The morphology of axonal and dendritic arbors in the immature cerebral cortex influences the degree of anisotropy in water diffusion. This enables cortical maturation to be monitored by the noninvasive technique of diffusion tensor magnetic resonance imaging (DTI). Herein, we utilized DTI of postmortem ferret brain to quantify regional and temporal patterns in cortical maturation. We found that diffusion anisotropy within the isocortex decreases over the first month of life, coinciding closely in time with expansion of axonal and dendritic cellular processes of pyramidal neurons. Regional patterns consist of differences between allocortex and isocortex, a regional anisotropy gradient that closely parallels the transverse neurogenetic gradient, and differences between primary and nonprimary isocortical areas. By combining the temporal and regional factors, the isocortical developmental gradient magnitude corresponds to a 5-day difference in maturity between relatively developed rostral/caudal isocortex at the gradient source and less mature isocortex at the occipital pole. Additionally, the developmental trajectory of primary areas precedes nonprimary areas by 2.7 days. These quantitative estimates coincide with previous histological studies of ferret development. Similarities in cerebral cortical diffusion anisotropy observed between ferret and other species suggest the framework developed here is of general potential relevance.

Keywords: brain, development, DTI, dendritic branching, ferret

Immediately after pyramidal neurons of the isocortex migrate from telencephalic ventricular zones to the cortical plate, they begin to differentiate (Rakic 1995). Dendrites, cell bodies, and axons begin as simple elongated structures, oriented perpendicular to the pial surface, and then gain structural complexity as they form interconnected, functional neural circuits (Conel 1939). This period of morphological development can be studied using diffusion tensor imaging (DTI) by monitoring the loss of water diffusion anisotropy with age (Huppi et al. 1998; Neil et al. 1998; Mori et al. 2001; McKinstry et al. 2002; Maas et al. 2004; Partridge et al. 2004). Structures such as axons and dendrites preferentially restrict water diffusion parallel to the pial surface in immature tissue, but water diffusion is restricted nearly uniformly in all directions in mature isocortex. Thus, as the isocortex matures, isocortical diffusion anisotropy decreases (Neil et al. 1998; McKinstry et al. 2002; Maas et al. 2004; Kroenke et al. 2007; Huang et al. 2008).

Recently, it has become recognized that, in addition to the temporal reduction of cortical diffusion anisotropy with development, spatial patterns throughout the isocortical sheet also reflect diversity in morphological structure at individual stages of development (delpolyi et al. 2005; Kroenke et al. 2005, 2007; Sizzenko et al. 2007; Huang et al. 2008). Although the biological source of regional variation in cortical diffusion anisotropy is not known, consistencies have been noted with other aspects of development. A rostrolateral to caudal/medial gradient in cortical diffusion anisotropy has been observed (Kroenke et al. 2007; Huang et al. 2008) that parallels known patterns of neurogenesis and synapse formation. Second, it has been observed that primary sensory/motor cortical areas precede nonprimary cortices in reduction in diffusion anisotropy (Kroenke et al. 2007). The overall objectives of the study described here are to test the hypothesis that regional diffusion anisotropy patterns within the ferret isocortex parallel previously described patterns of neurogenesis, synaptogenesis, and morphological development, and to develop a quantitative framework to characterize regional patterns of cortical development using DTI. In addition to regional variation in cortical diffusion anisotropy, the “inside-out” sequence of neurogenesis confers a laminar pattern of diffusion anisotropy in which superficial cortical layers exhibit highest diffusion anisotropy throughout early stages of cortical maturation (Kroenke et al. 2005, 2007; Sizzenko et al. 2007; Huang et al. 2008). In recognition of this, the analysis developed herein focuses on the temporal and regional trajectory of diffusion anisotropy within a consistent lamina (the least differentiated) across the deep to superficial gradient.

Temporal and regional patterns of isocortical development have previously been extensively characterized in the ferret (Mustela putorius furo) using histological and physiological methods. From embryonic days E24 to E40 (ferret gestation is approximately 42 days), a regional pattern of neurogenesis has been described (McSherry 1984; McSherry and Smart 1986). Neurons of the rostrolateral cortex are first to migrate to the cortical plate, neurons of the occipital pole are last, and throughout the intervening isocortex, a neurogenetic gradient exists. The pattern observed in ferret isocortical development is highly similar to the transverse neurogenetic gradient (TNG) described in detail in rodent species (Bayer and Altman 1991; Takahashi et al. 1999; Caviness et al. 2002; Tarui et al. 2005), and the gradient in isocortical maturation emanating from the insula reported in primate species (Sidman and Rakic 1982). This anterior/posterior gradient has been further confirmed in ferrets using birthdating experiments (Jackson et al. 1989; Noctor et al. 1997), and histochemical and electron micrographic characterization of synaptic development (Voigt et al. 1993). Quantitative golgi studies of primate cortical development have revealed a second factor of potential relevance to regional patterns of cortical diffusion anisotropy. Primary areas of the isocortex undergo morphological differentiation prior to...
nonprimary areas (Conel 1939; Travis et al. 2005). The boundaries of primary visual (Manger et al. 2002, 2004), auditory (Bizley et al. 2005), and somatosensory areas (Leclerc et al. 1993; Rice et al. 1993; McLaughlin et al. 1998) of the ferret isocortex have been described relative to anatomical landmarks, as well as specific nonprimary (Leclerc et al. 1993; Rice et al. 1993; Manger et al. 2002, 2004) and multimodal areas (Ramsay and Meredith, 2004; Bizley et al. 2007). Diffusion anisotropy measurements have therefore been performed on ferrets to characterize the influence of these factors on the trajectory of isocortical diffusion anisotropy loss with development. Findings are expected to provide comparative data that will extend previous findings of regional patterns of development reported in rat (Huang et al. 2008), baboon (Kroenke et al. 2007), and human (delpolyi et al. 2005). Additional reasons that ferrets have been chosen for this study are because they are more readily available than nonhuman primate subjects due to large litter sizes; they are born at an early stage of brain development, so that premature birth or in utero manipulations are not necessary to study the relevant period of development; and because they possess gyroencephalic brains similar to primate (but not rodent) species.

The 3 specific hypotheses generated in previous exploratory analyses of other species, which are to be tested in this study, are as follows: First, significant diffusion anisotropy is expected to be observable, and decrease with age, in the ferret isocortex over the first month of life. This developmental period corresponds approximately to the second half of gestation in primates (Mckinstry et al. 2002; Kroenke et al. 2007), and the first 10 days of life in rats (Huang et al. 2008). Throughout this period, significant diffusion anisotropy is expected to be exclusive to isocortex, and as observed in the baboon (Kroenke et al. 2007), a distinct high-to-low transition in fractional anisotropy (FA) is expected at the isocortical/allocortical border. Second, over the first postnatal month, a regional pattern of diffusion anisotropy is expected throughout the isocortex that parallels the regional pattern of the TNG (McSherry 1984; McSherry and Smart 1986; Noctor et al. 1997). Third, primary cortical areas are expected to exhibit lower cortical diffusion anisotropy than nonprimary areas due to their relatively early morphological development. The second objective is to provide a quantitative framework that simultaneously accounts for each of the factors that influence temporal and regional patterns of FA within the superficial isocortex. Comparisons between the results of the analysis and previous histology-based studies of ferret isocortical development are discussed to relate the comprehensive temporal and regional characterization of morphological differentiation presented here to the previously characterized context of regional patterns in neurogenesis and synapse formation.

**Materials and Methods**

**Animal Care**

Brains from 11 female ferrets of varying ages (listed in Table 1) were analyzed. Females were selected for the current study to avoid potential confounds associated with sex differences, though we are unaware of differences in morphological differentiation of the ferret cerebral cortex between normally developing males and females. Ferret litters were purchased from Marshall Bioresources (North Rose, NY) and delivered to the Department of Comparative Medicine of Washington University on postnatal day 4 (P4), or Oregon Health and Science University on P5. Ferrets were supplied with food (Purina, St. Louis, MI) and water ad libitum. From arrival to 2 months of age, kits were fed a high density ferret diet (51.1%) mixed with kitten milk replacer. Adults were fed the normal ferret diet (52.60). Litters were also supplemented with Nutri-Cal (EVSCO). All experiments were approved by local Institutional Animal Care and Use Committees, and carried out in accordance with the NIH “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 86-23, revised 1987).

**Tissue Collection**

Animals were injected with 0.5 mL euthasol (i.p.). Heparinized phosphate buffered saline (PBS) was injected into the left cardiac ventricle until the fluid of the right atria was clear. Phosphate buffered paraformaldehyde (% pH 7.4) was then perfused through the left ventricle for approximately 10 min. Eight of the 11 brains were extracted and placed in 4% paraformaldehyde indefinitely (Table 1). The remaining 3 brains (Table 1) were placed in 4% paraformaldehyde for 24 h, and then sectioned along the midsagittal plane and transferred to PBS at 4 °C for future histological processing. For these 3 brains, the right hemispheres were transferred to Fluorinert Electronic Liquid (FC-77, 3M, St. Paul, MN) immediately prior to imaging, and returned to PBS following magnetic resonance imaging (MRI) procedures.

**MRI Procedures**

Single-turn solenoidal coils, matched in size to each sample (Table 1), were utilized for radiofrequency transmission and reception. Experiments were performed using a 4.7 T magnet (Oxford, UK) interfaced with Nutri-Cal (EVSCO). All experiments were approved by local Institutional Animal Care and Use Committees, and carried out in accordance with the NIH “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 86-23, revised 1987).

**Table 1**

<table>
<thead>
<tr>
<th>Braina</th>
<th>Image resolution (mm)</th>
<th>MRI settings: TE/TR/NEX/B0b</th>
<th>Total time (h)</th>
<th>SNRC</th>
<th>RF coil diameter (cm)</th>
<th>Long-term storaged</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6a</td>
<td>0.25</td>
<td>0.067/4.0/8/4.7</td>
<td>14</td>
<td>35</td>
<td>1.3</td>
<td>PF</td>
</tr>
<tr>
<td>P10a</td>
<td>0.2</td>
<td>0.067/4.0/4.7</td>
<td>14</td>
<td>30</td>
<td>1.6</td>
<td>PF</td>
</tr>
<tr>
<td>P13a</td>
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<td>0.067/5.5/4.7</td>
<td>20</td>
<td>57</td>
<td>1.6</td>
<td>PF</td>
</tr>
<tr>
<td>P13b</td>
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<td>0.042/2.8/8/11.7</td>
<td>36</td>
<td>36</td>
<td>1.7</td>
<td>PBS</td>
</tr>
<tr>
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<td>0.3</td>
<td>0.067/5.8/6/4.7</td>
<td>31</td>
<td>20</td>
<td>22.5</td>
<td>PF</td>
</tr>
<tr>
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<td>0.042/10.5/6/11.7</td>
<td>36</td>
<td>67</td>
<td>1.7</td>
<td>PBS</td>
</tr>
<tr>
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<td>0.067/3.9/6/4.7</td>
<td>21</td>
<td>24</td>
<td>22.5</td>
<td>PF</td>
</tr>
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<td>0.035/3.5/6/4.7</td>
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<td>25</td>
<td>2.5</td>
<td>PF</td>
</tr>
<tr>
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<td>0.042/10.0/6/11.7</td>
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<td>72</td>
<td>1.7</td>
<td>PBS</td>
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<tr>
<td>P37b</td>
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<td>0.035/3.5/6/4.7</td>
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<td>50</td>
<td>3.0</td>
<td>PF</td>
</tr>
<tr>
<td>adult</td>
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<td>0.035/3.5/6/4.7</td>
<td>17</td>
<td>86</td>
<td>2.5</td>
<td>PF</td>
</tr>
</tbody>
</table>

a Brain name endings a and b denote use of DTT sensitization scheme A or B, respectively. Scheme B was used for the adult brain.
b TE and TR are in units of seconds, NEX is the number of repetitions, and B0 is in units of Tesla.
c SNR is defined as the mean signal intensity observed within cortical gray matter in a magnitude image in which b = 0, divided by the mean signal intensity within a region of the same image that is outside the sample and devoid of water.
d PF: 4% phosphate buffered paraformaldehyde, PBS: phosphate buffered saline.
with a Varian Inova console and a 10-cm inner diameter gradient coil (Varian, Palo Alto, CA), or an 11.7-T magnet interfaced with a 9-cm inner diameter magnetic field gradient coil (Bruker, Rheinstetten, Germany), as indicated in Table 1. A multi-slice spin-echo pulse sequence incorporating a Stejskal-Tanner diffusion sensitization gradient pair was used to acquire DTI data.

Two diffusion sensitization measurement schemes, herein termed scheme A and scheme B, were utilized to acquire DTI data in this study. Scheme A has previously been used by us to characterize cerebral cortical development in ex vivo nonhuman primate brain (Kroenke et al. 2007). In this series of experiments, each b value was unique in direction and amplitude, and the diffusion sensitization pulses were of 5 ms duration (δ), interpulse interval Δ of 50 ms, and magnitude G ranging from 0 to 38 G/cm. The TE value used in scheme A measurements was 67 ms. In more mature brain tissue, such as postnatal primate brain or subsequent to P31 in ferret, we have found that that reductions in the water H T2 value relative to earlier developmental stages results in insufficient signal to noise ratio (SNR) to optimally measure cortical diffusion anisotropy. Therefore, in order to incorporate measurements on tissue from more mature animals, it was necessary to perform additional measurements using a second experimental scheme. In scheme B, DTI measurements performed on brains from animals older than P31 were obtained by constraining TE to 42 ms, and b to 2.5 ms/μm². The remaining pulse sequence parameters were δ = 12 ms, Δ = 21 ms, and G = 11.6 G/cm. A 25-direction, isocohedral sampling scheme (Batchelor et al. 2003) was utilized for all experiments except for P37a and the adult brain, in which a reduced set of 22 directions were acquired. As described below, FA measurements that employed scheme A resulted in higher FA values relative to scheme B. Therefore, to quantitatively compare schemes A and B, measurements employing both schemes were also made on a subset of slices within a P51 hemisphere.

**DTI Analysis**

Diffusion tensor calculations were performed using nonlinear least-squares optimizations implemented in Matlab (The Mathworks Inc., Boston, MA). Data from each voxel were fitted to expressions corresponding to isotropic diffusion (a 2-parameter model), an axially symmetric diffusion tensor (a 5-parameter model) or a “standard” diffusion tensor, symmetric about the origin (a 7-parameter model) reported previously (Kroenke et al. 2006). A model selection algorithm, employing Akaike's information criterion corrected for small sample sizes (McQuarrie and Tsai 1998), was then used to select diffusion parameters from the appropriate diffusion model on an individual voxel basis. FA values were calculated from the eigenvalues of the diffusion tensor identified through the model selection algorithm using the standard formula (Basser and Pierpaoli 1996).

**Cortical Surface Generation and Surface-Based Analyses**

For each right hemisphere, surface models were constructed of inner (white matter to subplate or layer VI) and outer (layer I to the pial surface) boundaries of the cortex (olfactory bulbs were emitted at the lateral olfactory tract from all analyses). This was accomplished using standard functions in the CARET software package (www.brainvis.wustl.edu/caret) (Van Essen et al. 2001) and the manual editing approach previously described (Kroenke et al. 2007). Analyses were restricted to right hemispheres to avoid potential confounds associated with interhemispheric differences in cerebral cortical development (Dubois et al. 2008). Six anatomical landmarks, consisting of the coronalateral sulcus (CLS), Sylvian sulcus/presylvian sulcus (SS/PSS), suprasylvian sulcus (SSS), splenial sulcus (SpS), and cruciate sulcus (CS), and the anterior rhinal fissure (RF), shown in Supplemental Figure S1, were used to constrain the multiresolution morphing algorithm implemented in CARET to register the inner surface model to the outer surface model for each right hemisphere. Cortical mid-thickness surface models were then constructed from the set of surface coordinates midway between each coordinate pair in the inner-outer surface models. The mid-thickness surface was then registered to an adult mid-thickness surface, using the 6 anatomical landmarks described above. The adult mid-thickness surface was used as the atlas for all brains, on which surface regions of interest (ROIs; insula, primary visual, primary somatosensory, primary auditory, isocortex, allocortex, “unassigned” cortex, and the medial wall) were defined. The surface registration procedures enable surface ROIs delineated on the atlas model to be automatically projected onto each surface model for the hemispheres from younger animals.

Surface ROIs were defined on the atlas model relative to anatomical landmark structures. As described in the Results, the location of the insula was estimated to provide a first approximation of the source of the TNG. A surface node was chosen corresponding approximately to the center of the area designated insular cortex in the cat (Guldin et al. 1986; Clasca et al. 1997), as shown in Supplemental Figures S1, S2, and Figure 4. Surface ROIs were delineated on the atlas surface following published descriptions relative to gyral/sulcal landmarks for primary somatosensory (S1) (Rice et al. 1993; McLaughlin et al. 1998), auditory (A1) (Bialek et al. 2005), and visual (V1) (Manger et al. 2002, 2004) cortical areas, as shown in Supplemental Figure S2. Regions of the isocortex not included in these primary sensory areas were classified as nonprimary areas. The medial border of the isocortical ROI is located at the junction between the cingulate gyrus and the corpus callosum. Laterally, the isocortex borders allocortex, and the transition between these cortical regions is located at the fundus of the RF (Dennis and Kerr 1975; Lockard 1983, 1985). No obvious anatomical landmark can be used to reference the position of the caudal lateral border of the isocortex. Therefore, a conservative border that tends dorsally is drawn in this region to ensure that allocortical areas are not included in the isocortex (see white arrowheads, Fig. 1). The allocortex is bounded rostrally by the lateral olfactory tract (not included in the surface models presented here), and medially by periamygdaloid cortex. The boundary between allocortical areas and medial nuclear gray matter structures such as the amygdala is not possible to identify in the MRI data. Therefore, the approach adopted in a previous study of baboon cortical development (Kroenke et al. 2007) is taken here, in which regions of the cortical surface models medial to the ventral-most part of the pyriform lobe are termed “unassigned.”

**Figure 1.** In the first 10 days of life, the isocortex/allocortex boundary coincides with a transition from high-to-low FA. (a-h), Cortical FA is projected onto P6a and P10a cortical surface models, respectively. The lateral isocortical/allocortical boundary is marked by light blue spheres on each surface. As described in the text, the caudal lateral boundary (marked by white arrowheads) is drawn conservatively dorsal to avoid including allocortex within the isocortical surface ROI in subsequent analysis. Examination of T2-weighted images of the P10a brain (data not shown) indicate it was damaged at the rostral site marked by asterisks as it was being removed from the skull. (b-d, f-h) Coronal slices of FA parameter maps are shown for each brain at the 3 positions indicated with black lines. The blue trace marks the intersection of the inner (cortical/developing white matter) cortical surface model with each image plane. The isocortex/allocortex boundary is located at the fundus of the RF (blue arrows). At this location, a sharp transition is observed from high (isocortical) to low (allocortical) FA throughout the rostral/caudal extent of both brains. Left and right color scales are for surface models and coronal slices, respectively.
Laminar gradients in FA have been described in previous studies of developing rodent and baboon isocortex (Kroenke et al. 2007; Sizonenko et al. 2007; Huang et al. 2008). Such gradients correspond with histological observations that, in early-developing isocortex, cell bodies are more elongated (Dieni et al. 2004) and have less differentiated axonal/dendritic processes than neurons found in deep layers (Rakic 1972; Sidman and Rakic 1982). Therefore, in order to compare diffusion anisotropy between regions throughout the isocortical sheet, care must be taken to consistently sample diffusion anisotropy with respect to position in the laminar gradient. Cortical diffusion anisotropy is defined herein, for each node on the cortical mid-thickness surface, as the maximum FA value observed within the set of voxels intersected by 11 equally spaced points along a line segment from the inner cortical surface to the outer cortical surface, perpendicular to the cortical mid-thickness surface. The number of points was chosen to deliberately over-sample the number of voxels in the FA map that span the cortical wall, in order to ensure that each voxel along this normal segment was queried. Two approaches were taken to confirm the expectation that cortical FA values obtained using this procedure are located superficially in the developing cerebral cortex. First, the position of maximal FA along the 11 points spanning the inner to outer cortical surfaces was recorded for each isocortical voxel. This position corresponds to the superficial half of the cortex for 88% of voxels in brains of animals ranging in age from P6 to P24 and for 70% of voxels for brains from older animals. Second, voxels corresponding to isocortical FA values were highlighted in FA parameter maps and visualized using 3D-volume rendering Matlab functions. Consistent delineation of superficial cortex was observed in each of the 11 brains. All brains were devoid of obvious regional patterns in the superficial-to-deep position of maximal FA.

Computations involving the geodesic distance along the cortical mid-thickness were performed using standard functionalities of CARET software, or an implementation of Dijkstra’s algorithm (Cormen et al. 2001) within the Matlab programing environment. To control for differences in sizes of brains with age, geodesic distance is quantified in terms of a relative value, by dividing each geodesic distance by the largest geodesic distance observed within the hemisphere. Surface curvature was also determined at each surface node. This was done for a given node (reference node, j) by determining the tangent plane to the surface at that node, and computing the height, z, from the surface to the tangent plane for all other nodes (i = 1, 2, …, N) within a given radius of node j. The set of heights, (z), were fitted to a quadratic polynomial, \( P(x,y) \), of tangent plane variables x and y. The second derivatives of P with respect to variables x and y form a 2x2 matrix. The maximum principal curvature is the eigenvalue of this matrix with the largest magnitude: It represents the curvature of the surface in the “most curved” direction, according to a sign convention in which negative values indicate inward-facing curves (gyral tips) and positive values are outward-facing (sulcal troughs).

For all analyses investigating spatial and temporal patterns of cortical anisotropy, each cortical FA value was assigned attributes of age (a, in postnatal days), relative geodesic distance from a given surface node (d, ranging from 0 to 1), classification of primary or nonprimary isocortex (P = 1 or 0, respectively), and diffusion measurement scheme (S = 0 or 1 for schemes A and B, respectively). In all analyses, care was taken to avoid repeated sampling of individual voxels in the FA parameter map. If more than 1 cortical mid-thickness surface node corresponded to any 1 voxel in an FA parameter map, distance from the insula was recorded as the average distance of all coordinates sharing the same voxel. To resolve cases in which surface coordinates from multiple surface ROIs share a single voxel, the cortical FA value was attributed to the area with the largest number of coordinates. The number of unique voxels in the FA parameter map queried in this way by surface nodes within the isocortical ROI is given for each brain in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Brain</th>
<th>N°</th>
<th>Offset</th>
<th>Slope</th>
<th>( F_{\text{area}} )</th>
<th>( F_{\text{est}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6a</td>
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<td>0.0255</td>
<td>−0.0387</td>
<td>29.54</td>
<td>19.85</td>
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<td>0.1432</td>
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<td>0.1567</td>
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<td>0.0440</td>
<td>0.0031</td>
<td>112.5</td>
<td>0.2054</td>
</tr>
</tbody>
</table>

* N° is the number of voxels in the FA map identified for each brain.
* Offset is the (positive) difference between nonprimary and primary isocortical FA at a given point in the TNG.
* Slope is the change in isocortical FA per unit increase in relative geodesic distance from the TNG source.
* \( F_{\text{area}} \) is the F value calculated to compare the null and alternative hypotheses for the effects of area on cortical FA, accounting for distance. Degrees of freedom for all F values are (1, n – 3).
* \( F_{\text{est}} \) is the F value calculated to test whether geodesic distance from the TNG source significantly affects isocortical FA, taking differences between primary and nonprimary areas into account. Degrees of freedom for all F values are (1, n – 3).

The analysis of covariance (ANCOVA) was used to determine the effects of isocortical area (primary vs. nonprimary) on FA, taking into account geodesic distance from the estimated source points obtained from the collection of hemispheres were fitted to the expression

\[
\text{FA} = \left( \frac{x_j + \beta_3 \gamma_5}{x_j + (z_j - \gamma_5) \exp(- (a - \gamma_7)/x_j) + \gamma_2} \right) \quad \text{if} \quad a < \gamma_7, \\
\text{FA} = x_j + \beta_1 + \beta_2 a + P \text{or } \text{B} \quad \text{if} \quad a \geq \gamma_7, 
\]

in which

\[
\beta_7 = x_j + \beta_1 + \beta_2 a + P \text{or } \text{B} 
\]

using the nonlinear least-squares optimization function "lsqcurvefit" available through the Matlab Optimization Toolbox. The biological/ biophysical significance of the 7 adjustable model parameters \( x_j \) are as follows: At early stages of development (e.g., the day of birth), cortical FA is most pronounced, is uniform throughout the isocortex, and is given by \( x_j \). For each isocortical location, there is an age, \( \beta_7 \), in which neuronal differentiation begins to cause cortical diffusion anisotropy to undergo exponential decay at a rate characterized by the time constant \( x_j \). The threshold age \( \beta_7 \) is determined by \( x_j \), the age at which cortical FA begins to decay within nonprimary cortex located at the source of the TNG; \( \gamma_5 \), the difference in developmental stage between cortex located closest and furthest from the source of the TNG; and \( \gamma_7 \), the difference in developmental stage between primary and nonprimary cerebral cortex. The parameter \( \gamma_1 \) is the systematic difference in cortical FA values observed between measurements performed using diffusion anisotropy measurement scheme A versus scheme B. Herein, \( x_j \) are expressed in units of (postnatal) days; and \( x_j \) are unitless as they refer to FA values (Basser and Pierpaoli 1996).

Three variants of equation (1) were also considered. In the first variant, isocortical FA differences between primary and nonprimary areas are considered to be independent of age. In the second, primary and nonprimary cortical areas differ only in that primary isocortical FA decays at an accelerated rate. In the third, isocortical FA is assumed to decay linearly with age, rather than exponentially. The specific formulas for these variants to equation (1) are given in the Appendix.

**Statistics**

For statistical analyses, the FA value for each voxel was treated as an independent measure, as no interpolation or other transformations were performed to affect voxel intensity values in the DTI data. A 1-way, fixed effects analysis of covariance (ANCOVA) was used to determine the effects of isocortical area (primary vs. nonprimary) on FA, taking into account geodesic distance from the estimated source.
of the FA gradient. The null hypothesis was that there are no effects of primary/nonprimary area on isocortical FA taking geodesic distance from the FA gradient source into account. The alternative hypothesis for the model was that area has an effect on isocortical FA taking geodesic distance into account. For each age, F-statistics were calculated to compare the null and alternative hypotheses for the effects of primary/nonprimary cortical area on isocortical FA, accounting for geodesic distance from the FA gradient source. These F values are reported in Table 2 under the heading $F_{area}$. Additionally, F-statistics were calculated to test whether geodesic distance from the FA source significantly affects isocortical FA, taking differences between primary and nonprimary areas into account. These $F$ values are reported in Table 2 under the heading $F_{dist}$.

A further analysis was performed to evaluate whether surface curvature additionally influences cortical FA. Variables expressing geodesic distance, primary/nonprimary classification, and/or surface curvature were included in a multiple regression model if they met criteria of the stepwise multiple regression analysis implemented in SPSS. Stepwise inclusion and stepwise exclusion criteria for the $F$ statistics of the FA gradient. The null hypothesis was that there are no effects of area on isocortical FA when considering the TNG and differences between primary and nonprimary areas.

### Results

#### Cortical Diffusion Anisotropy in the Early Postnatal Isocortex and Allocortex

On postnatal days 6 and 10, cortical diffusion anisotropy is pronounced (mean isocortical FA $> 0.7$) and nearly homogeneous throughout the isocortex, as shown in the cortical mid-thickness surface models in Figure 1a,e. In contrast, diffusion is strikingly less anisotropic within the cerebral cortex ventral to the RF fundus coincides with the lateral isocortical/allocortical border (Dennis and Kerr 1975; Lockard 1983, 1985).

#### Reduction in Isocortical Diffusion Anisotropy over the First Postnatal Month

In Figure 2, mean isocortical FA is plotted versus age. Early in life (P6–P10), ferret isocortical diffusion anisotropy is high (0.74), and it decreases with age toward a value of 0.33 at maturity, with the majority of changes being observed from P10 to P31. In addition, FA changes with age, the Figure 2 data illustrate differences observed between FA measurements employing diffusion sensitization schemes A and B. Due to the inclusion of larger $b$ values in scheme A relative to scheme B, FA values recorded using scheme B (filled circles, Fig. 2) are systematically lower than those obtained using scheme A. To account for this offset, analysis using equation (1) includes a parameter $\beta_2$ that corresponds to the offset between diffusion measurement schemes, which is estimated to be 0.065 (described below, Table 3). To provide further confirmation that differences in FA between scheme A and scheme B measurements have been properly taken in to account, DTI data were acquired using both schemes on a P31 brain (data not shown). It was found that FA within the cerebral cortex was 0.063 larger when measured using $b$ values ranging from 0 to 12.5 ms/μm$^2$ (as in scheme A) compared with the 25 or 22-direction measurements employing a single $b$ value of 2.5 ms/μm$^2$ (scheme B), which is in close agreement with the $z_7$ estimate of 0.065. The gray curve in Figure 2 illustrates the general trend for isocortical FA to decrease exponentially after approximately P10 toward the FA value observed at maturity.

#### Regional Isocortical FA Heterogeneity within Individual Brains: Manifestation of the TNG and Differences between Primary and Nonprimary Isocortex

Figure 3 shows selected axial slices of FA parameter maps, and projections of cortical FA onto cortical mid-thickness surface models for brains P13b and P20b. As exemplified in the 2 brains, increasing isocortical FA from rostral to caudal positions along the cortical surface is consistently observed in all brains in the P10–P24 age range (Supplemental Fig. S2). Comparisons between isocortical caudal to the PSS, indicated by red arrowheads, and the TNG, cortical diffusion anisotropy was analyzed as a function of relative geodesic distance from a surface node located within the insula. The observed rostral/caudal isocortical FA gradient exhibits regional variation that is similar to the TNG, present in ferrets approximately 1 month prior (McSherry 1984; Takahashi et al. 1999). The TNG source has been proposed to originate near insular cortex (Sidman and Rakic 1982; Smart 1983). To further characterize the similarity between the isocortical FA gradient and the TNG, cortical diffusion anisotropy was analyzed as a function of relative geodesic distance from a surface node located within the insula. Results from ANCOVA calculations are given in Table 2. Highly significant effects of position within the isocortex on isocortical FA are observed throughout the first month of life.

In addition to the rostral/caudal gradient in isocortical FA, the Figure 3 FA parameter maps also show differences in diffusion anisotropy between primary and nonprimary cortical...
### Table 3
Dynamical parameters characterizing ferret isocortical FA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Equation (1)</th>
<th>Equation (2)</th>
<th>Equation (3)</th>
<th>Equation (4)</th>
</tr>
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<tbody>
<tr>
<td>$a_1$</td>
<td>Isocortical FA prior to reduction due to axonal/dendritic differentiation</td>
<td>0.742</td>
<td>0.745</td>
<td>0.741</td>
<td>0.741</td>
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<tr>
<td>$a_2$</td>
<td>Isocortical FA within mature cortex</td>
<td>0.327</td>
<td>0.339</td>
<td>0.331</td>
<td>0.371</td>
</tr>
<tr>
<td>$a_3$</td>
<td>Exponential time constant (days) reflecting the rate of reduction in isocortical FA</td>
<td>9.1</td>
<td>9.1</td>
<td>8.8</td>
<td>9.5</td>
</tr>
<tr>
<td>$a_3$, Equation (4)</td>
<td>Rate (1/days) of linear decrease in isocortical FA with age</td>
<td>8.2 (−0.023)</td>
<td>8.0</td>
<td>8.1</td>
<td>6.4</td>
</tr>
<tr>
<td>$a_4$</td>
<td>Postnatal age (days) in which reduction in a nonprimary isocortical area located at the TNG source begins</td>
<td>5.0</td>
<td>4.8</td>
<td>4.6</td>
<td>6.3</td>
</tr>
<tr>
<td>$a_5$</td>
<td>Difference (days) in development between cortex located at the source of the TNG versus at the occipital extreme</td>
<td>2.7</td>
<td>4.8</td>
<td>4.6</td>
<td>6.3</td>
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<tr>
<td>$a_6$, Equation (2)</td>
<td>Amount primary isocortical FA is reduced relative to nonprimary isocortex</td>
<td>0.046</td>
<td></td>
<td>3.2</td>
<td></td>
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<tr>
<td>$a_6$, Equation (3)</td>
<td>Amount (days) primary isocortical exponential time constant is reduced relative to nonprimary isocortex</td>
<td></td>
<td></td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>$a_7$</td>
<td>Difference in FA between measurements performed using DTI Scheme A and Scheme B</td>
<td>0.065</td>
<td>0.066</td>
<td>0.065</td>
<td>0.083</td>
</tr>
</tbody>
</table>

* Annotation in Figures 2 and 5 refers to equation (1) parameters.

---

**Figure 3.** Regional variability in isocortical FA. (a,f) Cortical FA is projected onto P13b and P20b cortical surface models, respectively. The isocortical/allocortical boundary (light blue), as well as primary visual (green), auditory (red) and somatosensory (yellow) area boundaries, are marked by color-coded spheres on each surface. Following annotations on the atlas surface, the locations of all boundaries were determined automatically through surface registration procedures as described in the text. (b-e,g-j) Horizontal slices of FA parameter maps for each position are indicated with black lines. Traces of the inner (cortical/white matter) surface model are shown in blue. Intersections between the horizontal slices and primary cortical areas are indicated with a colored line near the pial surface. Regional patterns are observable in which rostral regions (red arrows) are reduced in cortical FA relative to caudal regions (yellow arrows) at each age. In addition, cortical FA in primary areas is reduced relative to nonprimary areas.
areas. Primary somatosensory, auditory, and visual areas are delineated with yellow, red, and green borders, respectively, in Figure 3a,f and Supplemental Figure S2. In Figure 3b–e,g,j, primary cortical areas within the FA parameter maps are indicated with color-coded traces overlaying the pial surface. Isocortical FA within the primary visual cortex in Figure 3b,c,g,h, primary auditory cortex in Figure 3c,d,b,i, and primary somatosensory cortex in Figure 3b,c,h,i, is generally lower than within neighboring nonprimary areas. One noteworthy example is in the somatosensory cortex, in which the dorsally located forepaw and body representations (Leclerc et al. 1993; McLaughlin et al. 1998) are separated from the more ventral face representation by the intervening CLS (Leclerc et al. 1993; Rice et al. 1993). The CLS walls and fundus contain somatosensory cortex that is unresponsive to cutaneous stimulation (Leclerc et al. 1993) and is histologically distinct from the neighboring primary areas, possessing characteristics of callosally projecting nonprimary cortex (Rice et al. 1993). Figure 3c,b,i,h shows that cortical FA within the CLS is increased relative to the neighboring primary somatosensory areas in P13 and P20 brains. Additionally, a multi-sensory area has been described within the SS (Ramsay and Meredith 2004), and this region exhibits high isocortical FA relative to the primary cortical areas in Figure 3g–j. The ANCOVA results shown in Table 2 demonstrate that significant differences in isocortical FA exist between primary and nonprimary areas, provided covariation with geodesic distance is taken into account.

Ferret primary cortical areas tend to be located on gyral crowns. Therefore, the question of whether gyral/sulcal position contributes to isocortical FA was addressed. Surface curvature is a signed quantity that is large and positive at the trough of a sulcus, highly negative at the top of a gyrus, and near zero in flat regions of the cortex, such as along the wall of a sulcus. To examine whether surface curvature is related to isocortical FA, a stepwise linear regression model selection procedure was used. Significant correlations were observed between surface curvature and isocortical FA for most brains (brain P31a was the single exception), however, the degree of correlation is modest relative to geodesic distance and primary/nonprimary classification. For all but 2 brains (P6a and P31a, brains of age in which isocortical FA is relatively homogeneous), surface curvature accounted for less variance in FA than geodesic position or primary/nonprimary classification. Further, the direction of the surface curvature effect was not consistent throughout development. In brains P6a, P10a, P13b, P17a, P20b, and P31b, the correlation between isocortical FA and surface curvature is positive (i.e., FA is larger in sulcal funi than gyral crowns), and in the remaining brains the correlation is negative. The inconsistency in the direction of the effect in our data suggests a complex dependence of isocortical FA on position with respect to gyri and sulci. However, the magnitude of the effect indicates it is secondary to other factors being considered, and therefore subsequent calculations do not include effects of surface curvature.

**Determination of the Isocortical FA Gradient Source within Individual Brains**

Although the Table 2 results support the hypothesis that a rostral/caudal gradient in isocortical FA exists, a rigorous method for identifying the location of minimal FA is desired. Therefore, for each brain, ANCOVA calculations were performed treating each node in the isocortical surface ROI as the potential isocortical FA gradient source. For each calculation, the relative geodesic distance to all other nodes was determined and treated as an independent variable, with isocortical FA as a dependent variable. Figure 4a shows the sum of squared errors (SSE) for brain P13a that results from the ANCOVA calculations, projected onto each corresponding surface node. Lower SSE indicates better agreement with the ANCOVA model, which is interpreted as increased likelihood that the surface node under investigation is the isocortical FA gradient source. As with each brain within the age range P10–P24, a single minimum is observed in Figure 4a within the P13a rostralateral isocortex. In Figure 4b,c, locations of minimum SSE values following ANCOVA for brains P10 through P24 are projected onto the P13a surface model, and a flattened atlas model, respectively. Each sphere is color coded according to age as shown in the color bar. The identified surface nodes cluster in the rostral cortex, which is consistent with the isocortical FA gradient following the overall TNG pattern; however, a systematic difference is observable in which most ANCOVA minima are located dorsal to the insula (white spheres in Fig. 4).

**A Model of Cerebral Cortical Diffusion Anisotropy**

A model for the effects of age, position within the isocortical FA gradient, and identity as either primary or nonprimary isocortex, on cortical diffusion anisotropy is proposed in equation (1). According to this model, isocortical FA is initially maximal throughout the immature isocortex. Subsequently, once superficial cortical neurons begin to undergo morphological differentiation, isocortical FA decays exponentially toward isocortical FA at maturity. To determine the source of the isocortical FA gradient, SSE values were calculated following optimization of the equation (1) parameters.

**Figure 4.** Determination of the FA gradient source through analysis of residuals following ANCOVA (individual brains from P10 to P24 ferrets) and following equation (1) (all brains). (a) For each node in the P13a cortical mid-thickness surface, the SSE following ANCOVA is plotted according to the color scale (SSE values ranged from 6.53 × 10^{-3} to 8.78 × 10^{-3} for brain P13a). Low SSE indicates agreement between the data and the model incorporating effects of the TNG gradient. As with all brains, a single SSE minimum is observed, centered on the rostral lateral isocortex (red sphere). (b) TNG source estimates rendered on the P13a cortical mid-thickness surface. Estimates of the TNG source for individual brains from ferrets ranging in age from P10 to P24 (according to color bar) are shown as age color-encoded spheres. The TNG source estimated using SSE derived from equation (1) to analyze all 11 brains is indicated by a blue sphere (Node 4522). The initial estimate for the TNG source, located within the insula, is shown as a white sphere. (c) The same information presented in (b) is rendered on a flattened atlas surface.
simultaneously using data from all 11 brains, treating each node as the potential isocortical FA gradient source. The minimum SSE resulting from this analysis is Node 4522, shown as a blue sphere in Figure 4 and Supplemental Figure S2. In subsequent analyses, the origin of the isocortical FA gradient is held fixed at Node 4522.

Table 3 gives the results from optimizations using equation (1), and the Figure 5 surfaces illustrate the predicted isocortical FA behavior as a function of age and relative geodesic distance from Node 4522 for nonprimary (black surface), and primary (red surface) isocortex. According to the Table 3 parameters, morphological differentiation at the TNG source is first detectable on postnatal day 8.2 in nonprimary isocortex. Primary isocortical differentiation precedes nonprimary by 2.7 days. Thus, were a primary cortical area to overlap the isocortical FA gradient source, reductions in diffusion anisotropy would begin on postnatal day 5.5. Morphological differentiation in superficial cortex at the occipital pole, the developmentally least advanced location with respect to the TNG, begins 5.0 days later. Once begun, reductions in isocortical FA at any point in the isocortex follow an exponential decay, characterized by a 9.1 day time constant, toward the isocortical FA value at maturity. To provide a visual comparison between observed and predicted isocortical FA

![Figure 5. Modeling the combined effects of age, distance from the TNG source, and primary/nonprimary identity on isocortical diffusion anisotropy. Red and black surfaces correspond to primary and nonprimary cortical areas, respectively. Contours of the red surface are projected onto the age/relative geodesic distance plane (diagonal red lines). Correspondence between the surfaces and Table 3 parameters $a_t - a_b$ are shown.](image)

![Figure 6. Isocortical FA for primary (red) and nonprimary (black) areas versus relative geodesic distance from node 4522. Diffusion anisotropy increases with relative geodesic distance from node 4522, which is estimated to be the source of the FA gradient, and anisotropy is reduced in primary cortical areas relative to neighboring nonprimary areas. Solid lines are results from equation (1), using the fitted parameters listed in Table 3.](image)
values, the solid lines in Figure 6 show intersections of the red and black Figure 5 surfaces at 6 ages. Solid lines in Figure 7a show intersections of the Figure 5 surfaces as a function of age, for a fixed relative geodesic distance of 0.5. The root-mean-squared (RMS) residual for equation (1) is 0.0744, given in the Figure 7a inset.

Three variants of the equation (1) model were also considered. In the first variant, equation (1), primary/nonprimary differences are independent of age. An interpretation of this model is that inherent cytoarchitectonic differences between primary and nonprimary isocortex give rise to FA differences at all stages of development. As shown in Figure 7b, equation (1) provides a slightly poorer fit to the data than equation (1), with a corresponding RMS residual of 0.0771. In the second variant, equation (3), primary/nonprimary differences arise due to primary cortex maturing at an accelerated rate relative to nonprimary (but the initiation of differentiation is simultaneous with nonprimary cortex). This model is illustrated in Figure 7c, and also provides a slightly poorer fit to the data than equation (1), with a corresponding RMS residual of 0.0773. Last, a linear dependence of isocortical FA with age was investigated (eq. 4) as shown in Figure 7d. This functional form for the temporal decrease in FA with age was also found to provide a slightly poorer fit than the exponential decay of equation (1), resulting in an RMS residual of 0.0754. Each of the variants was therefore interpreted to be less likely than equation (1) based on the RMS residuals obtained. Optimization results from each variant are also given in Table 3.

**Discussion**

Water diffusion anisotropy within superficial ferret isocortex is sizeable in the first postnatal week, and decreases toward the value observed at maturity over the following month. These changes are interpreted here within the context of migration of pyramidal neurons from the ventricular surface to the cortical plate, and subsequent differentiation of these neurons to form mature cerebral cortex. Diffusion anisotropy measurements reveal regional heterogeneity in the pattern of cortical maturation. This heterogeneity reflects architectonic differences between isocortex and allocortex, a regional pattern in the age of neurons that results from the TNG, and differences in development between primary and nonprimary isocortex. Postmortem tissue has been used to provide a detailed characterization of these relationships because data may be collected on ex vivo samples over long periods of time (1-2 days), using customized MRI instrumentation, to obtain data possessing a high SNR. The high sensitivity achieved here was used to acquire data at high spatial resolution, or to acquire heavily diffusion-weighted images for purposes of maximizing precision in FA estimates. Previous systematic comparisons between DTI results obtained in vivo and with paraformaldehyde-fixed brains have documented that FA does not change with death (Guilfoyle et al. 2003; Sun et al. 2003; D’Arceuil and de Crespigny 2007; D’Arceuil et al. 2007). Ferrets are naturally born at an early stage of brain development, and are therefore amenable to in vivo studies of development by MRI (Neal et al. 2007). Therefore, it is anticipated that the framework

![Figure 7](image-url)
developed here will be of use in future longitudinal studies of development in vivo, within individual animals.

The brains investigated in this study were used to develop a framework for quantifying regional and temporal changes in isocortical FA with maturation. The patterns reported here for the ferret are consistent with previously observed patterns described for human (delCepo et al. 2005), baboon (Kroenke et al. 2007), and rat (Huang et al. 2008). Together, this comparative body of data is suggestive that a common framework can be used to interpret biological mechanisms underlying cortical FA changes with development. Application of DTI to ferret brain enables changes in isocortical FA to be precisely referenced to several critical stages of cerebral cortical development through comparisons between FA measurements and previous quantitative histological studies that have been performed in this species (Fig. 8).

Temporal Characteristics of Anisotropy Changes with Cortical Maturation

The data were analyzed in terms of a model in which isocortical FA evolves through 2 phases. In the first phase, newly born pyramidal neurons are migrating to the cortical plate. Isocortical FA does not change with age during this phase because the adopted analysis procedure extracts FA values specifically from the most anisotropic (i.e., the least mature) location between the inner and outer cortical surfaces, which is continually being replenished with new undifferentiated neurons. This phase corresponds to the flat region of the Figure 8 solid curves. In the second phase, morphological differentiation of axons and dendrites gives rise to reduction in cortical diffusion anisotropy with age, corresponding to the exponentially decaying regions of the Figure 8 curves.

Figure 8. Timeline comparison of isocortical FA changes to other developmental milestones. FA is plotted versus age for nonprimary isocortex at the source (blue curve) and distal-most extreme (green curve) of the FA gradient, using equation (1) parameters given in Table 3. The dashed portion of the curve prior to P6 is present only to guide the eye, as data from ages prior to P6 were not used in the equation (1) fitting procedure. Phases 1–6 of cortical histogenesis given by McSherry (1984) are represented as blue and green bars for isocortex at the TNG source, and distal extreme, respectively. Birthdates for layer II/III neurons are given for primary somatosensory (solid blue) and visual (solid green) areas. Dashed outlines extend 10 days following completion of neurogenesis to provide an estimate of neuronal migration periods. The onset of exponential decay in isocortical FA coincides with termination of neurogenesis and subsequent migration (arrows). Loss of isocortical diffusion anisotropy is associated with morphological differentiation of pyramidal neurons, and appearance of synaptophysin immunoreactivity (gray bars). References: 1) McSherry (1984), 2) Noctor et al. (1997), 3) Jackson et al. (1989), 4) Voigt et al. (1993), and 5) Zervas and Walkley (1999).

According to the parameters listed in Table 3, reductions in isocortical FA within primary and nonprimary areas located proximal to the neurogenetic gradient source begin at postnatal day 5.5 and 8.2, respectively. Neuronal birthdating studies of ferret somatosensory cortex (Noctor et al. 1997), a region that is located immediately adjacent to Node 4522 (Supplemental Fig. S2), indicate that layer II/III neurons are born over a period approximately spanning E35–E41. Equating E42 with postnatal day 0, this is 6.5–12.5 days prior to initiation of differentiation measured through changes in isocortical FA. A similar time interval separates birthdates for layer II/III neurons of visual cortex (P1–P8) (Jackson et al. 1989) and the earliest reductions in occipital cortex FA (P10–P13) (Table 3). It is estimated to require "several days" (Noctor et al. 1997), to "1–2 weeks" (Jackson et al. 1989) for ferret pyramidal neurons to migrate from ventricular zones to the cortical plate. Early reductions in isocortical FA therefore appear to coincide in time with pyramidal cell migration from ventricular zones to the cortical plate (Fig. 8 arrows). Initial reductions in cortical diffusion anisotropy also appear to be simultaneous with early phases of synaptogenesis, as made evident through the appearance of synaptophysin immunoreactivity, first noted within superficial layers of rostrally located ferret isocortex at approximately P7 (Voigt et al. 1993).

Isocortical FA decreases exponentially toward the value observed at maturity in concert with anatomical differentiation of pyramidal neurons. The FA value obtained here for mature isocortex, 0.33, is in close agreement with corresponding values obtained from rat on postnatal day 19 (Huang et al. 2008) and baboon at term (Kroenke et al. 2007). (Note that a relative anisotropy value of 0.20 reported for baboon corresponds to an FA value of 0.33.) The time constant characterizing the rate of reduction in anisotropy is 9.1 days (Table 3). Thus, anisotropy is within 5% of the mature value at P38 and P35.3 for nonprimary and primary isocortex located immediately within the TNG, respectively. It has been proposed that reductions in isocortical FA result from increases in complexity of axonal/dendritic arbors (McKinstry et al. 2002). Specifically, as the width of the distribution of axon and dendrite orientations increases with development, diffusion anisotropy is expected to decrease (Jespersen et al. 2007). Comparison of Figure 2 with pyramidal cell morphology studies of Zervas and Walkley (1999) demonstrate that reductions in isocortical FA are associated temporally with increases in the complexity of axonal and dendritic structure. Pyramidal neurons appear morphologically immature from P7 to P14, and approach maturity at approximately P35, which agrees closely with the timing of DTI parameters reported here (Fig. 8). This close agreement provides support for a link between anatomical differentiation of the cerebral cortex and the loss of diffusion anisotropy.

It is also noteworthy that changes in the laminar pattern of synaptophysin staining occur over a highly similar period of time, with immunoreactivity first being observed at P7, and the mature pattern being observed at approximately P29 (Voigt et al. 1993). Synaptic development per se is not expected to directly relate to changes in isocortical FA. However, synaptogenesis may be indirectly related to cortical diffusion anisotropy if it is regulated by common factors that stimulate cellular/anatomical development of the neuropil. Studies of primate species have generated varying viewpoints on the question of whether synaptic development exhibits regional variability similar to the...
TNG (Rakic et al. 1986; Granger et al. 1995; Huttenlocher and Dabholkar 1997). Although changes in isocortical FA also appear closely linked to the trajectory of synaptophysin immunoreactivity within the ferret isocortex (Voigt et al. 1993) (Fig. 8), this relationship is expected to be limited to the extent that synaptic development and morphological changes are influenced by the same regulatory mechanisms.

**Isocortex versus Allocortex**

A conspicuous transition in cortical FA was previously found in the medial temporal and frontal cortex of baboon (Kroenke et al. 2007), and was proposed to originate from differences between the developmental sequence leading to a 6-layered isocortex in comparison to the more primitive allocortex (Zilles 2004). The carnivore isocortex/allocortex boundary is located at the fundus of the RF (Dennis and Kerr 1975; Lockard 1983, 1985), and as a result the allocortex encompasses a much larger rostral/caudal extent of the brain than in primates (Brodmann 1909). A notable high-to-low transition in cortical diffusion anisotropy, similar to that observed in baboon, is observed within the P6 and P10 ferret brains at the fundus of the RF (Fig. 1). Evidence of the isocortical/allocortical distinction can also be seen in previously reported rodent brain data. For example, a dorsal/ventral transition from high-to-low FA within the developing rat cortex is observable in Figure 2 of Huang et al. (2008). Thus, despite interspecies differences in the position and extent of allocortex relative to the remaining cerebral cortex, the isocortical/allocortical boundary consistently coincides with a high/low transition in cortical FA.

**The Tranverse Neurogenetic Gradient**

Neurogenesis within the developing ferret isocortex is known to exhibit a "2D" rostral/caudal and lateral/dorsal gradient (McSherry 1984; McSherry and Smart 1986; Jackson et al. 1989; Noctor et al. 1997). In a previous exploratory DTI analysis of baboon (Kroenke et al. 2007), a regional gradient in isocortical FA was found to correspond with gradients in primate neurogenesis and synaptogenesis (Sidman and Rakic 1982; Granger et al. 1995). Additionally, regional patterns of cortical FA in developing rodent cortex (Huang et al. 2008), exhibit close similarity to the rodent TNG (Smart 1983). Therefore, in this study, the hypothesis was tested that ferret isocortical FA exhibits a gradient reflecting previously described regional variation in maturation. The TNG has been characterized in several species (Sidman and Rakic 1982; Smart 1983; McSherry 1984; Luskin and Shatz 1985; McSherry and Smart 1986; Bayer and Altman 1991; Takahashi et al. 1999; Smart et al. 2002; Tarui et al. 2005), and its origin has been proposed to be located near the insula (Sidman and Rakic 1982; Smart 1983; McSherry 1984), or at a site "opposite the internal capsule" (Marin-Padilla 1978). A significant increase in isocortical FA relative to allocortical FA was observed in brains ranging in age from P10 through P31 (Table 2). These findings therefore confirm our hypothesis and demonstrate an apparent close association between regional isocortical FA patterns and the TNG. This relationship was further explored in a quantitative manner to estimate the gradient origin and compare its magnitude with results obtained using other techniques.

The source of the isocortical FA gradient was determined as the cortical position in which the sum of squared residuals between observed isocortical FA, and values predicted by a model of the developmental gradient is minimal (Fig. 4). Due to the similarity between the isocortical FA gradient and the TNG, it is reasoned that the origins of these 2 regional patterns are approximately the same (however, this assumption will require further investigation to directly confirm). Analyses using the ANCOVA model for individual brains spanning 10--24 postnatal days of age consistently identified surface nodes on the rostral lateral surface as the TNG origin. Analysis of data from the 11 brains combined leads to identification of Node 4522, which could be regarded as an "averaged" position based on information from the collection of brains. Node 4522 lies dorsal to the initially hypothesized TNG source within the insula (compare white and blue spheres, Fig. 4 and Supplemental Fig. S2). However, the position of Node 4522 is actually more consistent with McSherry's Figure 9 (McSherry 1984) than is the node within the insula. McSherry describes the TNG source to be located approximately midway between the RF and the medial boundary of isocortex. Quantitative analysis of isocortical FA therefore provides a way to estimate the TNG source without requiring reference to an anatomical landmark.

The magnitude of the gradient in cortical maturation can be quantified as the difference in time taken to achieve a given stage of development between cortex located at the gradient source, and cortex located at the occipital pole. Using equation (1), this interval is estimated to be 5.0 days for ferret (parameter αs in Table 3). Previous independent studies have provided strikingly similar estimates of the TNG magnitude. McSherry (1984) performed an analysis in which cortical development was classified according to 10 stages, and the positions of contour curves representing regional fronts of each stage were determined as they coursed rostrally and ventrally over the period from E24 to E40 (Fig. 8). The complete progression throughout the cortex was reported for stages 1--6, in which contour curves for stages 2-4 spread throughout the cortex over periods of 4 days (E28--E32 for 2 and 3, E32--E36 for 4), whereas contour curves for stages 1, 5, and 6 spread throughout the cortex over periods of 8 days (E24--E32 for 1, and E32--E40 for 5 and 6). McSherry (1984) also noted "the progress of cortical plate across the surface of the cerebral vesicle is accomplished in about 5 days" in Marin-Padilla's (1978) study of early neocortical development in cats, a species whose brain develops at a highly similar rate to that of ferret (Issa et al. 1999). Additionally, Noctor et al. (1997) compared neuronal birthdates of somatosensory cortex (located adjacent to Node 4522) with those reported by Jackson et al. (1989) for visual cortex (located at the occipital pole) and found cortical-layer-dependent differences in development ranging from 0 (in deep layers) to 11 (in layers II/III, Fig. 8) days. Neglecting factors distinguishing primary and nonprimary cortex (described below), the close agreement between the TNG magnitude estimated in the above studies, and the isocortical FA gradient magnitude of 5.0 days (Table 3) supports an interpretation in which neuronal birth, migration to the cortical plate, and differentiation, all occur over an identical time period throughout the isocortex. Thus, the TNG magnitude gives rise to an equally sized developmental gradient in cortical differentiation, approximately 1 month after pyramidal neurons are born.

**Primary versus Nonprimary Cortical Areas**

Golgi studies of human cerebral cortex have identified differences between primary and nonprimary areas of the cerebral cortex.
cortex (Conel 1939; Sidman and Rakic 1982; Travis et al. 2005). The former exhibits a greater degree of morphological differentiation at a given point in prenatal development, indicating it develops prior to nonprimary isocortex. These differences correlate with measurable effects on cortical diffusion anisotropy in nonhuman primates (Kroenke et al. 2007). Within the framework of equation (1), our data is consistent with ferret primary cortical areas initiating differentiation 2.7 days prior to neighboring nonprimary areas.

Demonstration of differences between primary and nonprimary areas in the ferret is potentially complicated by the tendency of primary cortical areas to be located on the crowns of gyri. Multiple regression model selection procedures were therefore used to simultaneously account for primary/nonprimary classification and surface curvature effects. Although significant effects of both factors were observed in several brains, this analysis demonstrated that surface curvature effects are secondary to other factors under consideration. Further analysis of surface curvature was not pursued using this data set because the direction of the effect was not consistent throughout development.

The data were also fitted by models of alternate interpretations, such as isocortical FA being inherently different between primary and nonprimary cortex, perhaps due to distinct cytoarchitecture throughout all stages of development (Fig. 7b); or primary and nonprimary cortical areas simultaneously initiating the process of morphological differentiation, but with primary cortical differentiation occurring at a faster rate (Fig. 7c). These alternate interpretations were considered less likely than the equation (1) model, due to equation (1) providing the closest approximation of the data. The earlier initiation of cortical differentiation could be due to the early presence of afferents that invade primary cortical areas (Kostovic et al. 2002). Future studies could address the mechanism that gives rise to early reductions in primary isocortical FA by perturbing development of precociously developing axon systems.

Conclusions

Analysis of diffusion anisotropy within the developing cerebral cortex can be used to characterize several aspects of anatomical development. Specifically, the early pattern of cortical diffusion anisotropy delineates the border between isocortex and allocortex. Within the isocortex, diffusion anisotropy is substantial over the period in which pyramidal neurons migrate to the cortical plate. Cortical diffusion anisotropy begins to decline following the completion of neuronal migration. Comparisons with neuronal birthdating and other studies indicate that reductions in FA begin approximately 10 days following the completion of neurogenesis in the ventricular and subventricular zones. Regional variation in neurogenesis therefore confers regional variation in the loss of cortical diffusion anisotropy with development. Herein, the regional FA pattern was quantitatively analyzed to estimate the location of the TNG source in ferrets, and to estimate an age difference of 5.0 days between layer II/III neurons in isocortex at the TNG source and layer II/III neurons at the occipital pole. These calculations are buttressed by previously reported measurements made using other techniques. Additionally, the pattern of isocortical FA indicates differentiation of primary areas initiates approximately 2.7 days prior to other cortical areas in ferrets. This finding is consistent with previously reported Golgi studies of human tissue, and could arise from early innervation by axonal fibers specifically within primary cortical areas prior to other areas. The framework for analyzing temporal and regional patterns of cortical FA presented here gives rise to a consistent description with reports from other species, ranging from rodents to human. This consistency suggests the analysis developed here will be applicable in future studies of other species.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Appendix

Equation (1) may be modified to characterize 3 variant interpretations of temporal and regional patterns in isocortical FA. In the first variant, FA differences are considered to be independent of age. The formula for this modification is

$$\text{FA} = \begin{cases} a + S_2 + P_2 & \text{if } a < b, \\ a + (a - 2b) + P_2 & \text{if } a > b \end{cases} \qquad (2a)$$

in which

$$b = a_1 + a_2$$

and the parameter $S_2$ is a unitless difference in FA. In the second variant, primary and nonprimary cortical areas differ only in that primary isocortical FA decays at an accelerated rate

$$\text{FA} = \begin{cases} a_1 + S_3 & \text{if } a < b, \\ a_1 + (a - 2b) + S_3 + P_2 & \text{if } a > b \end{cases} \qquad (3a)$$

in which

$$b = a_1 + a_2$$

and the parameter $S_3$ has units of days. For equation (3), the decay time constant for nonprimary cortex, $a_2$, is expected (though not constrained in our calculations) to be greater than the decay time constant for nonprimary cortex, $a_2 - P_2$. In the third variant, isocortical FA is assumed to decay linearly with age, rather than exponentially

$$\text{FA} = \begin{cases} a_1 + S_4 & \text{if } a < b_1, \\ a_1 + (a - 2b_1) + S_4 & \text{if } b_1 < a < b_2, \\ a_1 + (a - 2b_2) & \text{if } a > b_2 \end{cases} \qquad (4a)$$

in which

$$b_1 = a_4 + d_3 + P_2, \\
$$

$$b_2 = b_1 - (a - 2b_2)$$

and the parameter $a_3$, the slope of FA reduction with time, has units of 1/day.


