Pediatric Neurology 95 (2019) 42–66

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Topical Review

Dysmaturation of Premature Brain: Importance, Cellular Mechanisms, and Potential Interventions

Joseph J. Volpe, MD [a, b, *]

[a] Department of Neurology, Harvard Medical School, Boston, Massachusetts
[b] Department of Pediatric Newborn Medicine, Harvard Medical School, Boston, Massachusetts

ARTICLE INFO

Article history:
Received 30 January 2019
Accepted 19 February 2019
Available online 27 February 2019

Keywords:
Prematurity
White matter injury
Preoligodendrocytes
Brain dysmaturation
Neurorestoration

ABSTRACT

Prematurity, especially preterm birth (less than 32 weeks' gestation), is common and associated with high rates of both survival and neurodevelopmental disability, especially apparent in cognitive spheres. The neuropathological substrate of this disability is now recognized to be related to a variety of dysmaturational disturbances of the brain. These disturbances follow initial brain injury, particularly cerebral white matter injury, and involve many of the extraordinary array of developmental events active in cerebral white and gray matter structures during the premature period. This review delineates these developmental events and the dysmaturational disturbances that occur in premature infants. The cellular mechanisms involved in the genesis of the dysmaturation are emphasized, with particular focus on the preoligodendrocyte. A central role for the diffusely distributed activated microglia and reactive astrocytes in the dysmaturation is now apparent. As these dysmaturational cellular mechanisms appear to occur over a relatively long time window, interventions to prevent or ameliorate the dysmaturation, that is, neurorestorative interventions, seem possible. Such interventions include pharmacologic agents, especially erythropoietin, and particular attention has also been paid to such nutritional factors as quality and source of milk, breastfeeding, polyunsaturated fatty acids, iron, and zinc. Recent studies also suggest a potent role for interventions directed at various experiential factors in the neonatal period and infancy, i.e., provision of optimal auditory and visual exposures, minimization of pain and stress, and a variety of other means of environmental behavioral enrichment, in enhancing brain development.

Introduction

Preterm birth (less than 37 weeks' gestation) is an enormous public health problem worldwide. According to the World Health Organization, approximately 15 million premature infants are born yearly and account for approximately one million deaths.[1] The United States ranks sixth among countries in terms of the number of preterm births. According to the Centers for Disease Control and Prevention, from 2014 to 2017 the preterm birth rate rose in the United States to approximately 10%. Of the approximately four million births in the United States, 1.4%, or about 56,000, are of very low birth weight (less than 1500 g).[2] Survival rates vary markedly as a function of gestational age but are at least 95% at 32 weeks', 90% at 28 weeks', and 60% to 65% at 24 weeks' gestation.[3-5] The substantial survival rates, unfortunately, are accompanied by relatively high incidences of neurological disability, for example, cerebral palsy in 5% to 10%, other motor disturbances in 25% to 40%, and cognitive, attentional, behavioral, and socialization disturbances in 25% to 50%.[6-16]

The neuropathological substrate of this disability in preterm infants, especially those very preterm (less than 32 weeks' gestation) and extremely preterm (less than 28 weeks' gestation), consists of a combination of cerebral white matter injury (WMI) and especially, subsequent dysmaturational events in both white matter and neuroaxonal structures (see later). This combination of WMI and disturbances of gray matter structures has been termed the *encephalopathy of prematurity*.[17] In the initial review describing this encephalopathy, a particular emphasis was placed on the initial injury.[17] Subsequent work now suggests that although WMI is an important and likely initiating event, multiple subsequent *dysmaturational events* are most critical in determining the outcomes (see

Conflicts of interest: None reported.

* Communications should be addressed to: Volpe; Department of Pediatric Newborn Medicine; Brigham and Women's Hospital; 221 Longwood Avenue, Room 343C; Boston, MA 02115 USA.
E-mail address: Joseph.volpe@childrens.harvard.edu.

https://doi.org/10.1016/j.pediatrneurol.2019.02.016
0887-8994/

later). Moreover, because these dysmaturational events evolve over a very prolonged period (many months), a relatively long time window exists for interventions to prevent, counteract, or ameliorate the dysmaturation, i.e., neurorestorative interventions (see later).

In the following discussion, I will review the multiple maturational events occurring in infant brain during the premature period; the dysmaturational events observed in premature infants, including the importance of the initiating cerebral WMI; the dysmaturational events that may occur without WMI; and the potential neuroprotective and neurorestorative interventions.

Brain maturation during the premature period

The brain dysmaturation that occurs in premature infants (see later) involves the multiple active developmental events occurring in human cerebrum during the period of 20 to 40 weeks' gestation and beyond. The rapidity and complexity of these cellular events underlie, to a considerable degree, their vulnerability to perturbations. The principal components involved include the oligodendroglial (OL) lineage, especially the preoligodendrocyte (pre-OL), cerebral white matter axons, subplate neurons, cerebral cortex, thalamus, and basal ganglia (Fig 1). In addition, microglia and astrocytes, especially in the white matter, are involved importantly in both normal development and dysmaturation of these principal components. The major developmental events during this period have been summarized in detail elsewhere.[18,19] A brief review of the temporal aspects of these events is appropriate here (Table 1).

Pre-OL as a principal cellular target

Pre-OLs are the principal cellular target in WMI of premature infants.[20-22] These cells are generated from OL progenitors and are the principal phase of the OL lineage during the premature period (Table 1, Fig 2). Pre-OLs account for 90% of the lineage during the peak period of WMI in premature infants. Even at term, pre-OLs account for 50% of the lineage in cerebral white matter, whereas approximately 50% of the lineage is the more differentiated "immature" OLs.[23] Mature, myelin-producing OLs do not develop in human cerebral white matter to an appreciable degree until postterm. The pre-OL begins ensheathment of white matter axons at approximately 30 weeks' gestation (Fig 3).[24] This process is critical for axonal differentiation[25-35] and, as a consequence, axonal function. The latter is the critical driving force for cerebral cortical development (see later), which evolves rapidly as an activity-dependent process during the third trimester of gestation.

The pre-OL is a highly vulnerable cell, with particular susceptibility to such insults as hypoxia, ischemia, and inflammation, which lead to death via excitotoxic and free-radical-mediated mechanisms.[20] The particular molecular characteristics that underlie this pre-OL vulnerability have been reviewed elsewhere.[22] Suffice it to say here, many experimental studies of acute pre-OL death produced by hypoxia, ischemia, and inflammation have shown protective benefit for such agents as antiexcitotoxic, anti-inflammatory, and antioxidant compounds (see later). Notably, however, as will be discussed later, in the premature infant with WMI, pre-OLs are *replenished* in the subacute period but *fail to differentiate* over the ensuing weeks or months to later phases of the OL lineage. As a result, hypomyelination is a hallmark of the disease.

Axons

Axonal development is remarkably active in the cerebrum during the premature period (and the early postnatal period)

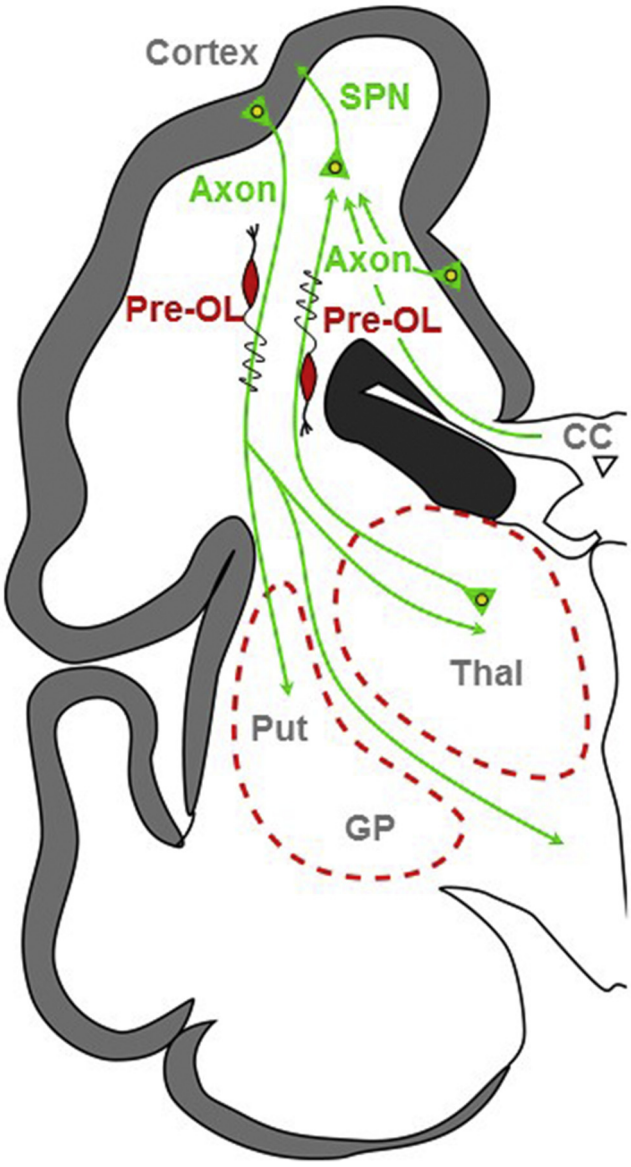

FIGURE 1. Schematic of major components involved in brain maturation in the premature period. See text for details. GP, globus pallidus; Pre-OL, pre-oligodendrocyte; Put, putamen; SPN, subplate neuron; Thal, thalamus.

(Table 1).[36] Utilizing immunostaining with GAP-43, a protein expressed on growing axons, Haynes et al.[36] showed marked expression in cerebral white matter to at least 37 weeks' gestation. Growing white matter axons reach approximately the subplate region at 20 weeks, the deep layers of the cortical plate at 27 weeks, and the entire cortex by 37 weeks (Fig 4). Axonal growth occurs primarily within the cortex after 37 weeks and into the first year of life. Based on work by Kostovic and coworkers,[37-40] the likely anatomic correlates of this progression in cerebral white matter during the premature period are growth of axons from thalamus to subplate neurons at 20 weeks and from subplate neurons to the cerebral cortex at 27 weeks (Table 1). Also, at 27 weeks, commissural and corticocortical cerebral white matter axons are actively growing, especially in the posterior periventricular regions, the so-called crossroads area. The increase in cerebral *cortical* expression of GAP-43 at 37 weeks may reflect a sum of continued cortical penetration from the subplate of thalamic ascending fibers and of commissural and corticocortical fibers (Fig 4). Thus it is apparent

TABLE 1.
Major Developmental Events During the Premature Period

20-24 weeks
Proliferation of OL progenitors
Cerebral white matter axons (projection, commissural, and association) grow actively
Subplate neuronal layer well established
Thalamic afferent axons synapse abundantly on subplate neurons
24-32 weeks
OL progenitor differentiation leads to prominence of pre-OLs in cerebral white matter
Cerebral white matter axons continue active growth
Pre-OLs begin ensheathment of cerebral white matter axons
Subplate reaches maximum size (several times thicker than cortical plate at 27-30 weeks)
Thalamocortical afferent axons depart subplate neurons and enter cerebral cortex
Cerebral cortical dendritic development and synaptogenesis become prominent
Callosal (commissural) and association (corticocortical) axons enter subplate
GABAergic neurons migrate into cerebral white matter
32-40 weeks
Pre-OLs remain the predominant cell of OL lineage in cerebral white matter until approximately 40 weeks when they and the more differentiated “immature” OL each account for approximately 50% of the OL lineage
Subplate layer gradually decreases
Callosal and corticocortical axons depart subplate and enter cerebral cortex
GABAergic neurons migrate to cerebral cortex and populate upper cortical layers
Cerebral cortical dendritic development and synaptogenesis become marked

Abbreviation:
OL = Oligodendroglial

that the premature period is one of extraordinarily rapid axonal development, especially in cerebral white matter. Axons during this rapid growth period are exquisitely vulnerable to multiple insults (see later).

Cerebral cortex-dendritic development, synaptogenesis

The cerebral cortex undergoes dramatic changes during the premature period. These events include attainment of proper alignment, orientation and layering of cortical neurons (six layers apparent by 30 gestational weeks), arrival of late migrating GABAergic neurons (principally to upper cortical layers), elaboration of dendritic and axonal ramifications (neurite outgrowth), onset of synaptogenesis, and a marked increase in cortical surface area with gyral development (Table 1).[18]

Neurite outgrowth, and particularly dendritic development, is most relevant in this context.[41-44] Dendritic development is especially rapid in the third trimester (Fig 5) and is correlated with the development of cortical activity (Table 1).[18] Importantly, in this context the progress of dendritic development depends on the *establishment of afferent input from cerebral white matter and then presumably synaptic activity*.[42,45-56] Thus axonal input from subplate neurons and then from thalamic, commissural, and corticocortical fibers are the principal driving forces underlying cortical dendritic development.[56] *The importance of synaptogenesis* in mediation of these effects of axonal input on cortical development has been emphasized in several studies (see [57] for review). In a seminal study, Sarnat and coworkers studied synaptic development in human cerebral cortex from 6 to 41 weeks' gestational age with the immunomarker synaptophysin, which identified maturation of synaptic vesicles in axonal terminals. Thalamocortical axons exhibited intense staining in frontal cortex at approximately 26 weeks' gestation, and diffuse and uniformly strong staining was apparent throughout the cortex from 34 weeks onward.[58] The findings integrate closely with measures of axonal development in the last trimester of gestation and with previous delineations of electrencephalographic maturation in premature infants. Functional synaptic activity via the axonal input to cortex is mediated principally through excitatory amino acid receptors, both the excitatory Ca^{++}-permeable *N*-methyl-D-aspartate and GluR2-deficient α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, which exhibit exuberant expression in developing human cortex during this period.[59,60] *This role of functional activity has implications for the effects of a variety of environmental stimuli on*

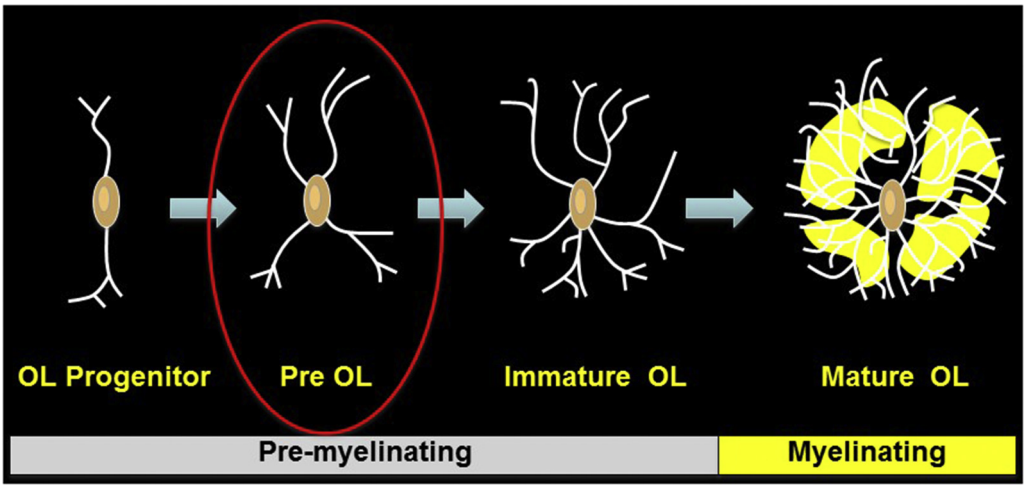

FIGURE 2. Major phases of the oligodendroglial lineage. The pre-OL (circled) is by far the predominant form during the premature period. OL, oligodendrocyte.

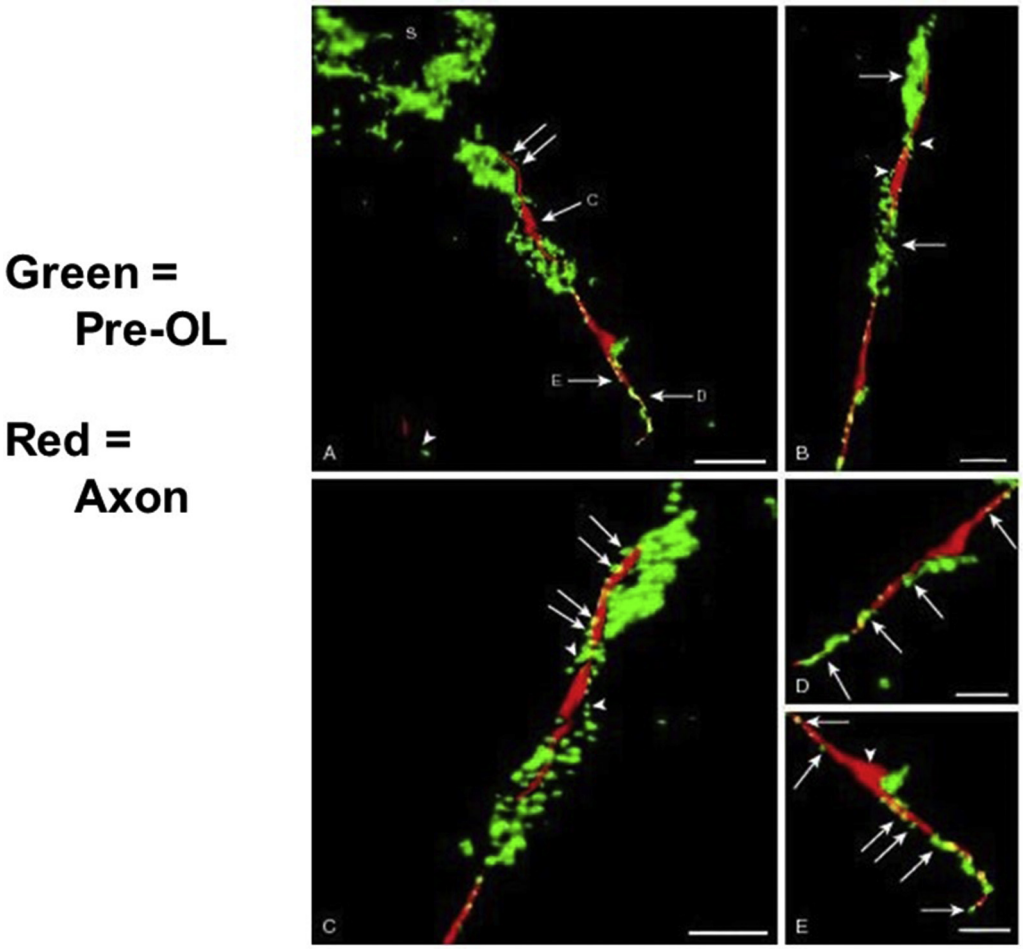

FIGURE 3. Pre-OL ensheathment of axons at 30 weeks' gestational age. Pre-OL is immunostained green, and axon, red. OL, oligodendrocyte. (From Back SA, Luo NL, Borenstein NS, Volpe JJ, Kinney HC. Arrested oligodendrocyte lineage progression during human cerebral white matter development: Dissociation between the timing of progenitor differentiation and myelinogenesis. J Neuropathol Exp Neurol. 2002; 61:197-211, with permission).

cortical development in the premature infant, and for potential neurorestorative roles for such stimuli in the context of brain injury and dysmaturation (see later).

Subplate neurons

This important transient population of neurons is well-established in subcortical white matter by 20 weeks' gestation.[37-40,61] During the important period of 24 to 32 weeks (the peak period for the occurrence of cerebral WMI) the subplate reaches maximum size (several times thicker than the cortical plate at 27 to 30 weeks) (Fig 1, Table 1). These neurons elaborate a dendritic arbor with spines, receive synaptic inputs from ascending afferents from thalamus and distant cortical sites,[18] and extend axon collaterals to the overlying cerebral cortex and to other cortical and subcortical sites (thalamus, other cortical regions, corpus callosum). The crucial organizational functions of these neurons include provision of a transient synaptic site for ascending afferents from thalamus and other cortical sites, i.e., these "waiting" afferents cannot synapse yet in cortex because their neuronal targets have not yet differentiated. These afferents would undergo degeneration if they did not have the subplate neurons as transient targets. Moreover, the subplate neurons extend axons to cortex to promote cortical differentiation and to guide the afferent axons to cortex when sufficient cortical differentiation has occurred. Subplate axon collaterals also descend to pioneer or guide the initial axonal projections from cerebral cortex toward subcortical sites (e.g., thalamus, corpus callosum, other cortical sites). The subplate neuronal layer gradually decreases after 36 to 40 weeks' gestation.

Late migrating GABAergic neurons

Particularly characteristic of human cerebral cortical development is the relatively late generation of GABAergic neurons from the dorsal telencephalic subventricular zone and from the ventral ganglionic eminence (Fig 6).[61-65] The origin of these late generated neurons is approximately 65% from the dorsal subventricular zone and 35% from the ventral ganglionic eminence. A substantial proportion of the ultimate population of GABAergic cortical neurons migrate through the cerebral white matter to the cortex in the third trimester. This migration peaks around term and then declines within the first six postnatal months.[66]

Microglia and astrocytes

Microglia and astrocytes are key players in the development of the white and gray matter structures just described. These glial elements also play a major causal role in the dysmaturational events that occur with cerebral WMI. Emphasis in this section is on the roles of microglia and astrocytes in normal development. Their role in dysmaturation is discussed later in the section on neuropathology.

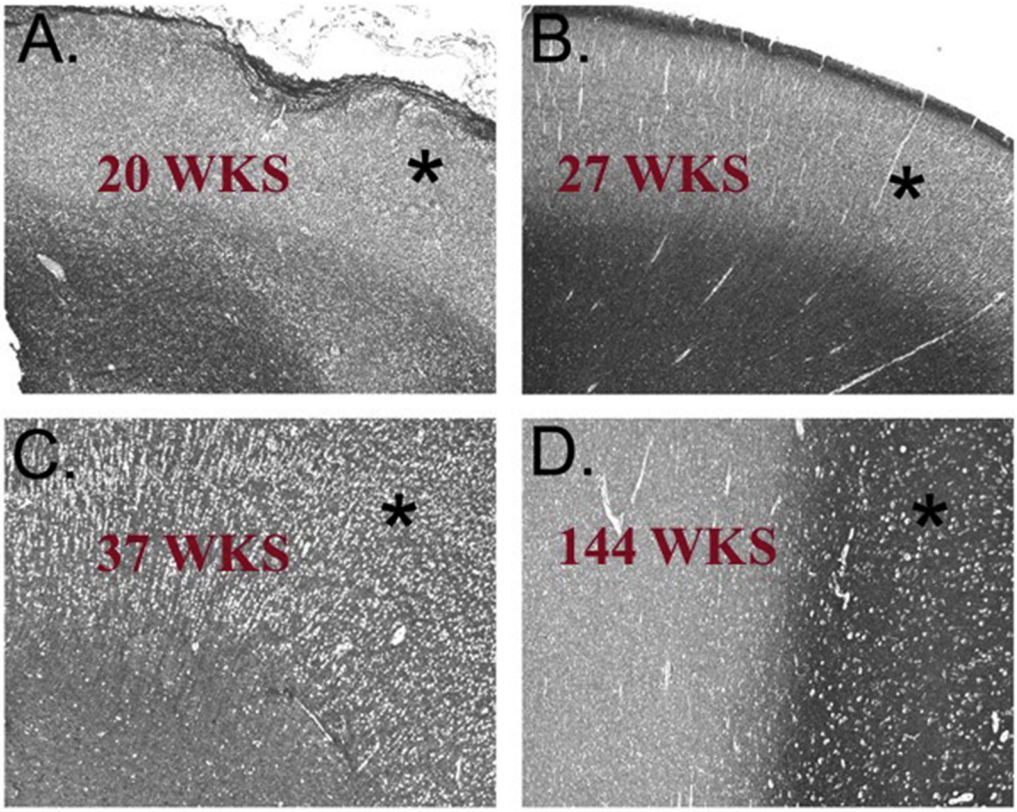

FIGURE 4. GAP-43 expression in developing human parietal white matter and cortex. Cortex is indicated by an asterisk. Note at 20 postconceptional (PC) weeks (A) there is strong expression in cerebral white matter to a region below the cortical plate, likely subplate neurons. At 27 PC weeks (B) the expression begins to enter the cerebral cortex and continues in white matter. By 37 weeks (C), diffuse expression in cortex as well as continued expression in white matter are apparent. At 144 PC weeks (approximately age two years) (D), expression is prominent in cortex but not in white matter. (From Haynes RL, Borenstein NS, DeSilva TM, Folkerth RD, Liu LG, Volpe JJ, et al. Axonal development in the cerebral white matter of the human fetus and infant. J Comp Neurol. 2005; 484:156-67, with permission).

Microglia

Microglia play important roles in such aspects of brain development such as axonal development, OL differentiation-myelination, vascularization, synaptogenesis, synaptic pruning, and neural circuit formation.[67-76] The roles in OL development involve microglial proteins that stimulate pre-OL proliferation, enhance pre-OL survival and provide iron for OL differentiation, and secrete cytokines that enhance differentiation.[75] These cells are also the principal neuroimmune cells involved in neuroinflammatory responses. As part of the neuroinflammatory responses microglia can be destructive to cellular elements, such as pre-OLs, principally by generating free radicals, secreting injurious cytokines, and enhancing excitotoxicity (see later).[73,77-85] Microglia have been characterized generally as pro-inflammatory (activated) (MI) or anti-inflammatory (M2). However, this bimodal characterization appears now to be too simplistic. Thus a recent landmark study in the developing mouse, utilizing molecular characterization methods, identified at least nine distinct microglial subpopulations with unique molecular signatures that changed over the course of development and exhibited marked spatial differences.[76] One distinct population was highly concentrated in axon tracts of the premyelinated brain. The molecular signatures of the microglial subpopulations in early development identified pathways associated with cell metabolism, growth, motility, and proliferation, among others. Studies in developing human brain will be of great interest.

Microglia become prominent in the human forebrain at 16 to 22 weeks' gestation and migrate progressively through the white matter from 20 to 35 weeks, and then to the cerebral cortex.[69,80,86,87] The critical point is that the cerebral white matter of the human premature infant is heavily populated with microglia during a period when various maturational events are occurring and when a variety of proinflammatory insults can lead to "activation" to destructive microglial phenotypes and WMI (see later). Moreover, because of the important role of microglial subpopulations in such important developmental events as OL development, axonal guidance, synaptogenesis, sculpting of neural networks, and cerebral connectivity, diversion of these normal cells to microglial phenotypes with primarily proinflammatory functions could contribute to disturbances in these maturational events observed in the premature brain.[72,73]

Astrocytes

The last half of human gestation also is a crucial time for astrocyte formation in the cerebrum.[18] Fibrous astrocytes (generated from radial glial fibers) increasingly populate the cerebral white matter. During development astrocytes are important in axonal guidance, angiogenesis, formation of the blood-brain barrier, synaptogenesis, neuronal survival, and axonal and synaptic pruning.[72] The molecular characteristics of astrocytes involved in facilitation of these events underlie such functions as expression of

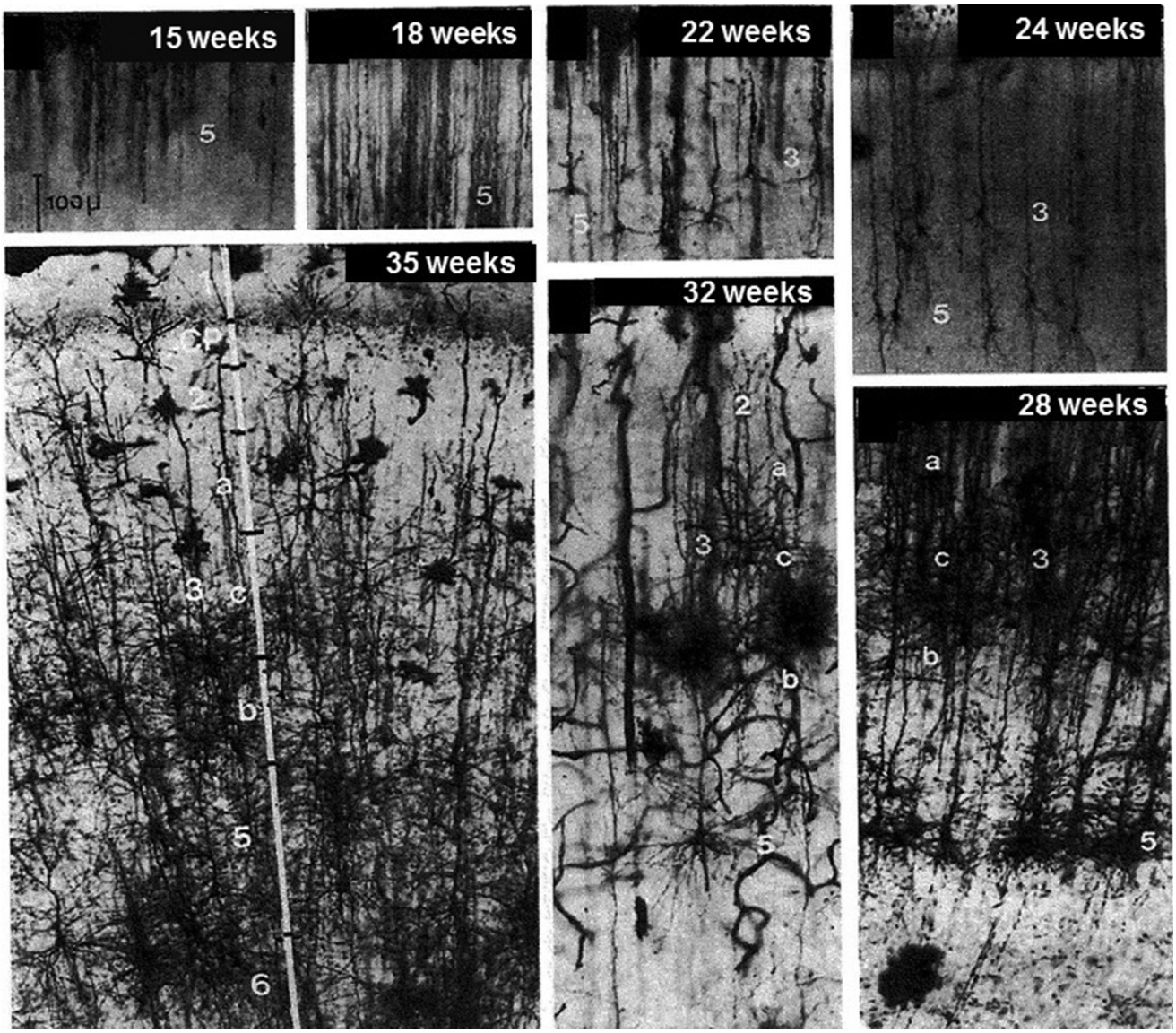

FIGURE 5. Cerebral cortical development from 15 to 35 weeks' gestation, Golgi-Cox preparations. Magnification is the same for each sample. Note the remarkable apical and basilar dendritic development, especially after 24 weeks' gestation. (From Marin-Padilla M: Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: A unifying theory, *J Comp Neurol* 321:233-240, 1992, with permission).

extracellular matrix (ECM) proteins and axonal guidance molecules, secretion of angiogenic factors, secretion of synaptogenesis molecules, clearance of extracellular glutamate, and secretion of various neurotrophic molecules. As will be discussed later, in the context of various brain insults (e.g., inflammation, hypoxia-ischemia), astrocytes can become "reactive" and exhibit a variety of metabolic changes that are deleterious to other white matter components, including pre-OLs.

Dysmaturation in premature brain

Overview

The principal manifestations of dysmaturation in premature brain have been elucidated by advanced magnetic resonance imaging (MRI) techniques in living infants (Table 2). Briefly, the abnormalities have by volumetric MRI, diminished regional volumes, especially of cerebral cortex, white matter, thalamus, and basal ganglia; by diffusion-based imaging, in cerebral white matter, decreased fractional anisotropy (FA) with relatively greater involvement of radial diffusivity (consistent with impairment of pre-OL ensheathment of axons), and in cerebral cortex, blunting of the normal decline in FA (consistent with impaired dendritic development); by surface-based MRI measures, decreased cerebral cortical surface area and cortical folding or gyrification; and by functional MRI, impaired development of measures of connectivity, including especially thalamocortical connectivity. The abnormalities have been elucidated most commonly at term equivalent age, but generally persist, or may increase later in infancy, childhood, adolescence, or adulthood.[88-93] The most common accompaniment by MRI has been cerebral WMI (see later). The dysmaturational events, in general, appear to be *secondary* to WMI (see later discussion in Mechanics of dysmaturation with cerebral white matter injury). The constellation of WMI and the accompanying disturbances of neuronal or axonal structures is generally referred to as the encephalopathy of prematurity.[17] However, recent work

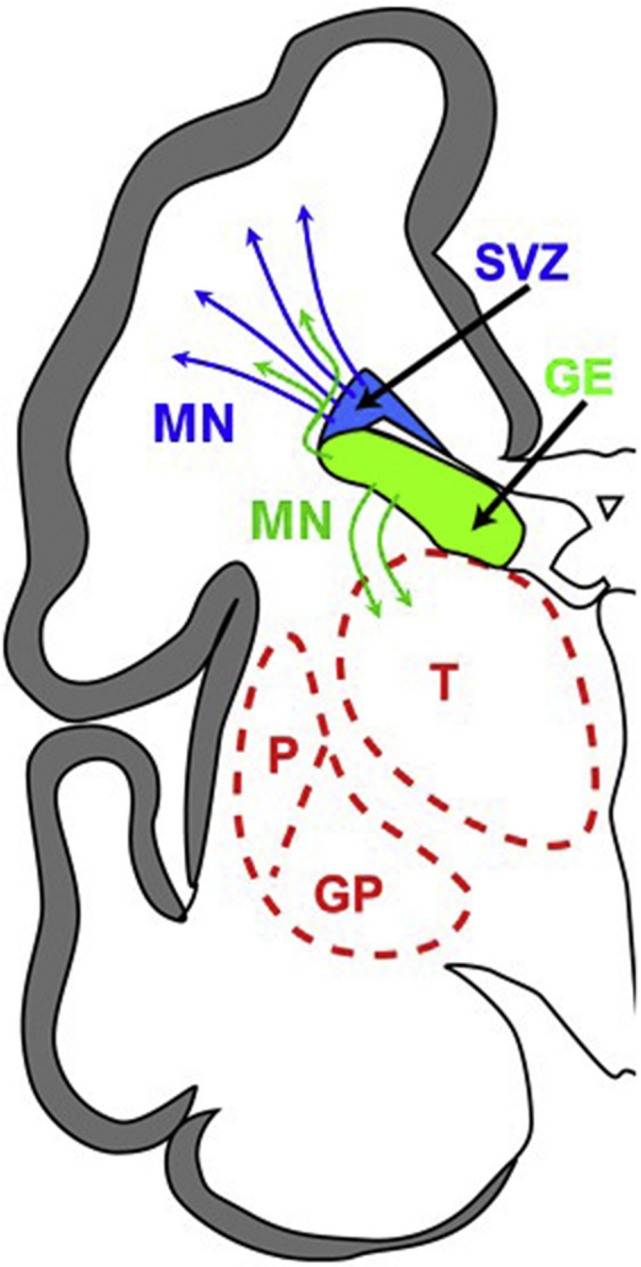

FIGURE 6. GABAergic neuron development during the premature period. Proliferation of GABAergic neurons occurs in the dorsal subventricular zone (SVZ) and ventral ganglionic eminence; migration proceeds radially and tangentially to cortex and thalamus, as shown.

suggests that some of the dysmaturational events documented in premature infants are not clearly related to WMI and perhaps are *primary* disturbances (see later). The emphasis in the following section is on the relation of cerebral WMI and dysmaturational events. Brief consideration of potentially primary dysmaturational events, perhaps independent of WMI, will then be presented.

Cerebral white matter injury: A spectrum

Neuropathology

Cerebral WMI encompasses a spectrum of neuropathology that ranges from overt periventricular leukomalacia (PVL) to diffuse white matter gliosis (DWMG) (without focal necroses) (Fig 7A-C). The two fundamental characteristics of PVL are focal necroses with loss of all cellular elements in periventricular white matter and a more diffuse lesion in cerebral white matter, consisting initially of death of early differentiating pre-OLs, accompanied by vigorous and persistent astrogliosis and microgliosis (Fig 8).[94-102] The focal necroses are essentially infarcts. Temporally, in the more diffuse lesion the pre-OL disturbance consists acutely of cell death, followed subacutely and chronically initially by proliferation of pre-OLs but then critically, by *a failure of maturation*.[103-108] As noted earlier, this pre-OL dysmaturation underlies the subsequent hypomyelination, a unifying feature of PVL. The mildest form of WMI, i.e., DWMG *without* focal necroses, now is the most common form of WMI in premature infants and is also accompanied by the pre-OL dysmaturation.[100-102]

The relative distribution of the spectrum of cerebral WMI in the modern era has been delineated best by neuropathological studies. The two largest, most recent series demonstrate that compared with earlier studies the areas of focal necrosis are smaller and, indeed, most cases have few or none.[100,101] In the series by Pierson et al. (n = 41), true PVL, i.e., with focal necroses, occurred in 17 (42%). Importantly, nearly all of these lesions were less than 1 mm in size. In an additional 17 (42%), only DWMG without focal necroses was observed. (Only seven of the 41 brains were free of white matter abnormality.) Critically, Busser and coworkers observed in association with DWMG the sequelae of pre-OL death, i.e., the excess of pre-OLs and failure of pre-OL maturation.[101] Thus the full spectrum of cerebral WMI can be illustrated as shown in Fig 7A-C.

Pathogenesis

The pathogenesis of the focal necroses characteristic of PVL relates primarily to decreases in cerebral blood flow, related to a variety of perinatal or neonatal events, and the presence in the periventricular area of vascular border zones and end zones.[20,22] The diffuse abnormality, DWMG, relates in considerable part to similar, albeit less severe clinical events (see later).

In the diffuse lesion the pathogenesis of the acute pre-OL injury or death likely relates in part to the acute insults, noted above, as well as the accompanying disturbances that may predispose the pre-OL to injury (e.g., intrauterine growth retardation, systemic infection, impaired nutrition) (see later). The stimulus for the subsequent proliferative response of OL progenitors to produce abundant pre-OLs remains unclear. The pathogenesis of the subacute and chronic failure of maturation of these newly generated pre-OLs appears to relate to deleterious effects of the abundant activated microglia and reactive astrocytes characteristic of the diffuse lesion. These components likely are involved in other dysmaturational events relating to axonal and neuronal structures, as described next.

Deleterious roles of microglia and reactive astrocytes. The *pro-inflammatory microglia* may impair pre-OL maturation by release of reactive oxygen or nitrogen species or cytokines (e.g., tumor necrosis factor-α, interleukin [IL]-1β) that then act on pre-OLs.[20] Recent studies of multiple sclerosis lesions have identified an inflammatory subpopulation of microglia that specifically targets myelin.[76] Whether such subpopulations are involved in pre-OL dysmaturation is unknown, but it is noteworthy that a large population of potentially activatable microglia are present in normal developing white matter during the premature period (see earlier). In addition, proinflammatory microglia have been shown recently to induce formation of neurotoxic reactive astrocytes.[109,110] As discussed next, such astrocytes are important in the pre-OL maturational failure. Finally, the shift in microglial phenotype from an antiinflammatory to a proinflammatory activated

TABLE 2.
Dysmaturational Features in Premature Brain Elucidated by Advanced MRI

Volumetric MRI	Decreased regional volumes, especially cerebral cortex, white matter, thalamus, basal ganglia
Diffusion imaging	In cerebral white matter, decreased FA, relatively increased radial diffusivity, variability altered axial diffusivity In cerebral cortex, blunting of the normal decline in FA
Surface-based MRI measures	Decreased cerebral cortical surface area and cortical folding/gyrification
Functional MRI	Impaired development of measures of connectivity, including especially thalamocortical connectivity

Abbreviations:
FA = Fractional anisotropy
MRI = Magnetic resonance imaging

phenotype diverts the critical roles of "normal" microglia in OL development described earlier.

The abundant *"reactive" astrocytes* (A1) in DWMG also likely play critical roles in the failure of pre-OL maturation.[108-111] The best established mechanism in the context of WMI is based on seminal work by Back and coworkers.[101,108,112] The likely sequence involves the generation by reactive astrocytes of high-molecular-weight forms of hyaluronic acid. Astrocyte-associated ECM is also involved in this generation. ECM is also a key source of hyaluronidases, which convert the high-molecular-weight forms of hyaluronic acid to lower-molecular-weight forms. The latter lead to failure of pre-OL maturation, probably by activating Toll-like receptor-2 receptors on pre-OLs.[113] The particular role of hyaluronan is supported by the observation that pharmacologic inhibition of hyaluronidases promotes pre-OL maturation and myelination (see later). Other products of reactive astrocytes may also be involved in the pre-OL dysmaturation. Thus, in human WMI reactive astrocytes express large amounts of interferon-γ, and pre-OLs express the interferon-γ receptor,[114] activation of which inhibits pre-OL differentiation.[20] Other products of astrocytes may contribute to the inhibition of pre-OL differentiation, for example, bone morphogenetic proteins and Notch ligand Jagged 1, but data on human preterm WMI are not yet available.[108] Finally, as noted for activated microglia, the shift in astrocyte phenotype from normal fibrous astrocytes to the toxic reactive phenotype also diverts the critical roles of astrocytes in the development of OLs (see earlier).

In view of the apparent critical roles of activated microglia and reactive astrocytes in disturbing pre-OL development (and likely also, aspects of axonal development), the question of the *duration* of DWMG in cerebral WMI of the premature infant becomes critical. Thus available evidence by MRI *in vivo* suggests that dysmaturation continues for many months and likely longer. Not unexpectedly, neuropathological data in human infants concerning duration of DWMG are somewhat scanty. However, available information suggests that DWMG is present for at least many months after the

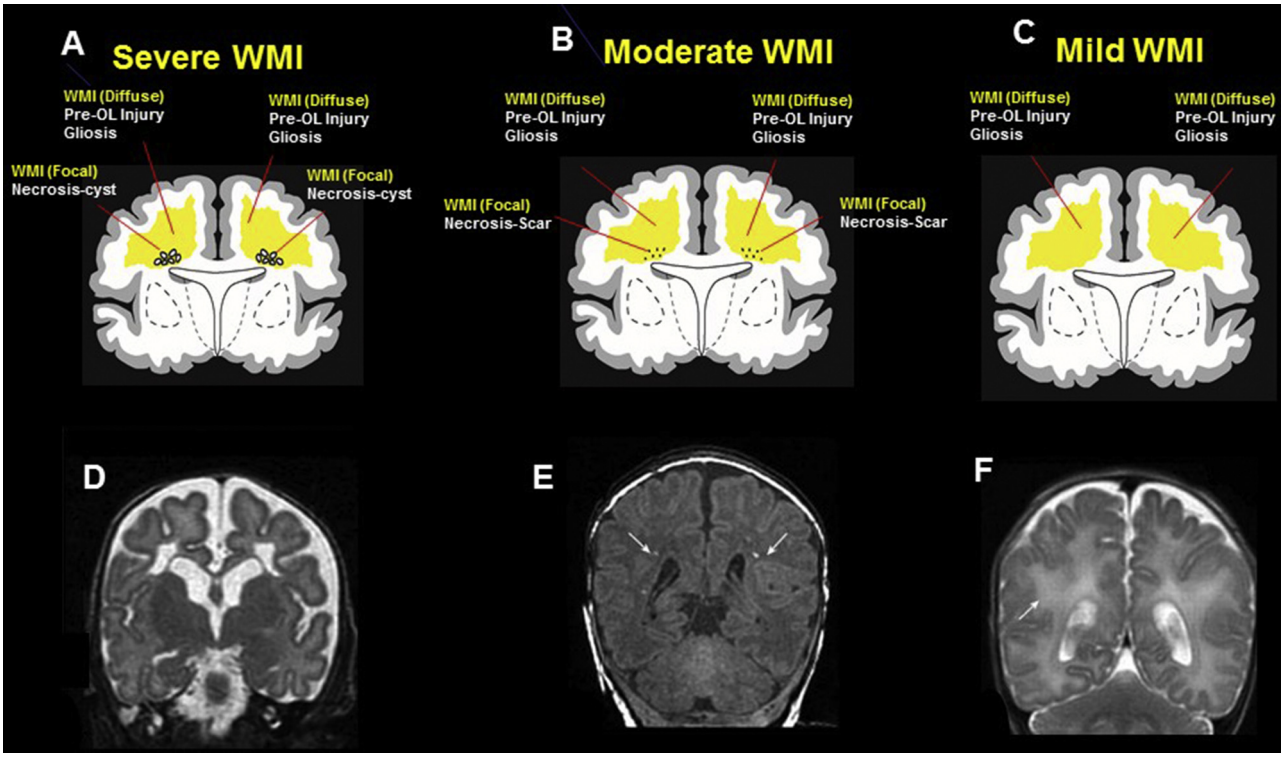

FIGURE 7. Spectrum of white matter injury (WMI) in premature infants. (A) to (C) illustrate the neuropathological spectrum of WMI. In severe WMI (A) the focal necrotic component consists of macroscopic areas of necrosis that result in cysts (i.e., "cystic" WMI). In moderate WMI (B) the focal necrotic component consists of small areas of necrosis that result in glial scars (i.e., "noncystic" WMI). In mild WMI (C) the focal component may be microscopic (less than 1 mm) or absent. In (A) through (C) the diffuse component of WMI consists of pre- oligodendrocyte (OL) injury or death (followed by pre-OL proliferation and maturation failure) and diffuse white matter gliosis (DWMG) involving activated microglia and reactive astrocytes. In panels (D) through (F) the magnetic resonance imaging correlates are shown. In (D), severe WMI, periventricular cysts are apparent; in (E), moderate WMI, punctate white matter lesions (PWMLs) but not cysts are seen; and in (F), mild WMI, only diffuse signal abnormality in white matter is apparent.

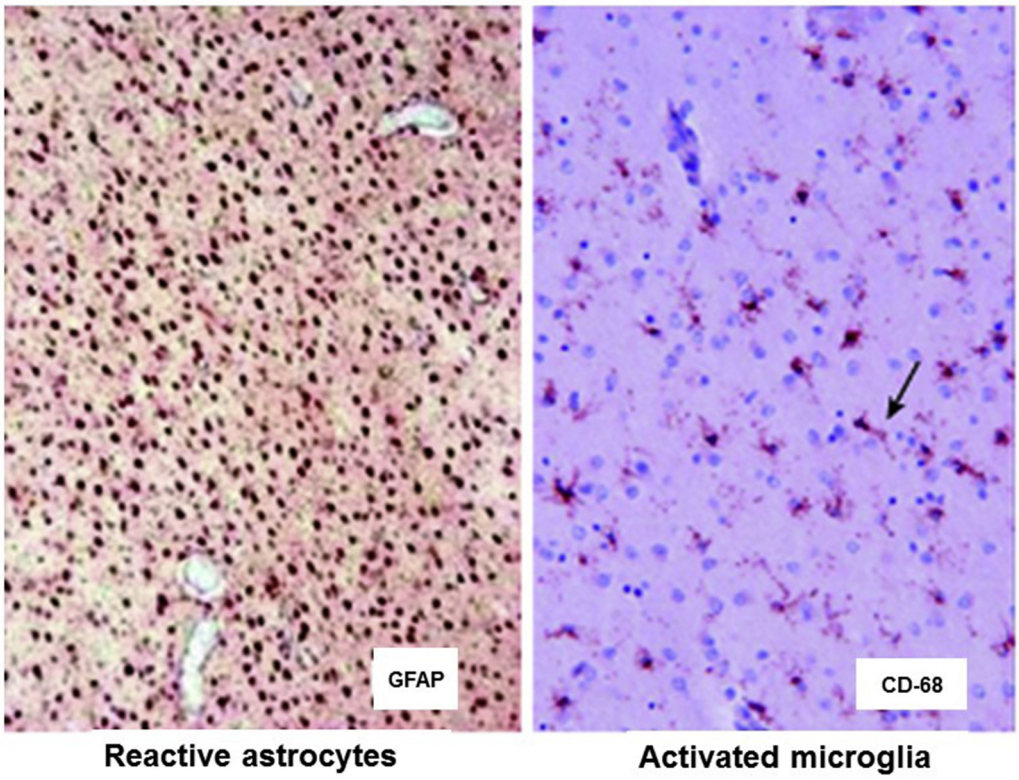

FIGURE 8. Diffuse reactive astrogliosis (GFAP immunostain) and diffuse activation of microglia (CD-68 immunostain) in cerebral white matter in all three varieties of white matter injury (WMI) illustrated in Fig 7. GFAP, glial fibrillary acidic protein.

premature period and likely longer.[98,100,103,115] There is precedent for microglia to be chronically activated in human neuropathology, for example, after traumatic brain injury.[116] In the latter setting, these cells are considered important in subsequent degeneration of axons and neurons years later and to play a role in the enhanced incidence of degenerative disorders, such as Alzheimer and Parkinson diseases.

Identification in vivo

Neuroradiological identification of the cerebral WMI spectrum *in vivo* is made best by MRI but is not entirely satisfactory. Thus the most severe end of the WMI spectrum, i.e., *severe WMI*, with large areas of necrosis and apparent cystic change, are readily identified as such (Fig 7D). However, such lesions are observed on MRI (and by neuropathology) in less than 5% of infants in modern neonatal intensive care facilities.[117,118] More common are small areas of necrosis (more than 1 mm) in periventricular and central cerebral white matter, seen at term equivalent age as (noncystic) punctate white matter lesions (PWMLs) in 15% to 25%, i.e., *moderate WMI* (Fig 7E).[119-122] Notably, this incidence of noncystic PVL (PWMLs) is appreciably higher if scans are performed early in the neonatal period—presumably the gliotic scars contract sufficiently to become invisible to MRI by term equivalent age. The least severe end of the WMI spectrum, i.e., *mild WMI*, is likely a heterogeneous group. Thus the large majority of focal necroses observed postmortem are less than 1 mm in size[100,101] and likely below the resolution of most conventional MRI scanners. In addition, the MRI correlate of the very common DWMG, *without focal necroses,* also is unknown. Importantly, as with the diffuse gliotic component of overt PVL, DWMG alone appears to lead to pre-OL death and subsequent dysmaturation,[101] and thus may be very important clinically. The frequent isolated finding of diffuse signal abnormality in cerebral white matter (Fig 7F) may be the MRI correlate of mild WMI, although both the reproducibility of this imaging finding and the relation to outcome remain unclear.[102] The few excellent studies that identify WMI without detectable focal necroses by the presence of diminished FA on diffusion-based MRI (see later) may be the best *in vivo* correlate of the admixture of the WMI spectrum that includes the two large groups of (1) focal necroses too small for identification (with DWMG) and (2) DWMG without any focal necroses, the two forms that we refer to as *mild WMI.*

Clinical importance

The clinical importance of the cerebral WMI spectrum relates to the motor and cognitive deficits associated with the lesion and the subsequent dysmaturation. The clinical phenomena associated with moderate and severe WMI have been described in detail elsewhere.[118] Identification of the neurodevelopmental sequelae of mild WMI is hindered by the difficulty in identifying the lesion by conventional MRI, as used in most large-scale studies. Large-scale MRI studies of premature infants show, as expected, worsening clinical outcomes as a function of severity of WMI. *However, it is noteworthy that infants with either no or "mild" abnormality in cerebral white matter by conventional MRI still exhibit neurological disability subsequently.* Although cognitive scales utilized among studies vary, cognitive scores for infants (less than 28 to 30 weeks' gestation) with no or "mild" WMI are approximately 85 to 93.[121,123-125] In a particularly well-characterized study of 480 extremely preterm infants (less than 28 weeks' gestation), 20% of infants with no apparent WMI by conventional MRI had cognitive scores less than 85.[124] The possibility that neuroaxonal dysmaturation with mild WMI (see mechanisms of dysmaturation with cerebral white matter injury)

is important in determination of these outcomes is suggested by follow-up studies that included assessment of gray matter abnormalities (as well as WMI).[126] As will be discussed later, studies that assess WMI by highly sensitive diffusion MRI measures show a clear association between mild WMI, dysmaturation, and subsequent cognitive disturbances.

Mechanisms of dysmaturation with cerebral white matter injury

The mechanisms of the dysmaturational features identified by MRI in premature brains (Table 2), especially in the context of WMI, are likely multiple. The prevailing theme is a sequence whereby *the initial insult (hypoxia, ischemia, inflammation, infection, etc.) leads to primary cellular injury or death, which in turn results in the subsequent replenishment of pre-OLs but secondary dysmaturation*. The cellular elements injured likely depend on the severity of the WMI. Thus in moderate to severe WMI (Fig 7A,B), all the rapidly developing cellular elements, as outlined next, appear to be injured, whereas in mild WMI (Fig 7C), the pre-OL may be the principal or only cellular element undergoing primary injury.

Dysmaturation with moderate-severe white matter injury

Pre-OL injury. Primary injury or death of the pre-OL, which is exquisitely vulnerable to hypoxic-ischemic, inflammatory, or related insults, is a consistent early feature of all forms of WMI.[21,108,127] Cell death, irreversible process loss, or both have been documented acutely.[21,103,105,108,127] *Subsequently, over the ensuing weeks replenishment of the pre-OL pool occurs but subsequent maturation to mature, myelin-producing OLs fails*. The important role of reactive astrocytes and activated microglia in this dysmaturation was described earlier. The result of this pre-OL dysmaturation is hypomyelination (Fig 9A). Also, however, pre-OL dysmaturation likely leads to failure of pre-OL ensheathment of axons, and as a consequence, impaired development, i.e., dysmaturation, of axons. The important trophic role of pre-OLs for axonal development, survival, and function was noted earlier. Indeed, this process is likely crucial for the exuberant axonal growth in cerebral white matter illustrated earlier (Fig 4) and the activity-dependent development of cerebral cortex (Fig 5). The consequences of the axonal disturbance would be diminished volumes of cerebral cortex and thalamus or basal ganglia, secondary to retrograde and anterograde (trans-synaptic) effects, i.e., involving projection fibers to and from the cortex, thalamus, and basal ganglia, i.e., thalamocortical, corticospinal, corticostriatal, and commissural and association fibers to and from the cortex, i.e., corticocortical (Fig 9A).

Axonal injury. Primary injury to the rapidly developing, vulnerable, premyelinating axons in cerebral white matter could be a primary event with WMI (Fig 9B). Although axonal injury is shown readily in the areas of focal necrosis, a more widespread degeneration of axons detected by the apoptotic marker, fractin, also has been identified.[128] This finding is consistent with related experimental observations concerning the vulnerability of developing axons.[129-133] The dysmaturational events subsequent to axonal injury (Fig 9B) by anterograde and retrograde effects would result in the impairments of cortical and thalamic development and related abnormalities detected by MRI (see Table 2). An impairment of pre-OL maturation would result from the loss of trophic axonal signals, with the ultimate consequence, hypomyelination. A contributory role for deleterious effects of activated microglia and reactive astrocytes (see earlier) also seems likely. Moreover, because of the role of both these glial types in normal axonal development, diversion to activated or reactive phenotypes may further impair axonal development.

Thalamic injury. Primary injury to thalamus is suggested by a neuropathological study of human infants with moderate to severe WMI and thalamic abnormalities (neuronal loss, gliosis, axonal degeneration) detected in approximately 60%.[100,134] A particular vulnerability of thalamus has also been shown in an experimental model.[135,136] Primary injury to thalamus could lead to degeneration of axons originating and terminating in the thalamus and, as a consequence, to pre-OL dysmaturation and hypomyelination (Fig 9C).

Subplate neuronal injury. Primary injury to subplate neurons would be expected to have major secondary dysmaturational effects on thalamus by retrograde degenerative effects on ascending thalamic axons ("waiting afferents"), as well as on cerebral cortex by anterograde effects via loss of subplate neuronal axons to cortex and on descending cortical axonal projections by loss of guidance from subplate axonal collaterals (Fig 9D). Considerable experimental data support these contentions.[37,137-144] With axonal degeneration, subsequent disturbances in pre-OL development would be expected (Fig 9D). Although data are not entirely consistent, experimental studies suggest that subplate neurons are particularly vulnerable to hypoxia-ischemia.[145] Two reports suggest a loss of subplate neurons in premature infants with moderate to severe WMI.[146,147]

Late migrating GABAergic neurons. Primary injury to late migrating GABAergic neurons seems possible because the migratory path of these late generated cells is from the dorsal subventricular zone through cerebral white matter to the cerebral cortex (Fig 6). Two neuropathological studies of moderate to severe WMI show a deficit in central white matter neurons consistent with late migrating GABAergic neurons.[146,147] The result of a disturbance in these neurons would be a deficit in cerebral cortical neurons, especially the upper cortical layers (Fig 9E). The MRI result would be diminished cerebral cortical volume, surface area, gyrification, and connectivity, as noted in advanced MRI studies (Table 2).

Conclusions. Thus, in moderate to severe WMI, i.e., identified by neonatal MRI by PWMLs (relatively common) or by cystic lesions (rare), several potential sequences of primary injury leading to dysmaturation and developmental impairments detected by advanced MRI techniques seem likely. Although pre-OL death and subsequent replenishment of pre-OLs, which then fail to mature, appear most consistent (Fig 9A), the other sequences depicted in Fig 9 may also occur to varying degrees, dependent in part on such factors as the gestational age of the infant; the nature, severity, and timing of the initiating insult(s); and the presence of other potentiating factors, for example, intrauterine adversity, postnatal infection, undernutrition, etc.

Dysmaturation with mild white matter injury

Mild WMI, as discussed earlier, is characterized by focal necrotic lesions less than approximately 1 mm in size, and thus undetectable by conventional MRI, or DWMG without focal necroses (Fig 7C). The dysmaturational features apparent subsequently *in vivo* by advanced MRI are similar in many respects to those described earlier for moderate and severe WMI (Table 2) but are less pronounced.[118,148-155] Thus a series of careful studies of premature infants without major WMI and utilizing diffusion-based MRI determinations of FA and related measures in cerebral white matter as a means to detect mild WMI, not readily apparent on conventional MRI, show at term equivalent age disturbances in volumetric development of cerebral cortex, cerebral white matter, thalamus, basal ganglia, cortical folding, cortical and white matter

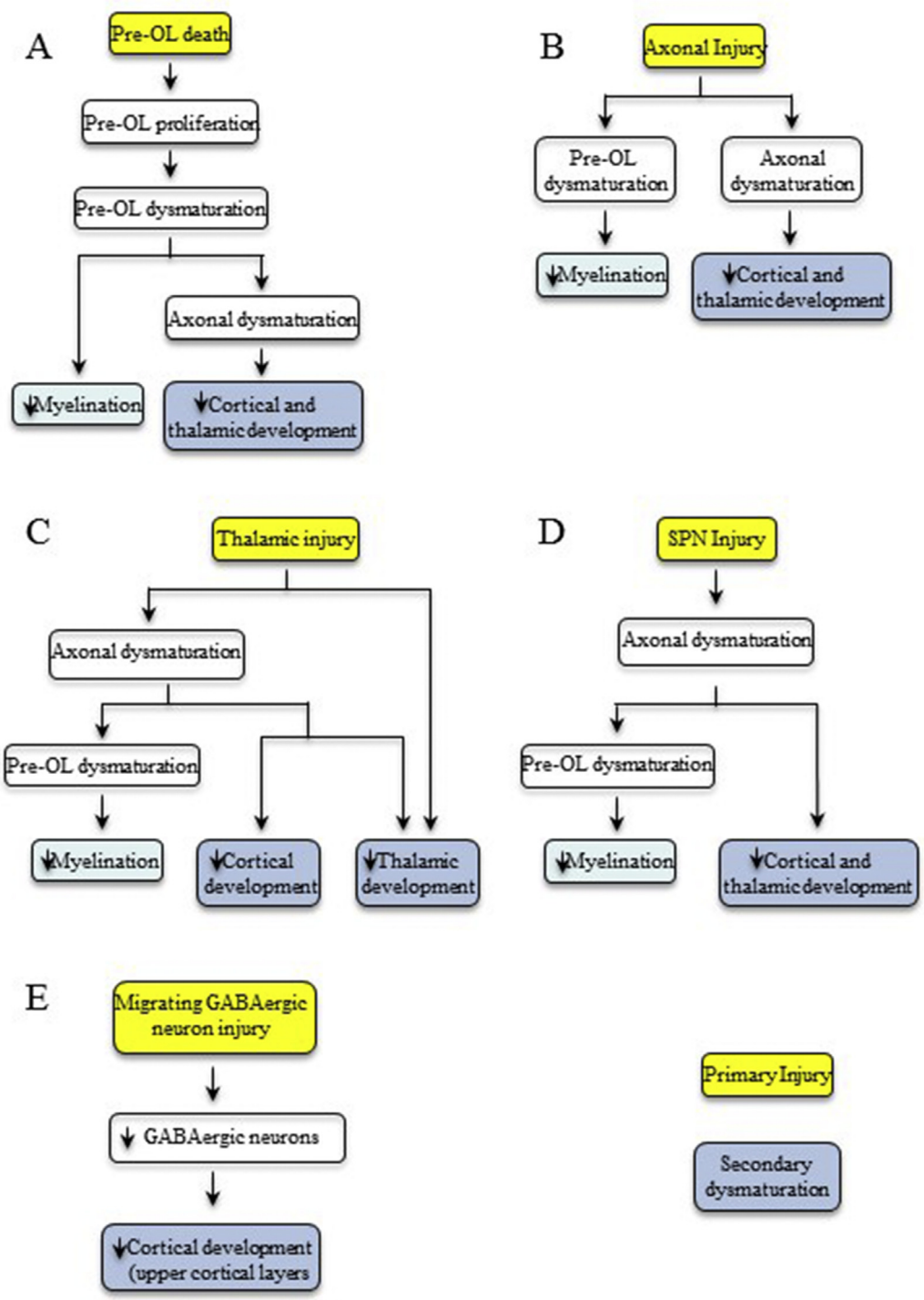

FIGURE 9. Mechanisms of dysmaturation following injury to pre- oligodendrocytes (OLs) (A), axons (B), thalamus (C), subplate neurons, (D) or migrating GABAergic neurons (E). For sequences (A–D), the initial injury leads to multiple dysmaturational events involving pre-OLs and axons. The principal outcomes are the disturbances of myelination and cortical and thalamic development, as shown *in vivo* by magnetic resonance imaging. See text for details.

microstructure, and thalamocortical connectivity.[148-153,155-157] In a particularly large, recent series (n = 491), Barnett et al. identified lower FA in cerebral white matter with particularly high radial (versus axial) diffusion (RD).[155] The high RD is consistent with an impairment of pre-OL ensheathment.[118,158] The findings suggest that impaired pre-OL maturation is the critical finding in mild cerebral WMI. The lower FA values were independently associated with increased number of days on ventilation, perhaps consistent with chronic hypoxia or related insults and with fetal growth restriction. The latter has been shown to be associated with a degree of hypoxia and in experimental studies to lead to delayed OL maturation[155,159]—recall that the pre-OL is exquisitely vulnerable to hypoxic and related insults (see earlier). The white matter findings also related to prolonged parenteral nutrition and suggest that impaired nutrition may lead to impaired pre-OL development (see later). Importantly, the abnormal FA values in the large study of Barnett et al. were associated with impaired neurodevelopmental performance at age 20 months.[155]

As noted earlier, two major neuropathological series indicate that mild WMI, as defined here, is at present the dominant form of cerebral WMI.[100,101] As noted earlier, detection of this milder but prevalent form of WMI cannot be made consistently by conventional MRI. The recent work just described with diffusion-based MRI indicates promise for detection *in vivo*.

Although the mechanisms for dysmaturation with mild WMI may overlap with those just described for moderate to severe WMI, major differences are likely. Thus, with mild WMI clear evidence for primary injury to components other than the pre-OL is lacking. *It is most likely that with mild WMI the deleterious effects of the abundant activated microglia and reactive astrocytes are the dominant mediators of dysmaturation, especially to the pre-OL, and perhaps also to axons.*

Pre-OL injury. Primary injury or death of the pre-OL with subsequent replenishment of pre-OLs but failure of maturation, as described for moderate to severe WMI (see earlier), may be the major mechanism for the widespread dysmaturation just described. The important role of activated microglia and reactive astrocytes was discussed earlier concerning moderate to severe WMI. The scenario to widespread dysmaturation, thus, would be similar to that described for moderate to severe WMI (Fig 9A).

Axonal injury. Although evidence for primary *injury* to axons in mild WMI is lacking, the deleterious effects of the abundant activated microglia and reactive astrocytes may disturb axonal *development*, separate from any effects on pre-OLs. In addition, as noted earlier, during normal development these glia are critical for axonal guidance and growth, and phenotypic diversion to activated or reactive cells could lead to dysmaturation. Thus a scenario similar to that depicted in Fig 9B seems possible.

Thalamic, subplate, late migrating GABAergic neuron injury. The scenarios described earlier for primary injury to these neural structures leading to dysmaturational gray matter disturbances in the setting of moderate to severe WMI (Fig 9C-E) cannot be ruled out in mild WMI but do not seem highly likely. For example, in the careful neuropathological study of Pierson et al.,[100] in the 17 infants with DWMG (and no focal necroses), neuronal loss in cortex, thalamus, and basal ganglia was observed in none.

Conclusions. The dysmaturational disturbances of white matter and gray matter structures apparent by advanced MRI methods in infants with mild WMI do not appear to be related to widespread injury. Pre-OL injury and dysmaturation do seem apparent, and thus the possibility of the multiple secondary developmental disturbances of gray and white matter structures described earlier (Fig 9A) is real. The abundant reactive astrocytes and activated microglia in cerebral white matter, i.e., DWMG, are also likely important in the pre-OL injury or dysmaturation. In addition, axonal injury and dysmaturation are also a potential consequence of the deleterious actions of these two glial types (Fig 9B).

Primary dysmaturation of gray matter structures

The possibility that the gray matter structures shown to exhibit secondary impaired development with encephalopathy of prematurity, as outlined in the preceding discussion, may exhibit *primary* dysmaturation is suggested by recent clinical and experimental studies. If primary dysmaturation does occur, the approaches to neuroprotection and neurorestoration (see later) could be quite different from those directed at secondary dysmaturation in the context of cerebral WMI.

Clinical data

Primary dysmaturation of cerebral cortex, in the absence of evidence for WMI, is suggested by a study of 95 premature infants studied by MRI at two time points in the neonatal period (32 and 40 weeks post-conception).[160] The principal finding was evidence for delayed microstructural development of cerebral cortical gray matter at multiple sites. Diffusion-based measurements showed delayed microstructural development in cerebral cortex, but *not cerebral white matter*, in association with *impaired somatic growth*. The expected normal developmental decline in FA in cortex was blunted, whereas the expected increase in FA in white matter was not. Thus no evidence for WMI or impaired white matter development could be identified. As described earlier,[18,161] FA decreases in the cortex principally with dendritic development. In the study of Vinall et al.[160] radial diffusion, and not axial diffusion in the cortex, was particularly affected, again most consistent with impaired dendritic development. The association with impaired somatic growth raises *the possibility that undernutrition is particularly involved,* although detailed data regarding nutrition, caloric intake, and feeding were not available. However, it is noteworthy that several studies of premature newborns with intrauterine growth retardation also show a particular involvement of cerebral cortical development, including reduced cortical volume, reduced cortical surface area, and impaired gyrification.[162-166] However, other studies of such infants have shown abnormalities in microstructural development of white matter.[155,167] Nonetheless, on balance, it does appear that disturbances in growth, perhaps secondary to undernutrition, either in the premature infant postnatally or *in utero* may have a primary dysmaturational effect on cerebral cortex. More data clearly are needed.

Experimental data

Three recent studies in a well-characterized preterm large animal (fetal sheep) model of cerebral ischemia raise the possibility of primary dysmaturation of cerebral cortex, subplate neurons, and caudate neurons.[168-170] Thus utilizing elegant neurobiological methods, Back and coworkers have shown disturbances in cortex, in dendritic development and synapse formation; in subplate neurons, in dendritic arborization and synaptic activity; and in caudate, in dendritic arborization, synaptogenesis, and synaptic activity.[168-170] These findings were apparent four weeks after the hypoxic-ischemic insult, but not after two weeks.[168] As the basic experimental paradigm was designed originally to replicate cerebral WMI of the premature infant, these examples of neuronal dysmaturation were accompanied by pre-OL degeneration and dysmaturation and diffuse gliosis with reactive astrocytes and reactive microglia. A reasonable question is whether the four-week period required for the evolution of the cortical, subplate, or caudate neuronal dysmaturation is necessary because the dysmaturation is secondary to the pre-OL degeneration and dysmaturation as described earlier (Fig 9A). In the absence of a definitive answer to this question, the possibility that the hypoxic-ischemic insult leads primarily and directly to neuronal dysmaturations is real. Coupled with the clinical study described earlier, the latter possibility demands further research.

Conclusions

The clinical studies of premature infants with impaired somatic growth and of those with intrauterine growth retardation raise the possibility that cerebral cortical development may be affected directly, i.e., *primarily*, perhaps by nutritional factors. In view of the rapid development of cortex during the premature period and therefore its likely vulnerability to neonatal insults, such a possibility seems reasonable. Experimental data also raise the possibility of a primary dysmaturational effect for hypoxia-ischemia on

TABLE 3.
Neuroprotective Interventions to Prevent Pre-OL Injury/Death

Mechanism Targeted	Interventions
Hypoxia-ischemia (perinatal or postnatal)	Multiple*
Systemic Infection (perinatal or postnatal)	Multiple†
Microglial Activation/ Inflammation	Minocycline Melatonin
Excitotoxicity	Memantine Topiramate
Reactive oxygen or nitrogen species	
Generation	Oxygenase/NOS inhibitors
Defenses	Antioxidative enzyme mimetics Free radical scavengers (N- acetylcysteine, vitamins E and K)
Multiple mechanisms	Erythropoietin‡,§ EGF§, IGF§ Estradiol

Abbreviations:
EGF = Epidermal growth factor
IGF = Insulin-like growth factor.
MRI = Magnetic resonance imaging
NOS = Nitric oxide synthase
* Interventions related primarily to management of pregnancy, neonatal resuscitation, mechanical ventilation, bronchopulmonary dysplasia.
† Interventions related to prevention and treatment of fetal and neonatal systemic infection.
‡ Studied in human preterm infants.
§ Also promotes pre-OL differentiation.

cortical, subplate, and caudate neurons. However, as discussed, the available data do not rule out a primary effect on pre-OLs with secondary neuronal dysmaturation.

Neuroprotective and neurorestorative interventions

As the pervasive theme in this review is that pre-OL *death* leads to subsequent *dysmaturation* of both white and gray matter structures, interventions are best considered (1) as preventative of the initial death (i.e., *neuroprotection*) or (2) as amelioration or prevention of the subsequent dysmaturation (i.e., *neurorestorative*). Although there is overlap in this categorization, the distinction best facilitates the discussion that follows.

Neuroprotective interventions

Neuroprotective interventions have focused on prevention of pre-OL injury or death. Many excellent recent reviews have addressed this issue, and therefore this will not be discussed further.[20,22,108,171] The principal neuroprotective interventions and the likely mechanism(s) affected in the cascade to pre-OL death are shown in Table 3. Most of the mechanisms are also relevant to those examples of WMI that are accompanied by direct injury to axons and neurons as well as to pre-OLs. Of the interventions shown in Table 3, only erythropoietin (EPO) has been studied in detail in human premature infants and will be discussed here.

Erythropoietin

As EPO has antiexcitotoxic, antioxidant, antiinflammatory, and antiapoptotic effects,[172] it is a prime candidate for the prevention of pre-OL injury or death, the critical initial event in genesis of preterm WMI. EPO has been shown to prevent or mitigate WMI in a variety of experimental models.[172-174] Although numerous studies of EPO in premature infants have been carried out, a recent meta-analysis of four randomized controlled trials (RCTs) comprising 1133 infants is especially useful.[175] Prophylactic EPO administration reduced the incidence of Mental Developmental Index scores of less than 70 (odds ratio 0.51 [0.31 to 0.81], $P < .005$) at 18 to 24 months. As the total numbers of infants with less than 28 weeks' gestational age were not large enough to assess adequately the outcome in this critical group, more data are needed. A large multicenter randomized controlled trial in the United States (Preterm Erythropoietin Neuroprotection Trial, NCT01378273) is focused on this critical group, and results should be available this year.

A closer assessment of the key EPO trials suggests that *the timing of EPO administration* may be critical in the likelihood of benefit. Thus, in one series of studies utilizing early, relatively brief administrations of EPO (atless than 3 hours, at 12 to 18 hours, and at 36 to 42 hours after birth), no significant differences in outcome at two years could be discerned[176] (although MRI at term equivalent age showed decreased WMI and better white matter maturation in the EPO-treated infants).[177,178] However, in a study utilizing EPO administration (as EPO or its higher glycosylated derivative darbepoetin) thrice weekly through 35 weeks' postconceptual age, the treated infants had better cognitive outcomes and less neurodevelopmental impairment at age 3.5 to four years, when compared with placebo-treated infants.[179] Thus the two different protocols with regard to the timing of EPO administration suggest that with the early, relatively brief approach, EPO was functioning only as a neuroprotective agent, whereas with the more prolonged approach the agent may have functioned *both* as a neuroprotective *and* a neurorestorative intervention. Perhaps consistent with this notion, the largest study to date randomized 800 infants of less than 32 weeks' gestation to placebo or EPO administered intravenously within 72 hours of birth and then once every other day for two weeks.[180] The rate of moderate or severe neurological disability at 18 months' corrected age was significantly lower in the EPO group (7.1%) versus the placebo group (18.8%) (odds ratio = 0.22, confidence interval, 0.19 to 0.55, $P < .001$). Dosing in the aforementioned Preterm Erythropoietin Neuroprotection Trial will be still more prolonged, i.e., initially, single doses intravenously, every other day, from day one to day 11, and subsequently, doses subcutaneously every other day until 32 weeks. The potential mechanisms for EPO's benefit concerning brain maturation, i.e., neurorestorative effects, will be discussed in the next section.

TABLE 4.
Neurorestorative Interventions - Experimental Studies

Major Interventions
EGF, IGF-1
Hyaluronidase inhibitors
Microglial or astrocytic manipulation
Stem cells
Exosomes
Dendrimers

Abbreviations:
EGF = Epidermal growth factor
IGF-1 = Insulin-like growth factor

Neurorestorative Interventions

The emphasis of this review has been on the evolution of the widespread dysmaturational events that follow the initial insult(s) and injury or death, especially to pre-OLs. These events develop over many weeks to months, and perhaps longer. This relatively protracted period raises the possibility of a long time window for interventions potentially capable of ameliorating or preventing the dysmaturation. I will term these interventions *neurorestorative*. The principal such interventions, shown in Tables 4 and 5, are classified based on their study in experimental settings only (Table 4) or in clinical settings with human infants, principally preterm infants (Table 5).

TABLE 5.
Neurorestorative Interventions - Clinical Studies

Major Interventions
Erythropoietin
Nutritional factors
Quality and source of milk
Components of milk
Breastfeeding
Polyunsaturated fatty acids
Iron
Zinc
Experiential factors
Neonatal period
Auditory
Visual
Pain, stress
Post-term
Early intervention programs
Parenting, educational, or social factors

Experimental studies

EGF and IGF-1. Both epidermal growth factor (EGF) and insulinlike growth factor (IGF-1) have beneficial effects in experimental models of preterm WMI (Table 4). The agents appear to exhibit both neuroprotective and neurorestorative properties. In a mouse model of preterm WMI, Scafidi et al. showed that either selective overexpression of human EGF receptor in OL lineage cells or the intranasal administration of EGF immediately after injury led to decreased OL death, enhanced generation of new OLs from progenitor cells, and promoted functional recovery.[181] The benign mode of administration of the EGF suggests potential clinical applicability.

IGF-1 has shown protective effects versus WMI both in neonatal animal models (hypoxia-ischemia, lipopolysaccharide-induced inflammation) and in cultured pre-OLs.[182-186] The agent also showed restorative effects, i.e., rescue of pre-OLs and promotion of myelination. Two issues limit enthusiasm for IGF-1: first, the peptide must be administered intraventricularly, and second, its effects are dose-related, with lower doses being effective but higher doses being toxic.

Hyaluronidase inhibitors. Pre-OL dysmaturation in chronic WMI appears related at least in considerable part to the astrocytic component of the diffuse gliosis characteristic of the lesion. Thus Back and coworkers have shown that reactive astrocytes synthesize high-molecular-weight forms of hyaluronic acid, which are readily detectable in the human lesion.[101,108] As described earlier, hyaluronic acid digestion products, generated from hyaluronidases in the disrupted ECM of WMI, lead to a block in pre-OL maturation. This block could be prevented by pharmacologic inhibition of hyaluronidase *in vitro* and in an animal model.[187] Whether use of a hyaluronidase inhibitor has value in preventing pre-OL dysmaturation in the human infant requires further study.

Microglial or astrocytic phenotypic manipulation. Abundant microglia are important components of the diffuse gliotic component of WMI (see earlier). These cells are principally in *an activated, pro-inflammatory state (M1 phenotype).* Their role in *acute* pre-OL injury or death likely relates to the generation of reactive oxygen and nitrogen species and secretion of injurious cytokines.[20] However, a variety of studies, performed in *in vitro* and *in vivo* models, including adult human lesions with failure of OL differentiation and myelin development (e.g., multiple sclerosis), suggest involvement of activated microglia in the subsequent pre-OL dysmaturation in human preterm WMI.[188-190] The data raise the possibility that interventions capable of converting microglia from a pro-inflammatory phenotype (M1) to an anti-inflammatory phenotype (M2) would have major potential as a neurorestorative therapy. Such *immunomodulatory* agents that cross the blood-brain barrier have been identified (e.g., minocycline, melatonin, minozac, etanercept) and are under study in human adult demyelinating diseases.[116,189,191] Their safety and efficacy in the premature infant for long-term use are not established.[192]

A recent relevant area of interest in the regulation of microglial phenotype involves *microRNAs.*[191,193] These components are short noncoding RNAs (18 to 22 nucleotides), which are transcriptional regulators of gene expression. Several microRNAs have been shown to promote or inhibit inflammatory responses in microglia. One prominent microRNA of activated microglia is mir-155, which is elevated in microglia in multiple sclerosis lesions. When silenced *in vivo* by intracerebral injection of IL-17 in early stages of experimental allergic encephalomyelitis, the pathologic and clinical effects of the demyelinating disease are blunted.[193] Thus the possibility of such systemic therapy seems real. Indeed, recent research shows that intravenous delivery of another microRNA (miR-124) that promotes polarization of microglia from an inflammatory (M1) to an anti-inflammatory (M2) phenotype via miR-124-enriched exosomes improves hippocampal neurogenesis and neurological function over four weeks after traumatic brain injury[194] (see also later discussion of exosomes). Notably, because the anti-inflammatory phenotype of microglia (M2) is important in the facilitation of many brain developmental events as described earlier, these avenues of research suggest a major neurorestorative possibility for *in vivo* manipulation of microglia phenotype.

Similar considerations concerning glial manipulation from a "harmful" to developmentally "helpful" phenotype apply to the *reactive astrocytes* in the diffuse gliotic component. Their involvement in pre-OL injury and dysmaturation and the potential value of hyaluronidase inhibitors were discussed earlier. Prevention of the microglial induction of harmful, "neurotoxic" astrocytes (A1 phenotype) is an area of active current research.[109,110] The valuable results would be inhibition of pre-OL death and preservation of the maturational effects of the developmentally beneficial astrocytic phenotype (A2). A variety of drugs and neutralizing antibodies (e.g., to tumor necrosis factor-α and IL-α from microglia) to prevent induction of harmful reactive astrocytes are under study in animal models and in adult human neurodegenerative disorders.[109]

Stem cells. Experimental studies of stroke and related ischemic brain injuries in neonatal animals suggest that stem cell therapies may be effective for restoration, particularly of OLs.[195-200] The major types of cells used thus far include neural, embryonic, mesenchymal, umbilical cord, and induced pluripotent cells. *In vitro* manipulation of neural precursor cells before transplantation can enhance their capacity to undergo OL differentiation and axonal remyelination.[201] A variety of routes of cell administration have been utilized, and *intranasal* administration may be the most efficient. Stem cells administered by this route appear to target the injury site after entering the brain via olfactory neural processes traversing the cribriform plate.[202] Studies of rodent models of preterm brain injury have shown that the intranasal route of administration can be effective not only for mitigating injury to myelin but also for improving behavioral outcome.[196,203]

Of particular relevance to cerebral WMI and pre-OL dysmaturation or myelination failure in the premature infant is a recent study of such injury produced by hypoxia-ischemia in the 3-day-old rat.[204] OL progenitor cells produced from embryonic stem cells were transplanted into the injured cerebrum. The transplanted cells survived, underwent differentiation, formed myelin sheaths, and stimulated proliferation of endogenous neural stem cells. Supporting a neurorestorative effect was the demonstration of

functional benefit after six weeks. It will be of particular interest if the results can be replicated after intranasal administration.

A relevant human study in this context involves the transplantation of human neural stem cells into the brains of four infants with connatal Pelizaeus-Merzbacher disease.[205] After one year, evidence of myelin ensheathment of axons was obtained by diffusion tensor imaging. Direct extrapolation to the human preterm infant with WMI is difficult because of the mode of administration used. Nevertheless, the findings suggest promise for stem cell therapy as a neurorestorative therapy in such infants.

Exosomes. The precise neuroprotective factors released by stem cells are not known with certainty and may vary as a function of the injury. Notably, however, effects on pre-OL and myelin development and on behavioral outcome in a rodent model of preterm WMI was achieved with *intravenous* administration of extracellular vesicles, i.e., *exosomes*, derived from stem cells, in lieu of stem cells per se.[206] (Exosomes are a type of extracellular vesicle and can carry membrane and cytosolic proteins, various types of RNA and lipids, and perhaps DNA).[207] Similar benefit from the use of exosomes has been demonstrated in other animal models of brain injury.[208-210] The great therapeutic potential of exosomes, isolated from blood, has been recognized only recently, and the capacity to induce OL differentiation and myelination could serve a crucial neurorestorative function in the premature infant. Human studies will be of great interest.

Dendrimers. Dendrimers are unique nanoparticles synthesized for a variety of functions, including targeted delivery of therapeutic agents to brain.[211,212] Their small size and tailorable surface functional groups make them valuable for this role. *Drugs, and perhaps ultimately, microRNAs or silencing RNAs can be attached to the dendrimer.* Several recent models of ischemia- or inflammation-induced neonatal or fetal brain injury have shown marked beneficial effects of dendrimer–N-acetylcysteine conjugates.[213-217] N-acetylcysteine is an antioxidant, and after intravenous administration of the conjugate, uptake into activated microglia, reactive astrocytes, and differentiating OLs could be demonstrated. Sustained prevention of OL injury and improved myelination were shown.[215] The principal cellular target appeared to be inflammatory microglia. Further studies will be of great interest.

Clinical studies

A burgeoning clinical literature suggests the possibility that the dysmaturation of both pre-OLs and gray matter structures after premature brain injury can be counteracted to a considerable extent. These neurorestorative interventions include pharmacologic agents, i.e., EPO, and modifications of nutrition and other environmental factors (Table 5). Implementation of these interventions during the vulnerable neonatal period, when the remarkable array of developmental events described earlier are proceeding most rapidly, is of critical importance. However, the beneficial effects of these interventions likely continue beyond the neonatal period (see later). The mechanisms of the benefits and the specific maturational events affected are not yet entirely understood. Our current understanding of these interventions is discussed next.

Erythropoietin. EPO was discussed earlier in relation to its neuroprotective effects. Notably as discussed earlier, current data suggest that more prolonged exposure to EPO is more beneficial than only early and brief exposure, thus suggesting that EPO has neurorestorative as well as neuroprotective properties.

Experimental data suggest that neurorestorative effects of EPO involve particularly OL development, although promotion of angiogenesis and neurogenesis may also occur.[118,172,218] In view of the likely importance of the failure of differentiation of pre-OLs in the genesis of axonal and neuronal dysmaturation, the decisive role of EPO in promoting pre-OL development after hypoxic-ischemic insults in experimental models is particularly relevant here.[219-221] *In vivo*, EPO appears to be generated primarily from astrocytes, abundantly present in the diffuse gliotic component of WMI. However, the EPO receptor is upregulated in pre-OLs after hypoxic-ischemic insults, and if sufficient endogenous EPO is not present, the unoccupied receptor leads to a failure of differentiation. The provision of abundant *exogenous* EPO may explain the benefit of EPO therapy vis-à-vis pre-OL differentiation. An indirect effect of EPO on pre-OL and neuronal or axonal maturation may also relate to its action of decreasing microglial recruitment, the other key glial element in DWMG, and thereby the deleterious effects of inflammation.[222] In addition, in an animal model,[223] EPO also promotes *cerebral cortical development* after hypoxia-ischemia and associated subplate neuronal loss, again consistent with its multifaceted effects on cellular development in brain. Recall that in moderate to severe WMI in premature infants, subplate neuronal loss and impaired cortical development are important features (see earlier).

A major question regarding EPO as a restorative therapy relates to the prolonged duration likely required. Reactive astrocytes and activated microglia are likely present for many months after the initial injury. More data are needed regarding the safety of such prolonged treatment with an agent with multifaceted developmental effects.

Nutritional factors. The importance of appropriate nutrition during the premature period for neurodevelopmental outcome and the deleterious effects of postnatal undernutrition are supported by a large corpus of clinical, epidemiologic, and experimental studies.[224-238] In the context of this section the data raise the possibility that optimal nutrition, *both* in the neonatal period *and* the subsequent posthospitalization period, could be a restorative intervention in premature infants with WMI and the encephalopathy of prematurity. The high prevalence of impaired nutrition in premature infants is illustrated by observations that 50% of very-low-birth-weight preterm infants had a discharge weight less than the tenth percentile for postmenstrual age and 27% had a discharge weight less than the third percentile.[239,240]

The *maturational events apparently vulnerable to nutritional disturbance* in infancy have been elucidated at least preliminarily, by several MRI studies.[155,160,241,242] Although results vary somewhat, impairments of cerebral cortical volumetric growth and microstructural differentiation, cerebral white matter microstructural maturation, and basal ganglia volumetric growth have been identified. Similar disturbances of brain maturation have been identified in infants born small for gestational age.[164,166,167] Impaired nutrition in the postnatal period in premature infants has been determined either directly (e.g., energy and lipid intake) or indirectly (e.g., deficient enteral nutrition, prolonged parenteral nutrition). Whether some of the dysmaturational events in these settings are primary or secondary to pre-OL dysmaturation is unclear. Nevertheless, the implication is that optimal nutrition could be restorative in this context.

The *quality of the milk provided to the infant* is another nutritional factor that appears important for neurological outcome and suggests a means for neurorestoration. Available data indicate particular value concerning neurodevelopmental outcome for fortified preterm versus term formula, for human milk versus formula, and for fortified human milk versus no fortification.[236,238,243-249]

Breastfeeding is of particular value, and several volumetric and diffusion tensor MRI studies have shown better white maturation in such infants.[235,236,244,247,248] Perhaps even more importantly,

breastfeeding beyond the neonatal period appears to have long-lasting beneficial effects on cerebral white matter. Thus, a study of 133 (healthy) term-born infants either exclusively breast-fed for a mean of 413 days or exclusively formula-fed or fed a mixture of formula and breast milk utilized advanced MRI measures at age 10 months to four years to assess white matter development.[247] The data show that early exclusive breastfeeding was associated with better development in relatively late maturing white matter regions, including frontal and temporal white matter, corticospinal tracts, and superior longitudinal and occipitofrontal fasciculi. Notably, several of these regions are important for specific higher-order cognitive domains, in which breast-fed infants have been shown to have improved performance.[247,250] Thus, current data suggest that breast-feeding could have neurorestorative potential and that the principal initial effect may involve OL development and myelination.

Polyunsaturated fatty acids (PUFAs) are crucial for brain development and are particularly concentrated in phospholipids of neural membranes, especially in the cerebral cortex and other gray matter structures.[251-253] Most brain PUFAs are acquired in the last trimester of gestation and first two years of life, during rapid brain growth.[254] Thus the preterm infant does not receive this critical transplacental transfer of PUFAs. Notably, however, breast milk is an excellent source of PUFAs. Two recent studies utilizing advanced MRI techniques and determinations of PUFA levels in red blood cells noted positive correlations between PUFA levels and microstructural maturation of cerebral white matter and macrostructural (volumetric) development of cerebral cortex and basal ganglia or thalamus near term equivalent age.[254,255] Concerning the potential value of supplementation with PUFAs, although not all findings are consistent, favorable effects on visual and cognitive function have been reported.[238,256,257] Supplementation to the lactating mother who is breastfeeding has been most effective. Thus the possibility of PUFA supplementation in this manner to the preterm infant, especially with the dysmaturational abnormalities of white and gray matter, is worthy of study as a neurorestorative intervention.

Iron is a critical nutrient for OL maturation. Iron deficiency can be an important contributing factor in causation of brain dysmaturation in premature infants, with particular involvement of OL maturation and subsequent myelination. Thus iron supplementation as needed can be considered a potential neurorestorative intervention. Although the data are not entirely consistent, most studies show impairments of motor, cognitive, and behavioral development in iron-deficient infants.[258-262] Iron deficiency in the neonatal period is usually related to dietary deficiency, particularly in the context of breastfeeding and prematurity.[263-265] Indeed, as many as 10% of infants in the first two years of life in the United States and 15% of breast-fed Canadian infants exhibit iron deficiency. As premature birth deprives the infant of the primary period of fetal iron deposition, i.e., the third trimester of gestation, the risks are still higher in such infants. Supportive of an effect on myelin development in iron-deficient infants is the finding on studies of auditory and visual evoked potentials of prolonged latencies, without impairment of amplitudes.[266-269] (The normal maturational decline in latencies relates to acquisition of myelin, whereas changes in amplitude relate more to neuronal development[161]). A recent study of delayed umbilical cord clamping (DCC) in full-term infants suggests a beneficial effect of iron on MRI-quantitated myelin at age four months.[270] Thus infants followed after delayed umbilical cord clamping had 48% higher serum ferritin levels and myelin content at age four months when compared with infants who had immediate cord clamping. Moreover, because infants with higher iron stores at age four months have been shown to persist with higher stores later, the findings suggest that the positive effect on myelination may persist.[271] More data are needed, but higher iron stores could represent an important neurorestorative goal in premature infants. The clinical findings regarding iron and myelination are consistent with experimental studies showing the crucial role of iron in the processes of OL differentiation and myelination.[263,265,272,273] As iron also plays an important role in neurotransmitter metabolism, the neural effects of iron deficiency may extend beyond an impairment of OL development or myelination.[274] *Quantitative* studies of myelination in living iron-deficient infants by advanced MRI measures would be of great interest.

Zinc is another critical nutrient for OL maturation. Zinc is critical for a variety of aspects of brain development, including OL development.[275-279] The effect is mediated particularly by an OL-specific zinc finger protein (Zfp 488) that functions as a transcriptional coregulator important for OL differentiation.[280] Recent work has shown that zinc concentrations in developing OLs are relatively high during differentiation and decline after maturation is achieved.[281] Moreover, altered zinc balance is involved in experimental models of ischemic and excitotoxic OL death.[282-284] The clinical relevance of this work remains to be clarified fully, but it is notable that preterm infants are vulnerable to zinc deficiency because of high zinc requirements, diminished zinc stores (most zinc stores are acquired in the last trimester of gestation), and suboptimal zinc absorption.[285-287] Moreover, zinc concentrations in human milk are highly variable, and current dietary guidelines for zinc intake for preterm infants are based on limited data.[286,288] More data are needed in human preterm infants on OL maturation, myelination, and neurological development, in relation to zinc status, and on ideal amounts of dietary zinc intake.

Experiential factors. Experiential factors (Table 5) can play a major role in regulation of OL differentiation or myelination and neuronal or axonal development in human infants.[18,19] Although emphasis is often placed on the *deleterious* effects of altered experience, especially in the neonatal period and particularly in the premature infant, the potential benefit of experiential factors as *restorative therapies* is important to consider. In this section, emphasis is placed on factors related to care in the neonatal period and later in infancy (Table 5).

A potential beneficial effect of modification of the *neonatal auditory environment* on cortical development and language outcome is suggested by recent clinical studies. The particular relevance of this work relates in part to the layout of neonatal intensive care units and associated attempts to minimize ambient noise. In part, because sound levels in many neonatal intensive care units, incubators, and ventilators can exceed current recommendations of the American Academy of Pediatrics,[289-293] many units have been designed to minimize such noise, often by maintaining infants in single rooms. Two recent studies suggest that this approach may have an adverse effect on language development and cerebral cortical development in premature infants.[294,295] In a study of 136 preterm infants assigned to either open-ward or single-room bedspaces, infants cared for in single-patient rooms had lower language scores at age two years, accompanied by abnormalities of cortical folding in the superior temporal area (after controlling for potential confounders) (Fig 10).[294] The difference in outcomes was attributed to differences in exposure to language in open areas versus single rooms. In a subsequent study of the role of maternal involvement with the infant, outcomes were assessed for infants from both single-patient rooms and open ward bedspaces while taking into account maternal involvement in the neonatal intensive care unit (NICU) care.[295] Infants with *high maternal involvement* from *both* single-patient and open ward bedspaces had higher cognitive and language scores at age 18 months than did infants with *low* maternal involvement. Notably, the effect size was

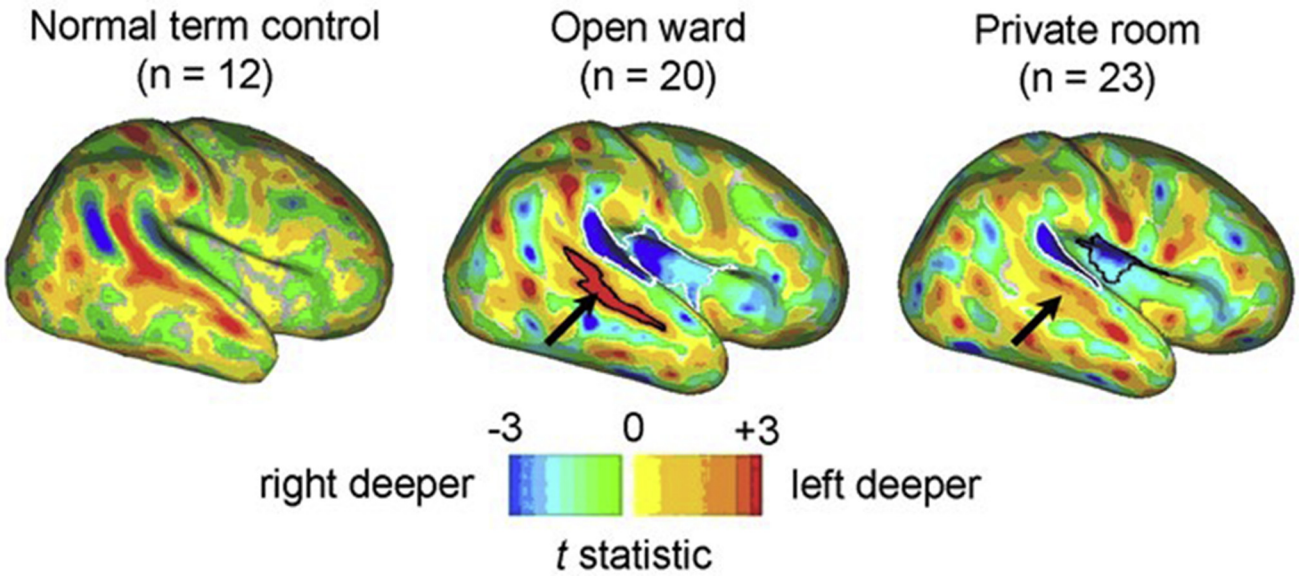

FIGURE 10. Hemispheric sulcal depth asymmetry t-statistic maps for normal term control infants and for premature infants maintained in an open ward (n = 20) or private room (n = 23). The superior temporal sulcus in normal term control infants shows that the superior temporal sulcus is deeper on the left. Premature infants raised in an open ward show a similar (or greater) depth on the left (arrow), whereas the premature infants raised in a private room show a more shallow depth (arrow). (From Pineda RG, Neil J, Dierker D, Smyser CD, Wallendorf M, Kidokoro H, et al. Alterations in brain structure and neurodevelopmental outcome in preterm infants hospitalized in different neonatal intensive care unit environments. J Pediatr. 2014; 164:52-60, with permission).

greater for children from single rooms. This second study suggests that the level of language exposure depends on a variety of factors, including room type and also other maternal characteristics that allow maternal verbal contact with the infant (e.g., availability of maternal leave, socioeconomic status, time spent with the infant, an NICU culture that encourages verbal contact from parents). These latter issues are also important subsequent to the infant's discharge (see later).

The underlying pathophysiology of these effects of auditory experience on language development relates in part to the striking development of the auditory system during the premature period. Thus connections between the cochlea and the brainstem are established by 24 to 25 weeks and connections to temporal lobe and auditory cortex by 30 to 31 weeks.[296,297] Subsequently, cortical development occurs rapidly. Thus it is biologically plausible that an important factor in the subsequent language impairment relates to a disruption of development of this key activity-dependent system. Consistent with this notion, functional connectivity MRI data show that disruption of brain networks detected at term equivalent age persists into childhood.[298]

The importance of the *nature of the auditory input* is shown by a recent study of the effects of different varieties of *music exposure* on cortical connectivity. A careful initial study utilizing functional MRI showed that music exposure in preterm infants had at term equivalent age lasting learning effects on music processing, with an increased connectivity between primary auditory cortex and brain regions involved in several aspects of music processing (e.g., temporal and cingulate cortex, basal ganglia).[299] Subsequent work will focus on the duration of such effects and effects on language processing. The initial findings suggest that details of the neonatal (and subsequent) auditory experience can have lasting beneficial effects on important areas of brain development.

Neonatal visual experience also appears to play an important role in the infant's brain development. As WMI in the premature infant tends to be most severe in the parieto-occipital region,[127] central visual impairment is a common neurological sequelae.[118] Notably, development of the axons of the geniculocalcarine tract, ensheathment of these axons by pre-OLs, early myelin formation of the tract, and development of visual cortex are very active during the premature period and into infancy.[18] Whether optimal visual experience in premature infants could capitalize on this actively developing system to counteract the deleterious central visual effects of WMI, i.e., have *restorative* properties, is important to consider. The data just reviewed regarding auditory experiences are encouraging in this regard. Experimental studies in normal and preterm monkeys showed that premature visual stimulation resulted in increases in size and proportions of synapses in visual cortex, presumably by activity-dependent alterations in synaptogenesis, synaptic modification, or synapse elimination.[300] Visual deprivation had opposite and unfavorable effects. The somewhat limited data available in human infants suggest that, as in monkeys, visual experiential effects are important mediators of cortical development. Thus visual experience of premature infants is associated with accentuation of the development of the visual evoked potential, a finding consistent with enhancement of axonal and dendritic development and synaptogenesis.[301,302] More data are needed to address how visual experience could enhance development of the cerebral visual system and perhaps counteract the effects of dysmaturation described earlier.

Pain and stress in the neonatal period, common experiences for the preterm infant, have been shown to have adverse effects on neurodevelopmental, behavioral, and cognitive outcomes.[303-308] Abnormalities of brain development accompany these effects (see next). The *findings suggest that reduction of pain and stress in these infants could serve as a neurorestorative intervention* (Table 5).

Several studies that quantitated pain and stress (either number of stressful events[309] or number of painful (skin breaks) procedures[307,308,310,311]) showed correlative disturbances in brain development involving cerebral cortex, thalamus, cerebral white matter, and functional connectivity. Functional connectivity disturbance has been demonstrated as early as term equivalent age (Fig 11).[309]

Although most abnormalities were detected at term equivalent age, thinning of cerebral cortex, especially in parietal and frontal areas, was identified at a mean age of 7.9 years.[311] Additional evidence concerning a relation of stress to cerebral white matter development emanates from a later randomized controlled clinical trial that evaluated the effectiveness of training parents in reducing stressful experiences in premature infants in the NICU.[312] At term equivalent age the infants of the mothers in the intervention group showed by advanced MRI significantly enhanced maturation and connectivity of cerebral white matter. These and related data caused the American Academy of Pediatrics to emphasize the need in neonatal intensive care facilities for "a pain-prevention program that includes strategies for minimizing the number of painful procedures performed."[313] *This evidence for a deleterious effect of pain and stress in the neonatal period suggests that interventions to reduce the responsible events could be neurorestorative*. Moreover, the potential importance of stress as a deleterious factor *beyond the neonatal period* and prevention or amelioration of such stress as a neurorestorative intervention will be discussed later.

One approach to diminish neonatal stress has been the individualized developmental care approach pioneered by Als.[314] The

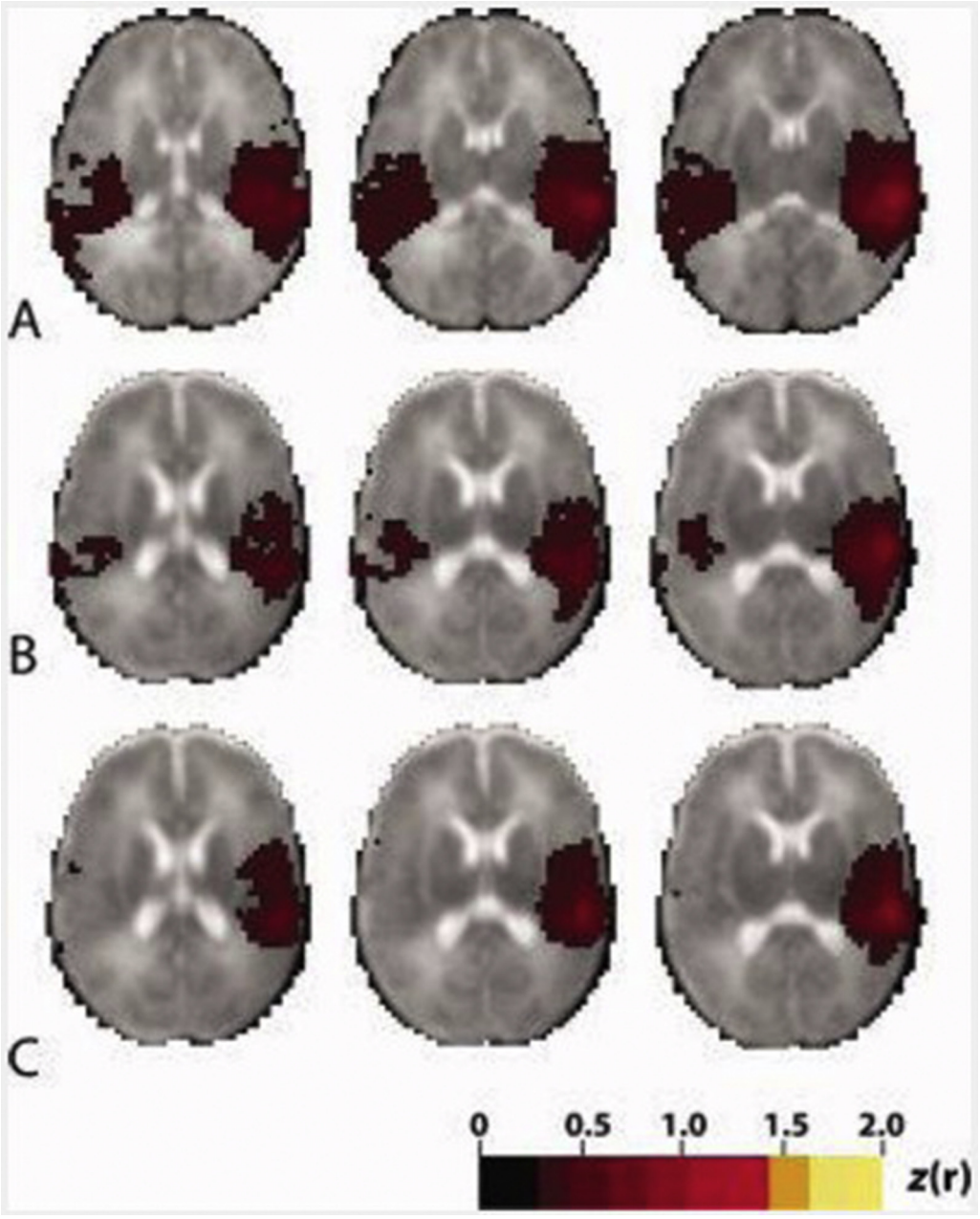

FIGURE 11. Functional connectivity correlation maps using a right temporal lobe seed in (A) term control infants (n = 10), (B) premature infants at term equivalent age and previously exposed to low stress (n = 10), and (C) premature infants exposed to high stress (n = 10). Note that the average correlation map in the infants with high stress exposures (C) does not demonstrate the interhemispheric correlation between the temporal lobes identified in the low stress (B) and control (A) infants. (From Smith GC, Gutovich J, Smyser C, Pineda R, Newnham C, Tjoeng TH, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. Ann Neurol. 2011; 70:541-549, with permission).

possible neurorestorative role of this approach was suggested by a randomized clinical trial of the Als care program, which showed at nine months' corrected age, improved neurobehavioral function, quantitative electroencephalographic evidence of enhanced maturation, and diffusion tensor MRI evidence of more advanced cerebral white matter fiber tract development.[315] Benefits were still present at age eight years.[316] Many elements of developmental care were identified, including patient positioning, light, sound, handling, approaches to feeding, and inclusion of parents in care.[317-319] The initial findings from this work regarding beneficial effects on brain maturation suggest the possibility that at least some of these approaches could lead to mitigation and recovery of the dysmaturational features of the encephalopathy of prematurity.

The *mechanisms for the deleterious effects of stress on brain maturation,* although not entirely understood in the human newborn and young infant, may involve brain microglia.[320] Such stress mediators as glucocorticoids and catecholamines are known to lead to proinflammatory activation of microglia in experimental studies. In view of the key role of microglia in various aspects of brain maturation in early life and the substantial number of microglia in cerebral white matter in the human infant, especially in the diffuse gliosis of WMI (see earlier), such modulation of microglia toward a proinflammatory state could lead to dysmaturational events. Moreover, this modulation of microglia has been related to a potentiated response to *subsequent* stress, for example, in infancy and beyond, with the exacerbation of dysmaturation and the promotion of cognitive and emotional disorders. Thus, in this context, elimination of stress would be a major neurorestorative intervention.

Parenting, educational, and social factors are critical in the infant's experience, especially beyond the neonatal period, and suggest opportunities for neurorestorative intervention (Table 5). Many studies in recent years have shown that factors relating to parenting, parental education, and socioeconomic context beyond the neonatal period are critical determinants of ultimate neurodevelopmental outcome, especially relating to cognition, language, and behavior.[10,321-329] Initial research with MRI suggests that the anatomical substrate for impaired neurodevelopment in this setting involves changes in volumetric growth of cortex, deep nuclear structures, and cerebral white matter,[328,330] and in functional network maturation.[328,331] *The particular importance of early parenting behavior, particularly maternal affective involvement, parent-child synchrony, and positive and responsive parenting, has been delineated and could represent a neurorestorative intervention.*[327-329]

Although the deleterious experiential effects just described begin in the neonatal period, their persistence into infancy and early childhood raises the possibility that *myelin development, a post-term event in human brain, could be especially affected*. This possibility is supported by recent elegant studies of infants institutionally reared in orphanages under circumstances associated with lack of parenting and neglect, including social, emotional, and linguistic deficiencies. Although disturbances in subsequent behavioral and cognitive functions in such infants are well known, impairments in macrostructural and microstructural white matter development have been shown only recently.[332-337] The potential to counteract this dysmaturation, i.e., a neurorestorative intervention, was shown by a large study of Romanian orphans randomly selected to remain in an institution or to be placed in supervised foster care at age two years.[338] Studies by diffusion tensor MRI at age eight years showed significant associations between *neglect in early life and impaired microstructural integrity of multiple white matter structures*. Particular affection of radial diffusivity suggested impaired myelin ensheathment of axons. Early intervention (i.e., foster care) promoted more nearly normative white matter development among the previously neglected children. *The particular link between environmental enrichment and white matter development* is reminiscent of studies of specific skill development, for example, musicians, which show a relation of enhanced skill to induced changes in white matter microstructure. Importantly, these changes are effected best during early sensitive periods, for example, in childhood, and, moreover, enhance additional practice-based changes in white matter microstructure and performance later in life.[339] Notably, a large experimental literature supports maturational value for environmental enrichment in many animal models.[328]

Randomized intervention studies of older infants and young children in lower socio-economic status conditions, albeit less severely neglected than the aforementioned orphans, also show benefit for development of language, emotional regulation, and cognitive skills.[328,340,341] The anatomical substrate of these effects remain to be established. However, the demonstrated benefit raises important and complex societal issues.

Conclusions

This major section focused on interventions directed at pre-OL death and subsequent dysmaturation of white and gray matter structures. Interventions directed at protection versus pre-OL *death*, termed *neuroprotective*, discussed in detail elsewhere, were not emphasized. Of these, only EPO has been studied in human infants, and prolonged therapy seems preferable to brief courses. Interventions considered to be promising for amelioration or prevention of the subsequent dysmaturation, termed *neurorestorative*, were emphasized. Promising preclinical data were reviewed. Clinical studies suggest value for EPO, optimal nutrition (concerning quality and source of milk, breastfeeding, PUFAs, iron, and zinc), and critical experiential interventions. The latter relate in the neonatal period to optimal auditory and visual experience and reduction of pain and stress. Importance for brain maturation can also be attributed to such interventions in infancy and early childhood as breastfeeding, carefully designed intervention programs, environmental enrichment, and parental, educational, and social factors.

Summary and Conclusions

The principal focus of this review has been the dysmaturation of brain in infants born prematurely, especially very preterm. The dysmaturational details have been identified primarily by advanced MRI studies. The review has delineated (1) the dysmaturational features involving both white and gray matter structures; (2) the context of their occurrence, i.e., the remarkable array of developmental events in the human brain during the premature period; (3) the spectrum of the cerebral WMI that appears to initiate the dysmaturational events; (4) the likely mechanisms by which the WMI, particularly the mild forms now most prevalent in neonatal intensive care facilities, lead to the dysmaturation; and (5) the potential neurorestorative interventions suggested by a burgeoning amount of recent work, both clinical and experimental.

References

1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388:3027–3035.
2. Murphy SL, Mathews T, Martin JA, et al. Annual summary of vital statistics: 2013 - 2014. Pediatrics. 2017;139:e20163239.

3. Ancel PY, Goffinet F, Kuhn P, et al. Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: Results of the EPIPAGE-2 cohort study. JAMA Pediatr. 2015:E1–E9.
4. Helenius K, Sjors G, Shah PS, et al. Survival in very preterm infants: An international comparison of 10 national neonatal networks. Pediatrics. 2017;140:e20171264.
5. Boghossian NS, Geraci M, Edwards EM, et al. Morbidity and mortality in small for gestational age infants at 22 to 29 weeks' gestation. Pediatrics. 2018;141:e20172533.
6. Kerstjens JM, de Winter AF, Bocca-Tjeertes IF, et al. Risk of developmental delay increases exponentially as gestational age of preterm infants decreases: a cohort study at age 4 years. Dev Med Child Neurol. 2012;54:1096–1101.
7. Hirvonen M, Ojala R, Korhonen P, et al. Cerebral palsy among children born moderately and late preterm. Pediatrics. 2014;134:e1584–e1593.
8. Peralta-Carcelen M, Carlo WA, Pappas A, et al. Behavioral problems and socioemotional competence at 18 to 22 months of extremely premature children. Pediatrics. 2017;139:e20161043.
9. Cheong JLY, Anderson PJ, Burnett AC, et al. Changing neurodevelopment at 8 years in children born extremely preterm since the 1990s. Pediatrics. 2017;139:e20164086.
10. Nguyen TN, Spencer-Smith M, Haebich KM, et al. Language trajectories of children born very preterm and full term from early to late childhood. J Pediatr. 2018;202:86–91.
11. Amer R, Moddemann D, Seshia M, et al. Neurodevelopmental outcomes of infants born at <29 weeks of gestation admitted to Canadian Neonatal Intensive Care Units based on location of birth. J Pediatr. 2018;196:31–37.
12. Broring T, Oostrom KJ, van Dijk-Lokkart EM, et al. Attention deficit hyperactivity disorder and autism spectrum disorder symptoms in school-age children born very preterm. Res Dev Disabil. 2018;74:103–112.
13. Twilhaar ES, Wade RM, de Kieviet JF, et al. Cognitive outcomes of children born extremely or very preterm since the 1990s and associated risk factors: A meta-analysis and meta-regression. JAMA Pediatr. 2018;172:361–367.
14. Burnett AC, Anderson PJ, Lee KJ, et al. Trends in executive functioning in extremely preterm children across 3 birth eras. Pediatrics. 2018;141:e20171958.
15. Spittle AJ, Cameron K, Doyle LW, et al. Motor impairment trends in extremely preterm children: 1991-2005. Pediatrics. 2018;141:e20173410.
16. Bolk J, Farooqi A, Hafstrom M, et al. Developmental coordination disorder and its association with developmental comorbidities at 6.5 years in apparently healthy children born extremely preterm. JAMA Pediatr. 2018;172:765–774.
17. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol. 2009;8:110–124.
18. Kinney HC, Volpe JJ. Organizational events. In: Volpe JJ, Inder TE, Darras BT, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 7. Philadelphia, PA: Elsevier; 2018:145–175.
19. Kinney HC, Volpe JJ. Myelination events. In: Volpe JJ, Inder TE, Darras BT, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 8. Philadelphia, PA: Elsevier; 2018:176–188.
20. Volpe JJ, Kinney HC, Jensen FE, et al. The developing oligodendrocyte: key cellular target in brain injury in the premature infant. Int J Devl Neurosci. 2011;29:423–440.
21. Back SA. Brain injury in the preterm infant: New horizons for pathogenesis and prevention. Pediatr Neurol. 2015;53:185–192.
22. Back SA, Volpe JJ. Encephalopathy of Prematurity: Pathophysiology. In: Volpe JJ, Inder TE, Darras BT, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 15. Philadelphia, PA: Elsevier; 2018:405–424.
23. Back SA, Luo NL, Borenstein NS, et al. Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. J Neurosci. 2001;21:1302–1312.
24. Back SA, Luo NL, Borenstein NS, et al. Arrested oligodendrocyte lineage progression during human cerebral white matter development: Dissociation between the timing of progenitor differentiation and myelinogenesis. J Neuropathol Exp Neurol. 2002;61:197–211.
25. Hardy R, Reynolds R. Neuron-oligodendroglial interactions during central nervous system development. J Neurosci Res. 1993;36:121–126.
26. Barres BA, Raff MC. Axonal control of oligodendrocyte development. J Cell Biol. 1999;147:1123–1128.
27. Yamazaki Y, Hozumi Y, Kaneko K, et al. Oligodendrocytes: facilitating axonal conduction by more than myelination. Neuroscientist. 2010;16:11–18.
28. Kremer D, Aktas O, Hartung HP, et al. The complex world of oligodendroglial differentiation inhibitors. Ann Neurol. 2011;69:602–618.
29. Kramer-Albers EM, White R. From axon-glial signalling to myelination: the integrating role of oligodendroglial Fyn kinase. Cell Mol Life Sci. 2011;68:2003–2012.
30. Lee Y, Morrison BM, Li Y, et al. Oligodendroglia metabolically support axons and contribute to neurodegeneration. Nature. 2012;487:443–448.
31. Oluich LJ, Stratton JA, Lulu Xing Y, et al. Targeted ablation of oligodendrocytes induces axonal pathology independent of overt demyelination. J Neurosci. 2012;32:8317–8330.
32. Berret E, Barron T, Xu J, et al. Oligodendroglial excitability mediated by glutamatergic inputs and Nav1.2 activation. Nat Commun. 2017;8:557.
33. Jiang C, Yang W, Fan Z, et al. AATYK is a novel regulator of oligodendrocyte differentiation and myelination. Neurosci Bull. 2018;34:527–533.
34. Santos AK, Vieira MS, Vasconcellos R, et al. Decoding cell signalling and regulation of oligodendrocyte differentiation [e-pub ahead of print]. Semin Cell Dev Biol. 2018. https://doi.org/10.1016/j.semcdb.2018.05.020.
35. Srivastava T, Diba P, Dean JM, et al. A TLR/AKT/Fox03 immune tolerance-like pathway disrupts the repair capacity of oligodendrocyte progenitors. J Clin Invest. 2018;128:2025–2041.
36. Haynes RL, Borenstein NS, DeSilva TM, et al. Axonal development in the cerebral white matter of the human fetus and infant. J Comp Neurol. 2005;484:156–167.
37. Kostovic I, Judas M. Correlation between the sequential ingrowth of afferents and transient patterns of cortical lamination in preterm infants. Anat Rec. 2002;267:1–6.
38. Kostovic I, Judas M, Rados M, et al. Laminar organization of the human fetal cerebrum revealed by histochemical markers and magnetic resonance imaging. Cereb Cortex. 2002;12:536–544.
39. Kostovic I, Jovanov-Milosevic N. The development of cerebral connections during the first 20-45 weeks' gestation. Semin Fetal Neonatal Med. 2006;11:415–422.
40. Kostovic I, Judas M. Transient patterns of cortical lamination during prenatal life: do they have implications for treatment? Neurosci Biobehav Rev. 2007;31:1157–1168.
41. Conel J. The Postnatal Development of the Human Cerebral Cortex. Cambridge: Harvard University Press; 1939.
42. Mrzljak L, Uylings H, Kostovic I, et al. Prenatal development of neurons in the human prefrontal cortex: I.A. qualitative Golgi study. J Comp Neurol. 1988;271:355–386.
43. Marin-Padilla M. Early ontogenesis of the human cerebral cortex. In: Peters A, Jones EG, eds. Cerebral Cortex. Vol 7. NY: Plenum Publishing Corp.; 1988:1–34.
44. Marin-Padilla M. Ontogenesis of the pyramidal cell of the mammalian neocortex and developmental cytoarchitectonics: A unifying theory. J Comp Neurol. 1992;321:233–240.
45. Marin-Padilla M. Prenatal and early postnatal ontogenesis of the human motor cortex: A Golgi study. I. The sequential development of the cortical layers. Brain Res. 1970;23:167–183.
46. Goodman CS, Shatz CJ. Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell. 1993;72:77–98.
47. Schlaggar BL, Fox K, Oleary DM. Postsynaptic control of plasticity in developing somatosensory cortex. Nature. 1993;364:623–626.
48. Johnston MV. Neurotransmitters and vulnerability of the developing brain. Brain Dev. 1995;17:301–306.
49. Flint AC, Liu XL, Kriegstein AR. Nonsynaptic glycine receptor activation during early neocortical development. Neuron. 1998;20:43–53.
50. Bardoul M, Levallois C, Konig N. Functional AMPA/kainate receptors in human embryonic and foetal central nervous system. J Chem Neuroanat. 1998;14:79–85.
51. Aizawa H, Hu SC, Bobb K, et al. Dendrite development regulated by CREST, a calcium-regulated transcriptional activator. Science. 2004;303:197–202.
52. Reiprich P, Kilb W, Luhmann HJ. Neonatal NMDA receptor blockade disturbs neuronal migration in rat somatosensory cortex in vivo. Cereb Cortex. 2005;15:349–358.
53. Konur S, Ghosh A. Calcium signaling and the control of dendritic development. Neuron. 2005;46:401–405.
54. Chen Y, Wang PY, Ghosh A. Regulation of cortical dendrite development by Rap1 signaling. Mol Cell Neurosci. 2005;28:215–228.
55. Chen YC, Ghosh A. Regulation of dendritic development by neuronal activity. J Neurobiol. 2005;64:4–10.
56. Yap EL, Greenberg ME. Activity-regulated transcription: bridging the gap between neural activity and behavior. Neuron. 2018;100:330–348.
57. Sarnat HB, Philippart M, Flores-Sarnat L, et al. Timing in neural maturation: arrest, delay, precociousness, and temporal determination of malformations. Pediatr Neurol. 2015;52:473–486.
58. Sarnat HB, Flores-Sarnat L, Trevenen CL. Synaptophysin immunoreactivity in the human hippocampus and neocortex from 6 to 41 weeks of gestation. J Neuropathol Exp Neurol. 2010;69:234–245.
59. Talos DM, Fishman RE, Park H, et al. Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. I. Rodent cerebral white matter and cortex. J Comp Neurol. 2006;497:42–60.
60. Talos DM, Follett PL, Folkerth RD, et al. Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol. 2006;497:61–77.
61. Bystron I, Blakemore C, Rakic P. Development of the human cerebral cortex: Boulder Committee revisited. Nat Rev Neurosci. 2008;9:110–122.
62. Zecevic N, Chen YH, Filipovic R. Contributions of cortical subventricular zone to the development of the human cerebral cortex. J Comp Neurol. 2005;491:109–122.
63. Letinic K, Zoncu R, Rakic P. Origin of GABAergic neurons in the human neocortex. Nature. 2002;417:645–649.
64. Rakic P. Developmental and evolutionary adaptations of cortical radial glia. Cereb Cortex. 2003;13:541–549.
65. Tan SS. Developmental neurobiology: cortical liars. Nature. 2002;417:605–606.

66. Xu G, Broadbelt KG, Haynes RL, et al. Late development of the GABAergic system in the human cerebral cortex and white matter. J Neuropathol Exp Neurol. 2011;70:841–858.
67. Hamilton SP, Rome LH. Stimulation of in vitro myelin synthesis by microglia. Glia. 1994;11:326–335.
68. Rezaie P, Male D. Differentiation, ramification and distribution of microglia within the central nervous system examined. Neuroembryology. 2002;1:29–43.
69. Rezaie P, Dean A, Male D, et al. Microglia in the cerebral wall of the human telencephalon at second trimester. Cereb Cortex. 2005;15:938–949.
70. Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron. 2012;74:691–705.
71. Squarzoni P, Oller G, Hoeffel G, et al. Microglia modulate wiring of the embryonic forebrain. Cell Rep. 2014;8:1271–1279.
72. Reemst K, Noctor SC, Lucassen PJ, et al. The indispensable roles of microglia and astrocytes during brain development. Front Hum Neurosci. 2016;10:1–28.
73. Hickman S, Izzy S, Sen P, et al. Microglia in neurodegeneration. Nat Neurosci. 2018;21:1359–1369.
74. Guttenplan K, Blum J, Bennett M. A role for microglia in retinal development. J Neurosci. 2018;38:9126–9128.
75. Hammond TR, Robinton D, Stevens B. Microglia and the brain: complementary partners in development and disease. Annu Rev Cell Dev Biol. 2018;34:523–544.
76. Hammond TR, Dufort C, Dissing-Olesen L, et al. Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity. 2019;50:1–19.
77. Agresti C, D'Urso D, Levi G. Reversible inhibitory effects of interferon-γ- and tumour necrosis factor-α on oligodendroglial lineage cell proliferation and differentiation in vitro. Eur J Neurosci. 1996;8:1106–1116.
78. Andrews T, Zhang P, Bhat NR. TNF-α potentiates IFNγ-induced cell death in oligodendrocyte progenitors. J Neurosci Res. 1998;54:574–583.
79. Xie Z, Wei M, Morgan TE, et al. Peroxynitrite mediates neurotoxicity of amyloid β-peptide 1-42- and lipopolysaccharide-activated microglia. J Neurosci. 2002;22:3484–3492.
80. Rivest S. Molecular insights on the cerebral innate immune system. Brain Behav Immun. 2003;17:13–19.
81. Lehnardt S, Massillon L, Follett P, et al. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci U S A. 2003;100:8514–8519.
82. Buntinx M, Moreels M, Vandenabeele F, et al. Cytokine-induced cell death in human oligodendroglial cell lines: I. Synergistic effects of IFN-gamma and TNF-alpha on apoptosis. J Neurosci Res. 2004;76:834–845.
83. Li J, Baud O, Vartanian T, et al. Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. Proc Natl Acad Sci U S A. 2005;102:9936–9941.
84. Pang Y, Cai ZW, Rhodes PG. Effect of tumor necrosis factor-alpha on developing optic nerve oligodendrocytes in culture. J Neurosci Res. 2005;80:226–234.
85. Larouche A, Roy M, Kadhim H, et al. Neuronal injuries induced by perinatal hypoxic-ischemic insults are potentiated by prenatal exposure to lipopolysaccharide: animal model for perinatally acquired encephalopathy. Dev Neurosci. 2005;27:134–142.
86. Monier A, Evrard P, Gressens P, et al. Distribution and differentiation of microglia in the human encephalon during the first two trimesters of gestation. J Comp Neurol. 2006;499:565–582.
87. Billiards SS, Haynes RL, Folkerth RD, et al. Development of microglia in the cerebral white matter of the human fetus and infant. J Comp Neurol. 2006;497:199–208.
88. Zhang Y, Inder TE, Neil JJ, et al. Cortical structural abnormalities in very preterm children at 7years of age. Neuroimage. 2015;109:469–479.
89. Smyser CD, Snyder AZ, Shimony JS, et al. Resting-state network complexity and magnitude are reduced in prematurely born infants. Cereb Cortex. 2016;26:322–333.
90. Rajagopalan V, Scott JA, Liu M, et al. Complementary cortical gray and white matter developmental patterns in healthy, preterm neonates. Hum Brain Mapp. 2017;38:4322–4336.
91. Batalle D, Hughes EJ, Zhang H, et al. Early development of structural networks and the impact of prematurity on brain connectivity. Neuroimage. 2017;149:379–392.
92. Neil JJ, Smyser CD. Recent advances in the use of MRI to assess early human cortical development. J Magn Reson. 2018;293:56–69.
93. Smyser CD, Wheelock MD, Limbrick Jr DD, et al. Neonatal brain injury and aberrant connectivity. Neuroimage. 2019;185:609–623.
94. Banker BQ, Larroche JC. Periventricular leukomalacia of infancy. A form of neonatal anoxic encephalopathy. Arch Neurol. 1962;7:386–410.
95. Gilles FH. Hypotensive brain stem necrosis. Selective symmetrical necrosis of tegmental neuronal aggregates following cardiac arrest. Arch Pathol. 1969;88:32–41.
96. Armstrong D, Norman MG. Periventricular leucomalacia in neonates. Complications and sequelae. Arch Dis Child. 1974;49:367–375.
97. Shuman RM, Selednik LJ. Periventricular leukomalacia. A one-year autopsy study. Arch Neurol. 1980;37:231–235.
98. Okoshi Y, Itoh M, Takashima S. Characteristic neuropathology and plasticity in periventricular leukomalacia. Pediatr Neurol. 2001;25:221–226.
99. Kinney HC, Armstrong DC. Perinatal neuropathology. In: Graham DI, Lantos PL, eds. Greenfield's Neuropathology. 7th ed. London: Arnold; 2002:543–551.
100. Pierson CR, Folkerth RD, Billards SS, et al. Gray matter injury associated with periventricular leukomalacia in the premature infant. Acta Neuropathol. 2007;114:619–631.
101. Buser JR, Maire J, Riddle A, et al. Arrested preoligodendrocyte maturation contributes to myelination failure in premature infants. Ann Neurol. 2012;71:93–109.
102. Volpe JJ. Confusions in nomenclature: "periventricular leukomalacia" and "white matter injury" - identical, distinct, or overlapping? Pediatr Neurol. 2017;73:3–6.
103. Haynes RL, Folkerth RD, Keefe R, et al. Nitrosative and oxidative injury to premyelinating oligodendrocytes is accompanied by microglial activation in periventricular leukomalacia in the human premature infant. J Neuropathol Exp Neurol. 2003;62:441–450.
104. Back SA, Luo NL, Mallinson RA, et al. Selective vulnerability of preterm white matter to oxidative damage defined by F(2)-isoprostanes. Ann Neurol. 2005;58:108–120.
105. Billiards SS, Haynes RL, Folkerth RD, et al. Myelin abnormalities without oligodendrocyte loss in periventricular leukomalacia. Brain Pathol. 2008;18:153–163.
106. Emery B, Agalliu D, Cahoy JD, et al. Myelin gene regulatory factor is a critical transcriptional regulator required for CNS myelination. Cell. 2009;138:172–185.
107. Fancy SP, Baranzini SE, Zhao C, et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev. 2009;23:1571–1585.
108. Back SA. White matter injury in the preterm infant: pathology and mechanisms. Acta Neuropathol. 2017;134:331–349.
109. Liddelow SA, Barres BA. Reactive Astrocytes: Production, Function, and Therapeutic Potential. Immunity. 2017;46:957–967.
110. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541:481–487.
111. Cekanaviciute E, Buckwalter MS. Astrocytes: integrative regulators of neuroinflammation in stroke and other neurological diseases. Neurotherapeutics. 2016;13:685–701.
112. Cargill R, Kohama SG, Struve J, et al. Astrocytes in aged nonhuman primate brain gray matter synthesize excess hyaluronan. Neurobiol Aging. 2012;33,830.e13-24.
113. Sloane JA, Batt C, Ma Y, et al. Hyaluronan blocks oligodendrocyte progenitor maturation and remyelination through TLR2. Proc Natl Acad Sci U S A. 2010;107:11555–11560.
114. Folkerth RD, Keefe RJ, Haynes RL, et al. Interferon-gamma expression in periventricular leukomalacia in the human brain. Brain Pathol. 2004;14:265–274.
115. Deguchi K, Oguchi K, Takashima S. Characteristic neuropathology of leukomalacia in extremely low birth weight infants. Pediatr Neurol. 1997;16:296–300.
116. Donat CK, Scott G, Gentleman SM, et al. Microglial activation in traumatic brain injury. Front Aging Neurosci. 2017;9:208.
117. Kwon SH, Vasung L, Ment LR, et al. The role of neuroimaging in predicting neurodevelopmental outcomes of preterm neonates. Clin Perinatol. 2014;41:257–283.
118. Neil JJ, Volpe JJ. Encephalopathy of prematurity: Clinical-neurological features, diagnosis, imaging, prognosis, therapy. In: Volpe JJ, Inder TE, du Plessis AJ, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 16. Philadelphia, PA: Elsevier; 2018:425–457.
119. Chau V, Synnes A, Grunau RE, et al. Abnormal brain maturation in preterm neonates associated with adverse neurodevelopmental outcomes. Neurology. 2013;81:2082–2089.
120. Kersbergen KJ, Benders MJ, Groenendaal F, et al. Different patterns of punctate white matter lesions in serially scanned preterm infants. PLoS One. 2014;9:e108904.
121. Guo T, Duerden EG, Adams E, et al. Quantitative assessment of white matter injury in preterm neonates: Association with outcomes. Neurology. 2017;88:614–622.
122. Tusor N, Benders MJ, Counsell SJ, et al. Punctate white matter lesions associated with altered brain development and adverse motor outcome in preterm infants. Sci Rep. 2017;7:13250.
123. Woodward LJ, Anderson PJ, Austin NC, et al. Neonatal MRI to predict neurodevelopmental outcomes in preterm infants. N Engl J Med. 2006;355:685–694.
124. Hintz SR, Barnes PD, Bulas D, et al. Neuroimaging and neurodevelopmental outcome in extremely preterm infants. Pediatrics. 2015;135:e32–e42.
125. Linsell L, Johnson S, Wolke D, et al. Cognitive trajectories from infancy to early adulthood following birth before 26 weeks of gestation: a prospective, population-based cohort study. Arch Dis Child. 2018;103:363–370.
126. Anderson PJ, Treyvaud K, Neil JJ, et al. Associations of newborn brain magnetic resonance imaging with long-term neurodevelopmental impairments in very preterm children. J Pediatr. 2017;187:58–65.e1.
127. Kinney HC, Volpe JJ. Encephalopathy of Prematurity: Neuropathology. In: Volpe JJ, Inder TE, Darras BT, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 14. Philadelphia, PA: Elsevier; 2018:389–404.

128. Haynes RL, Billiards SS, Borenstein NS, et al. Diffuse axonal injury in periventricular leukomalacia as determined by apoptotic marker fractin. Pediatr Res. 2008;63:656–661.
129. Tekkok SB, Goldberg MP. AMPA/kainate receptor activation mediates hypoxic oligodendrocyte death and axonal injury in cerebral white matter. J Neurosci. 2001;21:4237–4248.
130. Wakita H, Tomimoto H, Akiguchi I, et al. Axonal damage and demyelination in the white matter after chronic cerebral hypoperfusion in the rat. Brain Res. 2002;924:63–70.
131. Sizonenko SV, Sirimanne E, Mayall Y, et al. Selective cortical alteration after hypoxic-ischemic injury in the very immature rat brain. Pediatr Res. 2003;54:263–269.
132. McCarran WJ, Goldberg MP. White matter axon vulnerability to AMPA/kainate receptor-mediated ischemic injury is developmentally regulated. J Neurosci. 2007;27:4220–4229.
133. Alix JJ, Zammit C, Riddle A, et al. Central axons preparing to myelinate are highly sensitivity to ischemic injury. Ann Neurol. 2012;72:936–951.
134. Ligam P, Haynes RL, Folkerth RD, et al. Thalamic damage in periventricular leukomalacia: Novel pathologic observations relevant to cognitive deficits in survivors of prematurity. Pediatr Res. 2009;65:524–529.
135. Northington FJ, Ferriero DM, Flock DL, et al. Delayed neurodegeneration in neonatal rat thalamus after hypoxia-ischemia is apoptosis. J Neurosci. 2001;21:1931–1938.
136. Northington FJ, Ferriero DM, Martin LJ. Neurodegeneration in the thalamus following neonatal hypoxia-ischemia is programmed cell death. Dev Neurosci. 2001;23:186–191.
137. McConnell SK, Ghosh A, Shatz CJ. Subplate neurons pioneer the first axon pathway from the cerebral cortex. Science. 1989;245:978–982.
138. Ghosh A, Antonini A, McConnell SK, et al. Requirement for subplate neurons in the formation of thalamocortical connections. Nature. 1990;347:179–181.
139. Ghosh A, Shatz CJ. Involvement of subplate neurons in the formation of ocular dominance columns. Science. 1992;255:1441–1443.
140. Ghosh A, Shatz CJ. A role for subplate neurons in the patterning of connections from thalamus to neocortex. Development. 1993;117:1031–1047.
141. Volpe JJ. Subplate neurons - missing link in brain injury of the premature infant? Pediatrics. 1996;97:112–113.
142. Kanold PO, Kara P, Reid RC, et al. Role of subplate neurons in functional maturation of visual cortical columns. Science. 2003;301:521–525.
143. Kanold PO. Transient microcircuits formed by subplate neurons and their role in functional development of thalamocortical connections. Neuroreport. 2004;15:2149–2153.
144. Bystron I, Molnar Z, Otellin V, et al. Tangential networks of precocious neurons and early axonal outgrowth in the embryonic human forebrain. J Neurosci. 2005;25:2781–2792.
145. McQuillen PS, Sheldon RA, Shatz CJ, et al. Selective vulnerability of subplate neurons after early neonatal hypoxia-ischemia. J Neurosci. 2003;23:3308–3315.
146. Robinson S, Li Q, Dechant A, et al. Neonatal loss of gamma-aminobutyric acid pathway expression after human perinatal brain injury. J Neurosurg. 2006;104:396–408.
147. Kinney HC, Haynes RL, Xu G, et al. Neuron deficit in the white matter and subplate in periventricular leukomalacia. Ann Neurol. 2012;71:397–406.
148. Boardman JP, Counsell SJ, Rueckert D, et al. Abnormal deep grey matter development following preterm birth detected using deformation based morphometry. NeuroImage. 2006;32:70–78.
149. Anjari M, Srinivasan L, Allsop JM, et al. Diffusion tensor imaging with tract-based spatial statistics reveals local white matter abnormalities in preterm infants. NeuroImage. 2007;35:1021–1027.
150. Dubois J, Benders M, Cachia A, et al. Mapping the early cortical folding process in the preterm newborn brain. Cereb Cortex. 2008;18:1444–1454.
151. Boardman JP, Craven C, Valappil S, et al. A common neonatal image phenotype predicts adverse neurodevelopmental outcome in children born preterm. Neuroimage. 2010;52:409–414.
152. Ball G, Boardman JP, Rueckert D, et al. The effect of preterm birth on thalamic and cortical development. Cereb Cortex. 2012;22:1016–1024.
153. Ball G, Boardman JP, Aljabar P, et al. The influence of preterm birth on the developing thalamocortical connectome. Cortex. 2013;49:1711–1721.
154. Ball G, Aljabar P, Nongena P, et al. Multimodal image analysis of clinical influences on preterm brain development. Ann Neurol. 2017;82:233–246.
155. Barnett ML, Tusor N, Ball G, et al. Exploring the multiple-hit hypothesis of preterm white matter damage using diffusion MRI. Neuroimage Clin. 2018;17:596–606.
156. Ajayi-Obe M, Saeed N, Cowan FM, et al. Reduced development of cerebral cortex in extremely preterm infants. Lancet. 2000;356:1162–1163.
157. Ball G, Pazderova L, Chew A, et al. Thalamocortical connectivity predicts cognition in children born preterm. Cereb Cortex. 2015;25:4310–4318.
158. Wimberger DM, Roberts TP, Barkovich AJ, et al. Identification of "premyelination" by diffusion-weighted MRI. J Comput Assist Tomogr. 1995;19:28–33.
159. Tolcos M, Bateman E, O'Dowd R, et al. Intrauterine growth restriction affects the maturation of myelin. Exp Neurol. 2011;232:53–65.
160. Vinall J, Grunau RE, Brant R, et al. Slower postnatal growth is associated with delayed cerebral cortical maturation in preterm newborns. Sci Transl Med. 2013;5:168ra8.
161. Neil JJ, Volpe JJ. Specialized neurological studies. In: Volpe JJ, Inder TE, Darras BT, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 10. Philadelphia, PA: Elsevier; 2018:222–254.
162. Tolsa CB, Zimine S, Warfield SK, et al. Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. Pediatr Res. 2004;56:132–138.
163. Dubois J, Benders M, Borradori-Tolsa C, et al. Primary cortical folding in the human newborn: an early marker of later functional development. Brain. 2008;131:2028–2041.
164. Lodygensky GA, Seghier ML, Warfield SK, et al. Intrauterine growth restriction affects the preterm infant's hippocampus. Pediatr Res. 2008;63:438–443.
165. Padilla N, Falcon C, Sanz-Cortes M, et al. Differential effects of intrauterine growth restriction on brain structure and development in preterm infants: a magnetic resonance imaging study. Brain Res. 2011;1382:98–108.
166. Xydis V, Drougia A, Giapros V, et al. Brain growth in preterm infants is affected by the degree of growth restriction at birth. J Matern Fetal Neonatal Med. 2013;26:673–679.
167. Eikenes L, Martinussen MP, Lund LK, et al. Being born small for gestational age reduces white matter integrity in adulthood: a prospective cohort study. Pediatr Res. 2012;72:649–654.
168. Dean JM, McClendon E, Hansen K, et al. Prenatal cerebral ischemia disrupts MRI-defined cortical microstructure through disturbances in neuronal arborization. Sci Transl Med. 2013;5:168ra7.
169. McClendon E, Chen K, Gong X, et al. Prenatal cerebral ischemia triggers dysmaturation of caudate projection neurons. Ann Neurol. 2014;75:508–524.
170. McClendon E, Shaver DC, Degener-O'Brien K, et al. Transient hypoxemia chronically disrupts maturation of preterm fetal ovine subplate neuron arborization and activitym. J Neurosci. 2017;37:11912–11929.
171. Inder TE, Volpe JJ. Pathophysiology: General Principles. In: Volpe JJ, Inder TE, Darras BT, et al., eds. Volpe's Neurology of the Newborn. 6th ed. Chapter 13. Philadelphia, PA: Elsevier; 2018:325–388.
172. Rangarajan V, Juul SE. Erythropoietin: emerging role of erythropoietin in neonatal neuroprotection. Pediatr Neurol. 2014;51:481–488.
173. Rees S, Hale N, De Matteo R, et al. Erythropoietin is neuroprotective in a preterm ovine model of endotoxin-induced brain injury. J Neuropathol Exp Neurol. 2010;69:306–319.
174. Mazur M, Miller RH, Robinson S. Postnatal erythropoietin treatment mitigates neural cell loss after systemic prenatal hypoxic-ischemic injury. J Neurosurg Pediatr. 2010;6:206–221.
175. Fischer HS, Reibel NJ, Buhrer C, et al. Prophylactic early erythropoietin for neuroprotection in preterm infants: A meta-analysis. Pediatrics. 2017;139:e20164317.
176. Natalucci G, Latal B, Koller B, et al. Effect of early prophylactic high-dose recombinant human erythropoietin in very preterm enfants on neurodevelopmental outcome at 2 years: a randomized clinical trial. JAMA. 2016;315:2079–2085.
177. Leuchter RH, Gui L, Poncet A, et al. Association between early administration of high-dose erythropoietin in preterm infants and brain MRI abnormality at term-equivalent age. JAMA. 2014;312:817–824.
178. O'Gorman RL, Bucher HU, Held U, et al. Tract-based spatial statistics to assess the neuroprotective effect of early erythropoietin on white matter development in preterm infants. Brain. 2015;138:388–397.
179. Ohls RK, Cannon DC, Phillips J, et al. Preschool assessment of preterm infants treated with darbepoetin and erythropoietin. Pediatrics. 2016;137:1–9.
180. Song J, Sun H, Xu F, et al. Recombinant human erythropoietin improves neurological outcomes in very preterm infants. Ann Neurol. 2016;80:24–34.
181. Scafidi J, Hammond TR, Scafidi S, et al. Intranasal epidermal growth factor treatment rescues neonatal brain injury. Nature. 2014;506:230–234.
182. Cao Y, Gunn AJ, Bennet L, et al. Insulin-like growth factor (IGF)-1 suppresses oligodendrocyte caspase-3 activation and increases glial proliferation after ischemia in near-term fetal sheep. J Cereb Blood Flow Metab. 2003;23:739–747.
183. Brywe KG, Mallard C, Gustavsson M, et al. IGF-I neuroprotection in the immature brain after hypoxia-ischemia, involvement of Akt and GSK3beta? Eur J Neurosci. 2005;21:1489–1502.
184. Wood TL, Loladze V, Altieri S, et al. Delayed IGF-1 administration rescues oligodendrocyte progenitors from glutamate-induced cell death and hypoxic-ischemic brain damage. Dev Neurosci. 2007;29:302–310.
185. Zhong J, Zhao L, Du Y, et al. Delayed IGF-1 treatment reduced long-term hypoxia-ischemia-induced brain damage and improved behavior recovery of immature rats. Neurol Res. 2009;31:483–489.
186. Pang Y, Zheng B, Campbell LR, et al. IGF-1 can either protect against or increase LPS-induced damage in the developing rat brain. Pediatr Res. 2010;67:579–584.
187. Preston M, Gong X, Su W, et al. Digestion products of the PH20 hyaluronidase inhibit remyelination. Ann Neurol. 2013;73:266–280.
188. Favrais G, van de Looij Y, Fleiss B, et al. Systemic inflammation disrupts the developmental program of the white matter. Ann Neurol. 2011;70:550–565.
189. Miron VE, Boyd A, Zhao JW, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci. 2013;16:1211–1218.
190. Krishnan ML, Van Steenwinckel J, Schang AL, et al. Integrative genomics of microglia implicates DLG4 (PSD95) in the white matter development of preterm infants. Nat Commun. 2017;8:428.

191. Michell-Robinson MA, Touil H, Healy LM, et al. Roles of microglia in brain development, tissue maintenance and repair. Brain. 2015;138:1138–1159.
192. Biran V, Phan Duy A, Decobert F, et al. Is melatonin ready to be used in preterm infants as a neuroprotectant? Dev Med Child Neurol. 2014;56:717–723.
193. Ksiazek-Winiarek D, Szpakowski P, Turniak M, et al. IL-17 exerts anti-apoptotic affect via miR-155-5p downregulation in experimental autoimmune encephalomyelitis. J Mol Neurosci. 2017;63:320–332.
194. Yang Y, Ye Y, Kong C, et al. MiR-124 Enriched exosomes promoted the M2 polarization of microglia and enhanced hippocampus neurogenesis after traumatic brain injury by inhibiting TLR4 pathway. Neurochem Res. 2019;44:811–828.
195. Titomanlio L, Kavelaars A, Dalous J, et al. Stem cell therapy for neonatal brain injury: perspectives and challenges. Ann Neurol. 2011;70:698–712.
196. van Velthoven CT, Sheldon RA, Kavelaars A, et al. Mesenchymal stem cell transplantation attenuates brain injury after neonatal stroke. Stroke. 2013;44:1426–1432.
197. Fleiss B, Guillot PV, Titomanlio L, et al. Stem cell therapy for neonatal brain injury. Clin Perinatol. 2014;41:133–148.
198. van Velthoven CT, Gonzalez F, Vexler ZS, et al. Stem cells for neonatal stroke-the future is here. Front Cell Neurosci. 2014;8:1–3.
199. Wei ZZ, Gu X, Ferdinand A, et al. Intranasal delivery of bone marrow mesenchymal stem cells improved neurovascular regeneration and rescued neuropsychiatric deficits after neonatal stroke in rats. Cell Transpl. 2015;24:391–402.
200. Li J, Yawno T, Sutherland A, et al. Term vs. preterm cord blood cells for the prevention of preterm brain injury. Pediatr Res. 2017;82:1030–1038.
201. Nagoshi N, Khazaei M, Ahlfors JE, et al. Human spinal oligodendrogenic neural progenitor cells promote functional recovery after spinal cord injury by axonal remyelination and tissue sparing. Stem Cells Transl Med. 2018;7:806–818.
202. Li YH, Feng L, Zhang GX, et al. Intranasal delivery of stem cells as therapy for central nervous system disease. Exp Mol Pathol. 2015;98:145–151.
203. Oppliger B, Joerger-Messerli M, Mueller M, et al. Intranasal delivery of umbilical cord-derived mesenchymal stem cells preserves myelination in perinatal brain damage. Stem Cells Dev. 2016;25:1234–1242.
204. Chen LX, Ma SM, Zhang P, et al. Neuroprotective effects of oligodendrocyte progenitor cell transplantation in premature rat brain following hypoxic-ischemic injury. PLoS One. 2015;10:e0115997.
205. Osorio MJ, Rowitch DH, Tesar P, et al. Concise review: Stem cell-based treatment of Pelizaeus-Merzbacher disease. Stem Cells. 2017;35:311–315.
206. Drommelschmidt K, Serdar M, Bendix I, et al. Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury. Brain Behav Immun. 2017;60:220–232.
207. Holm MM, Kaiser JR, Schwab ME. Extracellular vesicles: Multimodal envoys in neural maintenance and repair. Trends Neurosci. 2018;41:360–372.
208. Otero-Ortega L, Gomez de Frutos MC, Laso-Garcia F, et al. Exosomes promote restoration after an experimental animal model of intracerebral hemorrhage. J Cereb Blood Flow Metab. 2018;38:767–779.
209. Williams A, Dennahy IS, Bhatti UF, et al. Mesenchymal stem cell-derived exosomes provide neuroprotection and improve long-term neurologic outcomes in a swine model of traumatic brain injury and hemorrhagic shock. J Neurotrauma. 2019;36:54–60.
210. Zhao L, Jiang X, Shi J, et al. Exosomes derived from bone marrow mesenchymal stem cells overexpressing microRNA-25 protect spinal cords against transient ischemia. J Thorac Cardiovasc Surg. 2019;157:508–517.
211. Menjoge AR, Kannan RM, Tomalia DA. Dendrimer-based drug and imaging conjugates: design considerations for nanomedical applications. Drug Discov Today. 2010;15:171–185.
212. Kannan RM, Nance E, Kannan S, et al. Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications. J Intern Med. 2014;276:579–617.
213. Kannan S, Dai H, Navath RS, et al. Dendrimer-based postnatal therapy for neuroinflammation and cerebral palsy in a rabbit model. Sci Transl Med. 2012;4:130ra146.
214. Kim ID, Shin JH, Kim SW, et al. Intranasal delivery of HMGB1 siRNA confers target gene knockdown and robust neuroprotection in the postischemic brain. Mol Ther. 2012;20:829–839.
215. Nance E, Porambo M, Zhang F, et al. Systemic dendrimer-drug treatment of ischemia-induced neonatal white matter injury. J Control Release. 2015;214:112–120.
216. Nemeth CL, Drummond GT, Mishra MK, et al. Uptake of dendrimer-drug by different cell types in the hippocampus after hypoxic-ischemic insult in neonatal mice: Effects of injury, microglial activation and hypothermia. Nanomedicine. 2017;13:2359–2369.
217. Lei J, Rosenzweig JM, Mishra MK, et al. Maternal dendrimer-based therapy for inflammation-induced preterm birth and perinatal brain injury. Sci Rep. 2017;7:6106.
218. Weisglas-Kuperus N, Heersema DJ, Baerts W, et al. Visual functions in relation with neonatal cerebral ultrasound, neurology and cognitive development in very-low-birthweight children. Neuropediatrics. 1993;24:149–154.
219. Iwai M, Stetler RA, Xing J, et al. Enhanced oligodendrogenesis and recovery of neurological function by erythropoietin after neonatal hypoxic/ischemic brain injury. Stroke. 2010;41:1032–1037.
220. Kato S, Aoyama M, Kakita H, et al. Endogenous erythropoietin from astrocyte protects the oligodendrocyte precursor cell against hypoxic and reoxygenation injury. J Neurosci Res. 2011;89:1566–1574.
221. Jantzie LL, Miller RH, Robinson S. Erythropoietin signaling promotes oligodendrocyte development following prenatal systemic hypoxic-ischemic brain injury. Pediatr Res. 2013;74:658–667.
222. Liu W, Shen Y, Plane JM, et al. Neuroprotective potential of erythropoietin and its derivative carbamylated erythropoietin in periventricular leukomalacia. Exp Neurol. 2011;230:227–239.
223. Jantzie LL, Corbett CJ, Firl DJ, et al. Postnatal erythropoietin mitigates impaired cerebral cortical development following subplate loss from prenatal hypoxia-ischemia. Cereb Cortex. 2015;25:2683–2695.
224. Hayakawa M, Okumura A, Hayakawa F, et al. Nutritional state and growth and functional maturation of the brain in extremely low birth weight infants. Pediatrics. 2003;111:991–995.
225. Latal-Hajnal B, Von Siebenthal K, Kovari H, et al. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J Pediatr. 2003;143:163–170.
226. Ehrenkranz RA, Dusick AM, Vohr BR, et al. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics. 2006;117:1253–1261.
227. Lira PIC, Eickmann SE, Lima MC, et al. Early head growth: relation with IQ at 8 years and determinants in term infants of low and appropriate birthweight. Dev Med Child Neurol. 2010;52:40–46.
228. Belfort MB, Rifas-Shiman SL, Sullivan T, et al. Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics. 2011;128:e899–e906.
229. Neubauer V, Griesmaier E, Pehbock-Walser N, et al. Poor postnatal head growth in very preterm infants is associated with impaired neurodevelopment outcome. Acta Paediatr. 2013;102:883–888.
230. Belfort MB, Gillman MW, Buka SL, et al. Preterm infant linear growth and adiposity gain: trade-offs for later weight status and intelligence quotient. J Pediatr. 2013;163:1564–1569.e2.
231. Pyhala R, Hovi P, Lahti M, et al. Very low birth weight, infant growth, and autism-spectrum traits in adulthood. Pediatrics. 2014;134:1075–1083.
232. Leppanen M, Lapinleimu H, Lind A, et al. Antenatal and postnatal growth and 5-year cognitive outcome in very preterm infants. Pediatrics. 2014;133:63–70.
233. Frondas-Chauty A, Simon L, Branger B, et al. Early growth and neurodevelopmental outcome in very preterm infants: impact of gender. Arch Dis Child Fetal Neonatal Ed. 2014;99:F366–F372.
234. Sammallahti S, Pyhala R, Lahti M, et al. Infant growth after preterm birth and neurocognitive abilities in young adulthood. J Pediatr. 2014;165:1109–1115.
235. Elitt CM, Rosenberg PA. The challenge of understanding cerebral white matter injury in the premature infant. Neuroscience. 2014;276:216–238.
236. Belfort MB, Anderson PJ, Nowak VA, et al. Breast milk feeding, brain development, and neurocognitive outcomes: A 7-year longitudinal study in infants born at less than 30 weeks' gestation. J Pediatr. 2016;177:133–139.e131.
237. Cusick SE, Georgieff MK. The role of nutrition in brain development: the golden opportunity of the “first 1000 days”. J Pediatr. 2016;175:16–21.
238. Belfort MB, Ehrenkranz RA. Neurodevelopmental outcomes and nutritional strategies in very low birth weight infants. Semin Fetal Neonatal Med. 2017;22:42–48.
239. Horbar JD, Ehrenkranz RA, Badger GJ, et al. Weight growth velocity and postnatal growth failure in infants 501 to 1500 grams: 2000-2013. Pediatrics. 2015;136:e84–e92.
240. Griffin IJ, Tancredi DJ, Bertino E, et al. Postnatal growth failure in very low birthweight infants born between 2005 and 2012. Arch Dis Child Fetal Neonatal Ed. 2016;101:F50–F55.
241. Schneider J, Fischer Fumeaux CJ, Duerden EG, et al. Nutrient intake in the first two weeks of life and brain growth in preterm neonates. Pediatrics. 2018;141:e20172169.
242. Coviello C, Keunen K, Kersbergen KJ, et al. Effects of early nutrition and growth on brain volumes, white matter microstructure, and neurodevelopmental outcome in preterm newborns. Pediatr Res. 2018;83:102–110.
243. Tanaka K, Kon N, Ohkawa N, et al. Does breastfeeding in the neonatal period influence the cognitive function of very-low-birth-weight infants at 5 years of age? Brain Dev. 2009;31:288–293.
244. Isaacs EB, Fischl BR, Quinn BT, et al. Impact of breast milk on intelligence quotient, brain size, and white matter development. Pediatr Res. 2010;67:357–362.
245. Quigley MA, Hockley C, Carson C, et al. Breastfeeding is associated with improved child cognitive development: a population-based cohort study. J Pediatr. 2011;160:25–32.
246. Bernard JY, De Agostini M, Forhan A, et al. Breastfeeding duration and cognitive development at 2 and 3 years of age in the EDEN mother-child cohort. J Pediatr. 2013;163:36–42.e31.
247. Deoni SC, Dean 3rd DC, Piryatinsky I, et al. Breastfeeding and early white matter development: A cross-sectional study. Neuroimage. 2013;82:77–86.
248. Ou X, Andres A, Cleves MA, et al. Sex-specific association between infant diet and white matter integrity in 8-y-old children. Pediatr Res. 2014;76:535–543.

249. Gibertoni D, Corvaglia L, Vandini S, et al. Positive effect of human milk feeding during NICU hospitalization on 24 month neurodevelopment of very low birth weight infants: An Italian cohort study. PLoS One. 2015;10:e0116552.
250. Grossmann T, Johnson MH. The development of the social brain in human infancy. Eur J Neurosci. 2007;25:909–919.
251. Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr. 2003;143:S1–S58.
252. Xiao Y, Huang Y, Chen ZY. Distribution, depletion and recovery of docosahexaenoic acid are region-specific in the rat brain. Br J Nutr. 2005;94:544–550.
253. Innis SM. Fatty acids and early human development. Early Hum Dev. 2007;83:761–766.
254. Kamino D, Studholme C, Liu M, et al. Postnatal polyunsaturated fatty acids associated with larger preterm brain tissue volumes and better outcomes. Pediatr Res. 2018;83:93–101.
255. Tam EW, Chau V, Barkovich AJ, et al. Early postnatal docosahexaenoic acid levels and improved preterm brain development. Pediatr Res. 2016;79:723–730.
256. Makrides M, Gibson RA, McPhee AJ, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. JAMA. 2009;301:175–182.
257. Jensen CL, Voigt RG, Llorente AM, et al. Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. J Pediatr. 2010;157:900–905.
258. Friel JK, Aziz K, Andrews WL, et al. A double-masked randomized control trial of iron supplementation in early infancy in healthy term breast-fed infants. J Pediatr. 2003;143:582–586.
259. Lozoff B, De Andraca I, Castillo M, et al. Behavioral and developmental effects of preventing iron-deficiency anemia in health full-term infants. Pediarics. 2003;112:846–854.
260. Kon N, Tanaka K, Sekigawa M, et al. Association between iron status and neurodevelopmental outcomes among VLBW infants. Brain Dev. 2010;32:849–854.
261. Berglund SK, Westrup B, Hagglof B, et al. Effects of iron supplementation of LBW infants on cognition and behavior at 3 years. Pediatrics. 2013;131:47–55.
262. Berglund SK, Chmielewska A, Starnberg J, et al. Effects of iron supplementation of low-birth-weight infants on cognition and behavior at 7 years: a randomized controlled trial. Pediatr Res. 2018;83:111–118.
263. Yager JY, Hartfield DS. Neurologic manifestations of iron deficiency in childhood. Pediatr Neurol. 2002;27:85–92.
264. Gordon N. Iron deficiency and the intellect. Brain Development. 2003;25:3–8.
265. Georgieff MK, Innis SM. Controversial nutrients that potentially affect preterm neurodevelopment: essential fatty acids and iron. Pediatr Res. 2005;57:99R–103R.
266. Roncagliolo M, Garrido M, Walter T, et al. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. Am J Clin Nutr. 1998;68:683–690.
267. Sarici SU, Serdar MA, Dundaroz MR, et al. Brainstem auditory-evoked potentials in iron-deficiency anemia. Pediatr Neurol. 2001;24:205–208.
268. Algarin C, Peirano P, Garrido M, et al. Iron deficiency anemia in infancy: long-lasting effects on auditory and visual system functioning. Pediatr Res. 2003;53:217–223.
269. Amin SB, Orlando M, Wang H. Latent iron deficiency in utero is associated with abnormal auditory neural myelination in ≥35 weeks gestational age infants. J Pediatr. 2013;163:1267–1271.
270. Mercer JS, Erickson-Owens DA, Deoni SCL, et al. Effects of delayed cord clamping on 4-month ferritin levels, brain myelin content, and neurodevelopment: A randomized controlled trial. J Pediatr. 2018;203:266–272.
271. Rao R, Bora R. Timing of umbilical cord clamping and infant brain development. J Pediatr. 2018;203:8–10.
272. Connor JR, Benkovic SA. Iron regulation in the brain: histochemical, biochemical, and molecular considerations. Ann Neurol. 1992;32:S51–S61.
273. de Vellis J, Carpenter E. Development. In: Siegel GJ, Albers RW, Brady ST, et al., eds. Basic Neurochemistry, Molecular, Cellular, and Medical Aspects. 7 ed. London: Elsevier; 2006:437–458.
274. Rao R, Tkac I, Unger EL, et al. Iron supplementation dose for perinatal iron deficiency differentially alters the neurochemistry of the frontal cortex and hippocampus in adult rats. Pediatr Res. 2013;73:31–37.
275. Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. Nat Rev Neurosci. 2005;6:449–462.
276. Li Y, Maret W. Transient fluctuations of intracellular zinc ions in cell proliferation. Exp Cell Res. 2009;315:2463–2470.
277. Fukada T, Yamasaki S, Nishida K, et al. Zinc homeostasis and signaling in health and diseases: Zinc signaling. J Biol Inorg Chem. 2011;16:1123–1134.
278. Maret W. Zinc in cellular regulation: The nature and significance of “zinc signals”. Int J Mol Sci. 2017;18:2285.
279. Kuspert M, Wegner M. SomethiNG 2 talk about-Transcriptional regulation in embryonic and adult oligodendrocyte precursors. Brain Res. 2016;1638:167–182.
280. Wang SZ, Dulin J, Wu H, et al. An oligodendrocyte-specific zinc-finger transcription regulator cooperates with Olig2 to promote oligodendrocyte differentiation. Development. 2006;133:3389–3398.
281. Bourassa D, Elitt CM, McCallum AM, et al. Chromis-1, a ratiometric fluorescent probe optimized for two-photon microscopy reveals dynamic changes in labile Zn(II) in differentiating oligodendrocytes. ACS Sens. 2018;3:458–467.
282. Zhang Y, Aizenman E, DeFranco DB, et al. Intracellular zinc release, 12-lipoxygenase activation and MAPK dependent neuronal and oligodendroglial death. Mol Med. 2007;13:350–355.
283. Domercq M, Mato S, Soria FN, et al. Zn2+ -induced ERK activation mediates PARP-1-dependent ischemic-reoxygenation damage to oligodendrocytes. Glia. 2013;61:383–393.
284. Mato S, Sanchez-Gomez MV, Bernal-Chico A, et al. Cytosolic zinc accumulation contributes to excitotoxic oligodendroglial death. Glia. 2013;61:750–764.
285. Krebs NF, Miller LV, Hambidge KM. Zinc deficiency in infants and children: a review of its complex and synergistic interactions. Paediatr Int Child Health. 2014;34:279–288.
286. Terrin G, Berni Canani R, Di Chiara M, et al. Zinc in early life: A key element in the fetus and preterm neonate. Nutrients. 2015;7:10427–10446.
287. Ackland ML, Michalczyk AA. Zinc and infant nutrition. Arch Biochem Biophys. 2016;611:51–57.
288. Griffin IJ, Domellof M, Bhatia J, et al. Zinc and copper requirements in preterm infants: an examination of the current literature. Early Hum Dev. 2013;89:S29–S34.
289. American Academy of Pediatrics CoEH. Noise: a hazard for the fetus and newborn. American Academy of Pediatrics. Committee on Environmental Health. Pediatrics. 1997;100:724–727.
290. Etzel RA, Balk SJ, Bearer CF, et al. Noise: A hazard for the fetus and newborn. Pediatrics. 1997;100:724–727.
291. Roizen NJ. Nongenetic causes of hearing loss. Ment Retard Dev Disabil Res Rev. 2003;9:120–127.
292. Zimmerman E, Lahav A. Ototoxicity in preterm infants: effects of genetics, aminoglycosides, and loud environmental noise. J Perinatol. 2013;33:3–8.
293. Kazemizadeh Gol MA, Black A, Sidman J. Bone conduction noise exposure via ventilators in the neonatal intensive care unit. Laryngoscope. 2015;125:2388–2392.
294. Pineda RG, Neil J, Dierker D, et al. Alterations in brain structure and neurodevelopmental outcome in preterm infants hospitalized in different neonatal intensive care unit environments. J Pediatr. 2014;164:52–60.
295. Lester BM, Salisbury AL, Hawes K, et al. 18-month follow-up of infants cared for in a single-family room neonatal intensive care unit. J Pediatr. 2016;177:84–89.
296. Weitzman L, Graziani L, Duhamel L. Maturation and topography of the auditory evoked response of the prematurely born infant. Electroencephalogr Clin Neurophysiol. 1967;23:82–83.
297. Starr A, Amlie RN, Martin WH, et al. Development of auditory function in newborn infants revealed by auditory brainstem potentials. Pediatrics. 1977;60:831–839.
298. Kwon SH, Scheinost D, Vohr B, et al. Functional magnetic resonance connectivity studies in infants born preterm: suggestions of proximate and long-lasting changes in language organization. Dev Med Child Neurol. 2016;58 Suppl 4:28–34.
299. Lordier L, Loukas S, Grouiller F, et al. Music processing in preterm and full-term newborns: A psychophysiological interaction (PPI) approach in neonatal fMRI. Neuroimage. 2019;185:857–864.
300. Bourgeois JP, Jastreboff PJ, Rakic P. Synaptogenesis in visual cortex of normal and preterm monkeys: evidence for intrinsic regulation of synaptic overproduction. PNAS (USA). 1989;86:4297–4301.
301. Tsuneishi S, Casaer P. Effects of preterm extrauterine visual experience on the development of the human visual system: a flash VEP study. Dev Med Child Neurol. 2000;42:663–668.
302. Schwindt E, Giordano V, Rona Z, et al. The impact of extrauterine life on visual maturation in extremely preterm born infants. Pediatr Res. 2018;84:403–410.
303. Whitfield MF, Grunau RE. Behavior, pain perception, and the extremely low-birth weight survivor. Clin Perinatol. 2000;27:363–379.
304. Grunau R. Early pain in preterm infants. A model of long-term effects. Clin Perinatol. 2002;29:373–394. vii-viii.
305. Anand KJ, Aranda JV, Berde CB, et al. Analgesia and anesthesia for neonates: study design and ethical issues. Clin Ther. 2005;27:814–843.
306. Doesburg SM, Chau CM, Cheung TP, et al. Neonatal pain-related stress, functional cortical activity and visual-perceptual abilities in school-age children born at extremely low gestational age. Pain. 2013;154:1946–1952.
307. Duerden EG, Grunau RE, Guo T, et al. Early procedural pain is associated with regionally-specific alterations in thalamic development in preterm neonates. J Neurosci. 2018;38:878–886.
308. Schneider J, Duerden EG, Guo T, et al. Procedural pain and oral glucose in preterm neonates: brain development and sex-specific effects. Pain. 2018;159:515–525.
309. Smith GC, Gutovich J, Smyser C, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. Ann Neurol. 2011;70:541–549.
310. Brummelte S, Grunau RE, Chau V, et al. Procedural pain and brain development in premature newborns. Ann Neurol. 2012;71:385–396.
311. Ranger M, Chau CM, Garg A, et al. Neonatal pain-related stress predicts cortical thickness at age 7 years in children born very preterm. PLoS One. 2013;8:e76702.

312. Milgrom J, Newnham C, Anderson PJ, et al. Early sensitivity training for parents of preterm infants: impact on the developing brain. Pediatr Res. 2010;67:330–335.
313. Committee on Fetus and Newborn and Section on Anesthesiology and Pain Medicine. Prevention and management of procedural pain in the neonate: an update. Pediatrics. 2016;137:e20154271.
314. Als H, Lawhon G, Brown E. Individualized behavioral and environmental care for very low birth weight preterm infants at high risk for bronchopulmonary dysplasia: neonatal intensive care unit and developmental outcome. Pediatrics. 1986;78:1123–1132.
315. Als H, Duffy FH, McAnulty GB, et al. Early experience alters brain function and structure. Pediatrics. 2004;113:846–857.
316. McAnulty GB, Duffy FH, Butler SC, et al. Effects of the newborn individualized developmental care and assessment program (NIDCAP) at age 8 years: preliminary data. Clin Pediatr (phila). 2010;49:258–270.
317. Symington A, Pinelli J. Developmental care for promoting development and preventing morbidity in preterm infants. Cochrane Database Syst Rev. 2006:CD001814.
318. Lubbe W, Van der Walt CS, Klopper HC. Integrative literature review defining evidence-based neurodevelopmental supportive care of the preterm infant. J Perinat Neonatal Nurs. 2012;26:251–259.
319. Pineda R, Guth R, Herring A, et al. Enhancing sensory experiences for very preterm infants in the NICU: an integrative review. J Perinatol. 2017;37:323–332.
320. Wolf SA, Boddeke HW, Kettenmann H. Microglia in physiology and disease. Annu Rev Physiol. 2017;79:619–643.
321. Gibbs BG, Forste R. Breastfeeding, parenting, and early cognitive development. J Pediatr. 2014;164:487–493.
322. Brown HK, Speechley KN, Macnab J, et al. Mild prematurity, proximal social processes, and development. Pediatrics. 2014;134:e814–e824.
323. Hair NL, Hanson JL, Wolfe BL, et al. Association of child poverty, brain development, and academic achievement. JAMA Pediatr. 2015;169:822–829.
324. Doyle LW, Cheong JL, Burnett A, et al. Biological and social influences on outcomes of extreme-preterm/low-birth weight adolescents. Pediatrics. 2015;136:e1513–e1520.
325. Linsell L, Malouf R, Morris J, et al. Prognostic factors for poor cognitive development in children born very preterm or with very low birth weight: A systematic review. JAMA Pediatr. 2015;169:1162–1172.
326. Parker MG, Rybin DV, Heeren TC, et al. Postdischarge feeding interactions and neurodevelopmental outcome at 1-year corrected gestational age. J Pediatr. 2016;174:104–110.
327. Treyvaud K, Doyle LW, Lee KJ, et al. Parenting behavior at 2 years predicts school-age performance at 7 years in very preterm children. J Child Psychol Psychiatr. 2016;57:814–821.
328. Leijser LM, Siddiqi A, Miller SP. Imaging evidence of the effect of socioeconomic status on brain structure and development. Semin Pediatr Neurol. 2018;27:26–34.
329. Lean RE, Paul RA, Smyser TA, et al. Social adversity and cognitive, language, and motor development of very preterm children from 2 to 5 years of age. J Pediatr. 2018;203:177–184.e171.
330. Betancourt LM, Avants B, Farah MJ, et al. Effect of socioeconomic status (SES) disparity on neural development in female African-American infants at age 1 month. Dev Sci. 2016;19:947–956.
331. Gao W, Alcauter S, Elton A, et al. Functional network development during the first year: relative sequence and socioeconomic correlations. Cereb Cortex. 2015;25:2919–2928.
332. Eluvathingal TJ, Chugani HT, Behen ME, et al. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. Pediatrics. 2006;117:2093–2100.
333. Behen ME, Muzik O, Saporta AS, et al. Abnormal fronto-striatal connectivity in children with histories of early deprivation: A diffusion tensor imaging study. Brain Imaging Behav. 2009;3:292–297.
334. Govindan RM, Behen ME, Helder E, et al. Altered water diffusivity in cortical association tracts in children with early deprivation identified with Tract-Based Spatial Statistics (TBSS). Cereb Cortex. 2010;20:561–569.
335. Sheridan MA, Fox NA, Zeanah CH, et al. Variation in neural development as a result of exposure to institutionalization early in childhood. Proc Natl Acad Sci U S A. 2012;109:12927–12932.
336. Hanson JL, Adluru N, Chung MK, et al. Early neglect is associated with alterations in white matter integrity and cognitive functioning. Child Dev. 2013;84:1566–1578.
337. Kumar A, Behen ME, Singsoonsud P, et al. Microstructural abnormalities in language and limbic pathways in orphanage-reared children: a diffusion tensor imaging study. J Child Neurol. 2014;29:318–325.
338. Bick J, Zhu T, Stamoulis C, et al. Effect of early institutionalization and foster care on long-term white matter development: A randomized clinical trial. JAMA Pediatr. 2015:E1–E9.
339. Steele CJ, Zatorre RJ. Practice makes plasticity. Nat Neurosci. 2018;21:1645–1646.
340. Heckman J, Pinto R, Savelyev P. Understanding the mechanisms through which an influential early childhood program boosted adult outcomes. Am Econ Rev. 2013;103:2052–2086.
341. Campbell F, Conti G, Heckman JJ, et al. Early childhood investments substantially boost adult health. Science. 2014;343:1478–1485.