Brain Maturation After Preterm Birth

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Two translational studies—one in humans and one in sheep—suggest that (i) premature birth is associated with delayed maturation of grey matter in the cerebral cortex and (ii) medical care that prohibits impairment of growth in premature neonates may enhance cortical development and reduce neurological disabilities associated with preterm birth.

Development of the brain’s cerebral cortex is regulated by a complex interplay between an unfolding genetic program and the environment. Neuronal proliferation, migration, differentiation, and circuit formation follow defined time scales, and their choreography is controlled by extrinsic factors mediated through blood-brain and placental barriers (1). Premature birth can alter the normal developmental processes. If the preterm brain does not possess the ability to recover from these disruptions, then they may be associated with neurodevelopmental impairments, with some consequences not manifesting until years later. Understanding the timing and rationale for therapeutic intervention to prevent developmental abnormalities in these infants requires the detailed monitoring of clinical parameters and the study of mechanistic aspects in animals.

Two papers published in this issue of Science Translational Medicine characterize adverse neurodevelopmental outcomes in preterm newborn infants. Vinall and colleagues (2) examine the association between neonatal growth (weight, length, and head size) and diffusion tensor imaging (DTI) measures of cortical development in infants born preterm (between 24 and 32 weeks gestational ages). Dean and colleagues (3) use a sheep model to examine the consequences of prenatal cerebral ischemia on cortical volume and relate magnetic resonance imaging (MRI)—defined cortical microstructure with brain histological analysis. Both of these translational studies draw attention to grey matter damage and suggest that avoiding somatic growth impairment during neonatal care may allow cortical development to proceed optimally and thus reduce neurological disabilities related to preterm birth.

DECIPHERING THE DEVELOPING CORTEX

It is well documented that survivors of preterm birth show a reduction in cerebral cortical volume relative to infants born at term. Premature birth—even in the absence of overt hypoxic-ischemic injury—is associated with loss of both cortical and subcortical (thalamus and basal ganglia) grey-matter volume, and the more preterm the infant, the greater the reduction in volume (4). This reduction in the volumes of cortical and subcortical (thalamus, basal ganglia) grey matter is not always associated with overt signs of white matter volume loss or injury. These observations are supported by the study by Vinall et al. (2), which showed that lower gestational age is associated with higher cortical fractional anisotropy (FA)—a diffusion imaging measure that reflects axon density and diameter as well as extent of myelination (white matter). FA values change during development of the cerebral cortex and are higher at early developmental stages because the majority of processes are radial—that is, structures mature and migrate from sites of neurogenesis (Fig. 1). Later, after completion of neurogenesis, radial glia progenitors disappear or are transformed to astrocytes (star-shaped glial cells that support neurons of the brain and contribute to the blood-brain barrier); cortical connections that transport nerve impulses from sense organs (afferent) and to subcortical structures (efferent) mature; and neurons develop extensive branching and arborization (treelike arrangement of processes). These processes are reflected in a normal reduction in FA values with increasing age of the cortex.

The Vinall et al. (2) study used a noninvasive imaging method—diffusion tensor MRI [at 1.5 Tesla (T), a unit of measurement that indicates the strength of a magnetic field]—to map the diffusion of water in the developing brains of a large cohort (N = 95) of newborn preterm human infants (neonates). The diffusion patterns of water provide an indirect measure of the existing macromolecular and structural elements—“obstacles” that alter fluid flow in the organ being measured, decreasing anisotropy—and thus yield information about the state (normal or abnormal) of the tissue architecture. The authors investigated changes in the cortical microstructure of the preterm neonates over time; scan 1 was taken at ~32 weeks gestation and scan 2 at ~40 weeks postmenstrual age (term equivalent). From these scans, Vinall et al. documented the expected decreases in the FA of cortical grey matter with the increasing postmenstrual age of the preterm neonates and then sought to establish which clinical parameters influenced cortical development after preterm delivery. Although the authors’ term-equivalent cortical data could be compared with data from term-born control neonates, a complete understanding of the normality of the observed decreases in cortical FA over time after preterm delivery would ideally require a parallel study of normal fetal cortical development in utero. Fetal diffusion studies have been performed, but in order to produce comparable data on cortical anisotropy, the imaging techniques require considerable optimization to overcome the effects of maternal and fetal motion and of the inherently poor signal-to-noise ratio of the images.

MECHANISMS BEHIND THE MAYHEM

Although it is accepted that cortical development is altered by preterm delivery, the causal mechanisms remain unclear. There are several alternative hypotheses. According to the first hypothesis, cortical volume loss is a product of neuronal loss of function and death (primary neuronal degeneration), possibly accompanied by so-called secondary retrograde neuro-axonal degeneration, which results from primary injury to immature white matter and subplate, a common occurrence in preterm infants. The second hypothesis is that cortical volume loss arises because of a failure of neuronal maturation rather than cell death or axonal degeneration. A third hypothesis—which implies that neither neuronal degeneration nor aborted maturation accounts for the brain volume decrease—suggests that thalamic areas of the brain (Fig. 1) are the primary sites that show altered growth in preterm infants (Fig. 2).
The thalamic region—a conglomerate of several nuclei with cortical connections that sit between the telencephalon and midbrain—relays information between the cerebral cortex and the sensory organs and subcortical regions of the brain. Altered growth in the thalami would result in secondary alterations in cortical development.

Premature newborns who display intrathoracic growth restriction (birth weights in the <10th percentile) demonstrate altered cortical folding and reduced cortical volumes when compared to preterm infants born at an appropriate weight for gestational age or full-term controls (5–7). The studies behind these findings were significant at the time, but they all had shortcomings. Toft et al. (6) used suboptimal criteria to define intrathoracic growth restriction and did not acquire true volumetric MR sequences. The Dubois et al. study (5) was conducted on a relatively small cohort (N = 35) that was further subdivided into infants with (N = 10) and without brain lesions. The authors assessed the folding process of the developing cortex rather than specific brain volumetric parameters and showed an additional nonsignificant alteration in cortical development: the presence of small white matter lesions. The Tolsa et al. study (7) assessed the impact of intrathoracic growth restriction on cortical development in infants born preterm. The authors used an adequate definition of intrathoracic growth restriction and a true volumetric MR sequence to quantify brain parameters but had no term-born control group: The clinical neurological outcome was assessed only at term-equivalent age. Therefore, no relationships could be established between their brain parameters and later cognitive or behavioral scores.

Both postnatal somatic (body) growth and cortical development in neonates born very preterm (less than 30 weeks of gestation) have been directly associated with cognitive ability in later years (8, 9). Cerebral palsy (CP)—nonprogressive movement disorders caused by ischemic brain injury during pregnancy or birth—results from lesions in the brain, and it is unusual to find CP in children who were born preterm (so-called ex preterm) but do not show evidence of brain lesions or abnormality with high-quality MRI. However, ex preterm children may exhibit altered cortical connectivity and synchronization (assessed by functional MRI) during cognitive tasks relative to full-term control children, even in the absence of significant cognitive disability (10, 11). The etiology of altered cortical development and processing in ex preterm children is currently not known.

**SIZE MATTERS**

The Vinall et al. (2) human MRI study set out to examine the extent to which poor postnatal growth relates to the microstructure of the developing cerebral cortex in human infants...
born very preterm. In evaluating the relationship between neonatal (postbirth) growth and cortical development, it is important to consider the effects of multiple medical conditions in infants born very preterm that can confound experimental measurements in this cohort. Therefore, the authors included several such variables—evidence of intrauterine growth restriction, gestational age, and sex—in longitudinal multivariable models to relate postnatal growth restriction with cortical diffusion tensor imaging parameters.

The study revealed that neonatal growth predicted cortical grey matter maturation independent of gestational age, birth weight percentile, brain injury, and systemic illness. Changes in cortical FA of infants born very preterm with impaired postnatal growth reflected changes in the radial, but not the axial, diffusion axis, suggesting a delay in the formation of neuronal processes within the cerebral cortices. In contrast to the grey matter, FA values for the white matter were not associated with postnatal weight changes, suggesting that white matter maturation is spared from effects of postnatal growth restriction.

Previous studies have emphasized that altered cortical development is associated with intrauterine growth restriction as a subsequent result of deficits in myelination. Although this association may still be valid, the Vinall et al. (2) study turns the focus from cerebral white matter development to neonatal somatic growth and its associated cortical grey matter vulnerability. The new work also implies that somatic growth restrictions that are more pronounced than expected for a particular gestational age and that persist after birth are associated with lower than normal cerebral cortical volumes and altered brain microstructure early in life. Extending the work of Vinall et al. to prenatal stages could yield mechanistic information about different kinds of brain developmental aberrations. Intrauterine growth restriction that results from placental dysfunction may have different effects on the developing brain than the presence of poor postnatal growth in preterm infants, which is likely to be multifactorial.

The results of Vinall et al. have important implications for future basic, translational, and clinical research on brain development and function. Neonatal growth over and above birth weight, brain injury, and systemic illness all predict the extent of cortical grey matter maturation in infants who are assessed in neonatal intensive care units. Therefore, by establishing the optimal somatic growth rate for a preterm infant and how this is best

Fig. 2. Thalamocortical connections. Advanced diffusion tensor imaging study at 3 Tesla of a cohort of preterm-born human infants imaged at term-equivalent age (N = 47). Diffusion tractography was used to compare thalamocortical connectivity between preterm infants and healthy term-born controls (N = 18). The regions in the cortex where connections to the thalamus were significantly altered after preterm birth are shown in red and yellow. The larger the difference, the higher the value (yellow = highest). Cortical connectivity was calculated on a voxelwise basis, and the test statistics are corrected for multiple comparison across the cortex (P < 0.001). [Reproduced with permission from (16)]
achieved, clinicians have the opportunity to optimize environmental conditions to mimic those that permit cortical development to proceed normally in infants born very preterm. However, it remains difficult to dissociate isolated poor somatic growth from poor growth that occurs secondary to ongoing or earlier illnesses. Guidelines for optimal neonatal growth also must address studies that provide evidence that accelerated postnatal growth increases the risks of subsequent adverse metabolic, endocrine, and cardiovascular outcomes (12).

The Vinall et al. study demonstrates the value of measuring FA to assess cortical maturation in preterm newborns and emphasizes that FA of cortical grey matter decreases nonlinearly with increasing postmenstrual age. Numerous entities can lead to changes in MRI-defined cortical microstructure, and the relationship between MRI findings and their anatomical correlates of brain maturation is not clear from this study (Fig. 1). Indeed, researchers lack a basic understanding of the remodeling of these circuits and their imaging correlates.

COUNTING SHEEP
It would be of great interest to systematically compare various cortical areas during normal fetal development and then examine the effects of prematurity with or without acquired brain injury. Such studies require combined imaging and detailed anatomical investigations. The new research by Dean et al. (3) aims to do just that. This study made use of a fetal sheep model of preterm birth and revealed that prenatal cerebral ischemia reduced cortical volume and disrupted MRI-defined cortical microstructure through reduction in the development of dendritic arborization of cortical neurons rather than from a reduction in neuron numbers. The immature (0.65 gestation) fetal sheep is a global cerebral ischemia model from reversible bilateral carotid occlusion. Similar to humans, the sheep model has limited cerebral vascular autoregulation (that is, unable to maintain adequate and stable cerebral blood flow in conditions of over- or underperfusion), and it allows the monitoring of fetal heart rate, blood pressure, blood gases, and cerebral blood flow. The relative timing and general pattern of normal human and fetal sheep white-matter maturation and myelination are similar to those of the ischemic fetal sheep model used by Dean et al., which corresponded to an ~24- to 28-week-old human premature infant.

Dean and colleagues (3) show that fetal sheep that had undergone a single 37-min bilateral carotid artery occlusion inflicted at 91 days of gestation time displayed no loss of cortical neurons several weeks later after premature birth. However, these sheep did display reduced dendritic arborization 4 weeks after birth and an associated postmortem reduction in cortical MR FA values measured at 11.7 T in fixed cortical tissue, which suggest disrupted cortical maturation. The authors ruled out the possibility of significant neuronal loss by assessing cortical neuronal death with a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay.

The Dean et al. study (3) makes an important contribution toward understanding of why there is an apparent loss of cortical volume in human preterm infants when assessed with clinical MRI. Until now, there has been inadequate data to determine whether cortical volume loss results from neuronal loss and, if so, whether this loss is a primary effect or a downstream effect from primary white matter and oligodendrocyte loss, which was previously observed in the preterm sheep brain after hypoxia by the same authors in their previous studies. However, despite providing some much needed answers, the new work leaves us with more questions. The experimental data of Dean et al. are confined to the cortical plate. It is unfortunate that data are not presented on the subplate, a transient zone in the developing cortex that contains some of the earliest generated neurons and connections below the cortical plate (Fig. 1). Across the gestational window, the subplate is essential for setting up cortical neuronal synaptic networks while also being the subject of involution (of subplate neurons) through apoptosis (13). Even in other model organisms, it is not yet clear whether cortical dendritic arborization may be driven in part by the (regressing) subplate.

Dean et al. (3) convincingly show disturbances in dendritic arborization and spine elaboration in their sheep model and suggest that pyramidal neurons—the primary excitation units of the mammalian cortex—in the cortical plate are highly susceptible to abnormal development. The authors used Golgi staining to expose the complete somatodendritic (cell body and all its processes) morphology of selected neurons in the frontal cortex at the level of diffuse white matter injury in sheep brains four weeks after the hypoxic insult and analyzed dendritic length, branching complexity, and other measures of neuronal maturation. The reduction in dendritic arborizations in the hypoxic-insult model compared to controls was distributed across all cortical layers. Neuronal complexity was analyzed at multiple time points after the hypoxic event (at this time, normal dendritic arbor complexity is low). Four weeks later, in control brains, the arbor underwent rapid expansion consistent with the pronounced expansion in cortical volume. The authors suggest that dendritic abnormalities observed in the hypoxic-insult model are related to disrupted maturation in this four-week time window rather than to dieback of a previously formed arbor.

This analysis was performed on reconstructed cells from Golgi-stained preparations. Because of the nature of this analysis, the authors had to select pyramidal cells (which was performed blind) from stained specimens. In such comparisons, it is very important that the same cell types from the same layer and area are sampled and compared. There are hundreds of different cell types estimated in the cortex, making this a difficult task. The authors make the point that the “effect of ischemia on neuronal complexity was independent of cortical location.” The resolution of this statement was restricted to the supra- and infragranular layers of the cerebral cortex and was not examined in specific layers. Because maturation of the cortical fields follows a specific sequence, the study should be extended to several different cell types and cortical regions.

The disrupted dendritic maturation is consistent with the FA abnormalities observed 4 weeks after ischemic insult in the sheep (3) and clinically in preterm infants (2). However, it is not clear whether all neuronal types are affected or whether a particular class of pyramidal neurons is relevant to the study of cognitive disabilities seen so frequently in preterm survivors. If not all pyramidal neurons are affected, then what proportion of cortex pyramidal neuron abnormalities is sufficient to result in altered FA across the whole cortex? And are the alterations in arborization alone sufficient to result in the significant alterations in measurable cortical volumes demonstrated in preterm infants? Moreover, cortical circuit development is not a linear process in which simple, radially oriented elements progressively develop more and more vertically
extending dendrites. There are numerous regressive events during normal brain development during which dendritic arbors are remodeled (for example, pyramidal neurons lose apical dendrites, spiny stellate cells remodel their apical dendrites, subplate neurons retract dendrites) (14). Examination of these specific cell populations would be an interesting future direction.

**FORM AND FUNCTION**

The new studies (2, 3) highlight the need for basic research to provide mechanistic information on cerebral cortical circuit formation and the causes of cortical aberrations. The connectivities of several brain cell types change over time and are modeled according to precise phases of cortical circuit assembly, and many of these processes have not been deciphered even in the most common rodent model systems. We know even less about these changes when the developmental program is altered by external factors and the subsequent developmental program is derailed. The Dean et al. study (3) represents an example of the way forward: It employs a model that is, in many respects, similar to the human situation, but modern developmental biology and imaging tools can be used to gain mechanistic insights. The authors establish the first links between fine changes in cortical circuits (somatodendritic remodeling, spine formation, and progressive and regressive events) and MRI data in the same model. The current resolution obtainable with clinical imaging might not be sufficient to detect many of these changes, but it can begin to link clinical findings such as those of Vinall et al. (2) to mechanistic explorations in model systems. Linking cortical MRI parameters with functional MRI studies in preterm infants could help researchers ascertain the corresponding functional significance of these parameters for early and subsequent neuro-cognitive development.

The two new studies also illustrate the need for more intricate imaging of animals and patients and how mechanistic studies in animals can shed light on the human condition. The reduction in the development of dendritic arborization of cortical neurons characterized in the sheep model provides a new focus on the role of the environment in improving adverse neurodevelopmental outcomes in preterm newborn infants. It is possible that specific stimulation of the neonate may improve the development of dendritic arborization. Stimulus-driven alterations in brain development might explain the major observed effects of social class and maternal education on long-term cognitive outcomes (15).

**REFERENCES AND NOTES**