Understanding human brain development is clinically relevant since many neurobiological disorders and disabilities have their origin in early structural, functional development and plasticity. With the advent of magnetic resonance imaging (MRI), it has become possible to address the question of where, when and how adverse conditions in fetal and early postnatal life and prematurity relate to the brain maturation \(^1\). The integration of healthy infants in longitudinal studies has become possible thanks to the absence of irradiation or other side effects, thus allowing a wide and thorough analysis of brain development with the possibility to study processes such as cortical folding of the human brain in vivo both in fetuses as well as in newborns. This course presents advanced imaging and postprocessing modalities used to study the immature brain and discusses their potential for in vivo assessment of cerebral development both in human as well as in animal models.

Conventional MR imaging techniques, including mainly T1- and T2-weighted images, allow the assessment of brain development \textit{in-vivo} with the highly sensitive assessment of gray and white matter changes, as well as the differentiation of unmyelinated and myelinated white matter \(^2\).

The subplate zone can be clearly distinguished in ex-vivo T1 MRI images after 13 weeks of gestation as an area of hypointensity and on in vivo T2 MRI as an area of hyperintensity \(^3\)\(^4\) due to the higher water content of the extracellular matrix (ECM) between the bordering cortical plate and the intermediate zone. The presence of this transient subplate zone is one of the main signs of cortical immaturity.
The subplate is crucial in developmental processes such as the ingrowth of thalamo-cortical axons\(^5\), the reorganization of the fetal white matter and establishment of cortical layers. When neurons near their final destination, they start to produce axons and dendrites, allowing connection with distant cerebral structures. This ontogenic step occurs largely, but not exclusively, during the second half of gestation and extends into the postnatal period. This entire process is influenced by genetic guidance, neurotransmitters and trophic factors, and also the glycoproteins of the ECM in the subplate zone. As for neuronal production, some axonal projections are produced in excess, connecting too many structures or neurons. This initial phase is followed by a regressive phase where redundant or misconnected axons are eliminated or retracted, allowing the emergence of adequate and functional connections. This balance between the maintenance or the elimination of axons is regulated by different mechanisms. Obviously, the survival of the neuron is determinant in this decision. Furthermore, competition for available trophic factors interacts with the genome to modulate this balance. Also, electrical activity is a key determinant for the maintenance of axons. Accordingly, in utero and especially postnatal stimuli and experiences significantly shape the developing brain by modulating the maintenance or elimination of some axons\(^6,7\).

Making proper connections through white matter structures is probably one of the determining factors for further cortical organization. One major hypothesis for the morphogenetic mechanism of cortical folding is based on mechanical tension along axons in the white matter\(^8\), the other being that differential growth of the cortex leads to folding\(^9,10\).

MRI has in recent years helped researchers to tackle this unsolved question of brain development through evolution\(^11,12,13,14\). In animal models the combination of conventional MRI and diffusion tensor imaging (DTI has further allowed to depict the relationship of changes in intra-cortical layering and cortical folding\(^15,16\).

The basis for these MRI based measures are image processing techniques that allow fine quantification of specific brain tissues such as white matter, cortex, subplate, cerebrospinal fluid and the exact definition of their borders.

**MR Image analysis: Automated computational techniques**

High-resolution T1- and T2-weighted images are the basis for the application of mathematically-based segmentation techniques that allow volume measurement of total cortical grey matter, white matter, basal ganglia and cerebrospinal fluid.
Segmentation techniques for the developing brain are challenging due to: 1) an inversion of the contrast between gray and white matter and changes during development prohibiting the use adult segmentation toolkits; 2) a strong signal heterogeneity secondary to myelination affecting both cortical structures and white matter regions; 3) the small size of the brain requiring high resolution MRI, which leads to low signal-to-noise; 4) thin structures in particular the cortex, prone to partial volume effects complicating further any automated segmentation tool based on signal intensity. Volumetric analysis of MRI data sets is achieved by segmentation of the imaged volume into tissue types depending on their difference in signal intensity, contour and anatomical knowledge followed by three-dimensional renderings. A detailed discussion of the different methods used for neonatal brain segmentation would be beyond the scope of course. The combination of a signal-based k-means classification with a mathematical morphology approach for shape recognition is currently the preferred method for fetal and neonatal brain segmentation.

**Figure 1: New Methods of neonatal Segmentation:** Segmentation results and comparison with expert consensus segmentation and with a state-of-the-art segmentation method (4 subjects). Each row contains corresponding coronal slices of (a) the T2w image; (b) the expert consensus segmentation (c) our segmentation; (d) results with the method of Warfield et al. [1] (does not segment brainstem and cerebellum). Legend: gray - cortical gray matter, white - central gray matter, red - unmyelinated white matter, orange - myelinated white matter, yellow - cerebellum, green - brainstem (unmyelinated).
A striking increase in cerebral cortical volume accompanies the axonal and dendritic growth described previously. That this growth is particularly rapid between approximately 28 and 40 weeks’ gestational age has been shown by these quantitative three-dimensional MRI techniques with use of postacquisition image analysis. Overall brain volume more than doubles between 28 and 40 weeks’ gestation, and cortical gray matter volume increases fourfold in the same period. This increase is thought to relate primarily to neuronal differentiation rather than to an increase in the total number of neurons. Cortical surface, changing from smooth, lissencephalic to highly convoluted, increases fivefold between 28 and 40 weeks’ gestation.
**MR Image analysis: Mathematical Morphology: primary cortical folding**

One of several possible approaches to investigate cortical folding in preterm infants is based on a mathematical morphology approach that process and analyze shape. The computational approach quantifies both surface area and cortical gyration through curvature measurements from three-dimensional reconstruction of the interface between developing cortex and white matter \(^{22,23}\). In order to define inner cortical surface accurately, the interface between the cortical gray matter and the white matter must be identified. A smooth triangle-based mesh of the surface detected between the developing cortex and white matter zone is then computed and the global area of this inner cortical surface can be measured. Finally, the local surface curvature is estimated from the mesh local geometry: positive curvatures correspond to the gyrus’s top and negative curvatures to the fold’s bottom \(^{24}\). The sulci are then defined as connected components of negative curvature. For each newborn a sulcation index (SI), defined as the ratio between the areas of sulci from the lateral, ventral and vertex surfaces (except the sylvian fissure and the medial surface) and the closed surface of the cortex is computed (Figure 2).

**Figure 2.** Inner cortical surface reconstructions:
These sophisticated image analysis tools need high-resolution primary input data with no motion artifacts.

**Cortical folding in the fetus & preterm infants**

The emergence of the cortical foldings in the preterm newborn brain was recently studied by applying dedicated post-processing tools to high quality MR images acquired shortly after birth over a developmental period critical for the human cortex development\textsuperscript{23 25}. In these studies, the emergence of the cortical foldings in the preterm newborn brain was investigated, by applying dedicated post-processing tools to high quality MR images acquired shortly after birth over a developmental period critical for the human cortex development (26 to 36 weeks of gestational age). For the first time, the interface between the developing cortex and white matter zone was reconstructed coherently in 3D using an original approach, which enabled a quantitative and in vivo mapping of the individual sulci appearance. Through the 3D reconstruction of the developing inner cortical surface, a sulcation index was derived and allowed measurement of variations with age, gender and presence of brain lesions and mapped the individual sulci appearance, highlighted early inter-hemispherical structural asymmetries that may be related to the cortical functional specialisation of the brain. Females have lower cortical surface, and smaller volumes of cortex and white matter than males, but equivalent sulcation. The highest sulcal index is found in the central region, followed by the temporo-parieto-occipital region, with the lowest sulcation index in the frontal region, which confirms that the medial surface folds before the lateral surface, and that the morphological differentiation of sulci begins in the central region, and progresses in an occipito-rostral direction (see figure).
Figure 3: Sulci identification on the inner cortical surface for newborns of different gestational age and sulcation index $^{23}$
Over the last few years, such spatio-temporal differences in brain maturation have been described in detail also in older children through the analysis of cortical volume surface and thickness changes\textsuperscript{26, 27}. Conditions such as twinning have been shown to alter sulcation and decrease the sulcation index\textsuperscript{23}. This finding indicates that intrinsic (genetic), as well as extrinsic (environmental, epigenetic), factors affect cortical folding. A recent has therefore addressed the question if early alteration of cortex formation can be highlighted at birth according to intra-uterine environment, and if early brain morphology can be related to infants’ outcome at term equivalent age. To do so, development was compared among premature newborns who experienced different prenatal conditions: normal singletons, twins and newborns with intra-uterine growth restriction (IUGR). The surfacic and volumetric results at birth suggested that normal twins had a delayed maturation of cortical volume and gyrification according to age, but this delay was harmonious in regard to the developmental profile. IUGR newborns also demonstrated a delay, but the cortical growth and folding was discordant to the normal developmental trajectory, as the alterations in sulcation index were not proportional to the low surfacic and volumetric growth. Size and cortical morphology has further been shown to reflect abnormal functioning or vice versa, as indicated in correlations between surface, gyrification index at birth and the APIB score at term equivalent age which correlates with later neurobehavioral deficits\textsuperscript{22}.

So far, the precise mechanisms responsible for such alteration in cortical phenotype during intra-uterine or post-natal development are still poorly understood, therefore studies of cortical folding in selected animal models will provide further insight into the mechanisms underlying cortical folding\textsuperscript{14}.

Preterm birth, a modulator of developmentally expected environment, may be responsible for the delay that was observed in sulci appearance in comparison with post-mortem and foetal studies, as both cortical volume\textsuperscript{28} and surface area\textsuperscript{29} of extremely preterm infants imaged at term equivalent age are decreased and less complex than in normal infants, and this impairment seems to increase with decreasing gestational age at birth\textsuperscript{30}. Several authors have investigated the influence of preterm birth on primary cortical folding. Biagioni studied preterm infants of $< 30$ weeks GA and demonstrated that the degree of cortical folding significantly increased with postmenstrual age\textsuperscript{29}. PT neonates with intrauterine growth restriction, major gestational pathology that alters metabolic substrate availability to the fetus but also changes its endocrine and growth
factor environment had more pronounced reduction of volume in relation to surface area and increased sulcation with resultant changes in cortical thickness; these values were found to correlate with impaired behavioral functions\textsuperscript{22}. That these early changes persist into childhood and potentially adulthood has been shown. Kesler noted abnormalities in sulcation in the temporal lobes of prematurely born children when compared to term control subjects at school age\textsuperscript{31}.

Infants with early white matter lesions showed a trend to increased gyrification in overlaying cortex\textsuperscript{32} and abnormalities in primary cortical folding have been associated with both functional development in PT infants at term equivalent and school age\textsuperscript{23}. Ramenghi quantitatively assessed brain development in PT infants with MRI-diagnosed WMI and matched PT controls with a normal MR appearance at term equivalent age\textsuperscript{33}. Both groups were comparable concerning GA, BW and age at imaging, but both myelination and cortical folding were significantly delayed in infants with WMI.

Cortical folding: Asymmetries in cortical folding during development
Specific early brain asymmetries have been described in post-mortem brains but in order to study their relation to functional development in vivo assessment of these asymmetries are required. With the studies in the preterm population we were able to show that the right hemisphere presents gyral complexity earlier than the left, which is particularly evident at the level of the superior temporal sulcus (STS), which parallels early functional competence in response to auditory stimuli in preterm newborns. In order to assess asymmetries without apriori a voxel-based analyses of cortical and white matter masks has been proposed on a set of preterm newborns from 26- to 36-weeks of gestational age\textsuperscript{23}. Inter-individual variations associated with increasing age were first detected in large cerebral regions, with a prevalence of the right hemisphere in comparison with the left. Asymmetries were further highlighted in three specific regions over the external cortical surface\textsuperscript{34}. Confirming the results from the previous qualitative studies, the authors observed deeper STS on the right side, and larger posterior region of the sylvian fissure on the left side, close to planum temporale. For the first time, this rater independent approach also detected larger anterior region of the sylvian fissure on the left side, close to Broca’s region.
Cortical folding: Voxel based analysis of cortical folding and Inter-individual variations

During the last trimester of human pregnancy the macroscopic morphology of the human brain changes greatly and quickly through the formation of sulci and gyri within the cortex as shown above. The high complexity observed in the adult brain is present in the term newborn, and more specifically sulcal patterns become variable across individuals. When comparing appearance of sulci central sulci showed less inter-individual variations than parietal, temporal and frontal sulci. In order to evaluate changes in sulcation over age on a voxel based approach a schema of sulcus folding was proposed (figure 5).

edges and bottoms of the sulcus for the oldest newborns. This led to an “apparent increase” in cortex and an “apparent decrease” in white matter.
Such observations were performed cross-sectionally over a group of newborns at different gestational ages. Figure 6 illustrates regions of inter-individual variations over age.

**Figure 6 Interindividual variations over age**

- **a:** Increases in cortex
- **b:** Decreases in white matter
- **c:** Superimposed clusters

Regions with the most acute variations over this age range are to be the first cortical places to fold, as well as the most spatially stable regions across individuals. It may represent the “sulcal roots” from where the primary sulci fold. These new methodologies aim at evaluating further local growth changes by applying optical flow algorithms (measuring optical flow between two depths maps) and local deformation fields with a Helmholtz decomposition to study the origins of sulci on the surface of the brain. Thereby defining sulcal roots that are elementary atoms around which the brain folding organizes itself.

*Fig. 7. Depth maps of two surfaces of the same subject at two different ages (birth, birth + 4 weeks).*
These data has contributed to a new extended framework for modelling cortical folding presented recently 12. It is based on a system of reaction-diffusion equations defined on a surface that evolves through the action of morphogens, this model allows to introduce noise (just like in biology of situations such as IUGR, brain lesions) that will lead to morphological variability in the brain sulcal pattern.

These new ways of studying brain development and cortical folding will finally allow us to evaluate the influence of genes and environment on the structural-functional relationship and its modulation throughout development. One day we will understand what made our brains grow globular and expand our frontal lobe a significant difference between neandertal and human brains from newborn to 1 year38.

Reference List


Ref Type: Generic


Ref Type: Generic


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