Spatial and Temporal Variations of Cortical Growth during Gyrogenesis in the Developing Ferret Brain

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Spatial and temporal variations in cortical growth were studied in the neonatal ferret to illuminate the mechanisms of folding of the cerebral cortex. Cortical surface representations were created from magnetic resonance images acquired between postnatal day 4 and 35. Global measures of shape (e.g., surface area, normalized curvature, and sulcal depth) were calculated. In 2 ferrets, relative cortical growth was calculated between surfaces created from in vivo images acquired at P14, P21, and P28. The isocortical surface area transitions from a slower (12.7 mm²/day per hemisphere) to a higher rate of growth (36.7 mm²/day per hemisphere) approximately 13 days after birth, which coincides with the time of transition from neuronal proliferation to cellular morphological differentiation. Relative cortical growth increases as a function of relative geodesic distance from the origin of the transverse neurogenetic gradient and is related to the change in fractional diffusion anisotropy over the same time period. The methods presented here can be applied to study cortical growth during development in other animal models or human infants. Our results provide a quantitative spatial and temporal description of folding in cerebral cortex of the developing ferret brain, which will be important to understand the underlying mechanisms that drive folding.

Keywords: curvature, fractional anisotropy, intrasubject registration, MRI

Introduction

In gyroencephalic species, such as humans, expansion of the cerebral cortical surface during brain development is associated with the mechanical process of gyral and sulcal folding (Welker 1990). Although empirical observations have been reported that abnormal cerebral cortical folding is associated with various neurodevelopmental disorders, the cellular and biomechanical mechanisms that underlie this process are not well understood. Experimental evidence does exist, however, on the interdependence of cortical folding and other critical central nervous system (CNS) developmental events. For example, Chenn and Walsh (2002) have caused gyral and sulcal-like structures in normally lissencephalic mice by inhibiting neuronal precursors’ exit from the cell cycle. Additionally, a mitogenic influence of thalamic axons on cortical neuronal precursors (Dehay et al. 2001) has been proposed to underlie the effect of early enucleation on altered cortical folding patterns observed in rhesus macaques (Rakic 1988) and ferrets (Reillo et al. 2011). The potential link between cerebral cortical morphology at maturity and its developmental history therefore is a motivating factor to more fully understand the mechanical factors that drive folding.

Cerebral cortical neurons originate in transient zones (e.g., the ventricular and subventricular zones) and then migrate to their final destination (Bystron et al. 2008). Neural progenitor cells in the ventricular and subventricular zones regulate the number and future cortical location of neurons (Rakic 2009). The number of neural stem cells prior to the onset of neurogenesis is exponentially proportional to the number of neurons produced and, subsequently, to the surface area of the cortex (Rakic 1988; Chenn and Walsh 2003). The spatial and temporal pattern of neuronogenesis in the human is well described (reviewed in Bystron et al. 2008; Breunig et al. 2011). Early in development (e.g., human gestation weeks 5–20), expansion of the surface area of the cerebral cortex is mainly due to the addition of neurons (Nieuwenhuys et al. 2008). However, long after the completion of this proliferative phase (e.g., human gestation weeks 20 through beyond birth), the cerebral cortical surface area still continues to expand. Indeed, the greatest amount of cortical folding occurs following the conclusion of pyramidal cell neurogenesis (Nieuwenhuys et al. 2008).

Experimental determination of biomechanical characteristics of the cerebral cortical growth is critical for understanding the underlying mechanisms that drive cortical folding. In several previous mechanical modeling efforts, assumed values for quantities such as surface area expansion, material properties of cortical and subcortical structures, and tension generated by axons and glial cells (Richman et al. 1975; Todd 1982; Smart and McSherry 1986; Welker 1990; Van Essen 1997; Toro and Burnod 2005; Xu et al. 2010) have necessarily been adopted due to the lack of such measurements. The 2 most widely discussed hypotheses for cortical folding are mechanical buckling caused by differential growth between inner and outer cortical layers (Richman et al. 1975) and the axonal tension-based theory of morphogenesis (Van Essen 1997). Following the work of Smart and McSherry (McSherry 1984; McSherry and Smart 1986; Smart and McSherry 1986), we have made use of the ferret as an ideal animal model for investigating the relationship between cellular level and macroscopic changes associated with cortical folding and surface area expansion (Barnette et al. 2009; Kroenke et al. 2009; Xu et al. 2010). Not only are regional and temporal characteristics of cell proliferation (Jackson et al. 1989; Noctor et al. 1997; Reillo et al. 2011) and differentiation (Voigt et al. 1993; Zervas and Walkley 1999; Kroenke et al. 2009; Bock et al. 2010; Jespersen et al. 2011) known with high precision but also the gestational period is sufficiently short in this gyrencephalic species that cortical folding takes place during the postnatal period, which facilitates its characterization and experimental manipulation.
By measuring tension-induced morphological deformations following microdissections, we observed patterns of axonal tension that are inconsistent with the hypothesis of axonal tension-induced cerebral cortical folding (Xu et al. 2010). Instead, finite element models of cortical expansion suggest the existence of intracortical stress fields that are consistent with the tension measurements and can produce sulcal/gyral folds through a mechanical buckling process (Xu et al. 2010). However, in order to generate a consistent pattern of sulci/gyri, as is observed in ferrets, additional factors, such as regional variation in the development of stress patterns, are needed. Smart and McSherry (1986) previously noted qualitatively that folding of the ferret cerebral cortex follows a rostral/caudal pattern (Smart and McSherry 1986) that resembles the rostral/lateral to caudal/medial gradient in pyramidal cell neurogenesis (the transverse neurogenetic gradient, TNG) that they and others described for this species (McSherry 1984; McSherry and Smart 1986; Jackson et al. 1989; Noctor et al. 1997). Therefore, the possibility exists that regional patterns in surface area expansion could give rise to consistent stress patterns that are important for a consistent pattern of cerebral cortical folding.

Here, we have quantitatively characterized temporal and regional patterns of surface area expansion in the ferret cerebral cortex and related our observations to previous descriptions of neurogenesis and cellular morphological differentiation. In a cross-sectional study of cerebral cortical surface area expansion from postnatal ages ranging from 4 days (P4) to P35, we have determined the rate of areal expansion over the final phases of neurogenesis and cell migration from ventricular/subventricular zones to the cortical plane, as well as during the subsequent phase of neuronal morphological differentiation. In order to examine the relationship between cortical surface area expansion and previously characterized pattern of cellular morphological differentiation, as well as cortical folding, longitudinal measurements analyzed over the period from P14 to P28 are used to compare regional patterns in these attributes of cerebral cortical growth. The implications of these findings are discussed in the context of cortical surface area expansion rates measured in other species, including humans, and in relation to the mechanics of cerebral cortical folding.

Experimental Procedures

In vivo and ex vivo MR images were acquired at ages P4, P7, P10, P14, P17, P21, P24, P28, and P35, and cortical surface representations were created. Nondimensionalized sulcal depth and mean curvature were calculated along with surface area of the isocortex and allocortex for each surface. Spatial and temporal variations in growth were calculated from 2 ferrets from the same litter scanned at 1 week intervals (P14, P21, and P28).

Animal Care

Female ferret kits were obtained from the commercial vendor Marshall Bioresources (North Rose, NY). Only female kits were analyzed in this study to remove potential effects due to differences in sex, though we are not aware of any such differences in this species. Kits were delivered at P4–5 to a dedicated animal facility at Washington University (WU), where they remained for the duration of the study. All procedures were performed in accordance with NIH and institutional guidelines for the care and use of animals and approved by the WU Institutional Animal Care and Use Committee.

Image Acquisition

In vivo images were acquired at ages P7 (N = 2), P14 (N = 3), P21 (N = 3), P28 (N = 3), and P35 (N = 1). Each kit was initially anesthetized using 3.5% isoflurane in O2 in a vented anesthesia chamber and placed in a nose cone with a pallet bar or tooth bar, depending on its age. Anesthesia was maintained with 1.5% isoflurane in O2 (1.0 L/min). The animal’s head was kept still in the prone position using a custom-made head support. Pulse rate and oxygen saturation levels were monitored continuously by a magnetic resonance imaging (MRI)-compatible pulse oximeter (Nonin Medical, Plymouth, MN) taped to one of the hind paws. Body temperature was maintained by flowing temperature-controlled water through a heating pad located underneath the animal. Animals were kept sedated for a total of 120–180 min for these procedures.

Images were acquired using an 11.7T small animal MRI system controlled by a Varian INOVA console and equipped with separate transmit and receive radiofrequency (RF) coils. T2-weighted images were acquired using a multislice spin-echo pulse sequence. The imaging parameters echo time (TE) and repetition time (TR) were chosen to maximize signal to noise and contrast to noise in the images at each age (Barnette et al. 2009). The values of TR and TE ranged from 4 to 4.4 s and 55 to 80 ms, respectively. Images were acquired at a resolution of 250 × 250 × 250 μm (isotropic), which provided adequate signal-to-noise ratio while still allowing for the structure of the cortex to be identified.

To prepare tissue for ex vivo measurements, animals were injected with 0.5 mL euthasol (intraperitoneal). Heparinized phosphate buffered saline was injected into the left cardiac ventricle until the fluid of the right atria was clear. Phosphate buffered paraformaldehyde (4%, pH 7.4) was then perfused through the left ventricle for approximately 10 min. The brains were extracted and placed in 4% paraformaldehyde indefinitely.

Images of postmortem tissue were acquired from brains of P4 (N = 1), P10 (N = 1), P17 (N = 1), and P24 (N = 1) animals, using a 4.7T small animal MRI system controlled by a Varian INOVA console. Single-turn solenoidal transmit/receive RF coils used were matched to brain sizes. Imaging parameters were chosen to maximize signal and contrast at each age. Image resolution was increased as a function of brain size from 150 μm isotropic to 350 μm isotropic. The TR and TE ranged from 5.5 to 11.8 s and 85 to 100 ms, respectively.

Image Segmentation and Surface Generation

CARET software (Van Essen et al. 2001) was used for image segmentation and surface generation. Images were segmented manually at the pial surface, which is the boundary between gray matter and cerebrospinal fluid. The segmentation volume was eroded by one voxel so that the boundary of the segmentation was within the cortex, approximating a midcortical surface. A midcortical surface is advantageous because a region of surface area represents approximately the same cortical volume whether the region is located on a gyrus or sulcus (Van Essen 2005). The olfactory bulbs were removed from the segmentation to avoid errors in the surface-based analyses (e.g., inflating the cortical surface to a sphere). A single slice of a T2-weighted image with the segmentation result overlaid on the right hemisphere from a single kit at 2 time points is shown in Figure 1.4.
A mesh representation of each cortical surface was generated by CARET from the segmentation volume, using a smoothing filter (strength = 0.1; iterations = 15). A cortical surface from each age is shown in Figure 2A. Each surface consists of approximately 10 000–30 000 points in space connected by a triangular mesh of approximately 20 000–60 000 faces. The medial wall was manually identified using the anatomical MR images and the cortical surface. The coordinates that reside on the medial wall are not part of the cortex and are excluded from the analyses described herein.

Calculation of Curvature and Sulcal Depth
At a point on a surface, curvature represents the deviation of the surface from the tangent plane. A tensor is required to describe curvature of a surface. Local estimates of principal curvature were calculated using an approach described in Filas et al. (2008). Mean curvature, \( K \), is given by

\[
K = \frac{1}{2}(K_1 + K_2),
\]

where \( K_1 \) and \( K_2 \) are the first and second principal curvatures, respectively. Each curvature value \( K \) is the inverse of the radius of curvature of a curve formed by the intersection of the surface with a normal plane (a plane perpendicular to the tangent plane at the point). The first principal curvature describes the curve in the normal plane in which this curvature is greatest. The second principal curvature describes the curve in the binormal plane (perpendicular to both the tangent plane and the normal plane of maximum curvature). Curvature has units of length\(^{-1}\).

Sulcal depth, \( \Delta \), is a measure of the distance from a point on the cortical surface to the nearest point on a convex hull. The convex hull for a cortical surface is the convex surface with the smallest area that encapsulates the entire cortical surface (i.e., imagine the surface formed by stretching an elastic balloon around a convoluted surface). Sulcal depth is calculated using CARET software (Van Essen 2005).

Mean curvature and sulcal depth provide local measures of shape on the surface but can also be used to generate global measures of shape. A variety of global measures of shape have been defined previously based on mean curvature, Gaussian curvature, individual principal curvatures, and surface area (Van Essen and Drury 1997; Magnotta et al. 1999; Batchelor et al. 2002; Rodriguez-Carranza et al. 2008). However, a single global measure may not be sufficient when looking at the brain; for example, both amplitude and frequency are required to accurately characterize a sine wave. Accordingly, we use an average of sulcal depth (analogous to amplitude) and an average of mean curvature (analogous to frequency) to provide a global description of cortical shape.

Rodriguez-Carranza et al. (2008) note that a good global measure of shape should be size independent. Both sulcal depth and curvature are dependent on size; for example, a circle of radius one has a different value of curvature (\( k = 1 \)) than a circle of radius 2 (\( k = 0.5 \)). Nondimensionalization of mean curvature and sulcal depth account for differences in size:

\[
\Lambda' = \frac{\Lambda}{L_c}, \quad K' = \frac{K}{L_c},
\]

where \( L_c \) is a characteristic length, which is defined here as the square root of the surface area divided by \( 4\pi \). The average of normalized sulcal depth, \( \Lambda' \), and the average of normalized mean curvature, \( K' \), are calculated by integrating each value over the surface and dividing by the surface area:

\[
\bar{\Lambda'} = \frac{\int |\Lambda'|dA}{A}, \quad \bar{K'} = \frac{\int |K'|dA}{A}.
\]

Surface integrals are approximated using an extension of the trapezoidal method. Supplementary Figure S1 provides examples of normalized mean curvature and normalized sulcal depth values for 4 shapes. To provide a reference for a known shape, for a sphere, the normalized mean curvature is \( K' = 1 \) and the normalized sulcal depth is \( \bar{\Lambda}' = 0 \).

Longitudinal Analysis
In vivo images of 2 kits from the same litter were acquired at P14, P21, and P28. Cortical surfaces of both the left and right hemisphere were created by manual segmentation as described above. In order to estimate temporal and spatial variations in growth, a point-to-point correspondence is required between surfaces. The Landmark Correspondence and Relaxation Of Surface Strain (LACROSS) registration approach (Knutsen et al. 2010) was applied to determine a point-to-point correspondence.
This procedure entails the use of the finite element method to solve a partial differential equation on a parameterized surface (i.e., a sphere). An optimal correspondence between the 2 surfaces is identified by minimizing an energy function that is based on distortions between the surfaces (i.e., surface strain) and surface shape functions (i.e., mean curvature). A schematic of the approach is shown in Figure 1B. The LACROSS registration method uses COMSOL Multiphysics v3.5 (COMSOL, Inc., Burlington, MA) and custom functions written in MATLAB (Mathworks, Inc., Natick, MA). Hereafter, the 2 animals used for the longitudinal analysis will be referred to as ferrets A.1 and A.2.

A local measure of cortical growth between 2 surfaces is required. Specifically, we are interested in quantifying how a small region on a younger surface (e.g., P14) deforms over time into its corresponding region on the older surface (e.g., P21). We define relative cortical growth from a younger surface to an older surface as the change in surface area per unit area per day. This measure can be thought of conceptually by drawing a small circle with area $A_1$ at some location on the younger surface. Mapping the circle to the older surface will cause it to deform and grow, so that it now has an area of $A_2$. Relative cortical growth, $\Delta A$, is given by

$$\Delta A \equiv (J-1) \approx \frac{A_2 - A_1}{A_1},$$

where $J$ is the dilatation ratio between the older and younger surfaces. The derivation for relative cortical growth is provided in Appendix 1. The ratio of sulcal depth at each vertex at P14 and P21 to P28 was calculated within sulci. Points that reside on sulci were identified using the sulcal depth values at P28 by applying a threshold at $\Delta = 1$ mm. The threshold was determined by inspection.

The cortical location of neurons derived from the source of the TNG was estimated based upon the results obtained...
from McSherry (McSherry 1984; McSherry and Smart 1986) as described previously (Kroenke et al. 2009). The estimated location of the TNG origin was identified on surfaces of P28 animals. Using CARET software, the geodesic distance from each point on the surface to the origin of the TNG was determined and normalized based on the maximum calculated distance.

**Results**

**Surface Area Expansion**

Histogenesis of the allocortex differs from that of the isocortex. The former does not undergo the deep lamina first, superficial lamina last sequence of neuron birth and migration; and the laminar organization of allocortex differs from the 6-layered pattern observed in isocortex at maturity (Sidman and Rakic 1982). Therefore, we characterized surface area expansion of these regions separately, by taking advantage of the rhinal fissure as an anatomical landmark delineating the allocortical/isocortical border (Kroenke et al. 2009). As shown in Figure 2B, the surface area of both the isocortex and allocortex increase during the first 7 weeks of life. Consistent with our previous observations (Barnette et al. 2009), significant differences are not observed in surface area or in expansion rate between data acquired in vivo using post mortem tissue. The allocortex is smaller and expands in surface area at a lower rate than the isocortex. By fitting the allocortical data in Figure 2B to a line, we estimate the allocortical expansion rate over the period from P4 to P35 to be 3.8 mm²/day per hemisphere.

The isocortical surface area expansion data in Figure 2B reveals the possibility that the initial rate of surface area increase (e.g., P4 – Tᵣ, Fig. 2B) is lower than at later stages (e.g., Tᵣ – P35, Fig. 2B) in which Tᵣ represents the transition age between surface area rates of change. To investigate this, a model selection calculation was performed. A linear increase in surface area expression,

\[ A = m(\text{age}) + b \]  

(5)

in which the slope, \( m \), is the rate of area expansion, and the intercept, \( b \), is the isocortical surface area at postnatal day 0, was compared with a two-slope expression

\[ A = \begin{cases} 
  m₁(\text{age}) + b & \text{if age} \leq Tᵣ, \\
  m₂(\text{age} - Tᵣ) + b & \text{otherwise} 
\end{cases} \]  

(6)

in which the isocortical surface area is assumed to increase linearly at a rate \( m₁ \) over the age range prior to the fitted parameter “transition.” Afterward, isocortical surface area increases at rate \( m₂ \). It was found that the Akaike Information Criterion, corrected for small sample size (AICC) (McQuarrie and Tsai 1998) is smaller for the 4-parameter expression equation (6) (223) than for the 2-parameter expression equation (5) (238), which indicates equation (6) provides a significant improvement over equation (5) in the agreement between the data and the model expression. Fitting the data shown in Figure 2B to the equation (6) yields a value for transition of \( Tᵣ = P₁₂.7 \) days, an isocortical surface area expansion rate \( (m₁) \) of 14.6 mm²/day per hemisphere between ages P4 and P12.7 and an expansion rate \( (m₂) \) of 36.7 mm²/day per hemisphere between ages P12.7 and P35.

**Cerebral Cortical Folding**

Figure 2A contains surface representations of the cortex at different ages, ranging from P4 to P35. Qualitatively, the cortical surface in the first week of life is small and mostly devoid of folds, with only small indentations hinting at where future sulci and gyri will develop. As the cortex continues to grow, the folds deepen and increase in curvature.

To characterize folding of the cerebral cortex over this time period, normalized sulcal depth, \( \Delta' \), and normalized mean curvature, \( K' \), averaged over the isocortex, are plotted as a function of age in Figure 2B,C. These measures provide a quantitative description of the amplitude and frequency of the folds, respectively. As illustrated in the averaged normalized surface curvature, at P4 and P7, the earliest ages examined, the cortical surface is very smooth. For comparison, \( K' = 1 \) for a sphere, and \( K' = 1.49 \) and 1.44 at P4 and P7, respectively. The surfaces become markedly more folded from P10 to P17, which corresponds to the largest rate of increase in \( K' \). Normalized sulcal depth increases at a steady rate during the first 3 weeks of life, after which the rate of increase is much diminished. This corresponds to the development of folds and the relative deepening of the folds. Normalized sulcal depth increases at a lower rate after ~P21. From P17 to P35, the surface area continues to increase at a high rate, but the degree of folding increases at a much lower rate.

**Longitudinal Analysis of Surface Area Expansion**

To test the possibility that surface area expansion is linked to cellular-level morphological differentiation, we reasoned that regional patterns of differentiation should be reflected in a similar regional pattern of surface area expansion. In a previous study of changes of water diffusion anisotropy within the ferret cerebral cortex (Kroenke et al. 2009), we observed a rostral/lateral to caudal/medial gradient in cortical water diffusion fractional anisotropy (FA) that parallels the TNG and was interpreted to arise from a regional pattern in cellular morphological differentiation (McSherry 1984; McSherry and Smart 1986). Serial surface area measurements were therefore carried out on 2 ferrets at ages P14, P21, and P28. The LACROSS method (Knutsen et al. 2010) was employed to determine a point-to-point correspondence between cortical surfaces at different ages, which allows for the calculation of local measurements of surface area expansion. Relative cortical growth (eq. 4) was calculated as the change in local surface area from P14 to P21 and from P21 to P28, normalized by the local surface area on the younger surface. Cortical growth values from P14 to P21 and P21 to P28 were projected onto cerebral cortical surface models for one hemisphere of one animal in Figure 3A and for both hemispheres in each animal in Supplementary Figures S2 and S3. Relative cortical growth was found to exhibit a rostral/lateral to caudal/medial regional pattern, as shown in Figure 3B. To quantify this regional variation, relative cortical growth is plotted as a function of relative geodesic distance from the cortical site of neurons derived from the origin of the TNG (d”) in Figure 3B and Supplementary Figure S4. A linear model was fit to the data using a least-squares fitting algorithm in Matlab. Combining the results for each hemisphere, the slope of cortical growth as a function of d” is 0.245 with an intercept at 0.833 from P14 to P21 and 0.357 with an intercept of 0.763, from P21 to P28. Values of the intercept and slope for each of the animals and hemispheres are listed in Table 1. Note that the slope and intercept values are unit less, as they are derived.
from 2 unit-less quantities: relative cortical growth and relative geodesic distance.

In addition to longitudinal changes in relative cortical growth, we looked at the progression of sulcal depth at P14 and P21 relative to its value at P28 as a way to quantify the degree of folding on a regional level. Figure 4 and Supplementary Figure S5 show the ratio of sulcal depth at P14 and P21 relative to P28 as a function of $d/C_3$ for surface points that reside on sulci, which were identified by applying a threshold to sulcal depth values at P28 ($D = 1$ mm). The threshold was determined by inspection. Similar to above, a linear model was fit to the data using a least-squares fitting algorithm in Matlab. The slope of the ratio of sulcal depth as a function of $d/C_3$ from P14 to P28 (slope = -0.14) decreases at a rate 4.5 times higher than from P21 to P28 (slope = -0.03). The intercept of the modeled data from P21 to P28 (intercept = 0.82) is larger than from P14 to P28 (intercept = 0.55). The values of the slope and intercept for both animals and hemispheres are listed in Table 2.

Discussion

A kinematic description of folding is necessary to provide insight into the underlying mechanisms of cortical folding (Van Essen 1997). The goal of this study is to provide a quantitative description of global and local growth in the developing ferret brain. Expansion of cortical surface area is due to cellular proliferation and morphological differentiation. The surface area of the isocortex was measured on cortical surface representations that were created from anatomical MR images as a function of age (P4–P35). The ferret is an ideal animal model for investigating the relationship between cellular-level and macroscopic changes associated with cortical folding and surface area expansion. Due to the resulting flexibility of experimental procedures that can be used, temporal characteristics of cell proliferation (Jackson et al. 1989; Noctor et al. 1997; Reillo et al. 2011) and differentiation (Voigt et al. 1993; Zervas and Walkley 1999; Kroenke et al. 2009; Bock et al. 2010; Jespersen et al. 2011) are well characterized in this species. In particular, cell birthdating (Jackson et al. 1989; Noctor et al. 1997) and other histological methods (McSherry 1984; McSherry and Smart 1986) have established that isocortical pyramidal neuron production is essentially complete by post-conceptional day (PC)41 (the ferret gestational term is 42 days) in rostral/lateral cortex (Noctor et al. 1997), PC49 (postnatal...
day (P)8 in occipital cortex (Jackson et al. 1989), and intermediate at other locations according to a TNG (McSherry and Smart 1986; Kroenke et al. 2009). Allowing for a 7–10 day period for neurons to migrate from ventricular zones to the cortical plate (Roberts et al. 1993; Noctor et al. 1997), the cellular proliferation period of surface area expansion occurs until approximately P9–P17, whereas expansion after this period is associated with morphological differentiation on the cellular level.

An estimate of the transition from cellular proliferation to morphological differentiation is reflected in the observed transition in the rate of surface area expansion in the isocortex at $T_{tr} = 12.7$ days. The rate of cortical expansion associated with cellular proliferation (age $< T_{tr}$) is 12.7 mm$^2$/day per hemisphere, approximately one-third the value of 36.7 mm$^2$/day per hemisphere associated with morphological differentiation (age $> T_{tr}$).

Compared with other gyroencephalic species studied to date, the rate of increase in cerebral cortical area is slow if considered in absolute terms but rapid when considered as a fraction of the adult surface area. Cross-sectional studies of baboon postmortem fetal tissue from gestational days 90 through 146 (Kroenke et al. 2007) and in utero MRI procedures performed between 25 and 35 weeks gestation (Clouchoux et al. 2011), as well as data obtained from prematurely delivered human infants obtained over a 26–36 weeks postconception age range (Dubois et al. 2008) reported a cortical surface areas that expand at rates of 210 and 190 mm$^2$/day per hemisphere, respectively. Note that half the total cortical surface area expansion rates reported by Kochunov et al. (2010) and in the human studies are quoted here for a single hemisphere. For the human studies, expansion rates were not explicitly reported but were estimated based on surface area values reported in Figure 9 of Clouchoux et al. (2011) and Figure 3C of Dubois et al. (2008). Surface area expansion data for ferret and primate species are shown in Figure 5A.

Using values of 760 (this study and Bock et al. 2011), 9700 (Kochunov et al. 2009), and 94 100 (Hill et al. 2010) mm$^2$ per hemisphere as adult cerebral cortical surface areas, expansion rates of 4.2, 0.57, and 0.22% of the adult surface area per day are obtained for ferret, baboon, and human, respectively. However, as is recognized in other interspecies developmental studies (Breunig et al. 2011), quantitative comparisons require differences in the rate of CNS development to be acknowledged.

Table 2
Slope and intercept values for the ratio of sulcal depth values at P14 and P21 relative to P28 as a function of relative geodesic distance from the origin of the TNG (Fig. 4 and Supplementary Fig. S5)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hemisphere</th>
<th>$\frac{\Delta(P14)}{\Delta(P28)}$</th>
<th>Slope</th>
<th>Intercept</th>
<th>$\frac{\Delta(P21)}{\Delta(P28)}$</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.1</td>
<td>Left</td>
<td>−0.13</td>
<td>0.55</td>
<td>−0.06</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>−0.14</td>
<td>0.55</td>
<td>−0.012</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.2</td>
<td>Left</td>
<td>−0.12</td>
<td>0.55</td>
<td>−0.063</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>−0.15</td>
<td>0.57</td>
<td>0.018</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>−0.14</td>
<td>0.56</td>
<td>−0.030</td>
<td>0.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Ratio of sulcal depth at P14 and P21 relative to P28. Points residing in the 6 major sulci of the ferret were identified via an applied threshold of sulcal depth values at P28. The ratio of sulcal depth at P14 and P21 relative to P28 represents the progress in folding toward maturation. As relative distance from the origin of the TNG increase, the sulcal depth ratio from P14 to P28 decreases (slope = −0.14; intercept = 0.55) while the ratio from P21 to P28 decreases at a much lower rate (slope = −0.03; intercept = 0.82).

Figure 5. Inter-species comparison of surface area during development. (A) Surface area expansion data for ferret and primate species as a function of the number of days post conception. Ferret data (green) are from this study while surface area values in the baboon (dark red—Kroenke et al. 2007; light red—Kochunov et al. 2010) and human (dark blue—Dubois et al. 2008; light blue—Clouchoux et al. 2011) were obtained from previously published studies. (B) The ratio of surface area during development to surface area in the adult as a function of CNS developmental event score.
According to comparative analyses of CNS development (Clancy et al. 2007), the Figure 5A age ranges correspond to ferret P8-P37 for Kroenke et al. (2007), P25-P52 for Kochunov et al. (2010), P42-P56 for Clouchoux et al. (2011), and P41-P54 for Dubois et al. (2008), in which the approximation procedure described in Leigland and Kroenke (2011) for converting baboon to rhesus macaque developmental ages was used. Thus, the majority of the Kroenke et al. (2007) data, the entirety of the Kochunov et al. (2010), and human data correspond to ferret ages greater than $T_p$. In Figure 5B, postnatal ages are converted to event scores after correcting for between-species differences in the rate of CNS development (Clancy et al. 2007). As is evident in Figure 5B, the rate of ferret cortical surface area expansion (230% of adult surface per event) is faster than that of baboon and human (70% and 45% of adult surface per event, respectively), after correcting for species-specific developmental time scales. Interestingly, the surface area of the isocortex during development actually overshoots the surface area of the adult.

In humans, the developmental sequence from 25 to 40 weeks gestational age (GA) corresponds to the development time in the ferret from P10 to P21 (Barnette et al. 2009), and global shape analysis has been calculated both in vivo (Dubois et al. 2008; Rodriguez-Carranza et al. 2008) and ex vivo (Batchelor et al. 2002). Both Batchelor et al. (2002) and Rodriguez-Carranza et al. (2008) used 7 and 16 global shape metrics, respectively. The metrics used by Batchelor et al. (2002) are size dependent and are influenced by the large changes in scale during development. For comparison with the normalized mean curvature ($\hat{K}$) index presented in this study, normalized $L^2$ norm of mean curvature (MLN$_2$) (Rodriguez-Carranza et al. 2008) and Sulcalification Index (SI) (Dubois et al. 2008) are used. Both MLN$_2$ and SI increase linearly during the developmental time periods studied (28–37 weeks GA and 26–36 weeks GA, respectively). In the ferret, $\hat{K}$ increases at a higher rate from P10 to P21 compared with before P10 or after P21 (Fig. 2B2). After P21, $\hat{K}$ increases slowly. This suggests that while the isocortical surface area continues to expand at a constant rate (36.7 mm$^2$/day per hemisphere through P35), the brain is only becoming slightly more curved during this time. In addition, normalized sulcal depth ($\hat{\Delta}$) provides a size-independent shape index that complements $\hat{K}$, providing information on both the amplitude and frequency of the folding during development.

Regional changes in surface area and sulcal depth provide additional information to supplement surface area changes of the isocortex as a whole. While the surface area of the isocortex increases at a constant rate from $T_p$ to P35, the rate of change in shape as measured by relative cortical growth and ratio of sulcal depth varies both along and between sulci. In addition to spatial variations, differences are also seen as a function of age (Fig. 4 and Supplementary Fig. S5). The ratio of sulcal depth represents the progress of folding toward maturity at each surface coordinate that reside on a sulcus. The P28 surface was designated as being at maturity since it was the oldest surface obtained in the 2 animals studied longitudinally. The change in normalized mean sulcal depth is relatively small from P21 to P28 (and to P35) compared with P14 to P21. Relative cortical growth is calculated by registering (i.e., determining a one-to-one correspondence between local isocortical sites) 2 cortical surfaces created from images of the same animal acquired at different ages. Relative cortical growth is quantified by the change in a small region of surface area per unit area, and, in this study, is measured from P14 to P21 and P21 to P28 in 2 animals. The rate of relative cortical growth exhibits a regional pattern of low surface expansion rostrally and laterally, to high expansion medially and caudally, which is similar to the regional pattern of cortical water diffusion anisotropy within the developing ferret cerebral cortex (Kroenke et al. 2009).

The rostral/lateral to caudal/medial gradient in diffusion anisotropy has been interpreted to result from differences in cortical neuron age due to the TNG, and hence, extent of morphological differentiation (Kroenke et al. 2009). To examine the possibility that the regional pattern in relative cortical expansion arises as a consequence of regional patterns of cellular morphological differentiation, it was therefore determined whether relative cortical expansion also correlates with relative geodesic distance from the cortical site of neurons derived from the TNG source ($d^*$). The confirmatory results shown in Figures 3A and Supplementary Figure S3 and Table 1 demonstrate that relative cortical expansion is also positively correlated with ($d^*$). Cortical FA values obtained using expressions from Kroenke et al. (2009), as described in Appendix II, are listed in Table 3 for limiting cases in which $d^*=1$ and $d^*=0$. A larger decrease in FA is seen near the occipital pole ($d^*=1$) compared with the origin of the TNG ($d^*=0$) from both P14 to P21 and P21 to P28. Similarly, the decrease in FA is larger from P14 to P21 compared with the decrease from P21 to P28 in each corresponding region. As shown in Table 3, regional and temporal patterns in relative cortical surface area parallel the magnitude in reductions in cortical FA. In general, the common gradient observed in water diffusion anisotropy and relative cortical growth with respect to the TNG indicates that the amount of reduction in FA over a given time period directly relates to the amount of relative cortical growth over that time period. Different factors, such as variations in the number of glial cells, synaptogenesis and arborization may explain spatial and temporal variations in patterns of growth. For example, an increased number of intermediate radial glial cells are seen in regions with greater tangential cortical expansion (Reillo et al. 2011), though folding of the cortex is not ensured by the presence of proliferating basal radial glia cells (Hevner and Haydar 2012).

McSherry describes the cortical location of neurons derived from the source of the TNG in the ferret to reside approximately midway between the rhinal fissure and the medial boundary (McSherry 1984; McSherry and Smart 1986). Using FA data in the cortex during development, we previously identified the source of the anisotropy to be in the rostral cortex, dorsal to the center of the insula (Kroenke et al. 2009). Studies in different species propose the origin of the TNG to reside near the insula (Sidman and Rakic 1982; Smart 1983; McSherry 1984). The result from Kroenke et al. (2009) is more consistent with Figure 9 from McSherry (1984) compared with

<table>
<thead>
<tr>
<th>$d^*$</th>
<th>FA$^{P14} - \text{FA}^{P21}$</th>
<th>FA$^{P21} - \text{FA}^{P28}$</th>
<th>Relative cortical growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.158</td>
<td>0.074</td>
<td>0.833</td>
</tr>
<tr>
<td>1</td>
<td>0.178</td>
<td>0.127</td>
<td>0.178</td>
</tr>
</tbody>
</table>

The change in FA as a function of relative geodesic distance from the origin of the TNG ($d^*$) calculated using equation (A5)
a surface coordinate near the insula. For this study, a similar location of the source of the FA gradient located within the rostral cortex was selected as the origin of the TNG. To investigate the effect on the results obtained in this study of an error in the identification of the origin of the TNG, a point located at the approximate center of the insular cortex was selected and set as an alternative origin of the TNG. The relative geodesic distance from all surface coordinates to the alternative origin was calculated. Relative cortical growth as a function of normalized geodesic distance from an origin near the center of the insular cortex was calculated, and a linear model was fit to the data. The calculated slope and intercept values are given in Supplementary Table S1. The similarity of the resultant slope and intercept values (Table 1 and Supplementary Table S1) give confidence in the observed relationship between relative cortical growth and $d^*$. Ideally, cortical growth would have been calculated at earlier time points (i.e., from P7 to P14) in addition to the current rages. Unfortunately, images at P7 were only acquired in one of the kits used for the longitudinal portion of this study. In addition, the LACROSS registration algorithm uses mean curvature to constrain the registration between 2 surfaces, with the assumption that regions of high curvature remain so during development. At P7, the cortical surface is very smooth, with only small indentations that hint at the future locations of sulci and gyri. Indeed, $K^*$ averaged for the P7 cortical surfaces is 1.44, which is not much larger than that of a sphere ($K^* = 1$) and is much smaller than at P14 and P21 ($K^* = 2.76$ and 3.81, respectively). In order to obtain an accurate registration during this time, a different fiducial marker than mean curvature is necessary. One possibility for future studies would be to identify the intersection of blood vessels with the cortical surface using $T_2$-weighted MRI and to track these intersections over time. Methodological developments, such as this, are currently under investigation.

Registration algorithms, through a series of assumptions, provide an estimate of how one surface corresponds to another surface, or, in this case, how one surface grows over time. It is important to acknowledge that the calculations of cortical growth are directly dependent on the assumptions that drive the algorithm. The LACROSS registration algorithm has been tested on a series of artificial test cases and actual cases. The algorithm uses normalized mean curvature to drive the registration but also has a term that minimizes distortions between the surfaces. In addition, it is important to analyze the registration results carefully to make sure that the mapping is physically reasonable on a small scale. This was achieved by mapping small regions between the registered surfaces and visually examining them. While other assumptions could be made to drive the registration, the assumptions made provide a reasonable estimate of growth in the developing brain.

Using the information observed in this study and from previous work, a hypothesis of the pattern of cortical growth prior to P14 can be generated. Given that cellular morphological differentiation first occurs near the origin of the TNG prior to P14, cortical growth should initially be higher near the origin of the TNG. At some point between P7 and P14, a transition should occur where the regions further from the origin of the TNG should grow at a faster rate. It is during this time period that the cortical folds begin to form. The 2 most widely discussed hypotheses for the underlying mechanisms of cortical folding are differential growth (Richman et al. 1975) and tension-based morphogenesis (Van Essen 1997). While axonal tension may not be the main force that drives cortical folding (Xu et al. 2010), axonal connectivity has a strong influence on folding patterns (Barron 1950; Rakic 1988). The model presented by Richman et al. (1975) qualitatively reproduced normal and pathological folding, but used unrealistic material properties and did not provide an explanation for the consistent folding patterns seen between subjects. Xu et al. (2010) proposed a model for folding of the cortex based on differential growth between regions. Using a numerical (finite element) model of the mechanics of the cortex and subcortical regions, they showed that growth in one region followed by remodeling and growth in a neighboring region could cause consistent folds to develop. Measures of actual cortical growth, such as the data obtained from this study, are valuable input parameters for mathematical models of cortical folding.

Conclusions

Using MRI and surface-based analysis techniques, global and local measures of expansion of the cerebral cortex were examined as a function of postnatal age in the developing ferret brain. The surface area of the isocortex undergoes a transition from a lower growth rate ($12.7$ mm$^2$/day per hemisphere) to a higher rate ($36.7$ mm$^2$/day per hemisphere) at approximately 13 days after birth ($T_0 = P12.7$), which corresponds to the transition from cellular proliferation to morphological differentiation. Cortical expansion in the latter phase is two-thirds to one-half of the rate reported previously in nonhuman and human primates, respectively. Locally, relative cortical growth increases as a function of relative geodesic distance from the origin of the TNG. In addition, the amount of cortical growth is proportional to the change in FA over the same time period. Anatomical MRI and surface-based analyses of the cerebral cortex provide a noninvasive means to quantify folding of the cortex during development; such data will be important for parameterization and validation of mathematical models of cortical folding.

Supplementary Material

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

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Appendix 1

The normalized growth rate between 2 surfaces at different times is the change in local area per unit area per day. Let $X$ be the surface coordinates for the younger surface (i.e., P14) and $x$ be the surface coordinates for the older surface (i.e., P21). Surface registration provides a point-to-point correspondence between the 2 surfaces.
\[ \mathbf{x} = \mathbf{x}(\mathbf{X}). \]  
(A1)

The deformation gradient tensor, \( \mathbf{F} \), transforms a line element on the younger surface to a line element on the older surface (Taber 2004).

\[ \mathbf{F} = \frac{d\mathbf{X}}{d\mathbf{x}}. \]  
(A2)

In practice, \( \mathbf{F} \) is calculated between 2 corresponding surfaces using the approach described in Filas et al. (2008). Briefly, at each point on the surface, a second-order polynomial is fit in the least-squares sense to describe the coordinate components of the older surface in terms of the components of the younger surface over a small region. Derivatives are then calculated analytically.

The dilation ratio, \( J \), represents the ratio of the area of the older surface to the younger surface at each coordinate.

\[ J = \det(\mathbf{F}) \approx \frac{A_2}{A_1}, \]  
(A3)

where \( \det(\cdot) \) is the determinant, and \( A_1 \) and \( A_2 \) are the areas of small corresponding regions on the younger and older surfaces, respectively. Relative cortical growth is a function of \( J \) and is given by

\[ \Delta A = (J - 1) \approx \left( \frac{A_2 - A_1}{A_1} \right), \]  
(A4)

Appendix II

FA as a function of age (\( a \)), and relative geodesic distance from the origin of the TNG (\( d' \)) for nonprimary cerebral cortical areas, using diffusion sensitization scheme A (Kroenke et al. 2009)

\[ \text{FA} = \begin{cases} 0 & \text{if } a < \beta, \\ 2x_0 + (x_1 - x_2)\exp\left(-\frac{d'^2}{\gamma}ight) & \text{if } a > \beta, \end{cases} \]  
(A5)

where

\[ \beta = x_4 + d'x_5. \]  
(A6)

The optimal parameters that describe FA in the developing ferret brain are: \( x_0 = 0.742, x_2 = 0.327, x_1 = 9.1, x_4 = 8.2 \), and \( x_5 = 5.0 \). Table 3 contains the change in FA between P14 and P21 and P21 and P28 for \( d' = 0 \) and \( d' = 1 \).

References


