Methodological Issues Relating to In Vivo Cortical Myelography Using MRI

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Abstract: The relationship between neocortical structure and function is a key area of research in neuroscience. Most studies of neural function, whether using neurophysiology or neuroimaging methods, are interpreted with relation to the underlying cortical myelo- and cytoarchitecture. For functional neuroimaging studies this often means using cytoarchitectonic maps based on the study of a limited number of brains, despite evidence for substantial interindividual variation. Improvements in MR technology, resulting in wider availability of high-field MRI systems, have led to an increase in the achievable resolution in MR scans. Several groups have reported the in vivo detection of myelination patterns within the cortex, consistent with those observed in postmortem tissue. This leads to the possibility of predefining areas for fMRI analysis based on the cortical architecture. To do this it is essential to know, in a quantitative way, how reliably myeloarchitectonic areas and boundaries can be detected using MRI. Here we investigate the striate cortex, known to be coincident with V1, to assess the detectability of the stria of Gennari across V1 and across subjects. Under optimal conditions, 80% of the stria of Gennari was visualized using our methodology, although there was considerable variability in the level of detection across subjects. We discuss the limitations of the methodology and propose ways to improve the detection level of cortical myeloarchitecture more generally. *Hum Brain Mapp* 26:240–250, 2005. © 2005 Wiley-Liss, Inc.

Key words: myeloarchitecture; cortex; structure; fMRI; cortical layers; occipital lobe; visual cortex; parcellation

INTRODUCTION

Linking the functional characteristics of brain regions to their underlying structure has long been a goal in neuroscience. By histological staining of postmortem tissue sam-

ples to reveal the cellular or myelin densities within the different layers of the cortex, neuroscientists have attempted to parcellate the brain into regions with distinct structural identity. Some of the most widely cited work of this kind was done in the early 20th century by Brodmann [1909; Garey, 1999]. Brodmann Nissl-stained human and animal cortical tissue and described 52 distinct regions based on their cytoarchitectonics. Similarly, Elliot Smith [1907] and Vogt and Vogt [1919], following the observations of Bailarger [1840; von Bonin, 1860], used the patterns of myelination within the cortical layers, as observed on myelin-stained sections, to define myeloarchitectonic regions of the cortex. More recently, observer-independent, automated methods of analysis and parcellation of cortical architecture have been developed [Annese et al., 2004; Schleicher et al., 2000]. These methods use computational algorithms to identify architectonic boundaries in digital images of histologi-

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cally stained postmortem sections. Using such methods it has been possible to construct probabilistic maps of human cytoarchitectonics [Amunts et al., 2000; Rademacher et al., 2001], based on a number of subjects rather than just the single subjects used in the earlier work.

The definition of anatomical regions has been accompanied by attempts to assign corresponding functional specificity. Neurophysiology experiments, primarily on animals, have enabled neuroscientists to propose specific hypotheses about the functional role of these cyto- or myeloarchitectonic regions. The advent of noninvasive functional mapping techniques now allows even greater study of human functional localization. In order to match function to structure, fMRI data are often transformed into a standard space, such as that proposed by Talairach [1988], and foci of activation interpreted with respect to their proximity to Brodmann's cytoarchitectonics areas in this uniform space. The considerable variability in size and proximity of the architectonic regions in relation to sulci and gyri, however, means that such approaches are at best a generalization and severely limit the interpretation of such results [Amunts et al., 1999; Rademacher et al., 1993]. Interpretation is improved and given a more robust statistical footing in studies that use as a reference the probabilistic atlases of human cortical architecture. However, the ideal would be to make direct comparisons of functional localization and cortical cyto- and myeloarchitecture in the same subject. For human studies this requires noninvasive in vivo methods of detecting the same structural features used in histological staining studies.

The first attempts to carry out in vivo myeloarchitectonic analysis using magnetic resonance imaging (MRI) were carried out by Press et al. [1989] and Damasio et al. [1991]. These authors scanned the hippocampus at 1.5 T and showed preliminary data displaying slabs of low- and high-intensity signal of a scale similar to known patterns of columnar modules and myelin septa in the region. It was Clark et al. [1992] who first scanned in the occipital lobe and showed a hypointense band within the cortex on proton density-weighted spin-echo images. This region, Brodmann's Area 17 or striate cortex, is characterized by a dense concentration of myelinated fibers in cortical layer IVb, known as the stria of Gennari. Comparisons with postmortem tissue, scanned using the same MRI parameters, but additionally sectioned and stained, showed that the MR signal intensity pattern across the cortex matched the corresponding myelin stain transmittance intensity. The slice thickness used by Clark et al. was 3 mm, which was considerably larger than their 391- μ m in-plane resolution. However, applying the technique used by Press et al. [1989] they aligned their imaging plane to be orthogonal to the cortical surface, thereby reducing partial voluming of adjacent layers. One of the observations made by Clark et al. [1992] was that the stria of Gennari was more distinct in the apex of the gyri than in the inner portions of the calcarine sulcus. More recent studies at 3 T [Barbier et al., 2002; Clare et al., 2002] scanned the same region in multiple subjects, using an in-

version-recovery FLASH (SPGR) sequence. These studies used image registration and motion correction techniques to reduce the effect of subject motion between sequential scans, which were then averaged to achieve the necessary signal-to-noise ratio. Both studies demonstrated that the stria of Gennari is clearly visible on multiple slices and across several subjects, although the stripe appeared patchy and it was not possible to follow its full length. Eickhoff et al. [2005] demonstrated, using comparisons with histological data, that these observed MR intensity profiles correspond closely to myelin patterns in the cortex, and less strongly to the cytoarchitecture. Additionally, Walters et al. [2003a] demonstrated that in vivo imaging at 1.5 T could detect distinct myelination patterns, not only in the calcarine sulcus, but also in the occipital extension of the inferior temporal cortex, thought to be the human homolog of monkey middle temporal (MT) area. They used intensity profile comparisons to distinguish the cortical signature in this latter region from that of striate cortex. Additionally, they showed that it was included within a region functionally activated in response to moving visual stimuli used to identify the human MT complex (MT+). However, quantitative correspondence between functional activation and structure was not defined in detail. We have recently demonstrated that the boundary between primary and secondary visual cortices (V1 and V2), measured using fMRI, colocalizes to the borders of myelination patterns detected on high-resolution MRI [Bridge et al., 2005].

It is clear that in vivo MRI is able to uncover some of the myeloarchitecture of the human cortex, but the reproducibility of these measures and their quantitative relation to measures of function have yet to be defined. If the architectonic borders detected by MRI are to be used to interpret fMRI activity patterns, it is essential to have a quantitative measure of the confidence that may be placed in them. To quantify and attempt to optimize the detection of myelination patterns in MRI, we chose to investigate the extent of anatomically defined striate cortex and its correspondence to primary visual cortex (V1) that can be determined using retinotopic fMRI mapping. V1 is known to correspond to the striate cortex (Brodmann's Area 17) in both primates and humans [Clarke, 1993]. By using functionally defined V1 as an indication of the regions of cortex where we would expect to observe the stria of Gennari, we quantitatively measure the sensitivity of in vivo MRI for detecting striate cortex.

MATERIALS AND METHODS

Data Acquisition

Five healthy volunteers (two male) ages 24–31 years were scanned on a 3 T whole-body MRI scanner (Varian Unity Inova, San Fernando, CA), with a head insert gradient coil (Magnex Scientific, Abingdon, UK), giving a maximum gradient strength of 34 mT/m. A quadrature surface coil (NOVA Medical, Wakefield MA) covering the occipital pole was used for fMRI imaging, a brain volume coil (Varian)

used for whole-brain structural reference scan, and a four-channel receive-only surface array coil (NOVA Medical) was used for high-resolution structural scanning.

To detect the myelinated layer of the striate cortex, magnetization prepared 3D FLASH images with an in-plane resolution of $300 \times 300 \mu\text{m}$ and a slice thickness of 1.5 mm were used (matrix size 384×512). A 120° preparation pulse was followed 300 ms later by a train of 20° excitation pulses (TR = 30 ms, TE = 11 ms). There were no steady-state pulses in this train and k -space coverage was center-out. This combination was chosen to give the maximum contrast between gray and white matter, using T_1 values previously measured at 3 T [Clare and Jezzard, 2001]. However, this has the effect of introducing a slight spatial filtering of the data in the phase encode direction [Deichmann et al., 2000]. Simulations of this contrast showed that the full width at half maximum (FWHM) of the point spread function in this direction was $390 \mu\text{m}$. The data were not zero-filled in order to reduce any possible Gibbs ringing artifact. Three high-resolution scans with the above parameters were obtained over three scanning sessions for each subject. Different scan plane orientations were used for each session: 32 slices oriented perpendicular to the calcarine sulcus, 16 slices oriented parallel to the calcarine sulcus, and 32 midline sagittal slices. Ten (32 slice datasets) or 12 (16 slice datasets) averages were carried out sequentially for signal averaging resulting in a total scanning time of 40–75 min. To reduce blurring due to subject motion between scans, each repeat was linearly registered to the first using FLIRT [Jenkinson et al., 2002] and averaged. In these images white matter appears dark relative to gray matter, with the signal intensity being 30–40% of that in gray matter.

Functional MRI scans were acquired in a separate session. Echo planar images (EPI) oriented perpendicular to the calcarine sulcus were acquired using typical parameters (TR = 4 s, TE = 30 ms, $2.0 \times 2.0 \text{ mm}$ in-plane resolution, 32 slices 2 mm thick). Stimuli were presented using a VSG graphics card (Cambridge Research Systems, Cambridge, UK) with an XGA video projector (Sanyo). Subjects viewed the stimuli on a small screen at a distance of $\sim 400 \text{ mm}$, using mirrors above the head. The effective visual field was $15\text{--}20^\circ$ depending on the size and position of the subject in the scanner. To measure the polar angular component of the retinotopic map a contrast reversing (8 Hz) checkerboard wedge (45°) was used, moving 30° every TR (4 s), giving a cycle time of 48 s. The eccentricity component was defined using an expanding ring stimulus with eight different stimulus positions, giving a cycle time of 32 s. Six cycles of each stimulus were included in each scan, and each wedge paradigm was repeated six times and each ring paradigm four times.

In addition to the functional and high-resolution structural scans, two further T_1 -weighted (MP-FLASH, TR = 20 ms, TE = 5 ms, TI = 500 ms, flip = 12°) structural scans were acquired on each subject. The first had the same prescription as the EPI images (voxel size $1 \times 1 \times 1.5 \text{ mm}^3$) and was acquired at the same time as the fMRI using the surface RF

coil. The second structural scan (acquired during a separate scanning session) covered the whole brain (voxel size $1 \times 1 \times 1 \text{ mm}^3$), acquired using a volume RF coil, and was used for gray/white matter segmentation.

Data Analysis

All fMRI data analysis was performed using mrVISTA software from Stanford University (<http://white.stanford.edu/software>). The first stimulus cycle of each scan was discarded to minimize transient effects of magnetic saturation and to allow all the hemodynamics to reach steady state. The linear trend in the time series at each voxel was removed to compensate for slow signal drift [Smith et al., 1999]. The time series at each voxel was Fourier-transformed and the component at the frequency of the stimulus presentation used to determine the phase and strength of correlation of the signal to the stimulus. The correlation coefficient was used as a thresholding measure and the phase gave information about the angular position or the eccentricity of the retinotopic map to which the voxel responded. In order to define V1 the functional data were transformed onto a flattened representation of each subject's gray matter. This flattened representation was achieved first by segmenting the cortical gray matter from the whole brain scan using FAST and then manual editing using mrGray [Teo et al., 1997; Zhang et al., 2001]. This folded, 3-D image was then computationally flattened to a 2-D image using an algorithm designed to preserve interpoint distances [Wandell et al., 2000]. The boundaries of V1 were determined from the flattened retinotopic map using the phase reversals present in the rotating wedge data [DeYoe et al., 1996; Engel et al., 1997; Sereno et al., 1995].

The regions of the high-resolution scans that display a dark band within the cortex were identified manually by three experienced observers independently. Lines were drawn on the images over the dark band using MRICro (online at <http://www.mricro.com>), then exported as image files and dilated to a thickness of 2 mm, approximately covering the gray matter in that region. A final "striate" image was then generated as the intersection of any two of the three observers' individual images on a pixel-by-pixel basis.

Alignment of all datasets was achieved by linearly registering each dataset (three high-resolution scans and the T_1 structural scan that matched the fMRI scan data) to the whole brain T_1 structural scan. This was carried out in the mrVISTA code, mrAlign [Nestares and Heeger, 2000], and is a two-stage process where the slice orientation of the scans is first approximately aligned by eye using a graphical user interface, then the two images are fully aligned computationally. This code is specifically optimized to align partial-brain images acquired with surface coils to whole-brain images acquired with volume coils. Once all views were aligned to the whole-brain T_1 structural scan they could be transformed into the flattened cortical space, along with their corresponding binary striate image.

In order to calculate percentage of overlap between functionally defined V1 and the observed myelination, an approach based on the cortical modeling was used. First, the segmented whole-brain scan was used to generate a model of the folded cortex (single pixel thickness), located 2 mm from the gray/white matter boundary, approximating the central layer of the cortex. For every point on this cortical model, perpendiculars to the surface were calculated up to a distance of 3 mm either side of the model. The regions of observed myelination and the functionally defined V1 were transformed to the same space as the whole-brain structural. Points on the cortical model were labeled as corresponding to V1 if any part of the perpendicular projections intersected with the V1 region of interest, and points were labeled as cortical striation if the projections intersected with the striate region of interest. This procedure is the 3-D equivalent of marking a line along the center of the gray matter and measuring the length of the line that runs along the cortex assigned as striate, and the length of the line that lies within the region defined as V1.

To assess the level to which the partial volume effect obscures the ability to observe the myelination of layers in the cortex, a measure of “effective resolution” was calculated. This value varies from 0.3 mm (the in-plane resolution in the read direction) to 1.53 mm (the diagonal of the voxel) and depends on the angle between the cortex at any particular point and the slice orientation. This value was calculated by taking the cortical surface model generated by mrMesh, which is based on the white matter segmentation of the whole-brain structural scan. At each tessellation of the model the vector representing the normal to the cortical surface was rotated (using the transform obtained from the computed alignment parameters) back to the high-resolution imaging space and the effective resolution recorded as:

$$r = \min\left(\frac{V_x}{\text{abs}(N_x)}, \frac{V_y}{\text{abs}(N_y)}, \frac{V_z}{\text{abs}(N_z)}\right).$$

where V_x , V_y , and V_z represent the voxel dimension in x, y, and z direction and N_x , N_y , N_z represent the x, y, and z components of the unit normal vector. For our data, V was taken as [0.30, 0.39, 1.5]. These values were then transformed to the flattened cortical representation and for each orientation and for each subject, percentage striate detection rates were calculated for voxels with an effective resolution above 0.6 mm and for those below 0.6 mm. This value is approximately twice the thickness of the stria of Gennari; therefore, detection is unlikely in voxels with lower resolution than this. In a similar way a measure of the curvature of the mesh (approximated as $R/(\text{abs}(R) + \sqrt{A})/4$ where R is the signed difference between a point and the average plane of its connected neighbors, and A is the area of all the triangles featuring this point) was calculated as part of the cortical flattening process. These values were transformed to the flattened cortical representation and the striate detection rate at the fundus of the sulci (curvature < -0.1) was compared to the detection rate in the rest of the cortex in order

to test the detectability of myelination patterns deep within the sulcus.

RESULTS

In all high-resolution images it was possible to visualize regions of the cortex with intensity patterns consistent with a central hypointense band. Such a band is indicative of a focal region of dense myelination. Two examples of very high resolution structural images from one of the subjects are shown in Figure 1. The first image shows a slice oriented perpendicularly to the calcarine sulcus, with cerebrospinal fluid (CSF) appearing brightest and white matter appearing darkest. Within parts of the gray matter along the calcarine sulcus a dark band can clearly be seen (magnified region). The second image shows a sagittal slice through the calcarine sulcus, and again a dark band can be seen within the gray matter. No Gibbs ringing artifact was observed in the high-resolution scans.

On the right side of Figure 1 are example intensity profiles taken through the gray matter at different locations in the image (as indicated by white lines). For both example images, signal intensity profiles A are taken through a region where the observers clearly identified a hypointense band in the cortex. In these profiles a strong dip in signal intensity is present (indicated by the arrow). Profiles B are taken from regions of the cortex that were identified as being within the region functionally defined as V1 on the basis of the retinotopic mapping, but were not classified by observers as containing a dark band. In both these examples a small dip can be seen in the profile, indicating that myelination may be detectable, but is not identified by an observer because of the shallowness of the dip. Profiles C are taken from regions outside of functionally defined V1 and show no obvious dip in the signal intensity at the center of the cortex.

In order to visualize the entire occipital lobe, the data were transformed onto a flattened cortical sheet. Figure 2 shows data from the same subject in this format (left hemisphere). The top image shows the phase of the fMRI response to the rotating wedge stimulus, for response coherence values > 0.5 . The foveal representation was identified from the expanding rings stimulus (data not shown), and the borders between V1 and V2 were identified (white lines) based on the phase reversal that occurs when the stimulus reaches the vertical meridians. Note that the retinotopic mapping allows the definition of the lateral borders, but not the anterior or posterior extent (the anterior extent is limited by the size of the visual field viewed by the subject). Figure 2b shows the cortical sheet shaded such that regions deep within the sulci are dark gray and regions on the gyri are light gray. Overlaid on this anatomy in red is the region identified as the primary visual cortex on the basis of the retinotopic mapping. The V1/V2 boundaries of the primary visual cortex are shown as solid red lines on all the subsequent images. The next image (Fig. 2c) shows in blue the combined region from the coronal, sagittal, and axial scans where striate cortex

Figure 1.

Example high-resolution images ($0.3 \times 0.3 \times 1.5$ mm) showing the dark myelination within the gray matter surrounding the calcarine sulcus. Example profiles through the gray matter from the boundary with white matter (left) to the boundary with CSF (right) are shown at three points: (A) in regions where cortical striation (indicated by the arrow) was clearly seen; (B) in regions that lie within functionally defined V1 but where cortical striation was not identified by the observers; (C) in regions outside of functionally defined V1.

was identified. While a high proportion of the region identified as V1 shows striate myelination patterns, the overlap is not complete.

Of the five subjects scanned in this experiment, the total amount of striate cortex identified as a percentage of the V1 area derived from the functional scans varied from 81% for Subject 1 (the example dataset) to 33% for Subject 5 (mean = 56%). The summary data for all subjects are shown in Table I. The percentage of the region where striate cortex was identified within functionally defined V1 is given for each of the three views and for the combined data from the three scans. It should be noted that the volume for which all three structural scans intersect does not contain all of V1 (~50%). This means that the percentages in the "Combined"

column are in some cases greater than the sum of that for the three orientations.

To investigate the reasons why coverage is not complete, and varies between subjects, we looked at two measures that could affect these results: effective resolution and curvature of the cortical surface. Figure 3 provides a representation of the effective resolution for each voxel of the flattened cortical sheet. These values range from the best resolution of 0.3 mm (represented by green), to the worst of 1.53 mm (red). Overlaid in blue outline on these maps are the regions where striate cortex was identified in the particular scan. The red lines represent the V1/V2 border as determined by the retinotopic mapping. The three images in this figure represent data acquired parallel to the plane of the calcarine

TABLE I. Percentage of cortical surface area within functionally defined V1 where myelination was observed

	Coronal (%)	Sagittal (%)	Axial (%)	Combined (%)
Subject 1	50	40	31	81
Subject 2	27	11	11	41
Subject 3	44	13	20	65
Subject 4	40	6	12	60
Subject 5	5	19	7	33

sulcus (labeled axial), and acquired perpendicularly to this plane (labeled coronal and sagittal). The average detection rate in any one orientation, across all subjects, was 3.2 times greater in regions where the effective resolution was ≤ 0.6 mm compared to regions > 0.6 mm (22.9–7.1%, $P < 0.001$ using sign test).

One region that showed a consistently lower detection rate was the fundus of the calcarine sulcus. Figure 4a shows an example of this, where the stria of Gennari is clearly seen along the flanks of the sulcus, but stops being visible as the fundus of the sulcus is reached. This is further illustrated in Figure 4b–d, which shows a measure of curvature of the folded cortex at each point on its flattened representation in this and two further subjects. In this image green represents the areas where the curvature is positive (gyri) and the yellow and red patches represent regions where the curvature is negative (sulci). Overlaid again on this figure is the combined region where striate cortex was identified (blue outline) and the borders of V1 (red lines). Across all five subjects the striate detection rate was slightly higher in the gyri and flat portions of the cortex compared to the sulci (53.3–47.8%, $P = 0.06$ using sign test).

DISCUSSION

Quantification and Intersubject Variability

Using high-resolution in vivo MRI we have demonstrated that the cortical striation indicative of the stria of Gennari can be detected through over 80% of the volume of functionally defined V1, under optimal conditions. There is, however, a high level of variability in the amount of striate cortex that can be detected across subjects. This variability is partly due to the signal-to-noise ratio (SNR) in the individual images. There is a significant correlation between the mean SNR for the three anatomical scans and the total amount of striated cortex detected ($r = 0.88$, $P < 0.05$). Subject 5, in particular, had scans with significantly lower SNR than the other subjects. Due to the size and shape of this subject's head, it was not possible to position the surface RF receive coil as close to the occipital cortex as for the other subjects. While the use of surface receive coils is essential to obtain the necessary SNR for such studies, the variation in positioning that can result must be carefully considered when comparing myelination patterns across subjects.

More important for the detection of cortical myelination is the contrast-to-noise ratio (CNR) between the cortical gray

matter and the myelinated layers. This depends not only on the underlying SNR, but also the choice of image contrast and the amount of partial volume averaging that occurs. The optimization of CNR is therefore a complex trade-off between voxel resolution, subject movement, scan time, and scan coverage. We chose a relatively high in-plane resolution (300 μm) such that the signal from the layer IVb myelination, which is ~ 280 μm thick [von Economo and Koskinas, 1929], would not be significantly diluted by partial voluming effects. However our choice of center-out k -space ordering has an implicit filtering effect on the data, providing a small level of blurring in the phase encode direction [Deichmann et al., 2000; Mugler et al., 1992]. Center-out ordering was chosen in order to maximize the SNR, but this comes at the expense of some resolution. Simulations using appropriate values for proton density, T_1 and T_2 based on measured values at 3 T [Clare and Jefferies, 2001], showed that despite this blurring resolution of white matter structures of the thickness of the stria of Gennari should still be visible. Further improvements may be achieved by applying k -space filters [Deichmann et al., 2000] or by using variable flip angle pulses in the approach to steady-state [Mugler et al., 1992]. Our choice of slice thickness (1.5 mm) was based on SNR considerations, but also on the desire to include as much coverage of V1 as possible in a reasonable scan time. Since the scans with highest coverage lasted 75 min, there is little scope for increasing resolution further within a single-scan session. It is also important to remember that reduction of the slice thickness while maintaining the coverage and SNR will significantly increase the scan duration. SNR is directly proportional to voxel size but proportional to the square root of the number of averages. Therefore, reducing the slice thickness from 1.5 mm to 1 mm would require scan times that are over three times as long to maintain the same SNR and coverage.

We believe that a more effective way of increasing the area of detection is to use multiple slice orientations. As demonstrated by Figure 3 and Table I, each orientation is sensitive to different regions of cortex and the sum of each give more information than any scan alone. In the subject shown in Figure 3, the coronal view shows the highest amount of striate and the axial the lowest. The reason for this is clear from the values of effective resolution. Some of the significant gaps in the observed striate cortex lie in regions of the flattened cortex where the effective resolution is worst. These maps of effective resolution also demonstrate that, compared to other planes, the axial plane is not optimal in this subject for detecting striate cortex since there are more extensive regions where the effective resolution is worse than 0.6 mm. This measure of effective resolution depends on the particular pattern of cortical folding for the individual subject, but for all subjects the coronal view provided the most extensive areas of acceptable resolution. A further step would be to combine the data from the three orientations to produce a single subsampled image of higher resolution. This may aid a continuous detection of striate even as the cortex bends around the sulci.

Lack of sufficient resolution alone does not account for all the regions where the stria of Gennari is not observed, as

Figure 2.

Representation of the functional and structural data in flattened space. **a:** The retinotopic data from the rotating wedge stimulus. The phase of the fMRI response is shown in color and the VI/V2 boundaries identified by the white lines. **b:** The pattern of sulci, with dark gray representing the sulci and light gray the gyri. The VI/V2 boundaries are shown as the red shading. **c:** The sulcal pattern and VI/V2 borders (now as red lines), and the combined area where cortical striation was identified in blue. Scale bar = 10 mm.

Figure 3.

Representations of the effective resolution at each point of the flattened cortex in color from green (0.3 mm) to red (1.53 mm). The blue outline represents the regions where cortical striation was observed in the high-resolution scans.

Figure 4.

Example of the loss of detection of the stria of Gennari deep within the sulcus. **a:** The hypointense band indicating the stria of Gennari is clear along the flanks of the calcarine sulcus (indicated by the red arrows) but is significantly less detectable at the end of the sulcus. **b–d:** Representation of the curvature at each point on

the flattened cortex of three different subjects, in color from sulcus (red) to gyrus (green). The blue outline represents regions where cortical striation is detected in any of the three high-resolution scans.

there are clear regions of high resolution with no identified striation. In the examples shown in Figure 4, the striate is most clearly detected in regions where the cortex is relatively flat; at the bottom of the calcarine sulcus it is much less clear. While the difference in detection rate is small, there is a significant decrease in striate detection rate when the curvature is less than -0.1 , that is, in the fundus of the sulcus. This finding confirms the observation of Clark et al. [1992] in their studies of MR scans of postmortem brains. This reduction in detection rate could either be a property of the cortical tissue or be due to detection methods. One possibility is that it is due to a change in the thickness of the cortical layers in the tight folds of the cortex. Welker [1990] observed that outer layers of the cortex (I and II) are gener-

ally thicker and the deeper layers (V and VI) are thinner in the fundus of the sulcus as opposed to the crown of the gyrus. This could lead to less distinction of the stria of Gennari as it passes round the fundus. Alternatively, it may be that in order to identify a region as containing striation, observers demand several pixels in a line showing striate features and cannot identify striate cortex unequivocally when they see just one or two darker pixels within the cortex. In regions of high curvature the changing effective resolution and the increased likelihood of poor registration mean that the striate appears patchier than along some of the long flanks of the calcarine sulcus. This is hinted at by the cortical profiles in Figure 1 that show regions within V1 where striate cortex was identified (A) alongside regions

within V1 where striate was not observed (B). They both show similar patterns, indicating that the observer-based detection is likely to have been a weakness in our approach. The use of automated detection tools, discussed in more detail below, would potentially reduce such distinction. However, it is in these regions of high curvature that automated profile extraction will also perform less well.

In order to reliably detect myelination patterns more generally in the brain, and specifically to identify borders between areas, it will be important to quantify the level of sensitivity in any particular region. The measures of effective resolution and cortical curvature may be useful in this respect. In our data we did not find a clear threshold of curvature or effective resolution that could be used to predict the complete presence or absence of detection of myelination patterns. This may be due in some part to the inaccuracies that are introduced by the cortical modeling process and in projecting the regions where myelination was detected by the observers onto this cortical surface. However, our data demonstrates that these markers significantly affect the ability to detect myelination patterns.

Improvement in detection of myelination patterns will inevitably require better resolution and higher SNR. Strategies for increasing SNR include imaging at higher magnetic field strength, improved RF coil design, an increase in the number of receiver channels and enabling datasets acquired over multiple sessions to be reliably combined.

One of the most challenging aspects of using our methods for linking cortical myeloarchitecture to specific function is the registration of images acquired with different contrasts and resolutions to the same space. Since all the data are eventually interpreted in the flattened cortical space, the registration of both the very high resolution structural and the EPI functional images to the whole-brain structural scan is of critical importance. While at 3 T distortions in EPI images can be a significant problem in many areas of the brain, the occipital lobe does not suffer too greatly, due to its distance from the major sinuses. All registrations were examined visually and alignment of the EPI to structural in the cortex around the calcarine sulcus was consistently good. The alignment of the very high resolution structural scan to the whole-brain structural was less reliable. The combination of a novel contrast, signal drop-off due to acquisition with surface coils, and low SNR meant that the sulci did not always align well without careful manual intervention in the registration process. Image registration methods are often highly optimized for registering a particular type of image to another, and so with further work it is hoped that a reliable and fully automated method for accurate alignment of these scans can be found.

Observer-Independent Pattern Detection

The use of observer-based striate identification, while sensitive to the identification of hypointense bands in the convoluted cortex, is likely to lead to increased variance since observers implicitly use subjective criteria. These criteria include the continuity of the banding and the relative con-

trast between the myelinated and nonmyelinated layers. In Figure 1 the profiles (B) from places within functionally defined V1 show a small intensity dip within the cortex despite not being identified as striated cortex by observers. Such differences in intensity change could be a real variation in myelination level, but given the proximity of these voxels to areas of well-defined myelination, they are more likely to be the result of partial volume effects.

Methods using automated cortex profiling and characterization have been applied very successfully to digital images of histological sections [Annese et al., 2004; Schleicher et al., 2000]. Eickhoff et al. [2005] and Walters et al. [2003b] have demonstrated some success with a computational method using *in vivo* MRI data. Automated methods, however, require very accurate segmentation of the cortex, which, although achievable in histologically stained sections and high SNR postmortem MR images, is very difficult with *in vivo* MR scans. Our experience of segmentation of the high-resolution MR scans using the FAST algorithm [Zhang et al., 2001] is that, while it was moderately successful at identifying the cortex, this segmentation was not reliable enough to generate the cortical profiles necessary for automated pattern detection. These techniques tend to be applied to 2-D data, but due to the highly convoluted nature of the cortex and the relatively coarse resolution of even the best *in vivo* MR scans, a full 3D approach is necessary. Such an approach may well need to incorporate some form of modeling of the cortical surface, in particular to account for the variation in partial voluming effects that will occur even if the resolution is isotropic. Such partial voluming effects are not a problem in very high resolution postmortem tissue photographs, but are a significant hurdle in MR until resolutions well below the thickness of the cortical layers can be achieved. We do believe, however, that this problem is tractable, and will lead not only to the independent identification of myelination patterns, but also to the detection of different myelination patterns in other regions of the cortex.

Other Markers of Cortical Architectonics

The methods described in this article all rely on a detection of intensity changes based on the T_1 changes that occur with myelination. There are, however, other potential contrasts that should be sensitive to the myelo- and even cyto-architectonics of the cortex. The use of magnetization transfer (MT) contrast [Wolff et al., 1991] has shown to be very sensitive to myelination changes, and has been used to study demyelination in multiple sclerosis [Davies et al., 2003]. Application of MT pulses to high-resolution imaging sequences should increase the contrast discrimination between myelinated and nonmyelinated layers. Another issue is the time taken for acquisition. Recently, visualization of the stria of Gennari has been possible using a fast spin echo (FSE) sequence lasting only 6 min [Fernández-Seara et al., 2004]. These scans were acquired at 4.7 T, and show how higher field MRI systems can bring great gains in SNR, provided that issues of RF homogeneity can be addressed.

Another macroscopic marker for the underlying cortical architecture is the thickness of the cortex. There have been several attempts to measure such differences [Fischl and Dale, 2000; Hutton et al., 2003; Walters et al., 2004], although these are also very dependent on the quality of the image segmentation and cortical modeling.

Some of the most promising methods for imaging the cortical architecture are based on diffusion imaging. These methods map the degree to which water is hindered in its free diffusion in the brain. Since water can more freely diffuse along the direction of cortical fibers, it has been possible to infer connectivity from such measurements. Recent work has shown how it is possible to use this methodology to parcellate the cortex based on its connectivity as measured using this noninvasive in vivo approach [Behrens et al., 2003; Johansen-Berg et al., 2004]. Since myeloarchitectonic features are related to the connectivity, such methods may prove to be able to delineate cortical regions using MRI scanning at low resolution. Alternatively, it may be possible to use very high b-value diffusion imaging to directly detect myelo- and cytoarchitectonics based on local differences in diffusion anisotropy. Recent work has shown some success with this [Wedeen et al., 2004; Wiegell et al., 2003], and the availability not only of higher field strength scanners, but also those with higher gradient strengths will help to advance such an approach.

CONCLUSION

We have demonstrated that the myelination patterns of the stria of Gennari are detectable in 30–80% of the central region of the primary visual cortex. While there are some significant methodological issues still to be addressed, particularly in the automated detection of the patterns, we believe that MR cortical myelography has the potential to be used more widely within the brain, provided that appropriate measures for the likelihood of detection are found. By choosing appropriate orientation of imaging slice and by incorporating data from multiple scans, myeloarchitectonic boundaries within the cortex should be distinguishable. Automated methods for detecting and characterizing cortical profiles from the images will further improve the detection and reproducibility of myeloarchitectonics, but models of the partial volume effects will need to be incorporated. The gradual availability of very high field (>4 T) MRI scanners to the research community and the increase in the sophistication of multiple channel receiver coils will undoubtedly mean that higher resolution MR scans will be possible, enabling more widespread noninvasive mapping of cortical myelination.

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