2005; Elston et al. 2006). Layer V pyramidal neurons project to the basal ganglia, as principal cells of the associative cortex—basal ganglia circuit (McGuire et al. 1991; Yeterian and Pandya 1994; Pandya and Yeterian 1996; Wise et al. 1996; Groenewegen et al. 1997). Pyramidal neurons of the layer IIIC have long ipsi- and contralateral cortico-cortical (associative) projections (Schwartz and Goldman-Rakic 1984; Hof et al. 1995b; Barbas et al. 2005; Germuska et al. 2006). Therefore, we decided to investigate the pattern of lifespan dendritic changes in layer IIIC and V pyramidal neurons in Brodmann's area 9, as revealed in rapid Golgi impregnated sections. Few previous studies examined the development of these neurons in human (Schade and van Groenigen 1961; Mrzljak et al. 1988, 1992; Koenderink et al. 1994; Koenderink and Uylings 1995; Uylings et al. 2002; Vukšić et al. 2002), but the number of subjects quantitatively analyzed was low in these studies, especialy in the first postnatal years.

Therefore, in this study we increased the number of cases from birth to 10 years by more than 3 times and applied the rapid Golgi silver method. The present analysis of an enlarged sample of subjects yielded at least one novel and unexpected result. We found that large layer IIIC pyramidal neurons display a biphasic pattern of dendritic growth, with the second phase of significant dendritic elongation during the third postnatal year after an almost year-long "dormant" period of fine rearrangement of dendritic trees. To the best of our knowledge, such biphasic pattern of dendritic growth was not described previously for any class of cortical pyramidal neurons.

Materials and Methods

Twenty-five subjects, ranging in age from 1-week-old term newborn to 91 years, were quantitatively studied. None of the cases had clinical histories of neurological disorders, nor were any neuropathological alterations detected in their brains. All analyzed subjects had lived under normal environmental, socioeconomical conditions. All available historical data were collected from autopsy reports and medical records (see Table 1). In 2 cases (19 and 22 years) death was due to suicide. In these cases additional anamnestic data did not point to any psychiatric problems in their life history. The tissue was collected with the approval of the Ethical Committees and according to the regulations of the School of Medicine in Zagreb, Croatia. The prefrontal cortex tissue was

Table 1
Specification of tissue sample

						IIIC		V	
Age	Case number	Sex	PMD (h)	Cause of death	*T* (μm)	*N*	CS%	*N*	CS%
1 w	cd96	F	3	b.o.	140	16	4	16	9
1 m	cd147	M	4	SIDS	160	16	5	15	8
2.5 m	cd105	M	6	Pneumonia	140	14	8		
7 m	cd123	M	5	SIDS	165	15	22		
12 m	cd107	M	13	SIDS	170	14	16	12	13
15 m	cd143	F	5	b.o.	170	16	8	10	9
16 m	cd159	F	12	Mucoviscidosis	165	10	12		
2 y	cd238	F	15	Neuroblastoma	200	12	18	11	16
2.5 y	cd175	M	16	Car accident	165	16	11	16	12
5.5 y	cd125	F	15	Strangulation	180	10	23		
6 y	cd156	M	15	Car accident	165	18	16	16	22
9 y	cd157	M	16	Car accident	160	14	11	14	18
10 y	cd150	F	16	CO poisoning	175	15	14	10	7
16 y	co185	F	8	Car accident	185	14	14	10	14
17 y	co170	M	20	b.o.	175	15	14	13	14
19 y	co167	M	20	Strangulation[a]	165	15	13		
22 y	co198	M	20	Strangulation[a]	210	11	19		
28 y	co192	M	6	Car accident	180	12	16	13	19
30 y	co180	M	12	Car accident	180	15	14	14	13
52 y	co215	F	6	Car accident	200	10	12	10	15
59 y	91.172[b]	M	6	Embolia thoracis	120	16	19		
62 y	co246	F	11	ca. Pulmonis	180	15	12	10	16
82 y	92.01[b]	M	6	ca. Urogenitale	135	16	22	14	26
87 y	co171	M	8	b.o.	140	15	11	15	10
91 y	91.77[b]	M	4	CVI	120	12	25		

Note: Abbreviations: m, months; w, weeks; y, years; M, male; F, female; *N*, number of neurons quantitatively studied; b.o., brain morphology normal, no psychiatric and neurological diseases present in the anamnesis, the cause of death not described in the protocol. CS%, percentage of incomplete segments; *T*, section thickness; ca; carcinoma, CVI, cerebrovascular insult; SIDS, sudden infant death syndrome; PMD, postmortem delay (in hours).
[a]Two cases that committed suicide without psychiatric and neurologic history.
[b]Three cases that are from the Netherlands Brain Bank, Amsterdam.

studied in sections of the large Zagreb Neuroembryological Collection (Kostović et al. 1991), with exception of the sections of 3 elderly cases that came from the Netherlands Brain Bank (Table 1).

The parts of the prefrontal cortex examined included the superior and middle frontal gyrus, mainly defined as frontal granular and magnopyramidal Brodmann's area 9 (Rajkowska and Goldman-Rakic 1995). The 1-cm^3 blocks of tissue were sectioned perpendicular to the long axis of the frontal gyrus, in the majority of cases from the right hemisphere. The classical chrome-osmium rapid Golgi method was used (see Mrzljak et al. 1990). The pia was not removed. After sectioning, the cortical tissue was immediately immersed in rapid Golgi solution (0.3% osmium tetroxide and 3% potassium dichromate) and kept in the dark. After 7 days the dichromate solution was replaced by 1% silver nitrate for 2 days. Then tissue was dehydrated and embedded rapidly in 8% celloidin. After embedding, a microtome was used to serially section the blocks into coronal sections (mainly 160–200 μm). This section thickness was a compromise between cutting dendritic systems and maximal clarity of dendrites in a section. Nissl sections of neighboring blocks were cut at 30 μm to insure that area 9 was selected.

In accordance with the criteria for quantitative analysis described below, the 3-dimensional branching pattern of the basal dendrites of 10–18 randomly chosen pyramidal neurons was analyzed per layer and per subject in 17 subjects. In additional 8 subjects (see Table 1) at least 10 neurons for quantitative analysis were found only in layer IIIC. The measurements were performed with a semiautomatic dendrite measuring system developed at the Netherlands Institute for Brain Research (Overdijk et al. 1978; Uylings et al. 1986a) using a 63× oil immersion objective with a long working distance, equipped with the Lucivid miniature CRT from MicroBrightField, Inc., Colchester, VT.

The time interval between death and fixation of the tissue (i.e., the postmortem delay, PMD) was less than 8 h for early postnatal cases, less than 13 h for infants, less than 16 h for children, and less than 20 h for adults. All analyzed subjects died without preagonal state, so that the PMD actually represented the interval for neuron death. No staining artifacts due to PMD, described by Williams et al. (1978) and de Ruiter and Uylings (1987), for rapid Golgi staining were detected in the cases that were studied quantitatively. The criteria for cell selection for quantitative analysis were (Uylings et al. 1986a): 1) subjects whose histological sections contained heavy precipitations and signs of insufficient or artefactual impregnation in any of the cortical layers studied were excluded. No obvious signs of incomplete impregnation should be visible, not only in the analyzed neurons, but also in the neighboring ones in the same tissue block. 2) Basal dendritic processes of pyramidal cells had to be completely impregnated and not obscured by other clustered elements in the tissue section. 3) The soma had to lie near the center of the thickness of section, to reduce the number of cut segments at the section surface. 4) Apical dendrites had to be oriented perpendicularly toward the pial surface. 5) At least 100 μm of the apical main shaft had to be visible to confirm the orientation and position of the cell. 6) The whole dendritic surface had to be covered by spines, except the proximal segments arising directly from the cell soma. 7) The downwardly directed axon had to emerge from the base of the soma or from the origin of a basal dendrite. 8) The subject had to have at least 10 good neurons per layer to be incorporated in this quantitative study. Applying the above-mentioned criteria resulted in an inclusion of 25 subjects and an exclusion of 52 subjects for this quantitative study. The excluded subjects together with the selected ones were used for qualitative observation.

Layer V was detected in counterstained Nissl sections, and in Golgi sections as a 200-μm-thick layer below the transparent layer IV. Large layer IIIC pyramidal neurons are in the 200-μm zone above the layer IV. Only the neurons of typical pyramidal morphology were analyzed (Braak and Braak 1985; Petanjek et al. 1998, 2000). Modified pyramidal neurons were not included in the analysis.

The basal dendritic trees of pyramidal neurons were measured using the following variables (see Tables 2 and 3) (Uylings and van Pelt 2002): 1) number of basal dendrites per neuron, 2) total number of segments per neuron, indicating the branching frequency (topological complexity), 3) total dendritic length per neuron (sum of length of all traced segments) including the length of the individual incomplete segments (incomplete segments are those that are ending on the section border or are obscured by other elements in the section and could not be followed to their natural ending), 4) length of individual terminal and intermediate segments, 5) radial distance of terminal tips from the origin of the dendrite, and 6) somatic cell surface, that is, the area of the cell soma projected onto the plane of sectioning to indicate its size.

Terminal segments are segments between the terminal tip of dendrites and the last bifurcation point before the terminal tip. Intermediate segments are segments between the dendritic origin and the next bifurcation point, or between 2 consecutive bifurcation points. Furthermore, the intermediate segments were subdivided according to their degree, that is, the number of terminal segments of the subsequent peripheral subtree (Uylings and van Pelt 2002). Thus, a "degree-2" segment is an intermediate segment which branches into 2 terminal segments. The actual metrical values were not corrected for tissue shrinkage. In addition, we describe qualitatively apical oblique dendrites, that is, dendritic subtrees arising from the apical main shaft (Uylings and van Pelt 2002) and the age-span changes in dendritic spines.

Statistical Analysis

We applied the SPSS package for statistical analysis. The dendritic variables were tested separately for each layer with the one-way analysis of variance with parametric and nonparametric analysis (Conover and Iman 1981; Uylings et al. 1989) with age as a main effect. In the statistical analysis every subject represents a separate age. The a posteriori Student–Newman–Keuls test for multiple comparisons was applied to determine which subjects were significantly different. A P level lower than 0.05 was considered to be significant. Statistical analysis with parametric and nonparametric procedures did show comparable results. Additional analysis was performed to compare 2 groups of consecutive age periods. In this analysis we used a 2-tailed t-test, while we applied regression analysis to test the existence of a pattern of increase or decline, respectively, in a series of cases. For this analysis the mean values per subject were used.

Table 2
Layer IIIC pyramidal cells

Age	No. of basal dendrites	No. of segments	Total length (mm)	Terminal length (μm)	Intermediate length (μm)	Soma surface (μm^2)
1 w	6.1 (0.35)	20 (1.3)	1 (0.06)	75 (2)	10 (0.7)	157 (9)
1 m	6.3 (0.25)	53 (2.9)	2.3 (0.17)	68 (2)	16 (0.6)	294 (14)
2.5 m	6.1 (0.34)	45 (3.2)	2.8 (0.16)	104 (3)	16 (0.6)	280 (15)
7 m	5.8 (0.26)	48 (2.6)	2.5 (0.15)	96 (3)	20 (0.7)	368 (14)
12 m	6.6 (0.31)	49 (3.5)	2.9 (0.21)	101 (2)	25 (1.1)	401 (23)
15 m	5.9 (0.25)	53 (3.2)	3.3 (0.2)	106 (3)	21 (0.7)	385 (27)
16 m	5.6 (0.31)	42 (2.4)	2.4 (0.21)	98 (3)	22 (1.1)	323 (23)
2 y	5.8 (0.37)	50 (3.9)	3.2 (0.37)	121 (4)	22 (0.9)	380 (18)
2.5 y	5.8 (0.27)	52 (3)	4.2 (0.25)	144 (3)	23 (0.8)	408 (22)
5.5 y	5.9 (0.28)	41 (3.2)	3.2 (0.34)	162 (4)	26 (1.1)	588 (50)
6 y	5.6 (0.26)	38 (2.1)	3.1 (0.18)	145 (4)	27 (1.3)	556 (36)
9 y	6 (0.18)	46 (1.7)	4.6 (0.19)	178 (3)	26 (1)	415 (26)
10 y	6.2 (0.22)	52 (3)	4.1 (0.18)	143 (3)	27 (0.9)	400 (22)
16 y	5.9 (0.22)	49 (2.4)	3.8 (0.24)	137 (3)	28 (1.1)	401 (15)
17 y	5.9 (0.22)	52 (2.9)	3.9 (0.19)	138 (3)	26 (0.9)	412 (25)
19 y	5.7 (0.23)	47 (2.6)	4.1 (0.16)	164 (3)	22 (0.9)	390 (33)
22 y	5.6 (0.34)	61 (3.5)	4.5 (0.27)	150 (3)	22 (0.9)	403 (32)
28 y	6.1 (0.26)	51 (2.9)	4.3 (0.28)	163 (3)	21 (0.8)	376 (29)
30 y	6 (0.35)	51 (3.1)	4.5 (0.23)	167 (3)	25 (1.1)	408 (34)
52 y	5.5 (0.27)	45 (3.6)	4 (0.38)	164 (3)	20 (0.9)	352 (28)
59 y	6.3 (0.27)	62 (3.3)	4.7 (0.26)	156 (3)	25 (0.7)	392 (23)
62 y	6.1 (0.24)	55 (3.3)	4.5 (0.29)	156 (3)	21 (0.7)	410 (52)
82 y	6.4 (0.3)	59 (2.7)	4.6 (0.17)	169 (3)	22 (0.8)	503 (39)
87 y	5.7 (0.28)	40 (2.6)	3.4 (0.18)	149 (3)	22 (1)	340 (26)
91 y	5.8 (0.31)	51 (3.7)	3.7 (0.25)	162 (3)	21 (0.8)	424 (26)

Note: The mean value and standard error (in brackets) of basal dendritic tree parameters per neuron. Soma surface = the surface of somatic cross-sectional area. Abbreviations as in Table 1.

Table 3
Layer V pyramidal neurons

Age	No. of basal dendrites	No. of segments	Total length (mm)	Ratio TL III:V	Terminal length (μm)	Intermediate length (μm)	Soma surface (μm^2)	Ratio SS III:V
1 w	6.4 (0.34)	31 (2.4)	1.4 (0.1)	0.71	75 (3)	12 (0.5)	243 (11)	0.65
1 m	6 (0.24)	40 (1.9)	2.3 (0.18)	1.00	94 (3)	18 (0.8)	365 (23)	0.81
12 m	5.3 (0.37)	33 (2)	2.8 (0.15)	1.04	151 (5)	25 (1.5)	294 (17)	1.36
15 m	6.4 (0.31)	42 (3)	3.5 (0.22)	0.94	139 (5)	26 (1.7)	333 (38)	1.16
2 y	5.8 (0.33)	40 (2.9)	3.4 (0.24)	0.94	172 (6)	22 (1.1)	332 (27)	1.14
2.5 y	5.4 (0.22)	37 (2.6)	3.6 (0.3)	1.17	171 (6)	31 (4.4)	455 (41)	0.90
6 y	5.1 (0.36)	30 (2.7)	2.7 (0.29)	1.15	170 (7)	29 (1.9)	498 (27)	1.12
9 y	5.4 (0.27)	36 (2.9)	3.9 (0.27)	1.18	200 (5)	25 (1.4)	377 (34)	1.10
10 y	5.7 (0.21)	41 (3.4)	3.6 (0.29)	1.14	153 (5)	22 (1.6)	309 (28)	1.29
16 y	5.4 (0.33)	41 (2.5)	3.6 (0.17)	1.06	153 (4)	28 (1.7)	385 (31)	1.04
17 y	5.1 (0.18)	35 (2.6)	3.4 (0.4)	1.15	183 (6)	20 (1.3)	333 (35)	1.24
28 y	6 (0.36)	47 (2.2)	4.5 (0.36)	0.96	197 (4)	22 (1.1)	393 (44)	0.96
30 y	6.1 (0.44)	40 (2.2)	4.9 (0.37)	0.92	218 (6)	26 (1.7)	377 (21)	1.08
52 y	6 (0.45)	35 (3.7)	3.5 (0.33)	1.14	185 (4)	19 (1.2)	369 (46)	0.95
62 y	5.5 (0.4)	36 (1.5)	3.3 (0.23)	1.36	177 (5)	18 (0.9)	347 (33)	1.18
82 y	5.9 (0.33)	49 (3.5)	4.1 (0.26)	1.12	197 (5)	20 (1)	430 (30)	1.17
87 y	5.5 (0.31)	33 (2.4)	3 (0.25)	1.13	156 (5)	19 (1.1)	256 (21)	1.33

Note: Mean values and standard error (in brackets) of measured parameters of basal dendritic tree per neuron. For the total basal dendritic length and somatic size, the ratio (TL III:V, and SS III:V, respectively) between layer IIIC and layer V pyramidal cells is shown in the neighboring column. The number of analyzed neurons is shown in the Table 1.

Results

Qualitative Observations

Qualitative observations are restricted to large pyramidal neurons located in the deep part of layer III (layer IIIC) and typical large layer V pyramidal neurons. At birth, pyramidal neurons are immature, but during first 3 postnatal months neuronal morphology shows significant changes (Figs 1–3). Large elongation of basal and apical oblique dendrites was observed for both types of neurons until third month, as well as an expansion of the dendritic diameter and soma surface. At the third postnatal month dendritic morphology appears to be adult-like with respect to both the branching frequency and the dendritic orientation. Layer IIIC basal dendrites are predominantly vertically oriented and descend into the layer IV (Fig. 1*C*), whereas layer V basal dendrites now run mainly parallel to white matter (Fig. 1*I*). Apical oblique dendrites originate in the proximal part of the apical dendrite shaft, run horizontally, and do not bifurcate frequently. The main shaft of the apical dendrite is directed toward the pial surface and reaches layer II, where it bifurcates in a terminal tuft. From the third postnatal month onwards it is obvious that in comparison to layer IIIC, layer V pyramidal cells have fewer but longer segments (Figs 1*C–F*, *I–K*, 2*C–F*, 3*C*, *D*). In contrast to newborn stage, the thickness of dendrites is more regular at the third postnatal month, but the dendrites are still thinner than in adult (Figs 2 and 3).

At birth, dendritic spines are visible in layer V (Fig. 3*A*), but rarely in layer IIIC pyramidal neurons (Fig. 2*A*). Long, hair-like spines represent the dominant form during the first postnatal month, whereas at the age of 2.5 months dendritic spines already attained predominantly a mushroom morphology. During childhood and puberty period we observed (Figs 2 and 3) dendritic spine overproduction. During period of overproduction as in the adult, the dendritic spine density seems to be higher in layer IIIC than in layer V pyramidal cells (Figs 2 and 3).

Quantitative Analysis

The development in somatic size (Fig. 4*A,B*), the number of basal dendritic segments per neuron (Fig. 4*C,D*), and the total length of basal dendrites per cell are shown quantitatively. In addition to the total dendritic length per cell (Fig. 5*A,B*) we analyzed the mean length of individual terminal segments (Fig. 5*C–F*) and intermediate segments (Fig. 6) per subject, as variables to describe the increase in length of the dendritic arbor. The total length of basal dendritic tree was obtained as the sum of the length of all segments traced, including incomplete terminal segments. Thus, the total dendritic length is affected by cutting. When the branching period was finished, 80–90% of the total dendritic length was determined by the length of all individual terminal segments (Uylings and van Pelt 2002). The values of mean length of terminal segment are not affected by cutting and, therefore are a better indicator of changes in the total dendritic length, than the values of total length of a dendritic tree (Uylings and van Pelt 2002).

Layer IIIC Pyramidal Cells

Statistical analysis showed significant postnatal age effects for all measured dendritic variables, except for the number of basal dendrites (Table 2). The major phase of the postnatal dendritic growth seemed to occur between birth and 2.5 years (Fig. 5*A,C,E*). The pattern of dendritic growth displayed 2 growth spurts from birth to 2.5 years. The first growth spurt occurred from birth to 2.5 months; the second growth spurt occured between 16 months and 2.5 years, and a "dormant" period occured between 2.5 and 16 months.

From birth to 2.5 months, the total length of basal dendritic tree of layer IIIC pyramidal cells increased 3 times. The total number of dendritic segments was significantly smaller at birth in comparison to all other postnatal ages (Fig. 4*C*), but adult values of segment number were already reached at the age of 1

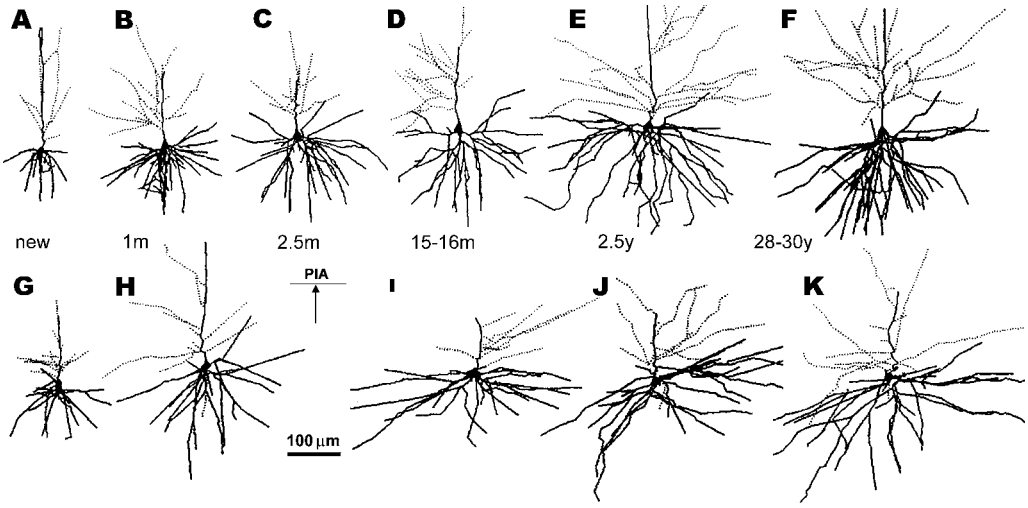

Figure 1. Three-dimensional reconstructions of basal and apical dendritic trees of rapid Golgi impregnated pyramidal cells projected onto the coronal plane. The orientation toward the pia is indicated by arrow. Oblique dendrites originate from the apical dendrite and are represented by dashed lines. All layer IIIC (upper row, *A–F*) and layer V (lower row, *G–K*) pyramidal cells are represented at the same magnification (bar scale = 100 μm) and at the following ages: newborn (*A*, *G*), 1-month-old (*B*, *H*), 2.5-month-old (*C*), 15-month-old (*I*), and 16-month-old infants (*D*), 2.5-year-old child (*E*, *J*), and 28-year-old (*F*) and 30-year-old (*K*) adults. Dendritic trees of layer IIIC pyramidal cells clearly increased between 16 months and 2.5 years of age. Note that there are no obvious differences between layer IIIC pyramidal cells of 2.5-month-old (*C*) and 16-month-old (*D*) infants as well as 2.5-year-old (*E*) and 28-year-old (*F*) subjects. On the other hand, no obvious differences were observed for layer V pyramidal cells of 15-month-old infant in comparison to all subsequent ages (*I–K*). Finally, note the differences in the complexity and orientation of basal dendritic trees of layer IIIC versus layer V pyramidal cells.

postnatal month. At birth, these cells acquired only one third of the total number of dendritic segments, implying that the remaining two thirds of segments appear postnatally, mostly during the first month. Thus, the significant increase in the total length of the basal dendritic tree detected after the first postnatal month is apparently caused by the elongation of dendritic segments and not by branching. The data on the total dendritic length (Fig. 5*A*) and on the length of individual terminal segments (Fig. 5*C,E*) show that basal dendrites reached approximately 60–70% of their adult total length at 2.5 months of age. Since the length at birth was just 20% of the total adult length, roughly the half of the dendritic growth in layer IIIC pyramidal cells occurred during the first 3 postnatal months.

No significant difference in the total length (values were between 2400 and 3300 μm) and mean length of individual terminal segments (values were between 96 and 106 μm) of the basal dendritic tree was found between any subject in the period from 2.5 to 16 months; however, a significant growth was detected between 16 months and 2.5 years because the total length of basal dendrites increased by approximately 30–40% of their adult size. This second growth spurt was especially evident on values of terminal segment length (Fig. 5*C*) and radial distance of terminal segments (Fig. 5*E*): all subjects younger than 2 years have significantly lower values compared to all subjects older than 2.5 years. We applied additional statistical analysis comparing subjects from these 2 groups. This analysis shows significantly lower values in the 2.5–16 months group ($P = 0.00001$) for both total length (2800 vs. 4100 μm) and mean length of terminal segment (101 vs. 156 μm). From 2.5 years onwards, the total length of the dendritic tree seem to fluctuate insignificantly around the stable value, thus implying that at 2.5 years it attained adult-like values and that dendrites do not undergo regressive changes thereafter. However, the data on the mean length of terminal segments might indicate that dendrites continue to elongate, albeit slightly, even after the age of 2.5 years. In 5 out of 7 subjects in the 2.5–17 years group, the values of terminal segment length were smaller than values in all the 10 subjects aged 19 years and older. It should be pointed out that the 9-year-old subject had the highest values of terminal segment length and that these values were significantly higher than in all other subjects analyzed. This makes it difficult to properly interpret results. When this subject was excluded from the analysis and the mean values per subject were compared in groups of subjects aged 2.5–17 years with group of subjects aged 19–86 years, the values in 2.5–17 years group were 10% lower than those in the 19–86 years group (145 vs. 160 μm), and this difference appeared to be significant ($P = 0.004$). The data on the total dendritic length are highly subject to the effect of cutting, and these values were quite low in 5.5- and 6-year-old subjects. To reduce the effect of cutting, we excluded the subjects with the highest and the lowest values in these 2 groups (i.e., subjects aged 5, 6, 9, 52, 62 years), and found that the difference in total dendritic length was again approximately 10% (4000 vs. 4400 μm, $P = 0.017$). Thus, we are inclined to conclude that dendritic differentiation may continue, but at a low rate, during the adolescence and early adulthood.

The elongation of individual intermediate segments (Fig. 6*A*) and of individual terminal segments (Fig. 5*C*) displayed different developmental trajectories. However, the summated length of all individual intermediate segments represents just 10% of the total length in the dendritic tree. Therefore, these changes are not so visible in the values of total dendritic length (Uylings et al. 1986a; Uylings and van Pelt 2002). The length of individual intermediate segments increased until the age of 6 years (Fig. 6*A*) when these segments become 2–3 times longer than at birth (Table 2). From 17 years onwards, the mean length of individual intermediate segments decreased significantly (Fig. 6*A*). The comparison of mean values per subject in a group of subjects aged 5–17 years versus the group of older subjects indicates the presence of a significant reduction in older subjects ($P = 0.0002$). This finding suggest that all types of intermediate segments of layer IIIC pyramidal cells may enter the period of transient overgrowth at some point between 5 and 17 years of age. The subsequent reduction in length of

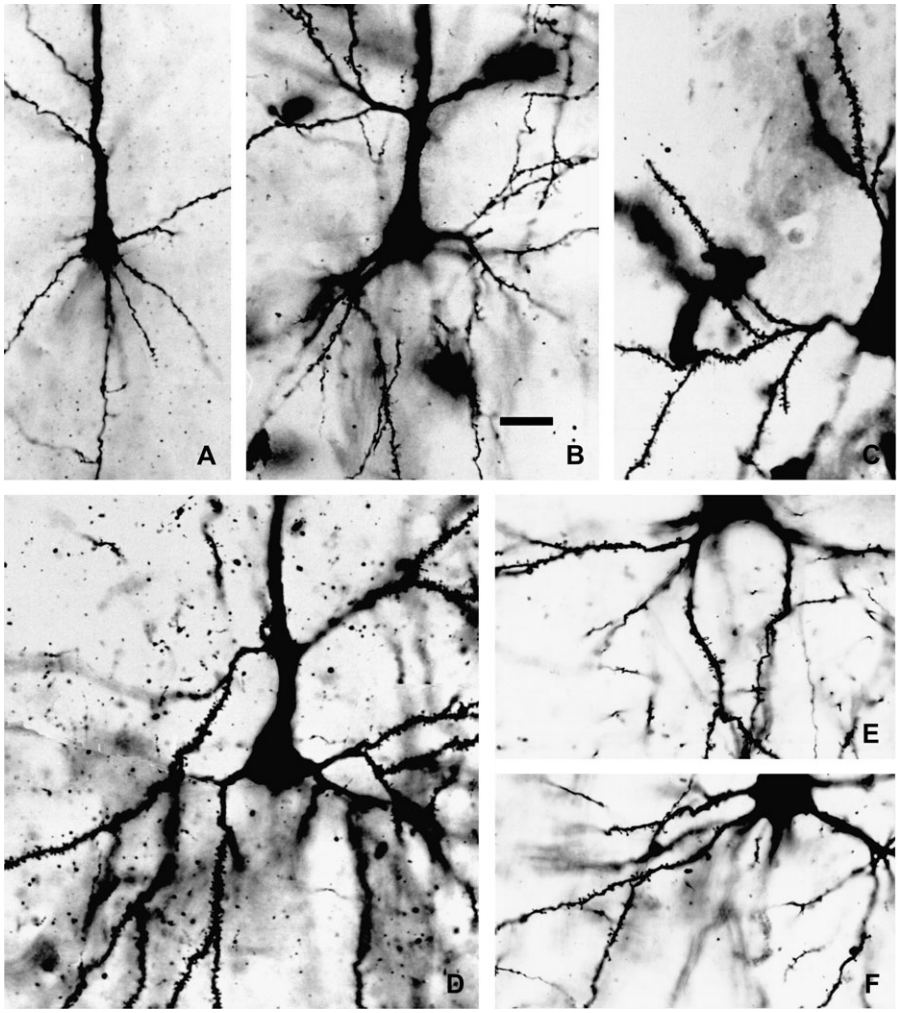

Figure 2. The morphology of rapid Golgi impregnated layer IIIC pyramidal cells of the dorsolateral prefrontal cortex in the newborn (*A*), infants aged 1 (*B*) and 16 (*C*) months, 2.5-year-old child (*D*), and 19-year-old (*E*) and 73-year-old (*F*) adults (the magnification is same for all microphotographs; bar scale = 20 μm). Note that very few dendritic spines are present in the newborn (*A*) and that the dendritic spine density increases until the age of 2.5 years (*B–D*), when most of the dendritic surface is covered with spines. Note also that an increase in the dendritic spine density occurs in parallel with an increase in the dendritic thickness (*A–D*). Even in these high-power microphotographs, the increase in dendritic complexity between newborn (*A*) and 1-month-old infant (*B*) is obvious.

intermediate segments was especially pronounced (approximately 30%) for degree-2 segments (Fig. 6*C*). This indicates that degree-2 segments may continue to branch after 17 years, but such a growth would be hard to detect because of its minor influence on the overall dendritic length and the total number of segments per cell.

The cross-sectional surface area of cell bodies of layer IIIC pyramidal cells increased until 7–12 months when adult-like values were reached (Fig. 4*A*). The values at 5 and 6 years suggest that at that age there was a transient and significant overgrowth of cell bodies. The frequency distribution of soma size for layer IIIC pyramidal cells was a unimodal one.

Layer V Pyramidal Cells

Statistical analysis showed a significant age effect for all measured dendritic variables (Table 3), except for the number of basal dendrites and the total number of segments in the basal dendritic tree (Fig. 4*D*). Thus, the formation of new dendritic segments in layer V pyramidal cells occurred only during the prenatal development (Mrzljak et al. 1992) and ceased around birth. After birth, only a significant elongation of dendrites of layer V pyramidal cells was detected (Fig. 5*B*,*D*,*F*).

At birth, both the total dendritic length per neuron (Fig. 5*B*) and the mean length of individual terminal segments (Fig. 5*D*,*F*) reached 40% of adult values. These values were significantly lower in newborn and 1-month-old infants than in all other analyzed subjects. At 12–15 months, the values of dendritic length become close to adult values, but it should be pointed out that no subject was available for quantitative analysis for the period from 1 month to 12 months of age. We were able to find 3 large layer V pyramidal neurons suitable for quantitative analysis in a 2.5-month-old subject, and the recorded values were similar to those observed in 12- to 15-month-old infants (the total basal dendritic length was 2900 μm, and the mean length of individual terminal segment was 154 μm). Both quantitative and qualitative findings suggest that the most intensive period of postnatal dendritic growth in layer V pyramidal cells occurs during the first 3 postnatal months. However, the quantitative data also indicate that dendritic growth might continue even after the age of 15 months, but to a much lower extent. This is suggested

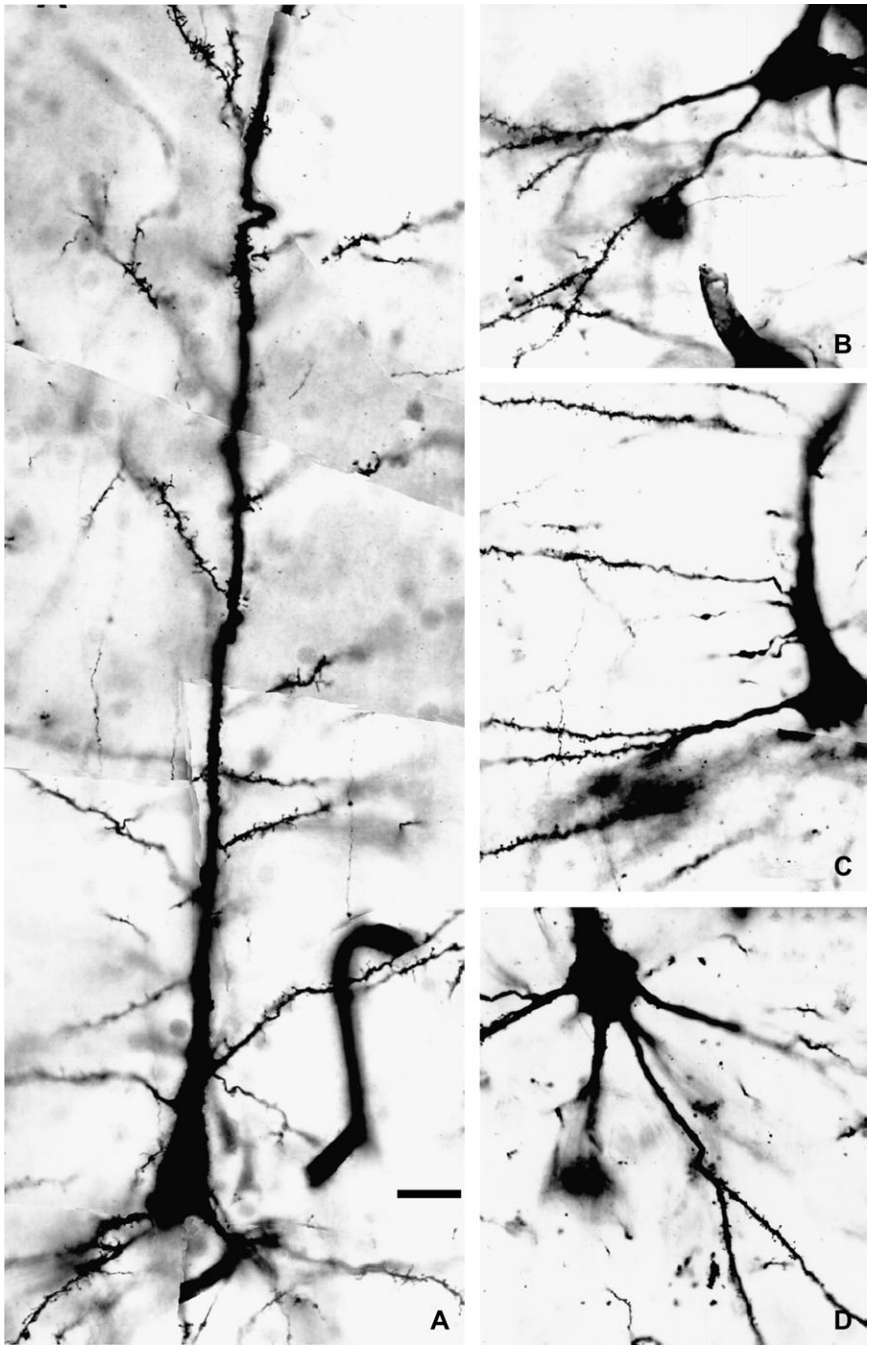

Figure 3. The morphology of rapid Golgi impregnated large layer V pyramidal cells of the dorsolateral prefrontal cortex in the newborn (*A*), 1-month-old infant (*B*), 19-year-old (*C*), and 49-year-old (*D*) subject (the magnification is the same for all microphotographs; bar scale = 20 μm). In comparison with layer IIIC pyramidal cells (Fig. 2*A*), the number of dendritic spines of layer V pyramidal cells in the newborn (*A*) is much higher.

by values of mean terminal segment length in 12- to 15-month-old infants, when compared with older subjects. The length values were 151 and 139 μm in 12- and 15-month-old infants, respectively (mean 145 μm), while the mean value of older subjects was 179 μm (i.e., 25% higher), ranging from 153 to 218 μm (Table 3). The individual intermediate segments elongated during the first and second postnatal year (Fig. 6*B*). Between 2.5 and 6 years of age, these segments were approximately 30% longer than in subjects older than 30 years. This finding is especially prominent for the degree-2 segments (Fig. 6*D*), suggesting that these dendritic segments of layer V pyramidal cells may continue to branch after puberty, as was noted for layer IIIC pyramidal cells also. On the other hand, all above-mentioned conclusions about growth after 15 months are weakened by the fact that, in comparison to layer IIIC pyramidal cells, layer V pyramidal cells were quantitatively analyzed in a smaller sample of subjects and also displayed a higher variability. Finally, as described for layer IIIC pyramidal cells, the cross-surface area of layer V pyramidal cells also displayed the highest values at ages of 2.5–6 years, but these values were not significantly higher in comparison to other ages to suggest the period of transient cell body overgrowth (Fig. 4*B*).

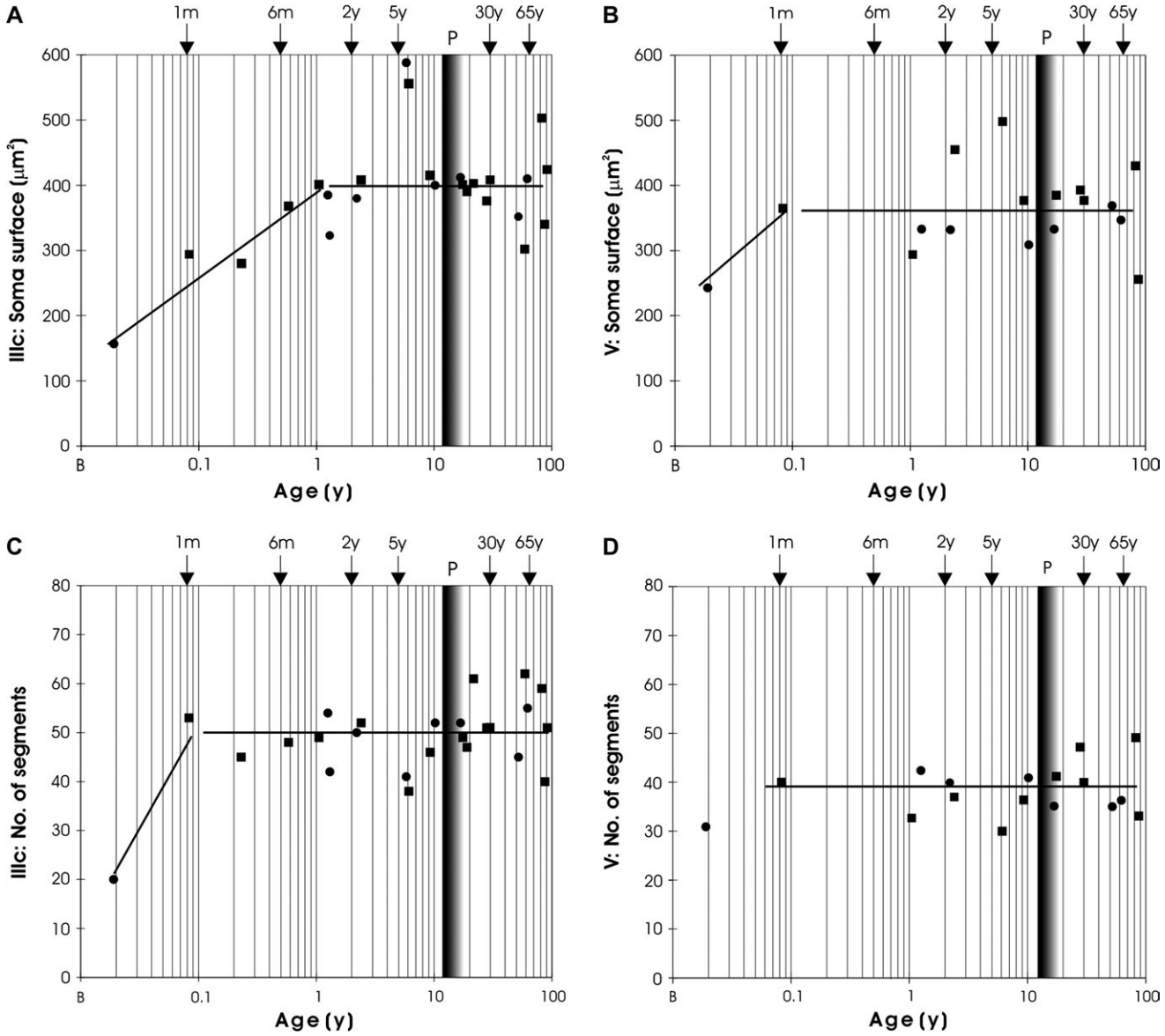

Figure 4. The somatic size, that is, the cross-sectional surface area of cell bodies (*A*, *B*) and the total number of segments (*C*, *D*) per neuron in layer IIIC (*A*, *C*) and layer V pyramidal cells (*B*, *D*). Note that in layer V pyramidal cells the somatic size is close to the adult value already at birth, whereas in layer IIIC pyramidal cells the adult values are reached around 7 months. Layer IIIC cell bodies in the newborn were several times smaller than in the adult. The putative somatic size overgrowth was less pronounced and appeared earlier in layer V pyramidal cells. The number of dendritic segments increased during the first postnatal month only in layer IIIC pyramidal neurons. The age is presented in postnatal years on a logarithmic scale in order to fit the entire human life span onto a single graph. The period of puberty is marked by a shaded bar. Squares represent males, and circles females. P, puberty; B, birth (because of logarithmic scale this is actually 4 postnatal days); m, months; y, years.

Morphological Differences of Layer IIIC and Layer V Pyramidal Cells

At birth, the dendritic tree of layer IIIC pyramidal cells seems to be significantly less mature than that of layer V pyramidal cells. However, these differences were no longer obvious at the age of 1 month (see in Table 3 the column, "ratio of total length between layer IIIC and V"). The basal dendritic tree of layer IIIC pyramidal cells becomes larger than the basal dendritic tree of layer V pyramidal cells from 2.5 years onwards. Table 3 shows that the basal dendritic tree of layer IIIC pyramidal cells was about 15% longer than that of layer V pyramidal cells in 75% of specimens in which both layers were analyzed. Thus, we conclude that the magnoypramidal character (with layer IIIC pyramidal cells being larger than layer V pyramidal cells) of the Brodmann's area 9 is established by 2.5 years of age.

However, the mean somatic size (Fig. 4*A*,*B*) was already larger in layer IIIC than in layer V pyramidal cells from 12 months onwards when already adult size is reached (see in Table 3 the column, "ratio of soma surface area between layer IIIC and V"). After the period of putative cell body overgrowth (5–6 years), the somatic size of layer IIIC pyramidal cells in subjects aged 9–62 years remained significantly (10%) larger than in layer V pyramidal cells ($P = 0.005$). In 3 (out of 8) subjects with both layers measured (group aged 9–62 years), the mean somatic size was approximately the same in both layer IIIC and layer V pyramidal cells (i.e., the layer IIIC–V ratio per subject varied

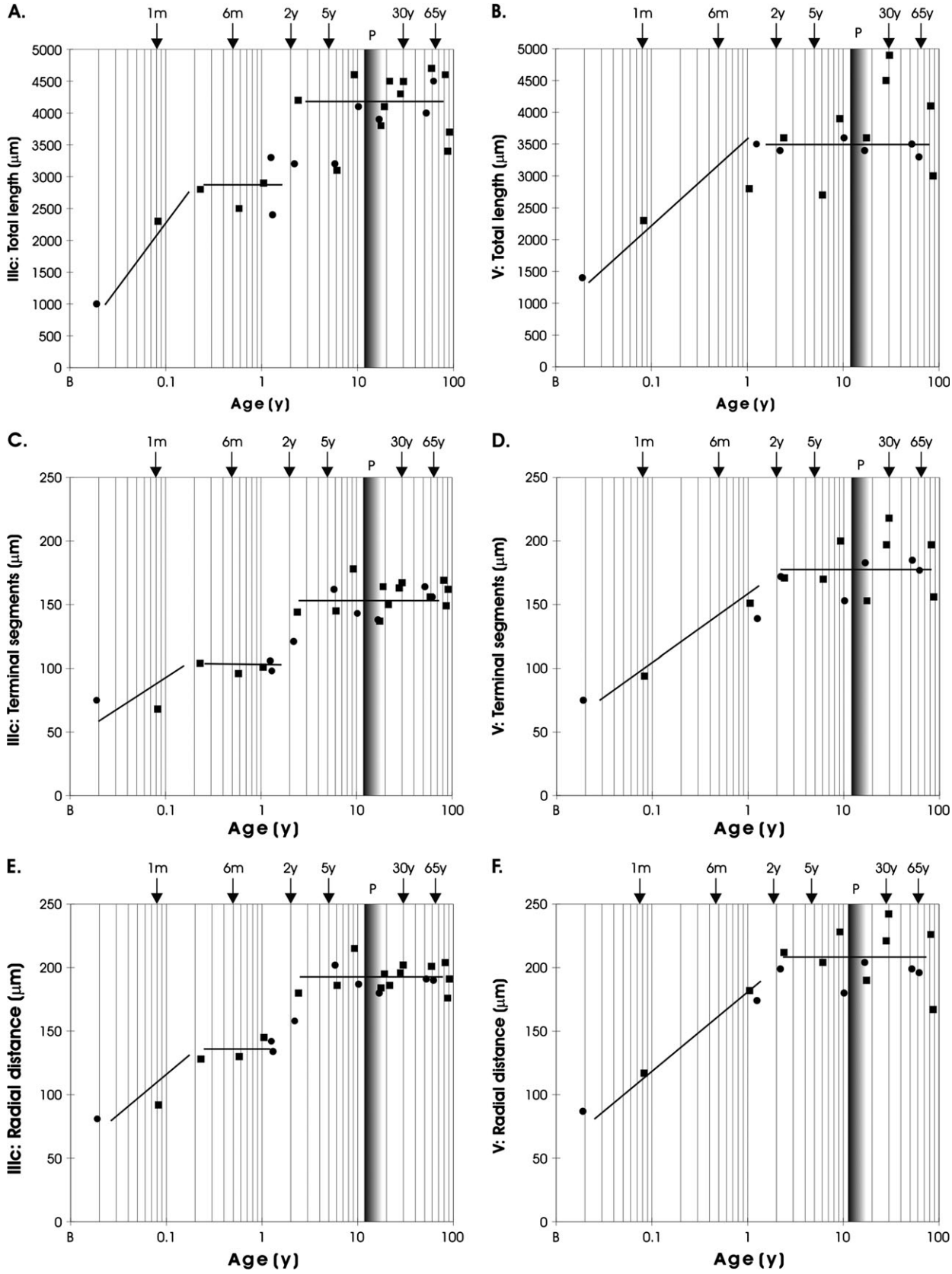

Figure 5. The total length of the basal dendritic tree per neuron (*A*, *B*) and the mean length (*C*, *D*) and radial distance (*E*, *F*) of terminal segments for layer IIIC (*A*, *C*, *E*) and layer V pyramidal cells (*B*, *D*, *E*). Note that the most rapid and intensive dendritic growth for both classes of neurons occurred between birth and 2.5 months. However, dendrites of layer V pyramidal cells grow until the ages of 16 months to 2.5 years, whereas dendrites of layer IIIC pyramidal neurons did not display significant growth between 2.5 and 16 months, but displayed a second growth spurt between 16 months and 2.5 years. This is especially conspicuous with data on the individual terminal segment length and radial distance. For abbreviations, see Figure 4.

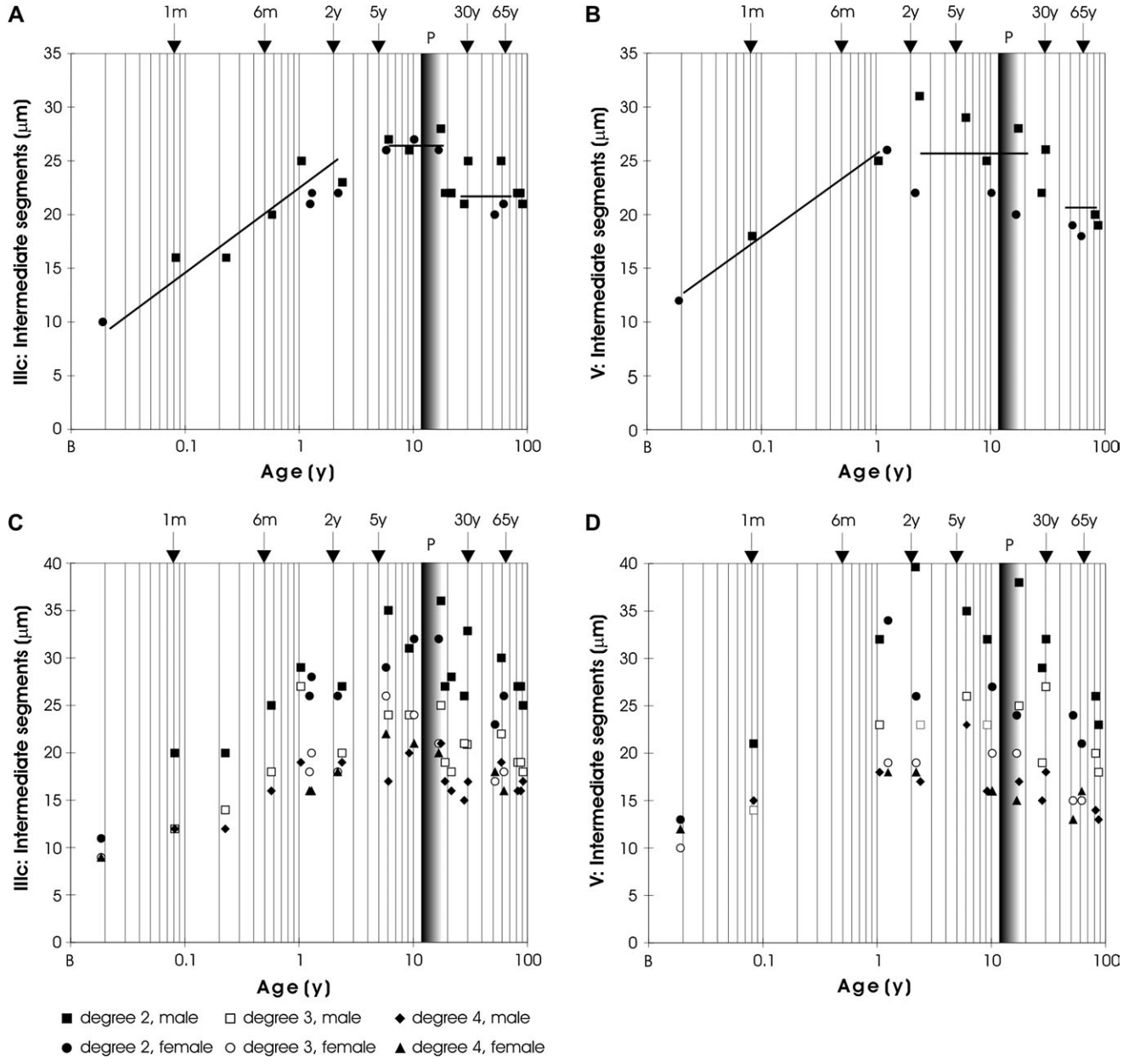

Figure 6. The mean length of individual intermediate segments independent of their degree (*A*, *B*), and the mean length for different degree types (*C*, *D*) for layer IIIC (*A*, *C*) and layer V pyramidal cells (*B*, *D*). Note that there is an overgrowth of the intermediate segment length until the puberty, independently of their degree type. Segments with a lower degree are on average longer than segments with a higher degree. For abbreviations, see Figure 4.

from 0.95 to 1.04); however, in all other subjects examined the somatic size of layer IIIC pyramidal cells was larger (i.e., the layer IIIC–V ratio per subject varied from 1.08 to 1.29). This was also the case for 2 old subjects (82 and 87 years), as for all subjects aged 12–24 months (Table 3).

Discussion

This study is a continuation of our previous quantitative analysis (Mrzljak et al. 1992; Vukšić et al. 2002) of prenatal and early postnatal development, from 13.5 postconceptional weeks (PCW) to the ninth postnatal week, analyzing the same 2 classes of prefrontal cortical pyramidal cells. However, in this study we increased the number of cases analyzed by more than 3 times in the age period from birth to 10 years.

The findings of the present study confirm and extend previous findings concerning the perinatal period as a phase of rapid dendritic growth of large pyramidal neurons of the human prefrontal cortex (Mrzljak et al. 1992; Vukšić et al. 2002). Thus, we found that by the third postnatal month layer V and layer IIIC pyramidal cells already attained 70–80% and 60% of the total adult size of their basal dendritic trees, respectively, and both classes of neurons already displayed an adult-like overall morphological dendritic phenotype. In addition, we present the following novel findings: 1) although at birth layer IIIC pyramidal cells were less developed than layer V pyramidal cells, they soon "catch-up" so that at about 1 month they already equal layer V pyramidal cells in absolute values of the total dendritic length and complexity; 2) layer V pyramidal cells attain at 12–15

months at least 90% of adult values, while after the third month layer IIIC pyramidal cells display a seemingly "dormant" period (with no significant overall growth) from 3 to 16 months followed by a second growth spurt from 16 months to 2.5 years; and 3) this second growth spurt for layer IIIC pyramidal cell dendrites (approximately 50% increase in total length in comparison to values at 16 months) represents a novel, hitherto undescribed feature, which is perhaps unique for "cognitive" layer IIIC pyramidal cells. Finally, our quantitative data suggest that both classes of neurons undergo a phase of transient somatic overgrowth at 5–6 years of age; however, these data were statistically significant only for layer IIIC pyramidal cells.

The present developmental curve of dendritic development in the human prefrontal cortex during the infancy and early childhood, derived from our rapid Golgi study (silver impregnation), is much more detailed than the one obtained from our previous Golgi Cox (mercury impregnation) studies (Koenderink et al. 1994; Koenderink and Uylings 1995). The growth patterns are compatible in both studies, but the absolute figures differ considerably. In the Golgi Cox study the figures for the number of basal dendrites per neuron in both layer IIIC and layer V pyramidal cells were similar, but the number of basal dendritic segments per neuron was about 30% higher in the Golgi Cox stained tissue (Koenderink and Uylings 1996). On the other hand, the Golgi Cox length values of the total dendritic length and of individual terminal segments were about 30% lower than the present rapid Golgi values. These differences are mainly due to a higher shrinkage in Golgi Cox staining, as noted earlier for older specimens (de Ruiter and Uylings 1987).

In this study we did not observed significant dendritic regression during aging, that is consistent with other rapid Golgi studies where no dramatic dendritic regression with aging was found (Jacobs and Scheibel 1993; Jacobs et al. 1997; Petanjek et al. 2000), in contrast to our Golgi Cox study that showed significant regression in layer V pyramidal cells but not in layer IIIC pyramidal cells (de Brabander et al. 1998; Uylings and de Brabander 2002). These differences might be explained by different affinity to stain different population of neurons by rapid Golgi and Golgi Cox method, as was mentioned previously (Buell 1982; Braak and Braak 1985). However, in our rapid Golgi preparations we noticed changes in layer V neurons with age only in that sense that the number of layer V cells which fulfill the criteria for quantitative analysis declined considerably, suggesting that some percentage of this population, which might undergo regression, was possibly not covered by our analysis.

Pyramidal Neurons of the Human Prefrontal Cortex Display 4 Phases of Dendritic Maturation during Prenatal and Postnatal Period

During the prenatal and early postnatal period, both layer V and layer IIIC pyramidal cells displayed 3 phases of dendritic growth which were also described in the majority of morphological and experimental studies (Mrzljak et al. 1990; Uylings et al. 1994; Cline 2001; van Pelt and Uylings 2002; Whitford et al. 2002). However, large layer IIIC pyramidal neurons also displayed a fourth, hitherto undescribed phase of late postnatal dendritic growth.

The first phase of dendritic growth begins immediately after the arrival of the neuron in the cortical plate, between 12 and 20 PCW in humans (Kostović and Rakic 1980; Kostović 1990b; Mrzljak et al. 1990; Rakic 2002). This phase is characterized by protrusion of primary dendrites, which includes the growth of primary basal dendrites and the apical dendrite with oblique dendrites. It is also characterized by large axon growth (Mrzljak et al. 1988, 1990), in agreement with findings of experimental in vivo and in vitro studies which demonstrated that an intensive axon growth precedes the dendritic growth during the neuronal differentiation (Cline 2001; Khazipov et al. 2001; Groc et al. 2002).

The second phase of dendritic growth occurs from 24 PCW to third postnatal month and is characterized by a rapid growth of the dendritic tree. This phase begins with an outgrowth of new dendritic segments, followed by a large elongation without significant formation of new dendrites, and coincides with the ingrowth of cortical afferent fibers into the cortical plate (Kostović and Goldman-Rakic 1983; Kostović and Judaš 2002; Judaš et al. 2005). For example, the intensive growth of dendrites of layer V pyramidal cells began around 24 PCW when thalamocortical afferents invade the cortical plate, and around 32 PCW for layer IIIC pyramidal cells, when cortico-cortical axons penetrate the cortical plate. As both thalamocortical and cortico-cortical afferents are glutamatergic, this finding is in agreement with experimental findings showing that an intensive dendritic differentiation started with the appearance of N-methyl-D-aspartate (NMDA) receptors on neurons (Cline 2001; Khazipov et al. 2001; Sin et al. 2002; Van Aelst and Cline 2004), that blocking of NMDA receptors resulted in arresting the dendritic growth (Cline 2001; Sin et al. 2002), and that activity was important for outgrowth of basal dendrite segments of pyramidal neurons (Groc et al. 2002, 2003; Van Aelst and Cline 2004). Above-mentioned data show that pyramidal neurons display an inside-out gradient of maturation during the prenatal and perinatal period (Kostović 1990b; Mrzljak et al. 1990; Uylings 2001).

The third phase of dendritic maturation is about a year-long, lasts between third and 16th postnatal month, occurs in parallel with a large increase in the dendritic spine density and the number of synapses (Bourgeois et al. 1994; Petanjek et al. 1994; Rakic et al. 1994; Anderson et al. 1995; Huttenlocher and Dabholkar 1997) and is characterized by fine dendritic rearangement with stabilization of dendritic structure in layer IIIC pyramidal cells. The fourth phase of dendritic growth occurred from 16 months to 2.5 years. During that phase, at one hand terminal dendritic segment length of layer V pyramidal cells show a further slight increase (up to 10%) to attain adult values. At the other hand, data on large layer IIIC pyramidal cells indicate that these cells display a second growth spurt and increase of about 50% (of values at 2.5 years) in comparison to values at 16 months. Such biphasic growth of layer IIIC dendritic arborization was not described in previous studies of dendritic development in humans nor in other primates during equivalent postnatal ages (Conel 1939–1967; Schade and van Groenigen 1961; Becker et al. 1984; Koenderink et al. 1994; Koenderink and Uylings 1995; Travis et al. 2005). The second growth spurt lasts about 4 times longer than the first, while the rate of dendritic elongation seems to be about 2 times slower, so the estimated rate of dendritic growth during the childhood in comparison to the perinatal period is at least 5 times slower for layer IIIC pyramidal cells.

Functional Implications of Differences in Tempo and Mode of Dendritic Differentiation of Supragranular and Infragranular Pyramidal Cells of the Human Prefrontal Cortex

Experimental data obtained in nonhuman primates have shown that layer IIIC pyramidal cells are key elements in circuitry

involved in working memory and other higher associative cognitive functions of the prefrontal cortex (Fuster et al. 2000; Elston et al. 2006; Wang et al. 2006). The selective regression and loss of these layer IIIC pyramidal cells is reported in various diseases and states characterized by a decline of higher cognitive functions (Morrison and Hof 2002; Pierri et al. 2003; Selemon et al. 2003). On the basis of their laminar distribution and comparable morphological appearance, large layer IIIC neurons impregnated by the rapid Golgi method in this study predominantly correspond to acetylcholinesterase (AChE) reactive and SMI-32–positive neurons (Kostović et al. 1988; Mesulam and Geula 1991a, 1991b; Mrzljak and Goldman-Rakic 1992; Hof et al. 1995a, 1995b). In the supragranular layers their number and density increased greatly during the primate evolution and they are not present in other nonprimate mammals (Campbell et al. 1991; Hof and Sherwood 2005). The strong AChE and SMI-32 reactivity in supragranular layers is more expressed in primate associative magnopyramidal areas (Kostović et al. 1988; Mesulam and Geula 1991b; Mrzljak and Goldman-Rakic 1992) and marks a subset of cortico-cortical neurons with long ipsi- and contralateral cortico-cortical (associative) projections with a columnar distribution of the terminal field within the cortex (Schwartz and Goldman-Rakic 1984; Campbell et al. 1991; Hof et al. 1995b; Barbas et al. 2005). They are also characterized by a high number of intracortical axonal collaterals that extend for several millimeters around the cells, also with a columnar distribution of their terminal field through layers II and III (Barbas and Pandya 1989; Kritzer and Goldman-Rakic 1995; Pucak et al. 1996; Melchitzky and Lewis 2003). Finally, the morphology, density, and number of large layer IIIC pyramidal cells in human magnopyramidal areas displays a great interindividual variability, more so than the cellular composition of other layers (Jacobs et al. 1993, 2001; Rajkowska and Goldman-Rakic 1995; Amunts et al. 2003; Uylings et al. 2005b; Elston et al. 2006). These data suggest that interindividual differences in size and internal composition of associative cortical areas are strongly involved in the biological basis of individuality.

The findings of this study indicate that infragranular (layer V) and supragranular (layer IIIC) pyramidal cells of the human prefrontal cortex display a common pattern of rapid dendritic growth during the first 3 postnatal months, and suggest differences in tempo of their dendritic differentiation thereafter. A rapid perinatal differentiation of layer V pyramidal cells may not be surprising, since these cells are the major neuronal elements for processing of early executive functions of the prefrontal cortex (Goldman-Rakic 1996; Robbins 1996; Herschkowitz et al. 1997; Fuster 2000; Monchi et al. 2006). An equally intense perinatal differentiation of layer IIIC pyramidal cells, however, is somewhat surprising because these cells are proposed to be the key elements involved in sophisticated, evolutionarily recent, and human-specific cognitive functions (Goldman-Rakic 1995; Fuster et al. 2000; Dubois and Levy 2004). Such an early functioning neural network may represent a neurobiological basis for those cognitive functions present already in newborns and young infants. The perinatal period is characterized by a rapid transformation and disappearance of fetal patterns of behavior (Kostović et al. 1995; Einspieler and Prechtl 2005; Hadders-Algra 2005) with concomitant appearance of certain aspects of cognitive functions (Diamond and Kirkham 2005; Werker and Yeung 2005). Of course, development of cognitive functions continues and accelerates during the early childhood (Davidson et al. 2006). Thus, human cognition develops both, early, in parallel with other cortical functions, and has a protracted continuous development until the third decade.

The findings of the present study show also that magnopyramidality (Braak 1980; Kostović et al. 1988; Kostović 1990a; Mrzljak et al. 1990; Hof et al. 1995a, 1995b; Rajkowska and Goldman-Rakic 1995; Pandya and Yeterian 1996; Semendeferi et al. 2001, 2002; Petrides 2005; Uylings et al. 2005a) of the dorsolateral prefrontal cortex begins to develop already at 1 year of age, when large layer IIIC pyramidal cells have reached or even exceeded the somatic size of layer V pyramidal cells. We had already suggested this before (Mrzljak et al. 1990) on the basis of qualitative analysis of rapid Golgi stained sections, as well as on the basis of semiquantitative analysis of Nissl stained sections (Conel 1939–1967). However, most other qualitative Nissl studies reported that the magnopyramidality becomes expressed much later, at the age of 4–5 years (Aldama 1930; Kononova 1940; Kostović 1990a; Amunts et al. 2003; Elston et al. 2006). The reason for this discrepancy might be that large layer IIIC pyramidal cells develop their characteristic intense Nissl staining later in childhood, when they appear to be the most prominent element of the dorsolateral prefrontal cortex. This correlates with the appearance of strong AChE (Kostović et al. 1988; Mesulam and Geula 1991b) and SMI-32 (Ang et al. 1991; Koenderink 1996) reactivity in large layer IIIC pyramidal cells between 2 and 4 years.

With respect to the second growth spurt of layer IIIC pyramidal cell dendrites, it is not clear what triggers further dendritic growth during the second and third year of life. The data on chemical development suggest that fine chemical maturation of large layer IIIC pyramidal cells occurs during the childhood (Kostović et al. 1988; Ang et al. 1991; Mesulam and Geula 1991b; Koenderink 1996; Uylings et al. 2002), in parallel with the maturation of dopaminergic innervation of projection prefrontal neurons (Goldman-Rakic and Brown 1982; Lidow and Rakic 1992; Lambe et al. 2000). Thus, intrinsic molecular maturation in combination with maturation of dopaminergic innervation might trigger an intensified dendritic growth during the period when social and educational environment exert a strong influence on the developing child. This opens the possibility that the activity, that is, the interaction with the social and educational environment of childhood may have a crucial role for proper dendritic rearrangement and final maturation of prefrontal supragranular pyramidal neurons.

We propose that the strategic position of large layer IIIC pyramidal cells within the circuitry of associative cortical areas leads to significant changes in their functional properties during postnatal development, and consequently significant changes in information processing throughout the entire brain, as has been suggested by data on functional brain development.

Protracted Development of Prefrontal Pyramidal Neurons until the Adulthood

With the exception of apical dendrites of small layer V pyramidal cells in the rat (Koester and O'Leary 1992), earlier cross-sectional studies of the development of pyramidal neurons in the neocortex and the hippocampus did not show an overgrowth in length and complexity of the dendritic arbor (Schade and van Groenigen 1961; Becker et al. 1984; Koenderink et al. 1994; Koenderink and Uylings 1995; Travis et al. 2005). It is possible that the absence of correction in the metrical data for

developmentally differential shrinkage (Uylings et al. 1986b) obscures a possible presence of a temporary dendritic overgrowth. The overgrowth, however, has been demonstrated in axonal tree (LaMantia and Rakic 1990; Woo et al. 1997; Innocenti and Price 2005) and number of synapses (Bourgeois et al. 1994; Petanjek et al. 1994; Rakic et al. 1994; Anderson et al. 1995; Huttenlocher and Dabholkar 1997). Our data show that during childhood and adolescence overgrowth of about 30% was observed on length of intermediate segments for both classes of neurons, but this is not reflected on total and terminal segment length. Qualitative observations suggest that this occurs in parallel with dendritic spine overproduction. In the prefrontal cortex the number of synapses (Huttenlocher and Dabholkar 1997) and the number of dendritic spines (Kostović et al. 1992, 1995; Petanjek et al. 1994) reached their highest levels at the age of 2.5–5 years, with a decrease later on in adolescence. Cognitive development continues during the rest of childhood and adolescence (Goldman-Rakic 1987; Diamond 2002; Fuster 2002; Shaw et al. 2006). The synaptic overproduction during that period is probably connected with synaptic pruning and stabilization, where activity plays a major role (Rakic et al. 1994). The greater synaptic overproduction in supragranular layers suggests that synaptic pruning and stabilization are more important for the maturation of cortico-cortical circuits (Woo et al. 1997). These data also point to the importance of the influence of education and social environment on the structural maturation of associative cortical circuitry during childhood and adolescence (Jacobs et al. 1993, 2001; Chugani 1998; Chugani et al. 2001; Raine et al. 2002; Grossman et al. 2003; Eluvathingal et al. 2006; Shaw et al. 2006; Uylings 2006). Some of our quantitative data suggest that further slight dendritic growth might occur at that time. However, these data have to be confirmed, so we can only tentatively conclude that fine dendritic and spine rearrangement occurs during childhood and adolescence, with final maturation in the third decade of life. These changes might represent a biological basis for protracted cognitive development.

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Notes

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