Quantitative Analysis of Basal Dendritic Tree of Layer IIIc Pyramidal Neurons in Different Areas of Adult Human Frontal Cortex

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ABSTRACT

Large long projecting (cortico-cortical) layer IIIc pyramidal neurons were recently disclosed to be in the basis of cognitive processing in primates. Therefore, we quantitatively examined the basal dendritic morphology of these neurons by using rapid Golgi and Golgi Cox impregnation methods among three distinct Brodmann areas (BA) of an adult human frontal cortex: the primary motor BA4 and the associative magnopyramidal BA9 from left hemisphere and the Broca's speech BA45 from both hemispheres. There was no statistically significant difference in basal dendritic length or complexity, as dendritic spine number or their density between analyzed BA's. In addition, we analyzed each of these BA's immunocytochemically for distribution of SMI-32, a marker of largest long distance projecting neurons. Within layer IIIc, the highest density of SMI-32 immunopositive pyramidal neurons was observed in associative BA9, while in primary BA4 they were sparse. Taken together, these data suggest that an increase in the complexity of cortico-cortical network within human frontal areas of different functional order may be principally based on the increase in density of large, SMI-32 immunopositive layer IIIc neurons, rather than by further increase in complexity of their dendritic tree and synaptic network.

Keywords: *cortico-cortical connections, working memory, schizophrenia, glutamatergic synapse, dendritic spine, magnopyramidal regions*

Introduction

The most complex cortical functions are involved in human cognition, but there is large controversy regarding mechanisms that enable humans to conceptualize, plan and prioritize, or why they are set apart from other animals in their cognitive abilities[1–3]. Increase in size of the brain was certainly the first step to make possible appearance of higher cognitive function. However, during evolution, there is a large linear increase in size of cerebral cortex in general, but for some regions this increase is exponential[1,2,4–6]. This is especially the case for size of granular frontal cortex (i.e. prefrontal cortex), that in humans occupies more than one fourth of cortical surface[6–8]. Numerous experimental and functional studies obtained in monkey and human brain showed that this region should be defined as associative frontal cortex, because it is a major region for the processing of the highest cognitive functions[5,9–11].

In addition to increased size of prefrontal cortex during evolution, changes appear also in internal composition. They are result of relative increase in the number of cortico-cortical neurons[6,11–14], as well as increase in their dendritic complexness[10,15]. Therefore, in contrast to the non-primate mammals, in primates the supragranular layers are thicker than the infragranular layers[6,16] and their internal composition varies highly between areas[15,17]. The most dramatic changes in internal composition are present in associative neocortical areas, where the large layer III pyramids are bigger than the large layer V

pyramids[16,18–23]. This feature is defined as magnopyramidality (magnocellularity) and is found only in higher primates[11]. The magnopyramidality is most pronounced in human brain, where it appears during childhood[3,5,24–28].

Experimental data obtained in nonhuman primates suggest that large pyramidal cells located deep in layer III (layer IIIc) are key elements in circuitry involved in working memory and other higher associative cognitive functions of the prefrontal cortex[10,29–31]. In line with this assumption are the data in humans, reporting selective regression and loss of layer IIIc pyramidal cells in parallel with decline of higher cognitive functions in various psychiatric diseases and states[32–34]. However, although the qualitative characteristics of cortical pyramidal neurons in the human brain have been relatively well documented for a long time[35], quantitative data studying individual dendrite morphology are limited, particularly data about regional variations[15,28,36–47]. Such data can serve as referent data in further research of pathological cases, and all findings together will be of importance in understanding neurobiology of human cognitive function. Therefore, the aim of the present study was to establish normal quantitative parameters of the largest layer IIIc pyramidal neurons among three distinct Brodmann cortical areas (BA): the primary motor cortex (BA4), the dorsolateral prefrontal region (BA9), Broca's motor speech area (BA45) of which the last mentioned BA was analyzed in both the left (L) and right (R) hemisphere.

Materials and Methods

We have analyzed postmortem brain tissue of 3 adult persons (2 males, age 37 and 45 years, 1 female, age 41 years) taken from magnopyramidal frontal region that corresponds to left primary motor area BA4, left dorsolateral prefrontal region BA9 and left and right Broca's area BA45[19,20]). None of the subjects had clinical record of neurological disorders, or any neuropathological deviations detected in their brains postmortem. The autopsies were approved by the Internal Review Board of the Ethical Committee at the School of Medicine, University of Zagreb in accordance with the Declaration of Helsinki from 2000. All tissue samples examined were part of the Zagreb Neuroembryological Collection[48].

The tissue blocks (1 cm^3) were sectioned perpendicular to the long axis of the gyrus; BA9 from left superior fronal gyrus, BA4 from the middle part of the left precentral gyrus, and BA45 from triangular part of inferior frontal gyrus from both hemispheres. The borders and positions of BA's have to be overlapped on all three major cytoarchitectonic maps of the human cortex[19]. The time between death and fixation of the tissue was less than ten hours. All analyzed subjects died without pre-agony state, so the post-mortem delay actually represented the interval for neuron death. The classical chrome-osmium rapid Golgi and Golgi Cox methods were used. The blocks of cortical tissue were immediately immersed in rapid Golgi (0.3% osmium tetroxide and 3% potassium dichromate) and Golgi Cox solutions (0.17% potassium

chromate, 0.2% potassium dichromate and 0.2% mercuric chloride) and kept in the dark. In the rapid Golgi procedure the dichromate solution was replaced after 7 days by 1% silver nitrate for the additional 2 days. Then tissue blocks were dehydrated and embedded rapidly in 8% celloidin. After embedding, a microtome was used to serially section the blocks into coronal sections (180–200 µm thick) which were dehydrated in alcohol, cleared in Histoclear (National Diagnostic) and cover-slipped with Histomount (National Diagnostic) mounting media. In the GolgiCox procedure the Cox solution was refreshed after 24 hours, but the fixation and immersion continued for 3 weeks, after which the tissue was dehydrated, embedded in celloidin and serially cut in coronal sections (180–200 µm thick). For developing of staining, sections were immersed in 20% ammonium hydroxide for 5 minutes and than transferred in 15% solution for 25 minutes. After rinsing they were further processed through 1% thiosulfate for 7 minutes, dehydrated in alcohol, cleared in Histoclear (National Diagnostic) and cover-slipped with Histomount (National Diagnostic) mounting media. Staining artifacts due to postmortem delay, described by Williams et al.[49] and De Ruiter and Uylings[50] were not detected in the cases that were studied quantitatively.

For immunocytochemical purposes, blocks of tissue (1 cm^3) were obtained from the same cortical regions (tissue adjacent to that taken for Golgi method) and fixed in the solution of 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS), pH 7.4, at room temperature during 6 hours. After rinsing in PBS during 1 hour, blocks were immersed in cryo-protective solution (20% sucrose in PBS) overnight at 4 °C and afterward frozen at –80 °C. Prior to immunocytochemical staining, sections of 60 µm thickness were gained cutting blocks on a cryostat in a coronal plane. Because of short postmortem period prior to fixation, and short period of fixation we did not found necessarily to apply antigen retrieval method[51,52]. Sections were pre-treated in 0.3% hydrogen peroxide in the mixture of methanol and bidistillated water (3:1) during 20 minutes and afterward washed (3×10 min in PBS) and incubated with blocking solution (PBS containing 3% BSA and 0.5% Triton X-100, all from Sigma, St. Louis, MO, USA) during 2 hours at the room temperature, to prevent non-specific staining. The sections were than incubated at 4 °C for 18 hours with the monoclonal anti nonphosphorylated-neurofilament H, SMI-32 (Stenberger Monoclonals, Inc., Lutherville, MD, USA), washed again and incubated for 1 hour at the room temperature with secondary biotinilated anti-mouse in blocking solution (1: 200) according to the manufacturer's protocol (Vectastain ABC kit, Vector Laboratories, Burlingame, USA). Following another washing, sections were incubated in Vectastain ABC reagent (streptavidin-peroxidase complex) 1 hour at room temperature, washed, and finally visualization of peroxidase activity was developed with Ni-3,3-diaminobenzidine (Sigma, St. Louis, MO, USA). The sections were washed in PBS, air-dried, dehydrated in a graded series of alcohol, cleared in xylene and cover- slipped with Histomount (National Diagnostic).

From each subject and per each Golgi method modification, five largest and best impregnated layer IIIc pyramidal neurons were chosen from single area (120 neurons in total), three-dimensionally reconstructed, and than quantitatively analyzed using specific computer-microscopic system Neurolucida 3.0[53,54]. The following variables were analyzed[55] (Figure 1): 1) soma cell surface,

i.e., the area of the cell soma projected onto the plane of sectioning to indicate its size (results of statistical analysis were presented in the table 1); from basal dendritic tree we analyzed per neuron: 2) number of basal dendrites (results of statistical analysis were presented in the table 1); 3) total number of basal segments, indicating the branching frequency, mean topological complex-
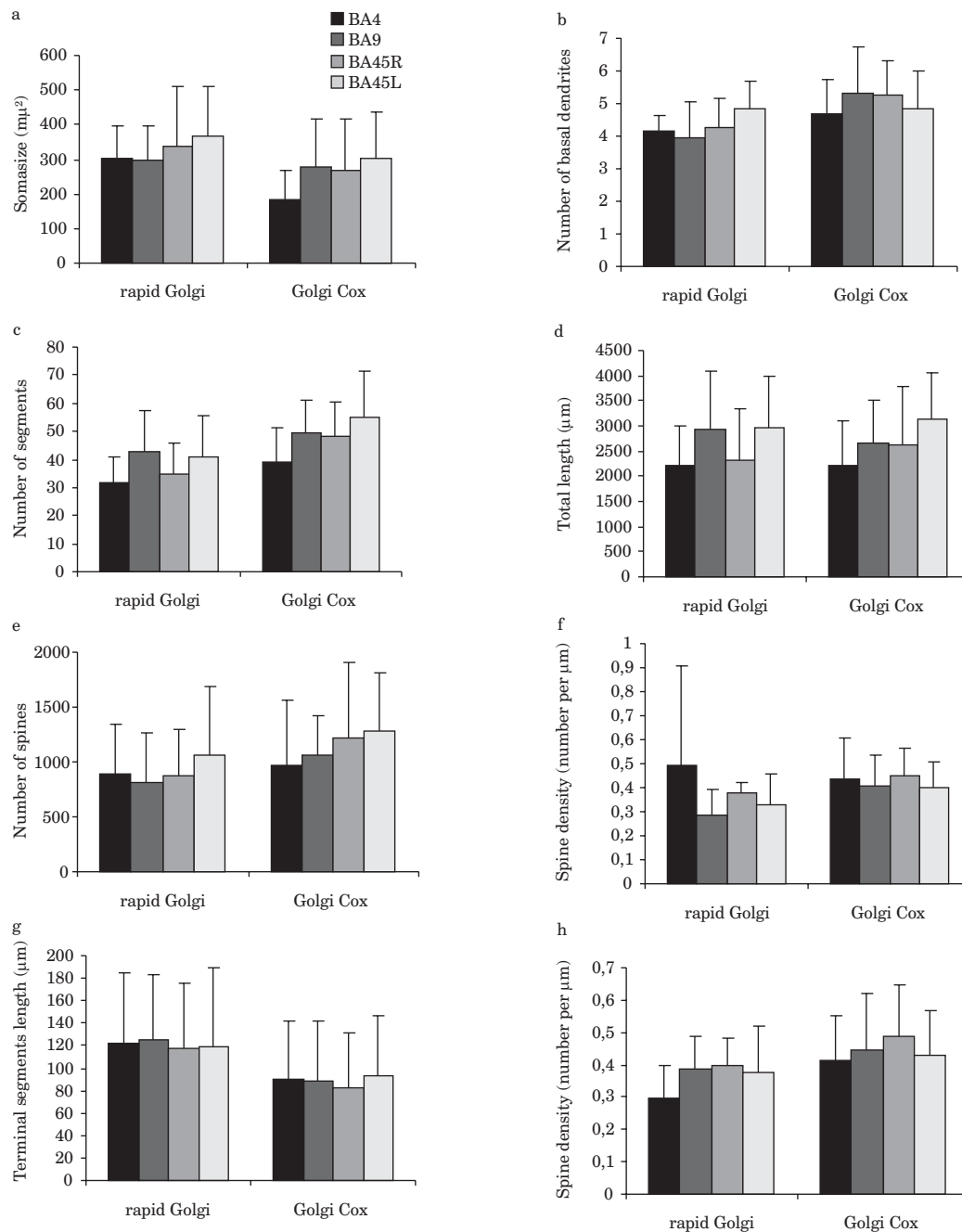

Fig. 1. *Quantitative data of layer IIIc basal dendrites of magnopyramidal neurons in human cortex (Brodman areas, BA) did not show significant regional difference for: a) soma size (mm2), b) number of basal dendrites per neuron, c) number of segments of basal dendritic tree per neuron, (d) total dendritic length per neuron (mm), e) number of dendritic spines per neuron on basal dendritic tree, f) total spine density per mm on basal dendritic tree, g) length of individual terminal segments (mm) on basal dendritic tree, h) spine density on terminal segments per mm on basal dendritic tree.*

ity (results of statistical analysis were presented in the table 2); 4) total basal dendritic length, including the length of individual incomplete segments (results of statistical analysis were presented in the table 2); 5) total number of dendritic spines (results of statistical analysis were presented in the table 3); 6) spine density (number of spines per μm) on basal dendritic tree as a whole (results of statistical analysis were presented in the table 3); 7) length of individual terminal segments (results of statistical analysis were presented in the table 4); 8) spine density on terminal segments (results of statistical analysis were presented in the table 4).

Incomplete segments (segments that are impossible to trace completely, since they were cut at the surface of the section or run into a precipitation) were excluded from the analysis of individual segment length. The actual values were not corrected for tissue shrinkage.

Statistical analysis. We applied the SPSS package for statistical analysis. The dendritic variables were tested separately with the one-way analysis of variance with region and method as a main effect. The a-posteriori Stu-dent-Newman-Keuls (SNK) test for multiple comparisons was applied to determine which parameters were significantly different. A p-level lower than 0.05 was considered as significant (Tables 1–4).

Results

Golgi analysis: qualitative observations

Qualitative observations are restricted to individual morphology of large pyramidal neurons located in the deep part of layer III (layer IIIc). The most characteristic feature is orientation of basal dendrites, where the majority of segments are running about perpendicular to pial surface in non-impregnated layer IV (Figure 2). The large layer IIIc neurons were the largest supragranular neurons regarding the size of soma and basal dendritic tree. However, in the primary motor cortex, the large pyramidal neurons of layer V were bigger than largest layer III neurons. The apical dendrite starts from the tip of the cell body, with side branches (between 5 to 10 dendrites) in their proximal part. When it was possible to follow, the

TABLE 1
ONE-WAY ANALYSIS OF VARIANCE (P-VALUES OF SNK TEST) FOR SOMATIC CELL SURFACE SIZE (LEFT-DOWN) AND NUMBER OF BASAL DENDRITES (RIGHT-UP)

somatic cell surface size ↓	C-A45L	R-A45R	C-A45R	R-A9	C-A9	R-A4	C-A4	
	1.00	0.25	0.53	0.10	0.60	0.21	0.59	R-A45L
C-A45L	0.39		0.38	0.29	0.13	0.43	0.29	0.85	C-A45L
R-A45R	0.56	0.46		0.06	0.65	0.06	0.72	0.29	R-A45R
C-A45R	0.39	0.95	0.69		0.009*	0.86	0.03	0.38	C-A45R
R-A9	0.57	0.98	0.80	0.85		0.006*	0.59	0.21	R-A9
C-A9	0.44	0.95	0.72	0.84	0.73		0.02*	0.39	C-A9
R-A4	0.55	1.00	0.74	0.89	0.87	0.86		0.33	R-A4
C-A4	0.004*	0.11	0.02*	0.07	0.08	0.10	0.08	basal dendrite number
	R-A45L	C-A45L	R-A45R	C-A45R	R-A9	C-A9	R-A4	

C – cox, R – rapid, * significantly different

TABLE 2
ONE-WAY ANALYSIS OF VARIANCE (P-VALUES OF SNK TEST) FOR TOTAL NUMBER OF BASAL SEGMENTS (LEFT-DOWN) AND TOTAL BASAL DENDRITIC LENGTH (RIGHT-UP)

total segment number ↓	C-A45L	R-A45R	C-A45R	R-A9	C-A9	R-A4	C-A4	
	0.66	0.35	0.75	0.93	0.68	0.32	0.36	R-A45L
C-A45L	0.02*		0.21	0.61	0.86	0.58	0.18	0.19	C-A45L
R-A45R	0.39	0.0007*		0.40	0.30	0.57	0.84	0.97	R-A45R
C-A45R	0.28	0.31	0.04*		0.63	0.87	0.55	0.69	C-A45R
R-A9	0.68	0.05	0.31	0.26		0.45	0.29	0.34	R-A9
C-A9	0.26	0.25	0.02*	0.76	0.33		0.62	0.72	C-A9
R-A4	0.21	0.0001*	0.52	0.008*	0.13	0.004*		0.96	R-A4
C-A4	0.74	0.01*	0.33	0.25	0.74	0.19	0.24	total basal dendritic length
	R-A45L	C-A45L	R-A45R	C-A45R	R-A9	C-A9	R-A4	

C – cox, R – rapid, * significantly different

TABLE 3
ONE-WAY ANALYSIS OF VARIANCE (P-VALUES OF SNK TEST) FOR TOTAL NUMBER (LEFT-DOWN) AND DENSITY IN BASAL DENDRITIC TREE (RIGHT-UP)

total number of spines ↓	C-A45L	R-A45R	C-A45R	R-A9	C-A9	R-A4	C-A4	
	0.49	0.47	0.49	0.58	0.63	0.21	0.53	*R-A45L*
C-A45L 0.49		0.68	0.92	0.34	0.95	0.71	0.91	*C-A45L*
R-A45R 0.87	0.36		0.83	0.42	0.88	0.54	0.83	*R-A45R*
C-A45R 0.39	0.78	0.45		0.24	0.84	0.53	0.83	*C-A45R*
R-A9 0.82	0.26	0.78	0.35		0.41	0.07	0.29	*R-A9*
C-A9 1.00	0.66	0.78	0.66	0.73		0.62	0.72	*C-A9*
R-A4 0.81	0.33	0.95	0.40	0.93	0.65		0.67	*R-A4*
C-A4 0.90	0.51	0.86	0.56	0.85	0.66	0.66	*spine density in basal dendrites*	
	R-A45L	*C-A45L*	*R-A45R*	*C-A45R*	*R-A9*	*C-A9*	*R-A4*	

C – cox, R – rapid

TABLE 4
ONE-WAY ANALYSIS OF VARIANCE (P-VALUES OF SNK TEST) FOR LENGTH OF INDIVIDUAL TERMINAL SEGMENTS (LEFT-DOWN) AND SPINE DENSITY ON THEM (RIGHT-UP)

individual length of terminal segment ↓	C-A45L	R-A45R	C-A45R	R-A9	C-A9	R-A4	C-A4	
	0.0001*	0.12	0.00003*	0.33	0.00002*	0.00001*	0.007*	*R-A45L*
C-A45L 0.00002*		0.03*	0.00004*	0.002*	0.35	0.00002*	0.21	*C-A45L*
R-A45R 0.85	0.00001*		0.00002*	0.32	0.003*	0.00001*	0.22	*R-A45R*
C-A45R 0.00002*	0.20	0.00002*		0.00002*	0.0003*	0.00003*	0.00001*	*C-A45R*
R-A9 0.54	0.00002*	0.59	0.00003*		0.0001*	0.00002*	0.07	*R-A9*
C-A9 0.00002*	0.76	0.00001*	0.21	0.00003*		0.00003*	0.07	*C-A9*
R-A4 0.57	0.00001*	0.73	0.00003*	0.62	0.00002*		0.00002*	*R-A4*
C-A4 0.00001*	0.67	0.00002*	0.27	0.00002*	0.78	0.00002*	*spine density on terminal segment*	
	R-A45L	*C-A45L*	*R-A45R*	*C-A45R*	*R-A9*	*C-A9*	*R-A4*	

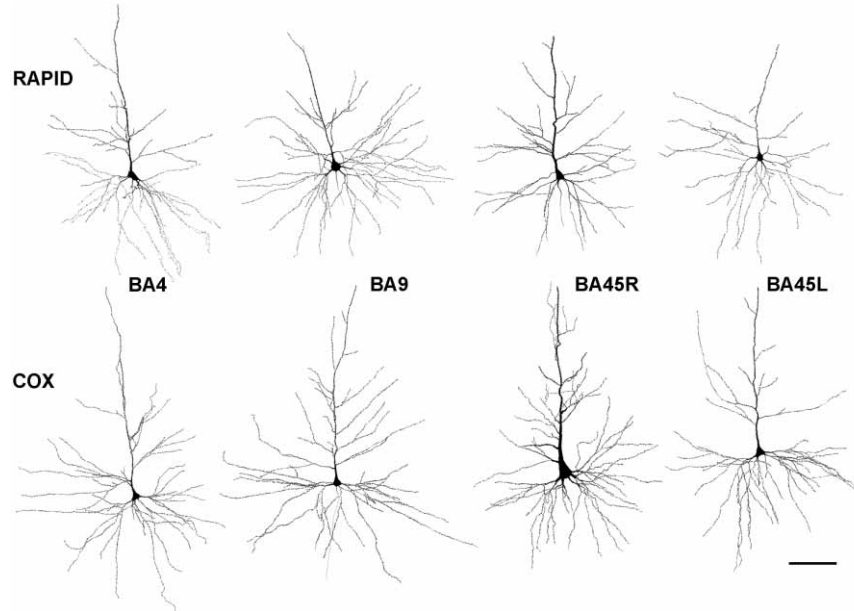C – cox, R – rapid, * significantly different

Fig. 2. Three-dimensional reconstructions of basal and apical dendritic trees of rapid Golgi (upper row) and Golgi Cox (lower row) impregnated layer IIIc pyramidal cells projected onto the coronal plane. All pyramidal cells are represented at the same magnification (bar scale = 100 μm) and are from the following regions (from left to right): motor cortex (left BA4), prefrontal cortex (left BA9), right (R) and left (L) Broca region (BA45).

main shaft of apical dendrite ends in layer I and II, ramifying in several thinner branches (terminal tuft).

Dendrites were covered by dendritic spines that were mostly mushroom-form. These spines differ in the length of stalk and size of bulb. Dendritic spines were not observed at the cell body, and also not on the most proximal part of apical dendrite and primary basal dendrites. The highest spine density was in the middle of the apical dendrite. On basal and on oblique dendrites, we were not able to observe qualitatively any gradient in dendritic spine density. The axon originates from the base of the cell body towards the white matter. However, in most cases, the axon was impregnated not more than $10–20\,\mu m$ in length. We were not able to observe qualitatively any regional differences in dendritic size and complexity, or the spine density and soma size between pyramidal neurons. However, we found that Golgi Cox method tend to show more complex basal dendritic tree than rapid Golgi method. This tendency was found in all analyzed areas.

Golgi analysis of basal dendrites: quantitative data

Statistical analysis of morphological parameters of basal dendritic tree did not show consistent differences in morphology of the dendritic tree, or in the spine number and spine density between different cortical areas (including left-right comparison of Broca's BA45) comparing separately neurons impregnated by different Golgi method modification (Figure 1).

However, statistical analysis showed significant differences in some parameters between neurons impregnated with different methods (rapid Golgi versus Golgi Cox), and that was consistent for all regions (Table 1–4). The following parameters differ: total number of segments in basal dendritic tree (regional variation were between 31–42 per neuron on rapid Golgi versus 38–54 on Golgi Cox impregnated neurons) and mean length of individual terminal segments (regional variation were between $119–125\,\mu m$ on rapid Golgi versus $83–90\,\mu m$ on Golgi Cox impregnated neurons). The spine density on terminal basal segments tends to be slightly lower in rapid Golgi than in Golgi Cox slices (regional variation were between 0.31–0.39 per μm length versus 0.41–0.48 per μm length), but without any significance. The mean size of cell body surface was larger on rapid Golgi than on Golgi Cox impregnated neurons (regional variations were between 290–370 μm^2 versus 190–300 μm^2), but this was not found to be statistically significant. All
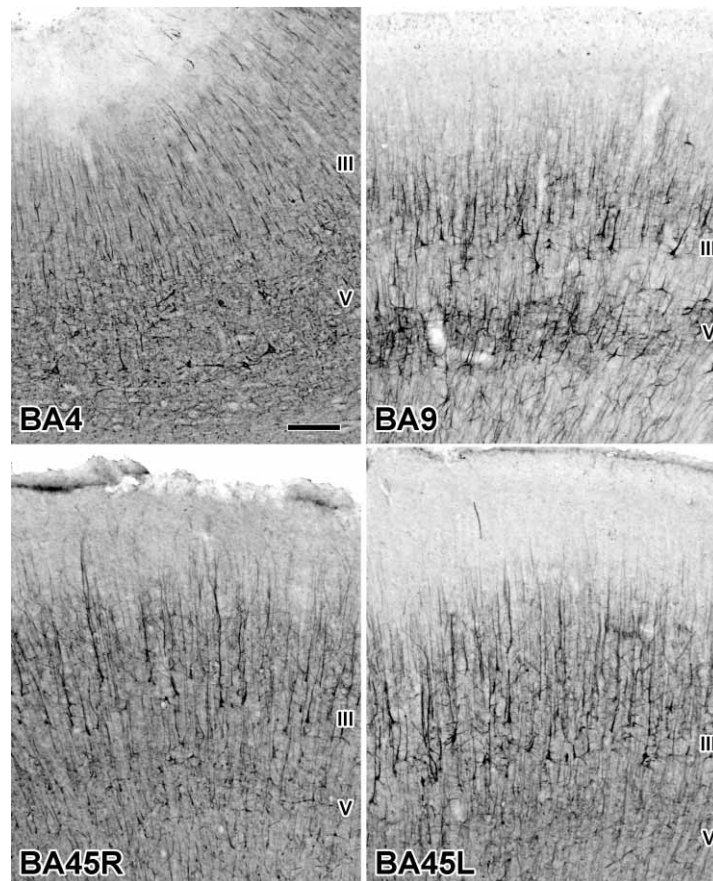

Fig. 3. Low power microphotography of the motor cortex (left BA4), dorsolateral prefrontal cortex (left BA9), right (R) and left (L) Broca's region (BA45) of sections immunocytochemically labeled with SMI-32 antibody at the same magnification (bar scale = 200 μm). III – layer IIIc, V – layer V.
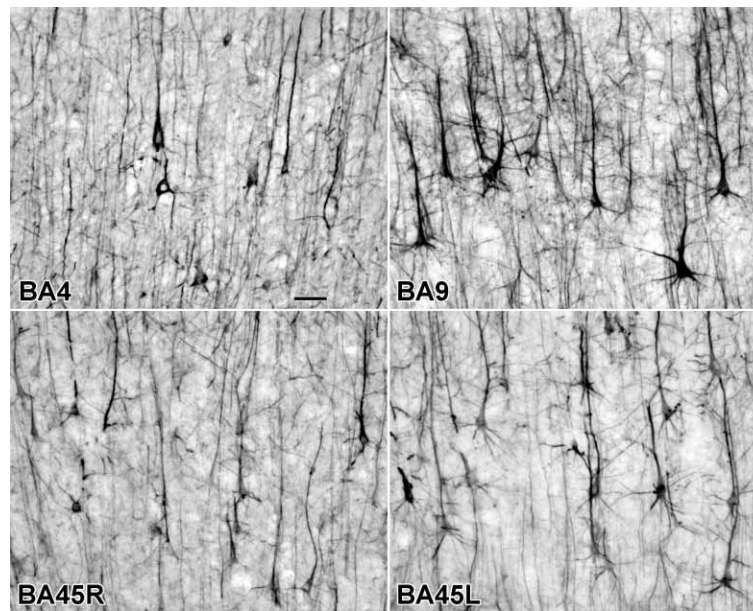
Fig. 4. High power microphotography in deep of layer III (layer IIIc) immunocytochemicaly labeled with SMI-32 antibody showed layer IIIC pyramidal cells of the motor cortex (left BA4), dorsolateral prefrontal cortex (left BA9), right (R) and left (L) Broca's region (BA45) at the same magnification (bar scale = 50 μm).

above mentioned data correlate with larger tissue shrinkage occurring in Golgi Cox stained tissue[50], so that larger part of dendritic tree will be covered in one slice of the same thickness. In addition, the best impregnated Golgi Cox neurons were in the middle of the sections, whereas in the rapid Golgi impregnations best impregnated neurons tend to be located more to the surface of the sections, so the number of segments which run out of the individual slices was higher in rapid Golgi method.

For other dendritic variables there was no statistically significant difference, neither tendency for differences between neurons impregnated by different modification of Golgi method. Mean number of primary basal dendrites per neuron was around 4–5 and total basal dendritic length varies between 2200–3100 μm. The spine density in the whole basal dendritic tree was for most of the regions between 0.33–0.41 per μm of dendritic length (for rapid Golgi impregnated neurons in the dorsolateral prefrontal BA9 was 0.29 and for motor BA4 was 0.51). Comparing individual region, the total spine number in basal dendritic tree was higher on Golgi Cox (980–1240 spines) than on rapid Golgi impregnated neurons (810–1040 spines), but without any significance.

Analysis of SMI-32 -immunocytochemicaly stained sections

Present literature data showed that in the human and monkey neocortex labeling with monoclonal antibody to nonphosphorylated neurofilament protein H (SMI-32) is found in subpopulation of pyramidal neurons with long projecting axon[56]. Previous data have demonstrated that the long association cortico-cortical pathways have a high representation of SMI-32-enriched pyramidal neu-

rons, whereas short cortico-cortical pathways are characterized by much lower numbers of such neurons[23]. That level of staining in individual neuron is proportional with its axon length, in supragranular layers the highest staining is present in largest neurons[57]. Therefore, we performed this analysis to compare qualitatively density between different BA.

Pyramidal neurons with heavy labeled cell body, partly apical dendrites and proximal basal dendrites, were found in the deep layer III (layer IIIc) in all examined BA (Figure 3). However, it was clear that the density of heavy stained cells was much lower in the primary motor region (BA4) comparing to other regions (Figure 4). Most of the heavy stained cells in the primary motor region were the largest layer III cells, but some small intensively label pyramids could also be seen. The characteristic of the BA4 was that SMI-32-staining was predominantly in infragranular layers (principally layer V) and less in supragranular layers (principally layer IIIc). The SMI-32-stained structures in layer III of primary motor cortex were mostly apical dendrites of infragranular pyramidal neurons running to the pial surface.

In other analyzed regions, staining was predominantly found in deep part of layer III. The highest density of heavy stained cells was found in BA9. The impression is that not only density of stained neurons, but that amount of dendritic tree stained per single neuron is higher and stronger in layer IIIc pyramids of BA9. The characteristic of the BA9 was that, in addition to largest layer IIIc neurons, some medium sized neurons were heavy stained too. In the Broca's area (BA45) distribution of the stained cells and the individual morphology was very reg-

ular. In BA9 and BA45 numerous faintly stained pyramidal cells could also be observed.

In summary, qualitative analysis of the SMI-32-stained section showed the most numerousness and most intensive staining of the neurons in BA9, a medium intensive staining of neurons in BA45, with impression of slightly higher staining in left than in right hemisphere, while neurons in primary motor BA4 were only sparsely stained (Figure 4).

Discussion

The results of this study indicate an absence of consistent differences in the dendritic complexity and spine number in large layer IIIc pyramidal neurons, among functionally and hierarchically different cortical areas in the frontal lobe. These findings were surprising, having in mind other comparative studies obtained in monkey[10] and human brain[15]. These studies showed increase in dendritic complexity and spine number of pyramidal neurons in higher ordered associative areas versus primary and unimodal sensory region. This dendritic progression was supposed to reflect significant differences in the nature of cortical processing, with spine-dense neurons at hierarchically higher association levels integrating a broader range of synaptic input than those at lower cortical levels. However, our results are in line with data of Hayes and Lewis[36], where no differences were observed in dendritic morphology of magnopyramidal neurons between left and right BA45.

Is it possible to make an integrative framework to pool results obtained in this study with opposing findings obtained in other studies? Larger cortex, as a result of higher number of neurons, will produce a broader range of connections, but to achieve demanding cognition it is crucial to establish proper integration inside numerically expanded circuitries. Comparative studies of pyramidal neuron morphology evolution[1,6,58] produced a widely accepted opinion that this is mainly due to increase in dendritic complexity and spine density at the level of individual neurons, where the most complex cells will attain the most extensive integration. We suggest additional point to this theory, supposing that at a certain level, further increase in dendritic complexity and synaptic density of most advanced neurons will not be of any functional importance, because integrative capacity of individual neuron will be exceeded.

So, if there is no possibility to increase integrative capacity of most complex neurons, and if further increase in neuron number and consequently exponential increase in number of connections occurred, how will it be possible to obtain increased need for integrative properties of newly expanded neuronal network? We suggest

that in this moment better integration that is needed for further functional complexness will be achieved by increase in ratio of morphologically most complex neurons. The same paradigm could be translated comparing the cortico-cortical network, that increased exponentionaly from primary to higher order associative areas of frontal lobe[6,11–14]. Absence of increase in morphological complexness of individual neuron that we have quantitatively observed on Golgi slices in parallel with increase in percentage (ratio) of most complex, associative large layer IIIc cells inside supragranular layers that was qualitative observed by SMI-32-immunocytochemistry, support our hypothesis. Herewith we have to mention that data supporting our hypothesis about increase of integrative capacity with expansion of cortico-cortical network, are at this moment very limited, and therefore highly speculative. However, in line with our hypothesis are the data showing evolutionary increase in diversity of non-pyramidal cells types in prefrontal cortex of primates, including humans[1,10,27,59].

It appears that large difference in metric results between the rapid Golgi staining and the Golgi Cox staining method exist. Flood et al.[60] and De Ruiter and Uylings[50] noticed that the dendritic extent in the Golgi Cox stained 200 μm sections was only 62% of the dendritic extent in the rapid Golgi stained 110 μm sections. They concluded that this difference might be attributed to a more substantial shrinkage of tissue stained with Golgi Cox. Our data are in accordance with this assumption, where on the sections of the same thickness, Golgi Cox neurons tend to show more, but shorter segments.

The morphology, density and number of large layer IIIc pyramidal cells in human magnopyramidal areas display the greatest interindividual variability[10,15,19,22,61]. It might be that interindividual differences in size and internal composition of cortico-cortical circuitries inside associative cortical areas are strongly involved in the biological basis of individuality and also connected with the cognitive ability of a single person[10,61]. There is a great possibility that interaction between psycho-social environments during brain development results in a large interindividual differences in brain structure observed later in adult human. Therefore, in the future studies, a larger number of analyzed subjects should be considered, whereas obtained morphological data should be correlated with psychological, educational and cognitive status of the individual person.

Acknowledgement

REFERENCES

1. UYLINGS HB, Eur J Morphol, 38 (2000) 309. — 2. KAAS JH, J Biosci, 30 (2005) 155. — 3. KOSTOVIĆ I, JUDAŠ M, PETANJEK Z, ŠIMIĆ G, Int J Psychophysiol, 19 (1995) 85. — 4. PANDYA DN, YETERIAN EH, Philos Trans R Soc Lond B Biol Sci, 351 (1996) 1423. — 5. KOSTOVIĆ I, PETANJEK Z, DELLALE I, JUDAŠ M, Developmental reorganization of the human association cortex during perinatal and postnatal

life. In: KOSTOVIĆ I, KNEŽEVIĆ S, WIESNIEWSKI HM, SPILICH GJ (Eds), Neurodevelopment, Aging and Cognition (Birkhäuser, 1992). — 6. UYLINGS HB, VAN EDEN CG, Prog Brain Res, 85 (1990) 31. — 7. SEMENDEFERI K, LU A, SCHENKER N, DAMASIO H, Nat Neurosci, 5 (2002) 272. — 8. UYLINGS HB, GROENEWEGEN HJ, KOLB B, Behav Brain Res, 146 (2003) 3. — 9. GROENEWEGEN HJ, UYLINGS HB, Prog Brain Res, 126 (2000) 3. — 10. ELSTON GN, BENAVIDES-PICCIONE R, ELSTON A, ZIETSCH B, DEFELIPE J, MANGER P, CASAGRANDE V, KAAS JH, Anat Rec A Discov Mol Cell Evol Biol, 288 (2006) 26. — 11. PETRIDES M, Philos Trans R Soc Lond B Biol Sci, 360 (2005) 781. — 12. JOVANOV-MILOŠEVIĆ N, BENJAK V, KOSTOVIĆ I, Coll Antropol, 30 (2006) 375. — 13. KOSTOVIĆ I, JUDAŠ M, Dev Med Child Neurol, 48 (2006) 388. — 14. RADOŠ M, JUDAŠ M, KOSTOVIĆ I, Eur J Radiol, 57 (2006) 187. — 15. JACOBS B, SCHALL M, PRATHER M, KAPLER E, DRISCOLL L, BACA S, JACOBS J, FORD K, WAINWRIGHT M, TREML M, Cereb Cortex, 11 (2001) 558. — 16. MRZLJAK L, GOLDMAN-RAKIC PS, J Comp Neurol, 324 (1992) 261. — 17. BENAVIDES-PICCIONE R, BALLESTEROS-YANEZ I, DEFELIPE J, YUSTE R, J Neurocytol, 31 (2002) 337. — 18. KOSTOVIĆ I, ŠKAVIĆ J, STRINOVIĆ D, Neurosci Lett, 90 (1988) 107. — 19. RAJKOWSKA G, GOLDMAN-RAKIC PS, Cereb Cortex, 5 (1995) 323. — 20. UYLINGS HB, JACOBSEN AM, ZILLES K, AMUNTS K, Cortex, 42 (2006) 652. — 21. AMUNTS K, SCHLEICHER A, BURGEL U, MOHLBERG H, UYLINGS HB, ZILLES K, J Comp Neurol, 412 (1999) 319. — 22. UYLINGS HB, RAJKOWSKA G, SANZ-ARIGITA E, AMUNTS K, ZILLES K, Anat Embryol (Berl), 210 (2005) 423. — 23. HOF PR, NIMCHINSKY EA, MORRISON JH, J Comp Neurol, 362 (1995) 109. — 24. AMUNTS K, SCHLEICHER A, DITTERICH A, ZILLES K, J Comp Neurol, 465 (2003) 72. — 25. JUDAŠ M, Perinatal cytoarchitectonic development of the motor speech region in the human frontal region, MS Thesis, in Croatian, (University of Zagreb, Zagreb, 1987). — 26. KOSTOVIĆ I, Prog Brain Res, 85 (1990) 223. — 27. MRZLJAK L, UYLINGS HB, VAN EDEN CG, JUDAŠ M, Prog Brain Res, 85 (1990) 185. — 28. PETANJEK Z, JUDAŠ M, KOSTOVIĆ I, UYLINGS HB, Cereb Cortex, (2007) — 29. BARBAS H, Brain Res Bull, 52 (2000) 319. — 30. WANG Y, MARKRAM H, GOODMAN PH, BERGER TK, MA J, GOLDMAN-RAKIC PS, Nat Neurosci, 9 (2006) 534. — 31. GOLDMAN-RAKIC PS, Proc Natl Acad Sci U S A, 93 (1996) 13473. — 32. MORRISON JH, HOF PR, Prog Brain Res, 136 (2002) 467. — 33. SELEMON LD, MRZLJAK J, KLEINMAN JE, HERMAN MM, GOLDMAN-RAKIC PS, Arch Gen Psychiatry, 60 (2003) 69. — 34. UYLINGS HB, DE BRA-BANDER JM, Brain Cogn, 49 (2002) 268. — 35. DEFELIPE J, JONES EG, Cajal on the Cerebral Cortex, An Annotated Translation of the Complete Writings (Oxford University Press, New York – Oxford, 1988). — 36. HAYES TL, LEWIS DA, Arch Neurol, 53 (1996) 1277. — 37. DUAN H, WEARNE SL, MORRISON JH, HOF PR, Neuroscience, 114 (2002) 349. — 38. SOLOWAY AS, PUCAK ML, MELCHITZKY DS, LEWIS DA, Neuroscience, 109 (2002) 461. — 39. PETANJEK Z, RAŠIN MR, DARMOPIL S, JOVANOV N, IVKIĆ G, Period Biol, 102 (2000) 411. — 40. PETANJEK Z, RAŠIN MR, JOVANOV N, KRSNIK Ž, Period Biol, 100 (1998) 221. — 41. VUKŠIĆ M, PETANJEK Z, RAŠIN MR, KOSTOVIĆ I, Pediatr Neurol, 27 (2002) 36. — 42. KOENDERINK MJ, UYLINGS HB, Brain Res, 678 (1995) 233. — 43. KOENDERINK MJ, UYLINGS HB, J Neurosci Methods, 64 (1996) 115. — 44. KOENDERINK MJ, UYLINGS HB, MRZLJAK L, Brain Res, 653 (1994) 173. — 45. PETANJEK Z, ŠIMIĆ G, JUDAŠ M, RADONIĆ E, KOSTOVIĆ I, Eur J Neurosci, suppl. No. 7 (1994) 49. — 46. PETANJEK Z, UYLINGS HBM, KOSTOVIĆ I, Eur J Neurosci, Suppl. 8 (1995) 187. — 47. UYLINGS HBM, PETANJEK Z, KOSTOVIĆ I, Postnatal development of layer IIIc pyramidal neurons in the human prefrontal cortex: a rapid Golgi study. In: Abstract Book vol. 21(3) p. 2021. (25th Society for Neuroscience Meeting, San Diego, 1995). — 48. KOSTOVIĆ I, JUDAŠ M, KOSTOVIĆ-KNEŽEVIĆ L, ŠIMIĆ G, DELALLE I, CHUDY D, SAJIN B, PETANJEK Z, Int J Dev Biol, 35 (1991) 215. — 49. WILLIAMS RS, FERRANTE RJ, CAVINESS VS, JR., J Neuropathol Exp Neurol, 37 (1978) 13. — 50. DE RUITER JP, UYLINGS HB, Brain Res, 402 (1987) 217. — 51. EVERS P, UYLINGS HB, J Neurosci Methods, 72 (1997) 197. — 52. EVERS P, UYLINGS HBM, Microwave-stimulated antigen retrieval in Neuroscience. In: TAYLOR CR, SHI SR (Eds), Antigen Retrieval Techniques: a revolutionary approach to routine immunohistochemistry (Eaton Publ, Natick, MA, USA, 2000). — 53. GROC L, PETANJEK Z, GUSTAFSSON B, BEN-ARI Y, HANSE E, KHAZIPOV R, Eur J Neurosci, 16 (2002) 1931. — 54. GROC L, PETANJEK Z, GUSTAFSSON B, BEN-ARI Y, KHAZIPOV R, HANSE E, Eur J Neurosci, 18 (2003) 1332. — 55. UYLINGS HB, VAN PELT J, Network, 13 (2002) 397. — 56. CAMPBELL MJ, HOF PR, MORRISON JH, Brain Res, 539 (1991) 133. — 57. MESULAM M, GEULA C, Brain Res, 544 (1991) 169. — 58. ELSTON GN, BENAVIDES-PICCIONE R, DEFELIPE J, J Neurosci, 21 (2001) RC163. — 59. LUND JS, LEWIS DA, J Comp Neurol, 328 (1993) 282. — 60. FLOOD DG, BUELL SJ, HORWITZ GJ, COLEMAN PD, Brain Res, 402 (1987) 205. — 61. JACOBS B, SCHALL M, SCHEIBEL AB, J Comp Neurol, 327 (1993) 97.

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KVANTITATIVNA ANALIZA BAZALNOG DENDRITIČKOG STABLA PIRAMIDNIH NEURONA SLOJA IIIc U RAZLIČITIMA AREJAMA FRONTALNOG REŽNJA MOZGA ODRASLA ČOVJEKA

S A Ž E T A K

Istraživanja mozga u primata ukazala su na dominantnu ulogu kortiko-kortikalnih piramidnih neurona IIIc sloja u neurobiologiji spoznajnih funkcija. U ovom radu prikazujemo rezultate usporedbene kvantitativne analize morfologije dendritičkog stabla najvećih neurona sloja IIIc impregniranih Golgi metodom, između tri različite Brodmannove areje (BA) frontalnog režnja mozga odraslog čovjeka: primarna motorna BA4, asocijativna magnopiramidalna BA9, te obostrano, govorna Brocina BA45. Statističkom analizom nismo pokazali značajne razlike u morfologiji dendritičnog stabla te broju i gustoći dendritičkih trnova između neurona različitih areja. Kvalitativna analiza laminarne distribucije i morfologije neurona označenih sa SMI-32 protutijelom, koje boji velike neurone sa dugim projekcijama, pokazala je da se u sloju III najveći broj SMI-32-imuno-reaktivnih neurona, kao i intenzitet reaktivnosti pojedinačnih neurona, nalazi u asocijativnom BA9, dok su u primarnom BA4 obojani samo poneki neuroni. Ovi podaci pokazuju da do povećanja složenosti kortiko-kortikalne mreže u funkcionalno različitim frontalnim regijama mozga dolazi prvenstveno povećanjem broja i gustoće velikih projekcijskih (kortiko-kortikalnih) neurona, a ne povećanjem njihove dendritičke i sinaptičke složenosti.