Cytoarchitectural Maps of the Human Brain
in Standard Anatomical Space

P.E. Roland,1* Stefan Geyer,2 Katrin Amunts,2 Thorsten Schormann,2 Axel Schleicher,2 Aleksander Malikovic,3 and Karl Zilles2

1Division of Human Brain Research, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden
2C. & O. Vogt Institut für Hirnforschung, Heinrich-Heine-University, D-40000 Düsseldorf, Germany
3Institute of Anatomy, University of Belgrade, YU-11000, Belgrade, Yugoslavia

Abstract: The remarkable intersubject variability of the human cerebral cortex poses major problems for the systematic study of functional-structural relationships. Lack of homology and macroscopical landmarks between brains implies that one cannot in three or two dimensions find which part of one gyrus or sulcus matches which part of another subject’s cerebral cortex. Furthermore, the frequent lack of correspondence between cytoarchitectural borders and the bottom of sulci invalidates correlations between gross morphology and microstructure. Therefore, we proposed that microstructural criteria should be used to define an anatomical space for comparison of individual brains and for establishing a probability map for each cytoarchitecturally defined area by quantitative means [Roland and Zilles, 1994; Trends Neurosci 17:458–467]. Here we examined the mapping of cytoarchitectural areas 4a, 4p, 3a, 3b, V1, and V2 into two commonly used anatomical standard reference spaces. Linear global transformations into Talairach space produced minimal overlap of corresponding cytoarchitectural areas. Global affine and nonaffine transformations into the anatomical space of the Human Brain Atlas (HBA) gave significantly larger volumes of overlap of corresponding cytoarchitectural areas. It is expected that local transformations can further improve the registration of corresponding cytoarchitectural areas and thereby define a common standard anatomical space in which to study variations in gross anatomical structure and function. Hum. Brain Mapping 5:222–227, 1997. © 1997 Wiley-Liss, Inc.

Key words: human brain; cytoarchitectonics; cerebral cortex; brain anatomy; image fusion; flow-field theory

INTRODUCTION

Functional mapping of the human brain is of little value if no structural or anatomical reference of functional activations can be achieved. Ultimately, brain-mapping scientists want to establish functional-anatomical relations of brain activations with structure. Although relations between the gross morphology of the brain, as depicted in anatomical magnetic resonance images (MRI), and functional activations of the human brain, as obtained by PET, f-MRI, MEG, or EEG, can be established in the single individual, such relations are of limited value due to the lack of true cortical landmarks and the lack of cytoarchitectural or other microstructural information [Roland and Zilles,
If one wants to make generalizations of functional mapping data or establish microstructural-functional relationships, one is forced to transform data into a three-dimensional (3D) or two-dimensional (2D) standard anatomical format. This is now done routinely at many brain-imaging centers.

Various anatomical standard formats exist and it is unclear in most cases by which criteria the standard anatomical format has been chosen. The Talairach standard format, the “quadrillage normaliseé,” is based “on the dimensions most frequently observed” in the radiological material studied by Talairach et al. [1967]. But in the 1988 version, another format is used in which the grid is adapted to a single normal female brain [Talairach and Tournoux, 1988]. Others have chosen a three-dimensional MRI of a young male to represent their standard anatomical space [Evans et al., 1988, 1991, 1992], whereas still others have chosen a single postmortem brain with no pathology and without excessively large distortions [Seitz et al., 1990; Greitz et al., 1991]. An attempt was made by Roland et al. [1994] to choose a representative male brain, deviating the least amount from 21 other young normal male brain MRIs. Although the brain in its outer dimensions, the ventricular system, and the central structures are representative of a population of 20–40-year-old normal male brains [Roland et al., 1994], the cortical gyri and sulci are still those of an individual brain and are not representative.

One may regard the choice of a standard anatomical format as unimportant, since it is possible to transform an image of one brain into the image of any other brain by global and local affine and nonaffine transformations. Nevertheless, it matters, because the standard anatomical format has also the format in which one may study variations in morphology, and variations in extent and localizations of functional fields. Furthermore, since transformations inevitably change voxel values, transformations should be kept at a minimum. Formal criteria for successful image fusion or image transformations can be established [Bajcsy and Kovacic, 1989; Pellizzari et al., 1989; Bookstein, 1991; Schormann et al., 1993, 1995; Davatzikos, 1996; Collins et al., 1995; Lancaster et al., 1995], but these criteria are all derived from gray values, macroscopic “landmarks,” or surface morphometrics. Although matching of brains so as to optimally match gray values in 3D images will automatically match also the cortical ribbon to a large extent, such matching does not rely on architectonic or other microstructural criteria. With the possible exception of the bottom of the central sulcus, no fixed relations exist between the sulcal geometry and microstructurally defined borders of cortical areas [Braak, 1980; Zilles, 1991; Roland and Zilles, 1994; Zilles et al., 1997]. Not even the topology of cytoarchitecturally defined areas is constant from brain to brain [Sarkissov et al., 1955]. Thus macroscopic landmarks in the human cerebral cortex with a biologically meaningful label are so sparse, that they cannot provide useful criteria for matching of brains or serve as definitions of standard anatomical space.

Clear biological criteria for optimal microstructural matching and optimal matching of functional fields are required. As the accurate extent of functional fields is an unresolved issue, we focus on establishing objective microstructural criteria for matching of brains. Cytoarchitectural borders, myeloarchitectural borders, and borders emerging from changes in laminar transmitter receptor densities can be objectively defined by quantitative methods [Geyer et al., 1996; Schleicher et al., 1986, 1995; Zilles and Schleicher, 1993]. This means that volumes of the cortex in single postmortem brains can be uniquely and objectively defined. Here we use objectively defined, quantitatively delimited cytoarchitectural volumes of the cerebral cortex, each representing a cytoarchitectonical area, a) to set objective criteria for matching of brains, and b) to examine two different approaches for transformations of brains into standard anatomical space, i.e., 1) the linear method of Talairach et al. [1967]; and 2) the affine and nonaffine global transformation of the Human Brain Atlas (HBA) into the standard anatomical format of the HBA [Roland et al., 1994].

**METHODS**

**Cytoarchitectural mapping**

In five postmortem brains, the borders of cytoarchitectural areas 4a, 4p [Geyer et al., 1996], 3a, 3b, 1-2, V1, and V2 were delineated by quantitative methods [Schleicher et al., 1995; Zilles and Schleicher, 1993]. Coronal 20-µm paraffin sections were stained by a modified Nissl method [Merker, 1983]. The volume density of neurons was estimated with the grey level index (GLI) procedure [Schleicher and Zilles, 1990]. Each pixel represents neuronal density in a field measuring 27 µm per side. Density profiles, oriented orthogonally to the laminae and extending from the boundary between layers I and II to that between layer VI and the white matter, were extracted from the images. These profiles were standardized to a cortical depth of 100%. Each profile was 11 pixels (297 µm)
wide, i.e., each value in a profile represents the average of 11 GLI values. Spacing between profiles was 297 μm. Shape-characterizing features were extracted from each profile. They included the mean amplitude and the first 4 moments (mean, standard deviation, skewness, and kurtosis). One mean feature vector $X_1$ was calculated from a block of 10 adjacent profiles, and another mean feature vector $X_2$ from a neighboring block of 10 profiles. The Mahalanobis distance [Dixon, 1988]

$$D^2 = (X_1 - X_2)'C^{-1}(X_1 - X_2)$$

was calculated from the vectors and the inverse of the pooled covariance matrix $C^{-1}$. Plotting the $D^2$ values as a function of the position of the blocks of profiles revealed maxima at locations at which regions covered by profiles showed major differences in laminar patterns. Statistical significance was established by computing Hotelling’s statistic

$$T^2 = \frac{D^2}{\frac{1}{n_1} + \frac{1}{n_2}}$$

in which $n_1 = n_2 = 10$, the number of profiles from which each of the mean vectors had been calculated. The corresponding $P$ value was not corrected for multiple comparisons, since the probability of getting three such maxima at the predicted locations in a set of adjoining sections is exceedingly small.

The five postmortem brains were scanned with a 3D-FLASH sequence in a 1.5 T Siemens MR-scanner. Flip angle was 40°, TR was 40 msec, and TE was 5 msec, giving a 3D array of $1 \text{ mm} \times 1 \text{ mm} \times 1 \text{ mm}$ voxels with a 1.5 T Siemens Magnetotome (Siemens, Erlangen, Germany). These 3D MR images were used for correcting the inevitable histological shrinkage and distortions in the Nissl-stained sections. Compensation for shrinkage and distortion was done by algorithms according to Schormann et al. [1993, 1995, 1996]. This software was also used for the 3D reconstruction based on the sections. Subsequently, the 3D-reconstructed brain was transformed into the format of the HBA.

**Transformations into standard anatomical formats**

For each cytoarchitectural area, the voxel values were set to one prime value (e.g., area 3a, 17; area V1, 41). This voxel value thus made it possible to uniquely define a particular cytoarchitectural area and locate the voxels which overlapped in a particular cytoarchitectural area of one, two, three, four, or five brains. Three types of transformation were done on the reconstructed 3D images. First, the images were translated and rotated in order to fit the anterior and posterior commissures (AC and PC) to the Talairach AC-PC plane. The anterior and posterior commissures of the selected standard brain were located, and the plane tangent to the upper surface of the anterior commissure and the lower surface of the posterior commissure was made. The vertical plane, tangent to the anterior commissure orthogonal to the CA-CP plane, was termed the VCA plane, for which $y = 0$ [Talairach et al., 1967]. Subsequently the images were scaled to accommodate the standard proportions of Talairach and Tournoux [1988]. In this Talairach format the overlaps of individual 4a areas were calculated. Similar overlaps were calculated for each of the other cytoarchitectural areas 4p, 3a, 3b, 1-2, V1, and V2.

Secondly, the images in Talairach format were transformed by the transformation tools of the HBA, i.e., further affine scaling, shear, and the nonaffine transformations asymmetric scaling, bending, compressing, and local shear [Roland et al., 1994]. This was done by adapting the structures of the atlas to the structures of the actual postmortem brain. This was done interactively by visual inspection of the display of the brains in sections on the computer monitor by two scientists who were very experienced with the use of the HBA. Care was especially taken to fit the central sulcus, the longitudinal fissure, and the calcarine sulcus in each brain with the structures of the HBA. Subsequently the images were reformatted into the format of the standard brain of the HBA, and the overlaps of corresponding cytoarchitectural areas were calculated.

**RESULTS**

Table I shows the mean volume of each of the seven cytoarchitectural areas in Talairach format, i.e., only after translation, rotation, and scaling of the 3D-reconstructed brains. The mean volumes are calculated as the mean of the total volumes of that cytoarchitectural area (i.e., the volume in the right hemisphere + the volume in the left hemisphere of each brain). Hemispheric differences are not the subject of this report, but in general the differences in individual brains between right and left hemisphere volume are small. It is also seen that the larger the cytoarchitectural area, the larger the standard deviation. These mean volumes thus give the upper limit for possible overlap. It is evident that simple transformation into Talairach space is insufficient for getting a reasonable alignment of
corresponding cytoarchitectural areas (Fig. 1). None of the areas lining the central sulcus have any space with 100% overlap. This is not due to peculiarities in the anatomical organization of one single brain, as seen in Figure 2. Even the space in which the majority of the brains (3 out of 5) overlapped is modest compared to the total volume of the areas (Table I).

The affine and nonaffine global transformations of the images into the standard brain format of the HBA provided significant 100% overlap of most cytoarchitectural areas. The thin primary somatosensory cortex i.e. areas 3a and 3b showed the least overlap (Table I). Compared to the total volumes, these 100% overlaps are modest. The space in which 3 out of 5 brains had overlap (60% overlap) was also substantially larger than those spaces obtained with the simple Talairach format (Table I). That the space of 100% overlap was modest was not due to peculiarities in the alignment of any single brain (Fig. 2).

**DISCUSSION AND CONCLUSIONS**

The most commonly used method of transforming images into Talairach format [Talairach and Tournoux, 1988] by linear operations is insufficient to provide a meaningful anatomical format of reference. This implies that the format (not the coordinate system) of Talairach and Tournoux [1988] is not suitable for the study of functional-structural relations. Filtering of the microstructural images will just increase the mixture of microstructurally defined regions. The HBA format is somewhat better, but still insufficient. The cytoarchitectural images are transformed into a format which is not optimal for securing 100% overlap of more than 20% of the volume of a cytoarchitectural area. This is because the HBA format was not chosen as an optimally defined microstructural format. Logically one cannot find an optimal format by matching gyri and sulci between individuals, because of the lack of macroscopi-

<table>
<thead>
<tr>
<th>Cytoarchitectural area</th>
<th>Total volume (mm³ ± SD)</th>
<th>Talairach 100% mm³ (%)</th>
<th>HBA 100% mm³ (%)</th>
<th>Talairach 60% mm³ (%)</th>
<th>HBA 60% mm³ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>11,435 ± 1,347</td>
<td>0 (0)</td>
<td>3,174 (27.5)</td>
<td>1,748 (15.2)</td>
<td>7,102 (64.7)</td>
</tr>
<tr>
<td>4p</td>
<td>8,609 ± 1,153</td>
<td>0 (0)</td>
<td>1,880 (21.8)</td>
<td>1,037 (12.0)</td>
<td>5,555 (64.5)</td>
</tr>
<tr>
<td>3a</td>
<td>3,677 ± 439</td>
<td>0 (0)</td>
<td>569 (15.5)</td>
<td>190 (5.2)</td>
<td>2,670 (72.6)</td>
</tr>
<tr>
<td>3b</td>
<td>6,454 ± 275</td>
<td>0 (0)</td>
<td>1,039 (16.6)</td>
<td>619 (9.6)</td>
<td>4,948 (76.6)</td>
</tr>
<tr>
<td>1 and 2</td>
<td>9,352 ± 2,638</td>
<td>171 (1.8)</td>
<td>3,699 (39.6)</td>
<td>1,805 (19.3)</td>
<td>5,933 (63.4)</td>
</tr>
<tr>
<td>V1</td>
<td>22,691 ± 3,463</td>
<td>513 (2.3)</td>
<td>11,804 (52.0)</td>
<td>4,048 (17.8)</td>
<td>12,392 (54.6)</td>
</tr>
<tr>
<td>V2</td>
<td>30,358 ± 6,722</td>
<td>200 (0.7)</td>
<td>12,464 (41.1)</td>
<td>4,073 (13.4)</td>
<td>17,453 (57.4)</td>
</tr>
</tbody>
</table>
cal landmarks and lack of correspondence between microstructure and gyral and sulcal anatomy. Our results also illustrate this. However, such an optimal format can now be defined as the format yielding maximal 100% cytoarchitectural overlap in a larger population of brains with quantitatively and objectively microstructurally defined areas. To transform images into this format, something better than global transformations will probably have to be used. We have experimented with an optical flow-derived algorithm giving a more than 85% match of visual areas [Lindeberg, 1997]. But other algorithms [Collins et al., 1995; Schormann et al., 1996] may be even better.

The borders of the quantitatively defined cytoarchitectural areas 4a, 4p, 3a, 3b, 1-2, V1, and V2 also coincided with the borders defined by quantitative laminar measurements of neurotransmitter receptor densities [Geyer et al., 1996, 1997]. Thus, microstructural borders used to define areas should be based on multiple quantitative criteria. If this condition is fulfilled, a maximal 100% overlap of respective microstructurally defined areas can be a meaningful standard reference format. For the areas chosen here, the Mahalanobis multivariate statistics defined sharp borders in individual brains, and consequently there were no uncertainties as to where the border was. Whether this will also apply in general remains to be seen.

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**REFERENCES**


