The classical cytoarchitectonic maps of human prefrontal areas produced by various cartographers in the early part of this century, though similar in gross topography, differ from one another in their descriptions of the size, shape, and precise location of specific regions within the frontal promontory. The current advances in human neurobiology stimulated us to re-investigate the cytoarchitecture of the human prefrontal cortex, beginning with areas 9 and 46, to establish a set of objective cytoarchitectonic criteria for identification of these areas. Nissl-stained and Gallyas-stained cellloidin-embedded sections were prepared from the left hemispheres of 17 human subjects 23–73 years old, without history of neurological disease. In eight cases, light microscopic observations were supplemented by morphometric data collected on a research microscope equipped with differential interference contrast optics and interfaced to a TV monitor with video mixing equipment and a microcomputer. We used the three-dimensional counting method of Williams and Rakic (1988) to measure (1) total cortical and relative laminar thickness, (2) neuronal packing density per 0.001 mm² in individual cortical layers, and (3) sizes of neuronal somata in selected cortical layers.

Light microscopic analysis confirmed that the cortical layers are more differentiated in area 46 than in area 9, particularly at the borders of layer IV. Layers III and V exhibit clearer sublamination in area 9, while layer IV is also somewhat wider in area 46 than in area 9 (9.3% vs 8.4% of cortical thickness); the overall thickness of the cortex is the same in both areas. Cytometric analysis revealed that layer IV neurons of area 46 are more densely packed than those in area 9 (55.38 ± 7.26 vs 45.80 ± 4.45 neurons/0.001 mm²), as are neurons in the supragranular layers II and III combined (53.51 ± 6.33 vs 45.69 ± 3.81 neurons/0.001 mm²). Finally, neurons in area 46 are more homogeneous in size than those in area 9. Differences in myeloarchitecture are also evident: each area contains numerous, well-stained radial striae and two pronounced bands of horizontal fibers, but in general, area 46 is less myelinated than area 9.

Objective cytometric methods can clearly distinguish two adjacent areas within the human prefrontal lobe. These findings may prove useful in the areal parcellation of the human cerebral cortex as well as provide a baseline for analysis of pathological changes in neurological and psychiatric disorders such as a schizophrenia, Huntington's or Alzheimer's diseases.

The anatomical parcellation of the prefrontal cortex is one of the challenges of human neuroanatomy. Despite the number of maps of the human cerebral cortex that have been available since the beginning of this century, there is neither a detailed written description nor a clear visual depiction of the prefrontal areas. Existing parcellation of the human cerebral cortex based on cytoarchitecture (Brodmann, 1909; von Economo and Koskinas, 1925; Kononova, 1949, 1955; Bailey and von Bonin, 1951; Sarkissov et al., 1955; Sanides, 1962), myeloarchitecture (Campbell, 1905; Vogt, 1927; Strasburger, 1937; Filimonoff, 1949; Sanides, 1964), and pigmented architecture (Braak, 1979, 1980) show general agreement in the definition and location of some frontal areas, for example, areas 4, 44, and 45. The delineation of other areas, such as areas 10, 9, and 46, differ markedly among the various maps.

These differences among the cytoarchitectonic maps raise questions about the validity of one map versus the other. Neuroscientists have tended to rely almost exclusively on Brodmann's map for localizing prefrontal (and other) areas, even though this map is based, as far as we know, on only one case and is not accompanied either by a description of the criteria used for distinguishing the areas charted or by photographic illustrations. Probably because English translations were not readily available, the cytoarchitectonic maps of von Economo and Koskinas (1925), Sarkissov et al. (1955), and Sanides (1962) have been largely ignored, even though their maps were based on a larger number of cases and defined parameters. Moreover, only a few attempts have been made to compare the various maps of prefrontal areas to determine if one is more useful than the other (Pakkenberg, 1955; Braak, 1980; Zilles, 1990). The probable reason for this is that cytoarchitectonic analysis is tedious and requires uniformly prepared tissue, standard methodology, and availability of human specimens—conditions that are not easily met. Moreover, researchers are understandably uncomfortable with the qualitative criteria most often used for cytoarchitectonic distinctions among cortical regions. Critical comments on the different criteria used by different investigators have been expressed throughout the century and the need for objective quantitative criteria in the analysis of cortical regions has been repeatedly emphasized (von Bonin, 1937; Lashley and Clark, 1946; Pakkenberg, 1955; Braak, 1980; Wiltson et al., 1992).

Thus, a reanalysis of human prefrontal cortex with objective quantitative methods is timely and necessary. Recent powerful imaging methods are now available for localizing functional activity in the living brain and there is more need than ever for reliable anatomical data on the human brain (Crick and Jones, 1993). The availability of an unbiased three-dimensional direct cell counting method coupled with Nissl optics and computer-assisted image analyzer (Williams and Rakic, 1988) has provided a reliable tool for unbiased quantification of cortical morphology in terms of cell density, neuron size, and laminar thickness. In the present study we have used this method to establish a set of cytometric criteria for the identification of areas 9 and 46 within the human prefrontal cortex. Little is known about the morphofunctional organization of these areas in humans, but the structure (Walker, 1940; Goldman-Rakic and Schwartz, 1982; Goldman-Rakic, 1984; Schwartz and Goldman-Rakic, 1988; Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989; McGuire et al., 1991; Preuss and Goldman-Rakic, 1991) and function (Goldman and Rosvold, 1970; Milner and Petrides, 1984; Funahashi et al., 1989; Fuster, 1989; Sawaguchi and Goldman-Rakic, 1991) of homologous areas, particularly area 46, have been well studied in nonhuman primates. In the following companion article (Rajkowska and Goldman-Rakic, 1995) we have used the cytometric criteria reported here to fully reconstruct the size, shape, and position of areas 9 and 46 in five human brains and to compare our maps with those of the classical cartographers. Reliable identification of prefront-
procedure for Nissl staining

Goldman-Rakic (1995) and then fixed in 10% phosphate-buffered formalin for sources: the Yakovlev Collection of the Armed Forces Institute of Neuropathology. The left frontal lobe was obtained at autopsy from 17 human subjects, including six individuals from Zagreb, eight from Boston, and three from New Haven. Four of these subjects had a history of neurological disease, and one had a history of psychiatric illness. The age of the subjects ranged from 23 to 75 years, and the postmortem delay was less than 24 hours. All brains were obtained from four sources: the Yakovlev Collection of the Armed Forces Institute of Pathology (AFIP), Washington, DC; The Brain Tissue Resource Center (BTRC), McLean Hospital, Boston, MA; The Department of Anatomy, University of Zagreb (Croatia); and The Department of Pathology, Yale-New Haven Hospital (YNHH), New Haven, CT. The brains available from the Yakovlev Collection consisted of celloidin-embedded 35-μm-thick serial sections cut through the whole hemisphere in the coronal plane (eight cases), sagittal (one case), or horizontal (one case) planes (see Table 1). For the present study we used only those sections from the frontal lobe. All other brains were processed in our laboratory as described below.

Cellidin Procedure for Nissl Staining

To obtain 35-40 μm Nissl stain sections from areas 9 and 46, the tissue obtained from Zagreb, Boston, and New Haven was dissected into 5 × 4 × 4 cm blocks containing the first two frontal gyri, from levels L4-L7 of Perry’s coronal maps (see Fig. 2 of Rajkowska and Goldman-Rakic, 1995) and then fixed in 10% phosphate-buffered formalin. The blocks were dehydrated initially in graded alcohol (12 d) and then in an EtOH/ether mixture (5 d). Infiltration with cellidin (2%, 4%, 6%, 12% Parlodion, Mallinkrodt) took place over a 4-week period. After hardening the blocks in chloroform vapors (12-14 d) and 70% EtOH for about 1 week, they were cut at 40 μm on a prototype sliding macrotome (LKB/Bromma). Every 10th section was stored in 70% EtOH and then defatted, rehydrated, and stained for 20 min in 0.025% thionin made up in 0.15 M acetate buffer (pH 5.1-5.5).

Gallyas Method for Myelin Staining

In five cases, a series of sections adjacent to Nissl-stained areas were stained for myelin by the Gallyas technique (Gallyas, 1979) modified for cellidin-embedded human tissue. After defatting and rehydrating, the sections were impregnated in 0.2% silver nitrate and 0.2% ammonium nitrate (pH 7.5) solution for 90 min and washed in 0.5% acetic acid. The sections were then placed for 5-20 min in a developing solution consisting of parts A (5% sodium carbonate), B (0.2% silver nitrate, 0.19% ammonium nitrate, and 1% silicotungstic acid), and C (solution B with 0.322% formalin) mixed in a 10:3:7 ratio. Following development, sections were washed in water, differentiated in Kodak E6 differentiator, washed again, and fixed in Kodak Ektalol fixer for 7 min. Dehydration to 95% EtOH and butanol and storage in a desiccator were the final steps before mounting.

Cytoarchitecture

In eight cases, light microscopic observations were supplemented by morphometric data collected on a computerized image processor with three-dimensional counting capability slightly modified for human studies (Williams and Rakic, 1988). The significant advantages of this method over other counting methods are discussed in detail in Williams and Rakic (1988). Briefly, a three-dimensional counting box is defined optically. Within the volume of each section, the "ceiling" and "floor" of a counting box is always smaller than the thickness of the section and a known distance from the cut surfaces of the section. The number of cells in the volume of the box is counted using standard three-dimensional exclusion criteria. Unlike conventional counting procedures, this approach allows cell size, shape, and orientation to be independent of section thickness. Therefore, this method does not require correction for sampling error as do conventional counting methods (Abercrombie, 1946).

Measurements were obtained in an uninterrupted series of counting boxes (50 μm × 70 μm × 25 μm) that spanned the entire depth of cortex from the overlying pial surface to the underlying white matter. Such an array of counting boxes, oriented perpendicular to the pia, will henceforth be referred to as a "probe." It is well recognized that the total thickness of the cortex and the thickness of individual layers vary in accordance with changes in the curvature of the surface of the cortex (Binkofski, 1936; Binkofski, 1959; Binkofski and Glezer, 1968). This is why, in the present study, we paid particular attention to the position of our measurements and took them only from uncurved portions of the cortical surfaces in both areas (Fig. 1). Prior to counting, selected sections were drawn under low magnification using a drawing tube attached to the stereomicroscope. Borders between layers and sublayers, between the cortex and underlying white matter as well as fiducial landmarks, such as fragments of blood vessels or perturbations of the pial surface, were marked on the drawings. These landmarks were used for orientation when counting under high power. All measurements were taken with a 100X oil objective at a final magnification of 2000× from cortical surfaces along the rims or flat crowns of gyri in areas 9 (located primarily on the superior frontal gyrus) and area 46 (located on the middle frontal gyrus, Fig. 1). The following parameters were measured in each area: (1) neuronal packing density that is, the number of neurons per 0.001 mm³; (2) total cortical thickness and relative thickness of each layer; and (3) size of neuronal soma in selected cortical layers.

Absolute values of laminar thickness can be affected by nonuniform shrinkage of tissue during histological processing, differences in postmortem interval, and variations between brains of sex and age. Therefore, we avoided laminar thickness as a percentage of total cortical thickness rather than in absolute terms. Numerical densities were analyzed in two ways. First, we compared densities per layer across areas 9 and 46 within the same brain. Second, comparisons were made for corresponding areas between brains. Neuron soma sizes were estimated by tracing outlines of cell profiles at the level of their equatorial transections and calculating them as the equivalent diameter of a circle that has the same area as the cell equatorial transection ("diameter circle," D-circle). Soma sizes were measured in four brains; in each, 150-200 somas were measured per cortical layer in each area.

Mean values for all parameters were measured from five to eight probes per cortical area taken from different rostrocaudal levels of each area (Fig. 1). All comparisons were evaluated by nonparametric statistical tests (Mann-Whitney U and Kruskal-Wallis).

Data on the density and soma sizes of glia were also collected in the same specimens using the same counting methods. These data are available upon request, but are not described further in the present report.

Results

Area 9

Cytoarchitecture

In all brains examined, area 9 was located on the first frontal gyrus covering both dorso lateral and dorsomedial surfaces of

| Table 1 Description of cases used for present analysis |
|----------------|----------------|---|---|---|---|
| Brain Code | Source | Age | Sex | PMI | Cause of death | Orientation |
| Mu | 160-67 | ARP | 58 | M | 6 | Cardiac arrest | Coronal |
| Mu | 66-64 | ARP | 60 | M | NA | Liver disease | Coronal |
| Mu | 85-64 | ARP | 67 | F | NA | Coronary occlusions | Coronal |
| Mu | 66-65 | ARP | 50 | M | 18 | Coronary occlusions | Coronal |
| STD V-57 | ARP | 44 | M | 6 | Lung cancer with metastases | Coronal |
| STD UV-54 | ARP | 32 | F | 2 | Vascular disease | Coronal |
| STD 1-42 | ARP | 28 | M | 3 | Cerebral cortical necrosis | Sagittal |
| V | 42-57 | ARP | 50 | F | NA | Myocarditis | Coronal |
| VNS | 18-84 | ARP | 23 | F | 12 | Pleural hemorrhage | Posterior |
| WT II | 87-78A | ARP | 25 | M | 2 | Respiratory failure, heart block | Coronal |
| B | 1824 | BTRC | 25 | M | 24 | Cardiac amyloidosis | Coronal |
| CO | 37 | Zagreb | 43 | F | 8 | Myocarditis | Coronal |
| CO | 133 | Zagreb | 29 | M | 12 | Myocarditis | Coronal |
| CO | 176 | Zagreb | 32 | M | 24 | Perforation of the duodenal ulcer | Coronal |
| CO | 188 | Zagreb | 23 | F | 7 | Myocarditis | Coronal |
| CO | 189 | Zagreb | 32 | M | 7 | Homicide | Coronal |
| A | 90-166 | YNHH | 67 | F | 14 | Post heart valve surgery | Coronal |

PMI, postmortem interval, in hours. NA, not ascertained. For source abbreviations, see Materials and Methods.
ponents were clearer dorsomedially (compare Figs. 44, 5/1).

horizontal stratification and radial disposition of cellular com-

eral surface due to a more uniform sizing of cells. Both the
sublayers of layer m were less distinct than on the dorsolat-

al gyrus. The cortex located dorsomedially was thicker and

ter is characterized by a gradual transition of gray matter to

3, 13). The border of layer VI with the underlying white mat-

contains fewer neurons and a higher density of glia (Figs. 24,

horizontally to the cortical surface. Two sublayers can be dis-

tinguished on the basis of differences in neuronal packing

Figs. 24, 3, 13)—Layer VI is composed of cells of different

are characterized by a gradual transition of gray matter to

3, 44, 13; Table 2). Although area 9 is granular, layer IV in this

region is neither very densely packed nor very wide (see Ta-

bles 2, 3; Figs. 2A, 3, 13) compared to layer IV in surrounding

areas 46 and 10 (see Tables 2, 3; Figs. 2A, 3, 13). The borders

separating layer IV from both adjacent layers are possibly not
distinct because this layer contains large pyramidal cells in-
termixed with small granular cells. In contrast, layer III is well
developed and has distinct sub-divisions, IIIa, IIb, and IIIc
(Figs. 2A, 3). Sublayer IIIa can be distinguished by small py-
ramidal cells that are densely packed; sublayer IIb has larger
cells and a lower packing density than in IIIa; finally, sublayer
IIIc contains the largest pyramidal cells intermixed with me-
dium- and small-sized pyramidal cells (see Table 13). Layer V has
two distinct sublayers: Va, which contains larger pyramidal
cells, and Vb, which contains less numerous and smaller py-
ramidal cells and has a fainter staining than adjacent layers,
reflecting the lower cell-packing density in this sublayer (see
Figs. 2A, 3, 13). Layer VI is composed of cells of different
shape and orientation including fusiform neurons oriented
horizontally to the cortical surface. Two sublayers can be dis-
tinguished on the basis of differences in neuronal packing
density: Vla contains a higher density of neurons and Vlb
contains fewer neurons and a higher density of glia (Figs. 2A,
3, 13). The border of layer VI with the underlying white mat-
ter is characterized by a gradual transition of gray matter to
white matter and is thus indistinct.

Some variations in the basic cytoarchitectural pattern of
area 9 were often evident on the dorsolateral and dorsomes-
dial surfaces and in the rostro-caudal axis of the superior fron-
tal gyrus. The cortex located dorsomedially was thicker and
sublayers of layer III were less distinct than on the dorsolat-
eral surface due to a more uniform sizing of cells. Both the
horizontal stratification and radial disposition of cellular com-
ponents were clearer dorsomedially (compare Figs. 4A, 5A).

Rostral portions of area 9 appeared to have higher neuronal
densities than more caudal sectors.

Myeloarchitecture

The myeloarchitecture of area 9 as revealed in the Gallyas-
stained material was distinguished by numerous, thick, well-
stained radial striae and two bands of more densely packed
horizontal fibers. The upper band corresponded to the cy-

toarchitectonic location of sublayer IIIc and layer IV and the
lower band corresponded to the cellular subdivision of layer
Vb (Fig. 4B). Sections on the dorsolateral aspect of the supe-
rior frontal gyrus appeared darkly stained and had very thick
radial striae while on the dorsomedial surface of this gyrus,
the striae were noticeably thinner (Figs. 4B, 5B).

Area 46

Cytoarchitecture

Area 46 was situated exclusively on the lateral convexity of
the frontal lobe covering central portions of one or more
convolutions of the middle frontal gyrus and extending to the
depth of the middle frontal sulcus in all cases examined (for
details, see Rajkowska and Goldman-Rakic, 1995).

Like area 9, area 46 is relatively thick (2.47 ± 0.29 mm)
but its distinguishing feature is the much higher average neu-
ronal packing density (55.38 ± 7.26 neurons/0.001 mm³)
comparing to area 9 (see Figs. 2, 3, 6, 13; Table 2). Area 46 can
also be distinguished from area 9 by the more distinct borders
between layers, a more pronounced horizontal stratification,
and a more conspicuous radial arrangement of cells (Figs. 2B,
3, 64, 7A). Neuron soma sizes are more uniform in compari-
son to those in area 9, especially in layer IV and the supra-
granular layers (see Figs. 2, 3, 6, 11-13). Layer IV is particularly
pronounced—it is relatively thick and has more distinct bor-
ders with adjacent layers than layer IV in area 9. On the sur-
faces of large gyri, neurons in layer IV have a very character-
istic regular arrangement in tangential rows (Figs. 2B, 3). In
layers III and V, sublayers can be distinguished but less easily
than in area 9, due to the more uniform sizes and compact
arrangement of neurons in these layers. Layer VI is thick with
two distinct sublayers (Figs. 2B, 64, 7A; Table 4). The border
between layer VI and underlying white matter is somewhat

Area 9

Area 9-46

Area 46

Figure 1. Line drawings of coronal sections of human prefrontal cortex (case MU 69-54) taken from five different rostro-caudal levels showing positions of cortical probes used to obtain layer-by-layer measurements, for example, cell density and laminar thickness, described in this study. Positions of coronal sections of area 9, area 46 as well as transitional area 9-46 are marked with different patterns. SFS, superior frontal sulcus; MFS, middle frontal sulcus; FIS, inferior frontal sulcus; SFG, superior frontal gyrus, MFG, middle frontal gyrus, SFG, transitional area showing combined features of both area 9 and 46.
Figure 2. Photomicrographs of the cytoarchitecture of areas 9 and 46 in coronal Nissl-stained sections from SOT II A-54. A, Area 9, from the lateral rim of the first frontal gyrus. B, Area 46, from the crown of the large gyrus of the second frontal convolution. Scale bar, 0.5 mm.

All these features give area 46 a more regular cytoarchitectonic appearance than area 9.

Not all portions of area 46 cortex are equally cytoarchitectonically distinct. Parts of area 46 located on the crown of smaller gyri or close to the fundus of sulci possess less regular arrangements of cellular components and layer IV in crevices has less distinct borders with adjacent layers compared to areas that cover the flatter surfaces of large gyri (compare Figs. 2B, 6A). Moreover, individual variations were observed in the structure of area 46. In some brains, small portions of the area contain large pyramids in layers III and V and exhibit less pronounced radial striations (Fig. 7B). Nevertheless, layer IV in this cortex is still wide and distinct and is densely packed with granular neurons of uniform size. In approximately half of the cases examined, neuronal density in supra-granular layers and in layer IV was higher in the more rostral portions of area 46 and decreased in the caudal portion.

Myeloarchitecture

In myelin-stained tissue, area 46 appears more lightly stained than adjacent areas 9 and 45 in most cases. Its radial striations are finer and longer while horizontal bands of fibers in layers
IIIc-IV and Vb appear more pronounced than in area 9 (Fig. 6B).

Transitional Areas

Transitional areas are defined here as cortical regions showing features of one or another adjacent area. The most commonly encountered transitional cortex was that bordering areas 9 and 46. Transitional areas were also observed at locations that exhibited combined features of adjacent areas 9-8, 9-45, 46-10, 46-45. All these transitional regions are treated as one archetype in Figures 5-11 of Rajkowska and Goldman-Rakic (1995).

Cytoarchitecture

Transitional area 9-46 was consistently found in the depths of the superior and middle frontal sulci and on portions of the middle frontal gyrus in 14 of the 17 cases examined. Only a very narrow strip of cortex with transitional features could be identified with confidence in the remaining three cases (see Fig. 7A).

The area designated 9-46 in Figure 1 contained cytoarchitectonic features of both areas 9 and 46 (Figs. 1, 3, 8A). Like area 9, this area was characterized by relatively low cell packing density and a pale sublayer Vb. Like area 46, layer IV is distinct and densely packed though not quite as prominently as in area 46 (see below). Another feature shared with area 46 is the uniform size of neurons in layers III and V. In addition this area was distinguished from both areas 9 and 46 by its thinner cortex and thinner layers.

Myeloarchitecture

We also observed transitional characteristics in the material prepared for myeloarchitecture. Area 9-46 had well-stained dark radial striae as had area 9 but these were thinner than those in area 9 and thicker than in area 46. This area was bistriate as were both areas 9 and 46 but had more pronounced and more compact bands of horizontal fibers corresponding to the external and internal stria of Baillarger (Fig. 8B).

Quantitative Cytometric Comparison of Areas 9 and 46

Neuronal Packing Density

Comparison of neuronal packing densities between the two areas showed that the number of neurons per 0.001 mm³ of cortical tissue was significantly higher in area 46 than in area 9 for all layers combined (55.38 ± 7.26 for area 46 and 45.80 ± 4.45 for area 9, p < 0.01; see Table 2). Layer-by-layer analysis revealed that the largest contribution to this overall difference was from layer IV (see Figs. 3, 7A, 13). The neuronal density in this layer of area 46 (107.63 ± 20.30 neurons/0.001 mm³) was significantly higher than in area 9 (82.56 ± 13.90 neurons/0.001 mm³, p < 0.05), in every case but one, (C0133) and ranged from 7% to 48% in individual cases.
Table 3). Area 46 also differed from area 9 when the densities for supragranular layers I, II, and III were combined (53.51 ± 6.33 neurons/0.001 mm³ vs 45.69 ± 3.81 neurons/0.001 mm³ for area 9, p < 0.01). However, the neuronal density of infragranular layers V and VI did not distinguish the two areas (Table 2, Fig 9). Comparisons between the neuronal densities of supra-versus infragranular layers were not statistically significant in either areas 9 or 46.

Relative Laminar Thickness
The relative laminar thickness of layer IV also differentiated the two cortices (see Fig. 13). In area 46, layer IV occupied a larger percentage of cortical thickness (9.30 ± 2.51%) than in area 9, where layer IV was only 6.41 ± 0.90% (p = 0.001; Figs. 3, 104; Table 4). Differences in relative laminar thickness of layer IV ranged from 26% to 38% in individual cases (Table 5). In one brain (A 90-166) these differences were only 9%.

Figure 4. Photomicrographs of the cyto- and myelo architecture of area 9 from brain Co 188. A and B show adjacent Nissl- and myelin-stained sections from the magnocellular subarea located on the dorsolateral rim of the superior frontal gyrus. Note numerous, thick radial striae and two bands of horizontal fibers. Scale bar, 0.5 mm.
Layer II, the external granular layer, also occupied a slightly larger percentage of cortical thickness in area 46 (7.50 ± 1.44%) than in area 9 (6.13 ± 0.70%; \( p < 0.05 \)). In contrast, pyramidal layer III was slightly wider in area 9 (35.93 ± 4.05%) than in area 46 (31.31 ± 2.51%; \( p = 0.05 \); Fig. 3). Despite these relative laminar differences, however, the entire cortical thickness was virtually identical in both areas (2.45 ± 0.28 mm in area 9 and 2.47 ± 0.29 mm in area 46; see Fig. 18). Analysis of the ratio of combined layer I-III versus layer V and VI relative thickness revealed that the supragranular layers are wider in both areas 9 and 46 (54.36 ± 4.51% vs 39.29 ± 4.71%, \( p < 0.001 \), for area 9; 49.21 ± 5.29% vs 41.71 ± 4.64%, \( p < 0.01 \), for area 46). The combined supragranular layers occupy a slightly larger percentage of cortical thickness in area 9 than in 46 (54.36 ± 4.51% vs 49.21 ± 5.29%, \( p = 0.05 \)).

Size of Neuronal Soma

Comparison of neuron soma sizes in pyramidal layers III and V confirm visual impressions of the distinctions between areas 9 and 46 (Figs. 3, 11–13). Soma diameters in both layers were significantly larger in area 9 (D-circle = 16.05 ± 0.59 μm for layer III and 16.88 ± 0.69 μm for layer V) than corresponding somal diameters in area 46 (D-circle = 13.45 ± 0.24 μm for layer III and 14.29 ± 0.48 μm for layer V; \( p < 0.001 \)). Also confirming light microscopic observations, soma sizes in layers III and V in area 9 were more heterogeneous than in area 46, reflecting the presence of large single neurons among numerous smaller ones (Figs. 3, 12, 13). Our analysis of neuron soma sizes in layer IV revealed a similar but non-significant difference (\( p > 0.05 \)) between the two areas: somal diameters in layer IV of area 9 were slightly higher than those in layer IV of area 46 (D-circle = 11.59 ± 0.67 μm for area 9 and 10.61 ± 0.85 μm for area 46). Since the latter analysis was based on neurons in only four cases, additional data may reveal larger differences.

Discussion

Cytoarchitectonic, myeloarchitectonic, and pigmentoarchitectonic descriptions of the human cerebral cortex, including the frontal regions, has existed for decades. The most comprehensive descriptions of the human prefrontal areas are those of von Economo and Koskinas (von Economo and Koskinas, 1925; von Economo, 1929), Sarkissov and co-workers (Sarkissov et al., 1955), and Sanides (1962, 1964). Although these cartographers described their criteria for distinguishing cortical areas and illustrated representative sections with high-resolution photographs, each arrived at a different parcellation of the frontal cortex and used a different nomenclature to designate cortical areas. Sanides (1962) distinguished approximately 60 frontal lobe subdivisions compared to the many fewer subdivisions published by Brodmann (1909), von Economo and Koskinas (1925), and Sarkissov et al. (1955). Although Zilles (1980) considered both Brodmann's (1909) and Sanides's (1962) frontal lobe maps to be in good agreement, Sanides himself stressed minor discrepancies between the maps. In our opinion, the frontal lobe maps of von Economo and Koskinas on the one hand and Sarkissov and co-workers on the other are rather more comparable to that of Brodmann than to the highly segmented map of Sanides. An especially complete and useful map of the human cerebral cortex is the last one published by Sarkissov and colleagues in 1955. The textbook, published in Russian and translated by one of us (G.R.), contains a detailed description of 10 major areas, 20 subareas, and 7 transitional zones within the frontal lobe with notes on their cytoarchitectonic variations. All this material is well illustrated by numerous photomicrographs. H. Braak's synopsis of the existing maps of the human telencephalic cortex commented that Sarkissov's atlas "is remarkably useful in and contrast to that of von Economo and Koskinas relatively easily accessible" (Braak, 1980).

Prior to the publication of the Sarkissov atlas, von Economo and Koskinas's textbook was the only one to contain notes on interindividual variability in areal and laminar patterns within cytoarchitectonic areas. Von Economo and Sarkissov also provided quantitative data on cortical and laminar thickness, surface areas, the numbers of cells per volume of tissue, ratio of gray matter volume to neuronal volume (so called Economo index), and the dimensions of pyramidal neurons. However, the counting methods available at that time were not unbiased and they introduced systematic errors in measurements, especially in the estimation of the number of cells per unit area in sectioned material. These errors arise because cell size, shape, and orientation as well as section thickness are not taken into account in older methods. Also, corrections were not performed to eliminate or reduce the possibility that one and the same nerve cell can fall within two adjacent sections and be counted twice. The problem of correction applies generally to the morphometric parameters found in many classical studies (von Bonin, 1937; Shariff, 1953; Bok, 1959; Pakkenberg, 1959; Sholl, 1959; Blinkov and Glezer, 1968; Colon, 1971; Coleman and Flood, 1987; Terry, 1987; Flood and Coleman, 1988). In addition, some of the measurements were indirect; for example, they were taken from photomicrographs (von Economo and Koskinas, 1925) or camera lucida drawings (von Bonin, 1937). Finally, more re-
Figure 5. Photomicrographs of adjacent Nissl-(A) and myelin-stained (B) sections from the variation in structure of area 9 located on the dorsomedial rim of the superior frontal gyrus. This cortex in comparison to the area 9 cortex located on the dorsolateral rim of the gyrus is thicker overall and sublayers of layers III and V are less distinct. In myelarchitecture, the dorsomedial subarea can be distinguished from the dorsolateral subarea by its thinner radial striae and less compact bands of horizontal fibers. Scale bar, 0.5 mm.

Recently, direct, unbiased cell counting methods have been used in studies of human frontal cortex (Henderson et al., 1980; Braendgaard et al., 1990; Uylings and van Eden, 1990). To date, these studies have been focused on estimation of global parameters such as total number of cells within the whole frontal cortex (Braendgaard et al., 1990) or volume of the entire prefrontal region (Uylings and van Eden, 1990). The present quantitative comparison of the cytoarchitecture of human prefrontal areas 9 and 46 is the first unbiased three-dimensional cytometric analysis of areal differences in human
brain and the first to provide a set of criteria for distinguishing between areas.

**Qualitative and Quantitative Cytoarchitectonic Distinctiveness of Areas 9 and 46**

Our analysis of area 9 confirms to a large extent the qualitative description of previous morphologists (von Economo and Koskinas, 1925; von Economo, 1929; Kononova, 1949, 1955; Sarkissov et al., 1955). Area 9 has a low packing density, a wide layer III with three distinct sublayers, and layer IV is indistinct. Also, layer V has two clear sublayers, and layer VI is heterogeneous. Layer VI was subdivided by von Economo into two sublayers, VIa and VIb, while Sarkissov and Kononova in their atlas treated the deepest sublayers as layers VI and VII, respectively. In our material the deepest layer of area 9 is also differentiated into an upper stratum that has a higher...
neuron packing density than the lower stratum. We refer to them, following von Economo, as sublayers VIa and VIb.

The large pyramidal cells among small- and medium-sized pyramids in sublayers IIC and Va are one of the most notable cytoarchitectural features of area 9. These cells were especially large on the dorsolateral aspect of area 9. Notes on structural differences between parts of area 9 located on the dorsolateral and dorsomedial aspects of the frontal lobe, respectively, can be found in Sarkissov's atlas of the human cerebral cortex and also in a study on the cyto- and myeloarchitecture of area 9 in macaque monkeys (Preuss and Goldman-Rakic, 1991). On the other hand, the widely used map of Brodmann contains no hint of any subdivision within area 9.

In the present study we observed a number of areas that did not conform either to the criteria of area 9 or 46. Because these areas were often found at the border regions between areas 9 and 46 or between 9 or 46 and the cortices bordering them, we have treated these areas as transitional areas and designated them by the areas with features they express. In virtually every case were generally the areas they bridged. For...
example, area 9–46 exhibited the low cell-packing density and pale sublayer Vb characteristics of area 9 and at the same time, the distinct, densely packed layer IV and the uniform soma sizes of layers III and V of area 46. Other cortical cartographers, particularly von Economo (1929) and Sarkissov et al. (1955), designated the exact area that we term area 9–46 as a parvocellular subdivision of area 9 (contrasting with the magnocellular archetype) on the basis of its smaller neurons in layers III and V. We also observed smaller neurons but considered the area in which they were found a type of transitional cortex in light of the fact that it shared features of both adjacent areas 9 and 46. Thus, our transitional zone, 9–46, probably corresponds to the parvocellular subdivision of area 9 of previous authors. These decisions are, to be sure, arbitrary and area 9–46 in the present study may ultimately be shown to be related mainly to area 9. We prefer the transitional designation as it is more neutral and leaves open this possibility, should additional criteria, for example, chemoarchitecture, allow finer classification of areas. Whether areas designated in this study as transitional turn out to be variations of classical areas or perhaps even new evolving structures as has been postulated as a mechanism of evolution.

Figure 8. Photomicrographs of adjacent Nissl-(A) and myelin-stained (B) sections from the transitional area 9–46 located on the crown of the first gyrus of the middle frontal convolution. Note well-stained radial striae and two pronounced bands of horizontal fibers; the upper band corresponds to cytoarchitectonic layers IIIc and IV while the lower one corresponds to sublayer Vb. Scale bar, 0.5 mm.
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In the contrast, pyramidal layer III was p < 0.05). In the contrast, pyramidal layer III was p < 0.05). Despite these laminar differences, the entire cortical thickness was virtually identical in both areas (145 ± 0.28 mm in area 9 and 2.47 ± 0.29 mm in area 46; see fig. 96); this finding differs from observations of previous authors (Sarkissov et al., 1955), who emphasized that area 46

The differences in layer IV thickness in areas 9 and 46 are highly significant (**, p < 0.01; *, p < 0.05, respectively). Values represent means ± standard deviation.

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Figure 9. Histogram showing neuronal density in areas 9 and 46. In area 46, layer IV is more densely packed (107.63 ± 20.30 neurons/100 mm²) than in area 9 (82.56 ± 13.90 neurons/100 mm²) and this difference is significant (**, p < 0.01). Also, the overall densities of cortical layers in area 46 and in the supragranular layers particularly, are higher than those in area 9 (**, *p < 0.01; *, p < 0.05, respectively). Values represent means ± standard deviation.

Figure 10. Histograms showing laminar (A) and cortical (B) thickness in areas 9 and 46. The differences in layer IV thickness in areas 9 and 46 are highly significant (***, p = 0.001). Layer IV occupied a much larger percentage in area 46 (9.30 ± 2.51% of total cortical thickness) than in area 9 (6.41 ± 0.9%). The other granular layer, layer II, also occupied a slightly larger percentage of cortical thickness in area 46 (7.50 ± 1.44%) than in area 9 (6.17 ± 0.75%); (*, p < 0.05). In the contrast, pyramidal layer III was represented by a slightly larger percentage of cortical thickness in area 9 (35.93 ± 4.05%) than in area 46 (31.31 ± 2.51%); (*, p = 0.05). Despite these laminar differences, however, the entire cortical thickness was virtually identical in both areas (2.45 ± 0.28 mm in area 9 and 2.47 ± 0.29 mm in area 46; see fig. 96); this finding differs from observations of previous authors (Sarkissov et al., 1955), who emphasized that area 46 can be distinguished from area 9 by its much thicker cortex. Values represent means ± standard deviation.

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(1988), they presumably have functional specializations that correlate with their morphological identities.

The structural features of area 46 have received much less attention in the literature. Moreover, different anatomists have emphasized different criteria for recognizing this area. Von Economo argued that the cortex covering the second frontal gyrus (area FD” on his map; area 46 on the maps of Brodmann and Sarkissov) was of the so-called "parietal" type, differing from the cortex covering the rest of the anterior part of the frontal lobe (i.e., areas FDM, FDP, and FE on von Economo’s map or areas 9 and 10 on the maps of Brodmann and Sarkissov). The cortex found in both prefrontal area 46 and the parietal region can be characterized as "thick, distinctly richer in smaller, more compactly arranged cells and considerably broad and densely packed granular layers II and IV" (von Economo, 1929). Kononova (1949) and Sarkissov et al. (1955) emphasized that area 46 could be distinguished from the surrounding area 9 by its thicker cortex and wider layer III. Unlike von Economo, these authors were not impressed with the thickness and cell-packing density of layer IV and failed to mention that it is well delineated from adjacent layers. The present observations and measurements in area 46 are therefore closer to von Economo’s description (and to Sanides, 1964) than to those of Kononova and Sarkissov.

In our material, a thick, distinct, and densely packed layer

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of heterogeneous population of cells of given layer ± standard deviation.

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Table 5

<table>
<thead>
<tr>
<th>Brain</th>
<th>Area 9</th>
<th>Area 46</th>
<th>Diff. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co 133</td>
<td>6.87 ± 2.25</td>
<td>10.57 ± 2.07</td>
<td>39.50%</td>
</tr>
<tr>
<td>Co 137</td>
<td>7 ± 1.41</td>
<td>10.5 ± 0.71</td>
<td>65.25%</td>
</tr>
<tr>
<td>Co 176</td>
<td>5.17 ± 2.79</td>
<td>8.25 ± 1.29</td>
<td>28.24%</td>
</tr>
<tr>
<td>Co 180</td>
<td>11.77 ± 0.75</td>
<td>6.6 ± 2.07</td>
<td>32.32%</td>
</tr>
<tr>
<td>Co 195</td>
<td>8.20 ± 3.56</td>
<td>8.4 ± 8.09</td>
<td>26.26%</td>
</tr>
<tr>
<td>Mu 169</td>
<td>5.25 ± 1.04</td>
<td>8.5 ± 1.03</td>
<td>28.16%</td>
</tr>
<tr>
<td>A 90-166</td>
<td>7.8 ± 2.17</td>
<td>8.6 ± 3.21</td>
<td>9.30%</td>
</tr>
<tr>
<td>B 1824</td>
<td>7 ± 2.05</td>
<td>11 ± 2.24</td>
<td>36.38%</td>
</tr>
</tbody>
</table>

Width of layer IV is expressed as a percentage of total cortical thickness at sites where measurements were taken. Values represent means from five to eight probes ± standard deviation.

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Figure 12. Histograms of neuron soma sizes in layer III (A) and V (B) of area 9 and layer III (C) and V (D) of area 46. Note wider range of neuron sizes in pyramidal layers of area 9 than those of area 46. The greater heterogeneity of soma sizes in area 9 reflects the presence of single large neurons among smaller ones.

IV appeared as the major delineating feature of this area. Our data show that among all cortical layers, only the neuronal packing density of layer IV differed significantly between areas 46 and 9; in addition, only the granular layers, II and IV, differed significantly in thickness. Notably, the two areas were not significantly different in their overall cortical thickness, contrary to the report of Kononova and Sarkissov. Also at variance with these authors is our observation that the relative thickness of layer III and supragranular layers is significantly higher in area 9 than in 46. Kononova and Sarkissov, in contrast, reported that area 46 could be distinguished from area 9 by its very wide layer III and wider overall cortical thickness. The only explanation we can think of for this discrepancy is that the uniformity of neuronal sizes in layer III can give a strong visual impression of greater thickness. However, our cytometric data disconfirms this visual impression and illustrates the value of quantification. Recent classification of prefrontal areas based solely on visual analysis (Chiavaras et al., 1993) is also not supported by the present cytometric measurements, probably for the same reason. In addition to wider and more densely packed granular layers, the higher neuronal packing density of the cortex in area 46 relative to area 9, and the smaller, more uniform sizes of its neurons were also confirmed by statistical analysis.

It has been noted that increasing granularization of prefrontal areas seems to be a progressive feature of the cytoarchitecture of the prefrontal cortex during the phylogeny. Prefrontal cortex in rodents is completely and in carnivores partially agranular and granulation of this area becomes prominent only in primates (Walker, 1940; Adrianov and Mering, 1959; Akert, 1964; Khokhyakova, 1977; Rajkowska and Kosmal, 1988; Barbas and Pandya, 1989; Preuss and Goldman-Rakic, 1991). Since granular layers II and IV in both monkey (Rajkowska and Goldman-Rakic, unpublished observations) and human (present results) are more pronounced in area 46 than in area 9, this area could be considered phylogenetically the more advanced of the two areas. Certainly increased granularization may convey a functional distinction not present or as well developed in nongranular regions.

**Myeloarchitecture**

Data on myelin staining in the human frontal lobe are scanty and can be found only in a few studies of the myeloarchitecture of the cerebral cortex (Campbell, 1905; Vogt, 1927; Strasburger, 1937; Filimonoff, 1949; Sanides, 1962, 1964). The most detailed notes on the myeloarchitecture of prefrontal regions corresponding to areas 9 and 46 are contained in studies of Vogt (1927), Filimonoff (1949), and Sanides (1964). Following Filimonoff, who based his description mainly on the classical map of Vogt, the myeloarchitectonic fields designated as 48 and 49, located on the superior frontal gyrus, collectively correspond to cytoarchitectonic area 9. According to Filimonoff, these fields have a slightly different pattern of staining than fields 52 and 54, which are located on the middle frontal gyrus and correspond, according to this author, to cytoarchitectonic area 46. Areas 48 and 49 belong to the so-called "unstriatal type" characterized by a pronounced external and poorly delineated internal stria of Bajal. In contrast, the more ventrally localized myeloarchitectonic fields, 52 and 54, have a clear bistriate pattern; that is, both external and inter-
nal striae are well pronounced. Similar observations were reported by Sanides (1964), who described a "paramotor zone," part of which corresponds to area 9, as less bistriate than a "paraopercular zone" located more ventrally and corresponding to area 46. In the present study we analyzed myeloarchitecture and cytoarchitecture on adjacent sections, and were able to observe slight differences in the bistriate pattern of staining in the two areas. Moreover, in our material, the transitional area 9-46 appeared to be "bistriate," too. Indeed, area 9-46 was characterized in some instances as having a particularly clear and compact stria of horizontal fibers, a finding not mentioned in any previous studies. We do not have a ready explanation for differences observed between our results and those of previous authors. However, some suggestive differences in myelin staining between areas can be found in Sanides's (1964) study of human cortex, where low fibrillarity corresponded to small neuronal somata (parvocellularity) whereas high fibrillarity always corresponded to the presence of large neurons (magnocellularity). Thus, the small neuronal soma in area 9-46 in our material may render the bands of

Figure 13. Computer print-outs of individual neurons (red) and glia (blue) and their histograms in 35 mm bins counted in complete representative probes through the cortical thickness of areas 9 and 46 from brain Co 188; layers and sublayers are indicated. Note that layer IV is much wider and more densely packed in area 46 than in area 9. Neurons in layers III, IV, and V of area 9 are more heterogeneous in size, as reflected by the presence of single large neurons among smaller ones.
horizontal fibers more pronounced in these areas in comparison to larger-celled area 9.

Additional observations made on the basis of myelin stain indicate that area 46 is more lightly stained than area 9, as could also be concluded from the description of these areas by Sanides (1964) and from the study of corresponding areas in the monkey prefrontal cortex (Preuss and Goldman, 1989). These two regions are among the areas that Yakovlev and Lecours (1967) considered the last to myelinate. We did not observe a major difference in myelination among cases from 23 to 73 years of age, either in area 9 or 46. Our findings rather suggest that different areas of frontal association cortex are not equally well myelinated in adult human cortex. The finding that area 46, in particular, appears less myelinated compared to surrounding areas may be a helpful marker for distinguishing areas within the human prefrontal cortex and homologous areas in various species (Preuss and Goldman, 1989). Further, this result challenges the long-held assumption originating in pathology between myelination and higher cortical function. Knowledge of detailed areal differences in adult cortex is thus essential for interpretation of developmental and age-related patterns of changes in morphological parameters such as myeloarchitecture.

Notes

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