Trends in Neurosciences

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REVIEWS

Review

Cortical Dysmaturation in Congenital Heart Disease

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Congenital heart disease (CHD) is among the most common birth defects. Children with CHD frequently display long-term intellectual and behavioral disability. Emerging evidence indicates that cardiac anomalies lead to a reduction in cerebral oxygenation, which appears to profoundly impact on the maturation of cerebral regions responsible for higher-order cognitive functions. In this review we focus on the potential mechanisms by which dysregulation of cortical neuronal development during early life may lead to the significant cognitive impairments that commonly occur in children with CHD. Further understanding of the mechanisms underlying cortical dysmaturation due to CHD will be necessary to identify strategies for neonatal neuroprotection and for mitigating developmental delays in this patient population.

Highlights

Patients with CHD frequently exhibit a broad spectrum of neurological deficits. Currently, the neurological deficits exhibited by CHD patients are irreversible.

Fetal cerebral hypoxemia is correlated with impaired cortical development in infants with CHD.

The perinatal period encompasses several critical windows for cortical maturation, including neurogenesis, neuronal migration, dendritic maturation, and circuit formation.

Recent animal studies show that both transient fetal hypoxemia and chronic perinatal hypoxemia have deleterious effects on neuronal development.

Studies in large animal models are essential to unravel the complex molecular and cellular mechanisms underlying cortical dysmaturation associated with CHD.

Why Study Disturbances in Cortical Development Associated with Congenital Heart Anomalies?

Human brain development is a highly dynamic process that involves appropriate timing and orchestration of cellular and molecular events. The unique cognitive abilities of humans likely result from a relative expansion of cortical regions responsible for intellectual and behavioral functions [1]. The frontal lobe, is particularly enlarged in humans, and is involved in higher-order executive functions such as planning, reasoning, decision making, attention, and personality. An appropriate balance between excitation and inhibition in the brain is crucial for its proper functioning [2]. Although most human cortical neurogenesis is completed in late second or early third trimesters of gestation, recent studies indicate that substantial cortical neurogenesis continues postnatally. New neurons migrate along the lateral ventricles to the frontal lobe in the early postnatal period. These neurons differentiate into inhibitory neurons and integrate into the frontal cortex, ultimately contributing to the development of cortical regions responsible for higher-order cognitive processes [3,4].

Advances in non-invasive neuroimaging have provided a detailed picture of the dynamic human cortical expansion that occurs during late fetal and early postnatal life (from 28 to 48 weeks of gestation age [5]). The brain is broadly vulnerable throughout this key developmental period. Various insults that can cause brain damage during this period include impairments associated with preterm birth, hypoxic–ischemic encephalopathy, and CHD [6–8]. Widespread disturbances in brain development associated with CHD affect the maturation of many regions, including the cortex, basal ganglia, thalamus, and cerebral white matter [9,10].

Recent magnetic resonance imaging (MRI) studies have demonstrated decreased cortical expansion and sulcation in the fetal and neonatal brain of individuals with severe and complex CHD (Table 1). Although the hospital mortality risk of neonatal surgery for severe and complex CHD has been greatly reduced over the past 2 decades, children frequently display long-term

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Table 1. Recent Imaging Studies[a] Assessing Preoperative Impairments in Cortical Maturation in CHD[b]

	Cohort	Gestational age	Cases (*n*)	Controls (*n*)	Technique	Findings
Fetal						
Zeng *et al.* 2015 [85]	Mixed	Second and third trimester	73	168	Ultrasound	Progressive ↓ frontal lobe volumefrom GW28
Schellen *et al.* 2015 [36]	TOF	25 weeks	24	24	MRI	↓ Cortical GM volume
Clouchoux *et al.* 2013 [35]	HLHS	25–37 weeks	18	30	MRI	↓ Cortical GM volume ↓ Cortical surface area ↓ GI Cortical sulcation delay
Masoller *et al.* 2016 [86]	Mixed	36–38 weeks	58	58	MRI	↓ Cortical sulcation
Kelly *et al.* 2017 [37]	Mixed	39 weeks	30	30	MRI	↓ Cortical GM volume ↓ GI
Postnatal preoperative						
Ortinau *et al.* 2013 [38]	Mixed	Term	15	12	MRI	↓ Cortical surface area ↓ GI
von Rhein *et al.* 2015 [27]	Mixed	Term	19	19	MRI	↓ Cortical GM volume ↓ Frontal, temporal, parietal, and occipital lobe volume
De Asis-Cruz *et al.* 2018 [39]	Mixed	Term	30	82	rs-fc MRI	Altered functional brain connectivity

[a]Studies within the past 5 years.
[b]Symbols and abbreviations: ↓, reduction; GI, gyrification index; GM, grey matter; GW, gestational week; HLHS, hypoplastic left heart syndrome; rs-fc MRI, resting state functional connectivity MRI; TOF, tetralogy of Fallot.

intellectual and behavioral disabilities after complex cardiac repair [11]. Thus, there is a crucial need to define the mechanisms underlying cortical disturbances to develop therapeutic interventions to lessen the impact of these developmental insults. Although cortical neurogenesis at fetal and adult stages has been widely studied (reviewed in [12,13]), the development of the frontal cortex during the perinatal period, particularly in humans, has only recently received greater attention as a result of methodological advances and the identification of ongoing postnatal neurogenesis in this region. It is crucial to understand the mechanisms involved in this process because dysregulation of cortical neurogenesis likely contributes to neurodevelopmental disabilities in children with CHD (Figure 1, Key Figure). Moreover, understanding the impact of CHD on the genesis, migration, maturation, and integration of newly migrated cortical neurons will be imperative to develop rational strategies for neonatal neuroprotection.

This review focuses on cortical maturation and the deleterious effects of CHD-induced hypoxemia on brain development during the critical window of perinatal development. We first discuss aberrant brain maturation in children with CHD and the associated neurodevelopmental disabilities. We then discuss current understanding of early postnatal neurogenesis in gyrencephalic species. We will focus on the impact of CHD on cortical maturation, particularly with respect to excitatory/inhibitory imbalance in developing cortex. We place special emphasis on the urgent need for well-designed animal studies to define disturbances in cortical neurogenesis caused by perinatal brain injury. Further study of the impact of hypoxemia on brain development is of broad relevance not only for children with CHD but for other populations in which perinatal hypoxic stress may cause long-term intellectual and behavioral dysfunctions.

Key Figure

Impact of Congenital Heart Disease on Perinatal Cortical Maturation and Subsequent Cerebral Impairments

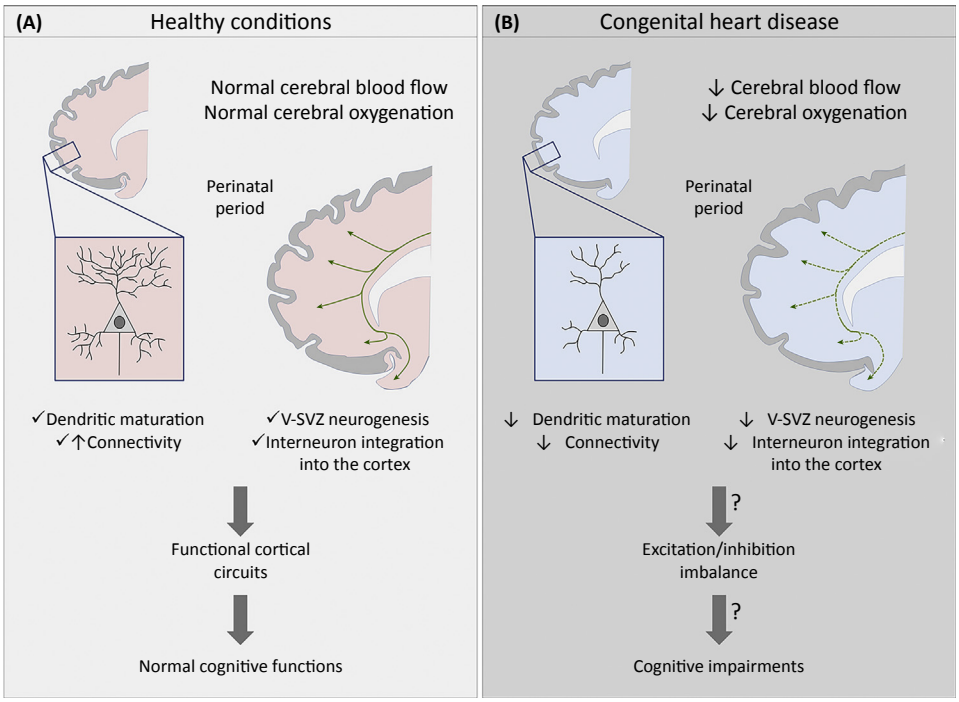

Figure 1. Cartoon illustrating perinatal cortical development in healthy conditions (A) and in congenital heart disease (CHD) (B). Before corrective surgery, CHD reduces cerebral blood flow and oxygenation. In animal models, hypoxemia is associated with a disruption of subplate neuron maturation and connectivity prenatally, and a decrease of ventricular–subventricular zone (V-SVZ) neurogenesis and interneuron populations in the frontal cortex postnatally. These alterations could result in a cortical excitation/inhibition imbalance and potentially explain the cellular basis for the spectrum of cognitive impairments observed in CHD patients. Green lines and arrows represent streams of neuroblasts in the V-SVZ migrating to the olfactory bulb and frontal lobe.

The CHD–Neurodevelopmental Axis

Neurodevelopmental Disabilities Associated with CHD

CHD is among the most common birth defects and represents the leading cause of infant mortality associated with birth defects [14]. Each year, CHD affects almost 1 in every 100 infants, of whom ~25% will need catheter intervention or cardiac surgery during their first year of life [15]. Improvements in surgical techniques and therapeutic interventions allow most patients to reach adulthood. In 2010, ~1.4 million adults and 1 million children were estimated to be living with CHD in the USA [16]. Although the hospital mortality risk is greatly reduced, children with CHD frequently display subsequent neurological disabilities affecting intellectual function, memory, executive function, speech and language, gross and fine motor skills, and visuospatial functions [17,18]. These disabilities often persist into adolescence and adulthood, and can ultimately represent long-term neurocognitive disabilities [19,20]. In addition to the

impact of the neurological morbidity on the patients themselves, the toll on families and society is immense. Accordingly, the Pediatric Heart Network of the National Heart, Lung, and Blood Institute has declared that 'one of the most important challenges in the 21st century for CHD is to improve neurological deficits' [21]. Elucidating the mechanisms underlying CHD-induced neurological impairments is therefore crucial for this growing population of patients.

Brain Maturation and Cortical Development in CHD

Historically, adverse neurological outcomes observed in CHD patients were mainly attributed to neonatal cardiac interventions. It is now clear that neurological deficits in CHD involve several complex factors, often combinatorial and cumulative, including (i) intrinsic genetic factors, (ii) preoperative factors related to abnormal fetal cerebral blood flow and oxygen and nutrient delivery, (iii) operative factors related to cardiopulmonary bypass or deep hypothermic circulatory arrest, and (iv) postoperative factors involving low cardiac output [11,17,19,22,23]. Recent advances in neonatal brain imaging revealed that CHD patients display antenatal and preoperative abnormalities in brain size, maturation, structure, metabolism, and connectivity [24,25]. During the third trimester of gestation, CHD fetuses display smaller total brain volume and intracranial volume compared with healthy fetuses [26]. This decrease is also observed at birth before any corrective operation [27]. In neonatal care, macroscopic brain development and maturation are estimated using a 'total maturation score'. This score is reduced preoperatively in neonates with CHD, which supports a link between CHD and impaired brain maturation that is independent of cardiac surgery [28–30]. Brain maturation can also be quantified using magnetic resonance spectroscopy through analysis of brain metabolite ratios. For instance, the ratio of *N*-acetyl aspartate (NAA) to choline increases with brain maturation, whereas the ratio of lactate to choline decreases. In the neonatal CHD brain, compared with control newborns, there is a 10% decrease in the NAA:choline ratio and a 28% increase in the lactate:choline ratio [9]. These findings, similar to those seen in premature infants, reflect a delay in brain development induced by CHD *in utero*.

In preterm birth, smaller cerebral volumes are associated with poor neurobehavioral outcomes in early childhood [31]. Specifically, cortical grey matter volume is negatively associated with motor performance and cognition at 24 months and with developmental quotient at age 3.5 years [32]. Given this established link between cortical development and neurobehavioral outcomes, we focus here specifically on disturbances in cortical development which are likely to be key contributors to cognitive and learning disabilities in CHD. It should be noted, however, that CHD patients also display a large spectrum of white matter injury [33], which likely plays a role in developmental delay associated with cardiac abnormalities, and possibly also with other forms of brain dysmaturation.

Synaptic connections and neuronal activity dramatically increase during the third trimester of pregnancy [34]. Moreover, neurons continue to migrate postnatally, particularly to the frontal lobe [3]. Deleterious factors during CHD, such as chronic hypoxemia, likely impair perinatal cortical maturation and disrupt long-term neurodevelopment (Table 1 for recent CHD studies that assessed the role of preoperative insults on cortical maturation). For example, fetuses with hypoplastic left heart syndrome (HLHS) and tetralogy of Fallot display reduced cortical grey matter volume [35,36]. Moreover, delays in cortical gyrification, in addition to reduced cortical grey matter volume, are observed prenatally and postnatally before corrective surgery in mixed cohorts of CHD [37,38]. Notably, a recent study using resting-state functional connectivity MRI (rs-fc MRI) reported alterations in connectivity in neonates with complex CHD before surgery and in the absence of brain parenchymal injury [39].

Disturbances in Cerebral Blood Flow in CHD

In contrast to the brain, which continues to undergo profound structural changes after birth, heart development (in terms of overall organ layout) is largely completed by gestational week seven [40]. During the third trimester, the blood supply to the brain increases and represents 25% of the combined ventricular output. Hence, normal cardiovascular function is crucial for optimal brain development. Cardiac defects cause modifications in the intracardiac circulation that result in changes in cerebral blood-flow characteristics [41,42]. A compensatory mechanism called 'brain sparing' is established during fetal development [43]. This autoregulation reduces cerebral vascular resistance and increases cerebral blood flow in fetuses with CHD [41,44]. Despite the protective function of brain sparing, this process is insufficient to ensure optimal blood supply to the brain in CHD [41], and its effectiveness also depends on the type of cardiac anomaly. For instance, brain sparing physiology seems to be impaired in the fetus with *dextro*-transposition of the great arteries (d-TGA) in whom somatic growth is preserved relative to head growth. By contrast, HLHS fetuses display notable cerebrovascular autoregulation [45]. Interestingly, lower cerebrovascular resistance in fetuses with single ventricle anomalies is associated with impairments in early neurological development [46]. Finally, an arterial spin-labeling MRI study found significant decreases in global cerebral blood flow in newborns with complex CHD, which suggests limitations in oxygen and nutrient delivery [42].

Chronic Cerebral Hypoxemia in CHD

Cerebral oxygenation is another crucial factor in brain development in the perinatal period. During fetal life, the brain consumes half of body oxygen [47]. In complex CHD (e.g., HLHS, d-TGA), oxygen supply to the brain is limited by decreased blood flow (ischemia) and also by desaturated blood streamed to the brain (hypoxemia) (see Figure I in Box 1). In addition, CHD is often associated with placental malformations such as abnormal maturation of villi, chorangiosis, inflammation, infarction, and fetal thrombotic vasculopathy [48]. Recently, non-invasive placental perfusion imaging demonstrated that global placental perfusion significantly decreases with advancing gestational age in pregnancies complicated by fetal CHD [49]. Overall, impairments in placental function further contribute to the fetal cerebral hypoxemia occurring in CHD. Blood saturation can be studied *in utero* with the blood oxygen level-dependent MRI signal, T2*. This signal is reduced in brains of fetuses with CHD, mainly due to the desaturation of blood streamed through their cerebral vasculature [50]. Fetuses with critical forms of CHD may have 10% less cerebral oxygenation compared to normal controls, and this is associated with a reduction in fetal brain volume [51]. This decrease of oxygen delivery to the brain also occurs in neonates with CHD before cardiac surgery [52,53]. Such neuroimaging studies indicate that aberrations in fetal circulation induce abnormal oxygen delivery that likely promotes developmental disturbances in CHD neonates. Although this relationship is not yet confirmed, recent advances in neonatal brain imaging have better defined brain injury mechanisms underlying CHD. It should be noted that hypoxemia also disrupts glucose delivery and metabolism, which may further contribute to aberrant cerebral development [54].

Impact of Hypoxemia on Cortical Development

Neonatal Neurogenesis in Humans

Originally believed to be complete before birth, neurogenesis and cortical maturation are now recognized to continue postnatally. In particular, early postnatal cortical development in gyrencephalic species seems to be determined by the neurogenic activity of the ventricular–subventricular zone (V-SVZ) [3,4]. In the mammalian brain, the V-SVZ is one of the most active neurogenic niches during the postnatal period. In rodents, neural stem cells residing in the walls of the lateral ventricles give rise throughout their lifespan to neuroblasts that migrate to the olfactory bulb via the rostral migratory stream (RMS) and differentiate into interneurons.

Box 1. *In Utero* Cerebral Hypoxemia in Complex Congenital Heart Defects

In the fetus, gas exchange takes place in the placenta and blood flow is distinctly different from the postnatal circulation. The stream containing the highly oxygenated blood from the placenta is preferentially directed across the foramen ovale to the left atrium, which favors the streaming of oxygen-rich blood to the brain (Figure IA). The unique blood supply to the fetal brain is influenced by many factors including the anatomic structure of the heart [87]. In many cases of complex CHD, these beneficial systems that regulate cerebral blood flow are altered *in utero* [42,51].

In *dextro*-transposition of the great arteries (d-TGA), the aorta arises from the right ventricle whereas the pulmonary trunk is connected to the left ventricle (the opposite of normal circulation). The oxygen-rich blood in the left ventricle is thus directed toward the body through the pulmonary trunk and ductus arteriosus. On the other hand, the brain receives relatively deoxygenated blood that streams from the superior vena cava through the right ventricle, which causes reduced cerebral oxygenation during the fetal period (Figure IB).

In hypoplastic left heart syndrome (HLHS), the left side of the heart is underdeveloped and dysfunctional. The oxygenated blood from the placenta and deoxygenated venous return mix in the right atrium. Blood with lower oxygen content is ejected into the pulmonary trunk and ductus arteriosus. In addition to reduced oxygenation, the blood supply to the brain is restricted by retrograde flow into the cerebral circulation and the small diameter of the aortic arch, which significantly decreases cerebral perfusion. In this syndrome, the fetal brain is therefore underperfused with hypoxic blood (Figure IC).

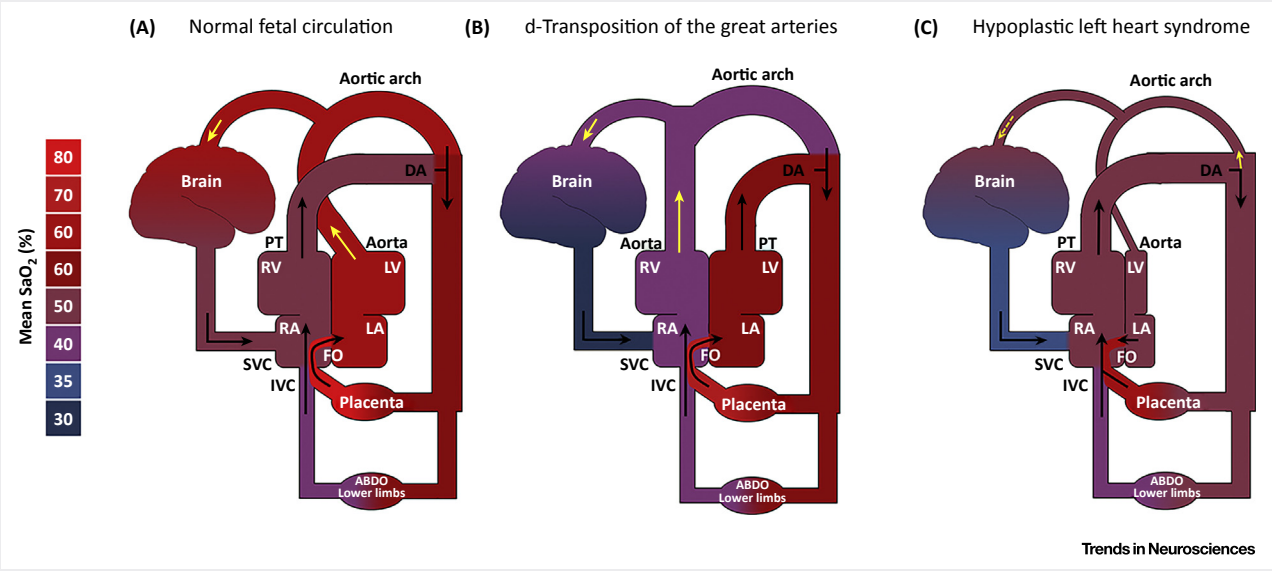

Figure I. Simplified Systemic Hemodynamics in (A) Normal Fetal Circulation, (B) *Dextro*-Transposition of the Great Arteries (d-TGA), and (C) Hypoplastic Left Heart Syndrome (HLHS). Black arrows represent the blood flow; yellow arrows represent the cerebral blood supply. The pulmonary circulation is not included because the blood flow to the lungs is highly limited owing to elevated pulmonary vascular resistance and relatively low lung volume before birth. Abbreviations: ABDO, abdomen; DA, ductus arteriosus; FO, foramen ovale; IVC, inferior vena cava; LA, left atrium; LV, left ventricle; PT, pulmonary trunk; RA, right atrium; RV, right ventricle; SVC, superior vena cava. Adapted, with permission, from Sun *et al.* [51].

Interestingly, this stream of migrating neurons is also observed in young infants [55]. In addition, the human infant brain displays another neuronal migration route, not reported in other vertebrates, called the medial migratory stream [55]. This route contains streams of migrating neuroblasts with elongated morphologies that divert from the proximal limb of the RMS to migrate to the frontal lobe. This supplementary stream provides interneurons to the ventromedial prefrontal cortex, an important area for social decision-making and risk processing [56,57]. Recently, numerous neuroblasts have been discovered in the frontal lobes. These cells seem to originate from various progenitor zones in the ventral forebrain, but their exact birth time and place are not yet defined (Box 2). These young neurons migrate tangentially along the lateral ventricles in the V-SVZ, and then radially to an extensive region of the anterior forebrain, including the cingulate gyrus and prefrontal cortex [3]. The migrating neuroblasts subsequently integrate within the frontal cortex. These cells differentiate into interneurons and seem to play a crucial role in postnatal plasticity (Box 3).

Box 2. Embryonic Origins and Diversity of Interneurons

In the mammalian developing brain, excitatory and inhibitory interneurons are generated separately from each other. Excitatory neurons originate from the dorsal telencephalon, whereas interneurons derive from specific transient germinal zones in the ventral telencephalon, namely the ganglionic eminences (GEs) and the preoptic area. The GEs are spatially subdivided into lateral, medial, and caudal domains (LGE, MGE, and CGE, respectively). Interneurons differentiate locally or migrate tangentially from the subpallium to their final destination. In mice, the LGE produces inhibitory projection neurons destined for the striatum as well as inhibitory interneurons destined for the olfactory bulb. The MGE generates local projection neurons for the globus pallidus and the amygdala, as well as interneurons migrating to the cortex, striatum, hippocampus, and amygdala. The CGE gives rise to local caudal striatal neurons as well as to interneurons migrating mainly to the cortex, striatum, and hippocampus ([88] for review).

Rodent cortical interneurons are mainly produced in the CGE and the MGE, and also in the preoptic area, although to a lesser extent. The MGE is responsible for 50–70% of cortical interneurons, which include two large non-overlapping subpopulations of interneurons expressing parvalbumin and somatostatin. Interestingly, the subtype of interneurons originating from the MGE depends on the temporal sequence of their generation. The CGE gives rise to late-born interneurons, which represents another population of cortical interneurons (30–40%) that mainly express the 5HT3a receptor but also other markers such as calretinin, vasoactive intestinal peptide, and reelin. Finally, the preoptic area produces a small fraction (5–10%) of different subtypes of cortical interneurons that frequently express neuropeptide Y [88].

Studies suggest that the neocortex contains >20 different classes of interneurons categorized based on their morphological, molecular, electrical, and synaptic properties ([89] for more on interneuron diversity). As discussed above, this considerable interneuron diversity is mainly shaped by developmental spatiotemporal determinants such as spatial origins and birthdate [90]. In addition, environmental cues play a crucial role in determining subtype-specific features of cortical interneurons including settling position, morphology, synapse specificity, and afferent and efferent connectivity [89]. Modification of the environmental input as a result of perinatal CHD-induced brain injury could therefore interfere with interneuron diversity and influence cortical maturation.

Impact of Hypoxemia on Neurogenesis and Interneuron Migration

There is a growing consensus that chronic antenatal and early postnatal insults contribute to long-term neurodevelopmental disabilities in children with CHD (Table 1). Hypoxemia during CHD affects the brain from the third trimester until after birth [50–53]. It is therefore possible that CHD disrupts the recruitment of these late-migrating neurons and their connectivity.

The impact of chronic hypoxemia on cortical neurogenesis was recently studied using the neonatal piglet [4]. This species is particularly relevant because the early postnatal porcine V-

Box 3. Roles of Interneurons in the Frontal Cortex

Although constituting a minority of cortical neurons (~20% in rodents) [91], GABAergic inhibitory interneurons play a crucial role in fine-tuning the activity of pyramidal neuron assemblies. To date, it is not clear if the late-migrating cortical GABAergic inhibitory interneurons play a different role from those that migrate earlier, or if they simply serve as an additional mechanism of postnatal plasticity. More than 20 different classes of interneurons have been identified in the cerebral cortex [59] that display distinct or partially overlapping functions, morphologies, and properties, and this makes their classification highly complex (reviewed in [89]). In particular, one criterion for the classification of GABAergic inhibitory interneurons is the expression of molecular markers including calcium-binding proteins (parvalbumin, calbindin, calretinin), neuropeptides (e.g., vasoactive intestinal peptide, neuropeptide Y, reelin, somatostatin), and receptors (e.g., 5HT3R, mGluR1, CB1). Interestingly, this interneuron diversity depends on the interaction of specific genetic and environmental factors (Box 2) [88,92]. The postnatal integration of interneurons into the frontal cortex could therefore influence this diversity and contribute to delayed plasticity during postnatal human development.

At early stages of brain development, GABA is thought to exert a depolarizing effect on neural cells [93], and plays a crucial role in circuit maturation and cortical plasticity. It allows cortical networks to generate oscillations of wider amplitude and faster frequencies that are distinctive features of circuit maturation. In addition, GABAergic transmission can shape the synaptic wiring and large-scale architecture of neuronal circuits. For instance, such transmission adjusts intracortical circuitry by inducing morphological changes at the level of dendritic spines [94]. Overall, GABAergic inhibitory interneurons play a crucial role in cortical development and maturation. They are particularly relevant in the context of perinatal brain injury because postnatal disruption of the GABAergic system may contribute to the etiology of neurodevelopmental disorders.

SVZ shares structural features with the postnatal human V-SVZ and displays a high density of proliferative neuroblasts [58]. Moreover, akin to that in human, young neurons in the porcine SVZ migrate postnatally to frontal cortices, where they mainly differentiate into calretinin-positive interneurons. After chronic hypoxemia, porcine brains are smaller and exhibit a significant reduction in cortical grey matter volume and gyrification index, which is also observed in the neonatal brain with CHD. Chronic hypoxic exposure also induces a significant decrease of proliferative and non-proliferative neural stem/progenitor cells as well as neuroblasts within the V-SVZ (Figure 2). Moreover, the impaired neurogenic activity in the piglet V-SVZ after chronic hypoxemia is associated with reduced interneuron populations within frontal cortices, primarily the prefrontal cortex, which may induce imbalances between excitatory and inhibitory neurons. Interestingly, the V-SVZ of human infants that sustained

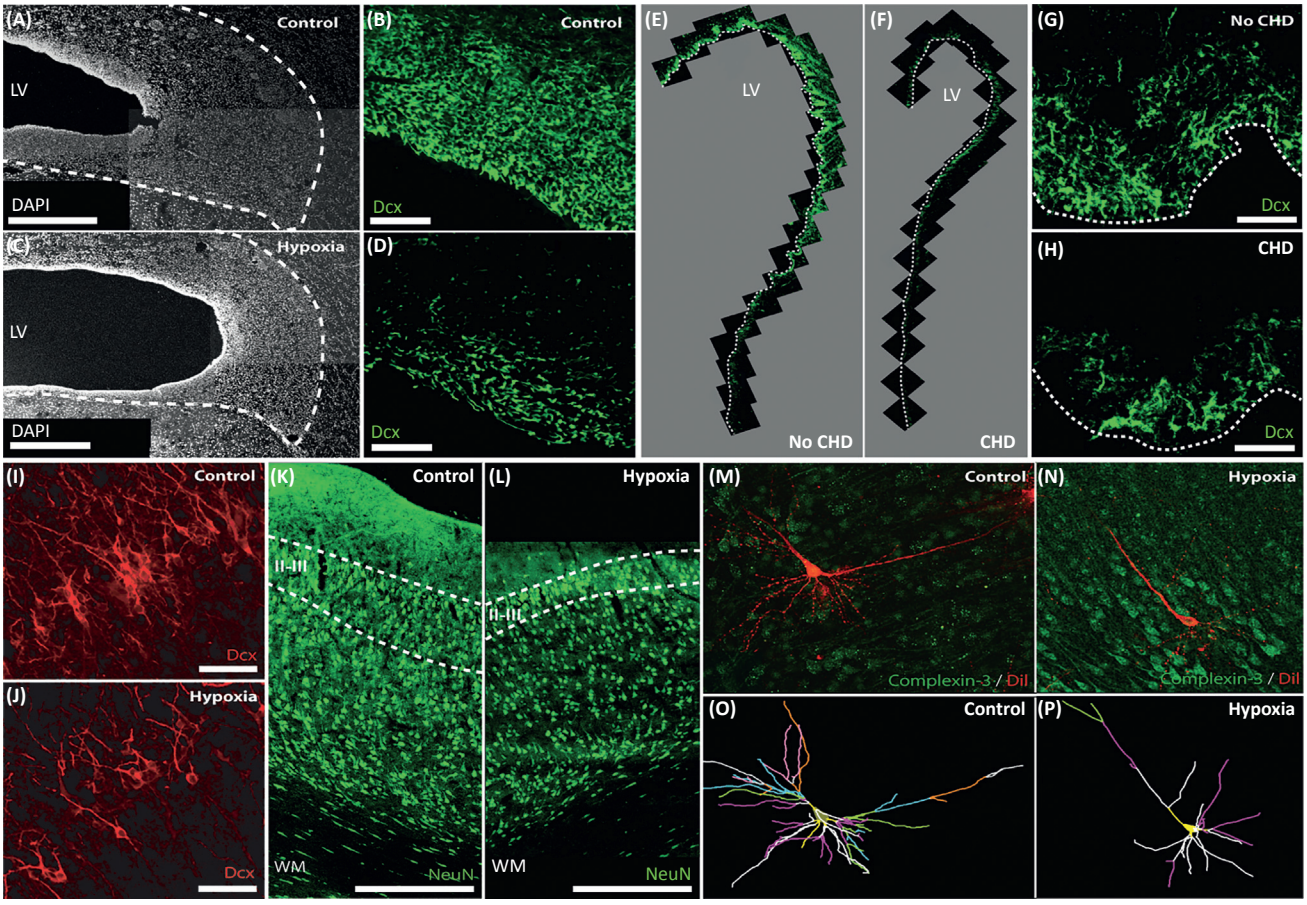

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Figure 2. Cellular Impairments in the Perinatal Brain Associated with Hypoxia and Congenital Heart Disease (CHD). Postnatal hypoxia in the piglet reduces the width of the subventricular zone (SVZ) (A,C; scale bars, 500 mm) and the number of neuroblasts (DCX^+) in the SVZ (B,D; scale bars, 50 mm). Similarly, CHD reduces neuroblast numbers (DCX^+) in the SVZ of human infants (E–H; Scale bars, 50 mm). Postnatal hypoxia in the piglet also reduces the number of DCX^+ immature neurons (I,J; scale bars, 50 mm) and $NeuN^+$ mature neurons (K,L; scale bars, 500 mm) in layers II/III of the frontal cortex. Prenatal hypoxia in the sheep results in a decrease in dendritic arborization of subplate neurons. 3D reconstructions of the soma and full basal and apical dendritic arbors (O,P) of representative sheep subplate neurons (M,N). Branch order rank is indicated by color: 1st order, bright yellow; 2nd order, white; 3rd order, purple/hot pink; 4th order, bright green; 5th order, cyan blue; 6th order, orange; 7th order, slate grey; 8th order, salmon pink; 9th order, forest green; 10th order, bright blue. Adapted, with permission, from Morton *et al.* [4] and McClendon *et al.* [75]. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; LV, lateral ventricle.

abnormal fetal cerebral blood flow as a result of CHD also displayed a pronounced depletion of neuroblasts [4] (Figure 2).

It is well documented that, in humans, intellectual and behavioral disabilities related to attention deficits, hyperactivity, learning, and working memory are significantly associated with imbalances between excitatory and inhibitory neurons in the frontal cortex [2,59]. These types of disabilities are also commonly observed in CHD patients [60]. For instance, neuropsychological assessment of school-aged children with CHD reveals lower scores in language, attention, executive functioning, and memory, even after successful cardiac surgical correction [20,61]. Attention-deficit/hyperactivity disorder symptoms are also more prevalent in children with CHD [62]. These findings suggest that developmental disturbances in the GABAergic system contribute to the neurodevelopmental disabilities seen in CHD [63] (Figure 1).

Impact of Hypoxemia on Prenatal Neuronal Maturation and Synaptic Activity

Transient or chronic disturbances in fetal oxygen delivery disrupt fetal brain development [64] and may also contribute to the pathogenesis of neurodevelopmental disabilities in CHD. Instrumented fetal sheep preparations provide unique access to analyze the impact of oxygenation on the fetal brain because of the feasibility to repeatedly sample fetal arterial blood gases and metabolites *in utero* in relation to a wide array of sensitive markers of disrupted brain development or injury [65]. Cerebral grey matter and white matter are differentially susceptible to fetal hypoxemia. White matter injury occurred infrequently in models of hypoxemia without cerebral ischemia in which a restriction in uteroplacental blood flow [66] or maternal hypoxemia [67] resulted in decreased fetal oxygen delivery and acidemia. However, moderate global cerebral ischemia in conjunction with hypoxemia appears to be a crucial factor to generate a spectrum of fetal ovine white matter injuries that range from diffuse non-necrotic lesions to severe cystic necrosis [68,69]. Late oligodendrocytes progenitors (pre-oligodendrocytes) are the primary cell type in the white matter that is lost in diffuse lesions arising from hypoxia–ischemia [70].

By contrast, severe global cerebral hypoxia–ischemia is necessary to induce pronounced fetal neuronal degeneration in cortical and subcortical grey matter structures [71]. In fact, immature fetal neurons are much less susceptible to cell death from hypoxia–ischemia than are pre-oligodendrocytes [71]. This neuronal resilience at the immature stage is also observed after middle cerebral artery stroke in preterm infants, who display remarkable cortical sparing in contrast to term neonates [72]. Despite the decreased susceptibility of immature neurons to fetal hypoxia–ischemia, cortical projection neurons nevertheless sustain disturbances in maturation of both the basal dendritic arbor and spines [73]. Reduced dendritic arborization contributes significantly to a reduction in cortical volume and accounts for disturbances in cortical anisotropy defined by high field MRI measurements [73]. Neuronal dysmaturation with similar morphometric features was observed in medium spiny projection neurons of the caudate, which also displayed significant disturbances in spine density and glutamatergic synaptic activity in response to hypoxia–ischemia [74].

A central role for hypoxemia in disrupted neuronal maturation was recently defined in the subplate of preterm fetal sheep where the response to a 25 minute hypoxic–ischemic insult was compared with that of exposure to hypoxemia alone [75]. The subplate resides directly beneath the cerebral cortex and comprises a unique neuronal population that is transiently present during development to provide guidance cues that establish thalamocortical connectivity [76]. Four weeks after the insult, subplate neurons displayed persistent disturbances in basal dendritic arborization that were accompanied by disturbances in subplate neuron excitability

and synaptic activity (Figure 2). Notably, subplate neuron dysmaturation was significantly linked to the magnitude of transient fetal hypoxemia. These findings suggest that fetal hypoxemia is sufficient to disrupt neuronal maturation. Although these findings argue that transient fetal hypoxemia can disrupt subplate neuron maturation, the response to chronic hypoxemia is unclear. Future studies will also be necessary to determine whether hypoxemia can disrupt the maturation of cortical projections neurons or interneurons in a fashion similar to subplate neurons (see Outstanding Questions).

Concluding Remarks and Future Perspectives

Because mortality rates among CHD patients have gradually decreased, there has been a greater research focus on improving neurological outcomes for the growing adult population with CHD. CHD affects cerebral maturation during a broad developmental window. Hypoxemia occurs both *in utero* and postnatally, and likely plays a crucial role in the cortical maturation disturbances that are frequently associated with CHD (Figure 1). In addition, multiple factors associated with cardiac surgery may injure the brain of infants with CHD, including inflammatory response, reoxygenation, reperfusion, and prolonged anesthesia exposure. Hence, in addition to chronic hypoxemia, cumulative pathological events in critical developmental time-periods could contribute to the high prevalence of neurodevelopmental disturbances in the CHD population [11,19,22].

Human pathology studies are technically challenging owing to limited access to autopsy tissues and the variability of postmortem tissue integrity. Hence, animal models are essential to elucidate the impact of CHD-induced insults on the developing brain. Despite the utility of rodent genetic tools, the differences between human and rodent brain development, especially regarding cortical maturation, greatly limit the study of CHD-induced disturbances. Larger mammals with gyrencephalic brains offer several advantages for elucidating the mechanisms of CHD-induced injury. Fetal sheep and neonatal piglets have been the key species used to study the prenatal and postnatal effects of chronic hypoxemia on brain development, respectively. Moreover, larger mammals often provide closer anatomical, physiological, and metabolic similarities to humans, which is valuable for evaluating neuroprotective strategies before potential clinical trials. As recent advances in genome editing, such as CRISPR/Cas9, become more available in larger mammals, it should be feasible to generate preclinical large animal models to access mechanistic questions related to the cumulative effects of hypoxemia on key developmental events including neuronal maturation and postnatal V-SVZ neurogenesis.

The development of human 'brain organoids' has dramatically advanced our ability to model human brain development *in vitro* [77]. These 3D structures are derived from human pluripotent cells (including induced pluripotent stem cells and embryonic stem cells) and recapitulate some key aspects of human brain development and function. For instance, neuronal cells organize in a multilaminar fashion in brain organoids. In addition, these structures generate an outer SVZ, a distinctive feature of the developing human SVZ [78]. Moreover, human cortical spheroids were recently fused to human subpallial spheroids to model the migration of interneurons from the subpallium to the cortex [79]. Interneurons functionally integrate with glutamatergic neurons to form a microphysiological system [79]. This system may provide novel access to study the effects of hypoxemia on perinatal circuit assembly, and help to elucidate cellular and molecular abnormalities occurring during the development of specific neural circuits.

Previous clinical trials in neonates and infants who underwent cardiac surgery have led to refinements in surgical methods that may improve the neurodevelopmental outcomes of CHD patients [80]. During the preparation of this manuscript an interventional clinical trial was

Outstanding Questions

How can we generate new animal models to study the effects of hypoxemia during gestation and during postnatal life?

During which developmental period, prenatal or postnatal, is the brain most sensitive to developmental and behavioral disabilities associated with hypoxemia?

How does prenatal dysmaturation of subplate neurons influence postnatal integration of interneurons into the cortex?

When and where are late-migrating interneurons generated? Does hypoxemia have an impact on the generation, migration, and maturation of these cells?

Does fetal neuronal dysmaturation play a role in the functional consequences of white matter injury in CHD patients?

How can we isolate the long-term neurological outcomes of CHD from the impact of corrective surgery on the brain?

To what extent can environmental enrichment play a beneficial role to improve disturbances in neuronal development arising *in utero* or postnatally during a highly plastic period in human brain maturation?

underway to test the effect of maternal hyperoxygenation in CHD (https://clinicaltrials.gov/ct2/show/NCT03136835). This approach likely results in a diffuse increase in fetal oxygenation which may influence fetal blood flow and heart dimensions [81,82]. Maternal hyperoxygenation may thus reduce the deleterious effects of hypoxemia on fetal systemic and brain development [81]. However, it should be noted that the response to maternal hyperoxygenation is highly cardiac lesion-dependent. For instance, the beneficial effect of hyperoxygenation is prevented by the presence a restrictive/intact atrial septum or ventricular septal defects [83,84].

Improving neurodevelopmental outcomes in CHD is one of the main challenges for pediatric cardiology. Recent advances in our understanding of fetal neuronal susceptibility to hypoxemia may have relevance to both infants with CHD and those with other causes of intermittent or chronic postnatal hypoxemia, such as apnea of prematurity or chronic lung disease. The identification of persistent human perinatal neurogenesis that targets the frontal cortex has further opened new opportunities that may lead to regeneration and repair of the dysmature cortex in children with CHD. The abundance of available tools to study the complexity of the cellular and molecular mechanisms underlying cortical development and dysmaturation will likely help to identify novel strategies to treat and improve outcomes in children suffering from CHD-related intellectual and behavioral disabilities.

Acknowledgments

The authors would like to kindly thank Drs Evan Goldstein and Joshua Corbin for critically reading the manuscript. This work was supported by R01HL139712 from the National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute (N. I.), 1RO1NS054044 and R37NS045737 from NIH/National Institute of Neurological Disorders and Stroke (NINDS) (S.A.B), 1R01AG031892-01 from NIH/National Institute on Aging (S.A.B), 17GRNT33370058 from the American Heart Association (S.A.B), District of Columbia Intellectual and Developmental Disabilities Research Center (DC-IDDRC) (V. G.), U54HD090257 from NIH/National Institute of Child Health and Human Development (V.G.), and R37NS109478 (Javits Award) from NIH/NINDS (V.G.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the DC-IDDRC or the NIH.

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