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What brakes the preterm brain? An arresting story

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Children surviving premature birth have a high risk of cognitive and learning disabilities and attention deficit. In turn, adverse outcomes are associated with persistent reductions in cerebral growth on magnetic resonance imaging (MRI). It is striking that modern care has been associated with a dramatic reduction in the risk of cystic white matter damage, but modest improvements in terms of neurodevelopmental impairment. This review will explore the hypothesis that the disability is primarily associated with impaired neural connectivity rather than cell death alone. Very preterm infants exhibit reduced thalamocortical connectivity and cortical neuroplasticity compared with term-born controls. In preterm fetal sheep, moderate cerebral ischemia with no neuronal loss, but significant diffuse failure of maturation of cortical pyramidal neurons, was associated with impaired dendritic growth and synapse formation, consistent with altered connectivity. These changes were associated with delayed decline in cortical fractional anisotropy (FA) on MRI. Supporting these preclinical findings, preterm human survivors showed similar enduring impairment of microstructural development of the cerebral cortex defined by FA, consistent with delayed formation of neuronal processes. These findings offer the promise that better understanding of impairment of neural connectivity may allow us to promote normal development and growth of the cortex after preterm birth.

Premature birth is one of the leading causes of morbidity and mortality. Approximately 6–13% of all births are preterm (1), and in the United States, this has been estimated to cost the community more than $26.2 billion in 2005 alone (1). Most of this cost is related not to acute care but to cerebral palsy and long-term neurodevelopmental disability in surviving very premature (≤30 wk gestation) infants. Disability is highly associated with greater prematurity (2,3), but even in "late preterm" infants at 34–36 wk gestation, the risks of injury and disability are increased sevenfold or more compared with term infants (4). Although there is evidence for modest overall improvements in survival without disability in recent cohorts (2,3), others found no apparent improvement in disability after extremely preterm (<25 wk gestation) birth (5).

Historically, preterm human autopsy cases typically showed severe necrotic white matter injury (WMI) with evidence of axonal degeneration and loss of cortical neurons (6,7). This necrosis in turn was associated with cerebral palsy. The greater risk of long-term disability in boys is correlated with greater risk of WMI (8). Encouragingly, there is now evidence of a progressive reduction in the severest form of cystic WMI over time (9). In modern cohorts, severe WMI is seen in only ~1% of cases, whereas less severe (nonnecrotic), diffuse WMI is now common (9,10).

It is striking that despite this apparent marked reduction in the severity of WMI, that even less severe forms of WMI injury are associated with impaired brain development and disability (10). Preterm survivors have a high risk of neurobehavioral disturbances and intellectual disabilities related to learning, cognition, visuospatial integration, attention deficit, and socialization (11–13). Given that the brain is still growing and maturing at this stage of life, we might predict that preterm infants would show a greater ability to return to normal (14). In marked contrast with this postulate, long-term follow-up up to 16 y of age shows that preterm infants without apparent neonatal brain injury show reduced verbal and performance intelligence quotient levels (11). Furthermore, modern imaging shows that preterm birth is associated with perturbations of the trajectory of cerebral development at least up to adolescence, with reduced thinning of the gray matter and reduced white matter growth (15), and evidence of long-lasting changes in connectivity (16).

These persistently adverse outcomes in the setting of diffuse WMI suggests the hypothesis that primary disturbances in neuronal processing may occur in multiple cortical and subcortical gray matter structures, in addition to disturbances in connectivity related to cerebral WMI. In the present review, we will critically dissect the underlying neuropathology of preterm brain injury, including the importance of acute white and gray matter damage in a subset of infants, the association of hypoxia–ischemia and infection/inflammation with adverse outcomes, the timing of injury, and the impact on the ability of the preterm brain to continue to develop after injury.

TIMING AND ETIOLOGY OF PRETERM BRAIN INJURY

In historical cohorts, the major lesions associated with premature birth were cystic periventricular leukomalacia and unilateral periventricular–intraventricular hemorrhage. These severe lesions damaged the descending white matter tracts and were associated with chorioamnionitis, prolonged rupture of membranes, asphyxia, sepsis, hypocarbia, and in turn with the diplegic form of cerebral palsy (6). Both lesions have become much less common in modern cohorts. Periventricular–intraventricular

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hemorrhage in particular may be reduced by maternal glucocorticoid therapy (17).

Early imaging and postmortem data suggest that cerebral injury occurs in the immediate perinatal period in approximately two-thirds of cases, and an appreciable number of cases occur before the onset of labor; in contrast, injury after the early neonatal period represents only ~10% of cases (18,19). Strongly consistent with this, acute electroencephalogram (EEG) abnormalities are reported in the early perinatal period in the majority of infants and were highly predictive of long-term outcome (20). The precise etiology of injury remains surprisingly unclear; however, it is likely to involve both hypoxia–ischemia and infection/inflammation (6).

Acute, profound asphyxia occurs in a small minority of premature births (21) and so cannot possibly account for the overall burden of disability after preterm birth. Nevertheless, there is some evidence for a role of subtle insults, as shown, e.g., by depressed Apgar scores at birth (22). More recently, the Extremely Low Gestational Age Newborns (ELGAN) study reported that severe intrauterine growth restriction and evidence of placental vascular thrombosis consistent with long-standing, prenatal hypoxemia were associated with impaired neurodevelopmental outcome at 2 y of age (23). After birth, although low blood pressure is common, the evidence for an association between hypotension and adverse outcomes is limited, as reviewed in ref. (24). The ELGAN study, for example, suggests that neither vasopressor treatment nor blood pressure in the lowest quartile for gestational age were independently associated with neurodevelopmental outcome (25).

There is increasing evidence that exposure to *in utero* infection at critical stages of brain development can significantly increase the risk of neurodevelopment abnormalities. As with asphyxia, although overt severe infection is strongly linked with neural injury, subclinical infection is more common and also highly associated with adverse outcomes (26). Clinical studies support a role for both prenatal and postnatal infection (27,28). For example, increased cord blood levels of tumor necrosis factor-α have been associated with depression of the EEG in the first few days of life, WMI on cranial ultrasound, and risk of handicap at 2 y of age (27). In the ELGAN study, although placental bacteria were not associated with neurodevelopmental outcome at 2 y of age (23), necrotizing enterocolitis, or bacteremia combined with ventilation on d 14 after birth were strongly associated with impaired development (29). Strikingly, the combination of intrauterine growth retardation with increased inflammatory markers in the first 2 wk of life was associated with greater increase in risk of severe neurodevelopmental impairment compared with either alone (30). These findings support that both hypoxia–ischemia and infection/inflammation impair brain development and may interact to increase damage.

THE PATTERN OF PRETERM BRAIN DAMAGE IN MODERN COHORTS

Long-term follow-up studies using quantitative magnetic resonance imaging (MRI) show that preterm birth is associated with long-term reductions in cortical surface area, volume and folding, and reductions in volume of subcortical regions (31,32). Neurodevelopmental outcomes are independently correlated with both WMI and the magnitude of the gray matter deficits (32). Similarly, decreased caudate, cortical, and hippocampal volumes were correlated with lower intelligence quotient in preterm infants at 7–8 y of age (33,34), and reduced hippocampal volume correlated with some aspects of memory impairment in children in early adolescence (35).

After long-term follow-up of preterm infants from 4 to 6 y of age, neurocognitive impairment was associated with preceding WMI (and reductions in gray matter volumes), whereas children who had not had WMI appeared to have normal neurocognitive development (36). It is unclear whether this relationship reflected a direct effect of WMI on cortical development or some common mechanism affecting both the white and the gray matter. Prematurely born children who had no overt neonatal WMI were reported to have altered neural connectivity at 12 y of age on white matter diffusion tensor imaging and volumetric imaging compared with term controls (16). Further follow-up up to 16 y of age confirmed that the preterm children showed extensive changes in both microstructure and total cerebral white matter volume and that measures of language development were closely related to microstructural integrity (11). Intriguingly, whereas at 12 y, there were marked regional sex differences in fractional anisotropy (FA) measurements that correlated with verbal intelligence quotient and vocabulary measures, few sex effects were found at 16 y (11). Similarly, at 6 y of age, others found little effect of adjustment for sex on risk of performance impairments (36). It is likely that much larger cohorts will be required to disentangle the effects of sex and the risk of diffuse WMI at birth on long-term outcomes.

DOES ACUTE NEURONAL LOSS CONTRIBUTE TO IMPAIRED CONNECTIVITY?

It is surprisingly unclear whether preterm birth is commonly associated with overt neuronal loss. Postmortem cases of infants dying in the early neonatal period show that at least the most severely affected infants have significant neuronal loss (19,37,38). MRI of preterm infants exposed to known severe perinatal hypoxia has demonstrated a consistent pattern of acute subcortical damage involving the thalamus and basal ganglia, in addition to cerebellar infarction combined with diffuse periventricular WMI, but sparing of the cortex (39).

Although now in the minority, infants with cystic WMI appear to have reduced numbers of cortical neurons compared with infants without cystic injury (7). Furthermore, a detailed postmortem analysis suggested that neuronal loss occurred in a third or more of cases of cystic WMI, whereas there was no apparent neuronal loss in infants with only chronic, noncystic WMI (40). Similarly, in human autopsy cases with acute noncystic diffuse WMI, few acutely degenerating cells were observed in the cerebral cortex in contrast to the situation in the cerebral white matter. A quantitative analysis of neuroprostanes, specific biomarkers of neuronal oxidative damage, found no evidence of acute neuronal degeneration in these

cases (41). It is unclear whether this cortical deficit represents neuronal injury or a secondary consequence of white matter damage. Regardless, the evidence that infants with diffuse WMI have little neuronal loss supports the hypothesis that additional mechanisms are involved in impaired gray matter development.

CONNECTIVITY AND THE "CONNECTOME"

A key area of modern research will be to understand how function relates to the changes in connectivity of the brain. A "connectome" is a map of neural connections in the brain and can be created by using advanced MRI-based tractography (42). Ball *et al.* recently reported that in term and preterm infants (median gestation at birth 28 wk), at term-equivalent age, there was a significant association between frontal and temporal lobe volumes and thalamic and cortical tissue reduction and the loss of microstructural integrity in the connective white matter tracts (43). Subsequent tractography demonstrated that connectivity between the thalamus and cortex was significantly reduced (44). Similarly, there was a close relationship between cerebellar injury in premature infants and subsequent reduced growth of the contralateral cerebral hemisphere by term-equivalent age (45). In a cross-sectional study, resting-state functional connectivity MRI demonstrated that term infants have strong resting-state networks involving the thalamus, sensorimotor cortex, brainstem, and cerebellar vermis, whereas preterm infants at term-equivalent age showed much more limited connections (46). Supporting these findings, recent evidence shows that both early and late premature birth were associated with reduced long-term depression-like responses to noninvasive transcranial magnetic brain stimulation in adolescence (47), consistent with impaired cortical neuroplasticity. Furthermore, prematurely born adolescents with MRI evidence of WMI showed reduced magnetoencephalographic brain activation over the right parietal cortex in response to specific stimuli (48), consistent with a link between early WMI and long-term altered cortical function.

PRECLINICAL EVIDENCE THAT MILD CEREBRAL ISCHEMIA IMPAIRS BRAIN DEVELOPMENT

Late fetal human development is associated with a dramatic expansion of the cerebral cortex, at least in part due to prolific sprouting of neuronal dendrites, which in turn mediates the interconnection of neurons (49). Dean *et al.* recently found that reversible cerebral ischemia in preterm fetal sheep at 0.65 gestation, which did not cause acute neuronal loss, was associated with impaired expansion of the dendritic arbor and reduced synaptic density of cortical projection neurons in all cortical layers after 4 wk recovery (as illustrated in **Figure 1**) (50). Strikingly, these histological changes were associated with impairment of cortical growth and the normal maturational decline in cortical FA on *ex vivo* MRI scans. Mathematically, these changes in FA after ischemia were consistent with the changes in the growth of dendritic arbor of cortical pyramidal neurons compared with

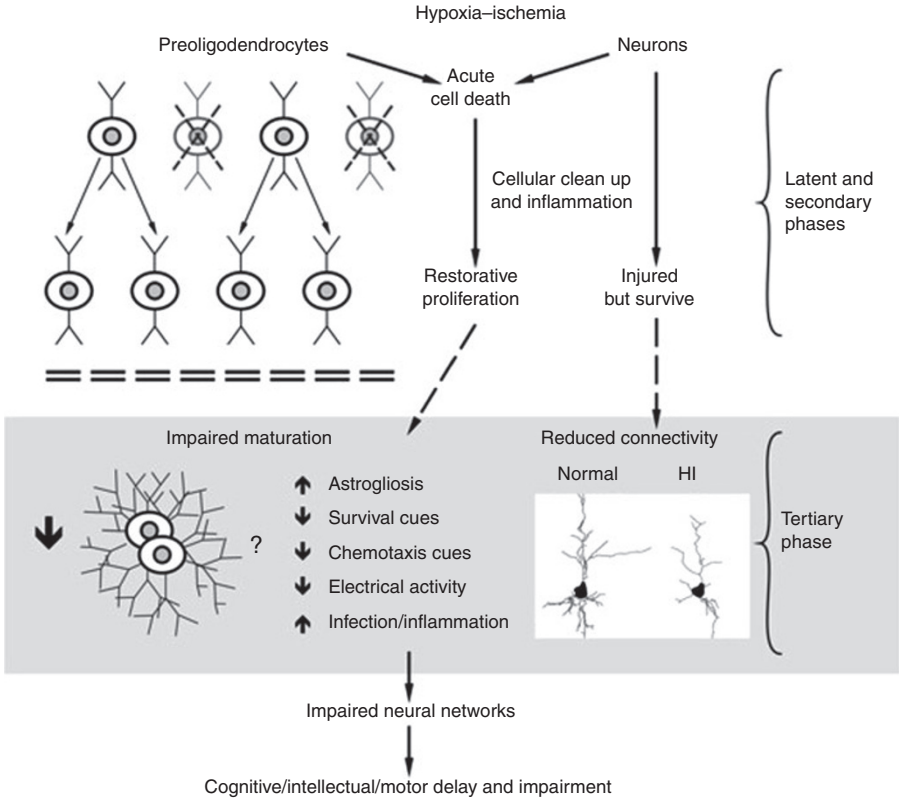

Figure 1. Flow diagram outlining the hypothesized relationship between hypoxia and infection/inflammation in the developing brain and long-term reduction in regional brain growth. These mechanisms combine to reduce neuronal arborization (see examples taken 4 wk after sham control or hypoxia–ischemia (HI) studies in preterm fetal sheep (50)), leading to persistently impaired connectivity.

that in controls. These findings are in contrast with the finding in P3 rats that unilateral hypoxia–ischemia leads to markedly reduced FA in the ipsilateral injured cortex (51). This severe, focal insult causes infarction of the cortex in the territory of the middle cerebral artery, with disruption of the radial organization, whereas there is no overt cortical injury in the sheep model or in typical preterm brain injury in human infants (52).

Supporting the findings in preterm sheep, human survivors of preterm birth show a similar delayed decline in cortical FA, consistent with impaired microdevelopment of cortical gray matter (53,54), whereas FA is reduced in white matter regions (16). The delayed maturational decline in cortical FA before 38 wk was proportional to the severity of prematurity and strongly associated both with impaired cortical growth and with neurodevelopmental outcomes at 2 y of age (54).

PRECLINICAL EVIDENCE THAT MILD/CHRONIC SYSTEMIC INFLAMMATION IMPAIRS BRAIN DEVELOPMENT

There is strong evidence for a causal linkage between acute, severe exposure to lipopolysaccharide (LPS), a purified polysaccharide from the outer wall of Gram-negative bacteria, and impaired neural development. For example, acute intravenous exposure of preterm fetal sheep to LPS was associated with substantial white and gray matter injury (55), followed by impaired brain growth and loss of the normal maturational increase in cortical EEG amplitude 10 d later (56). However, as discussed above, such overt infection is relatively uncommon in preterm infants. Recently, we reported that a stable, low-dose infusion of LPS to preterm fetal sheep older than 5 d, which did not perturb fetal blood gases, carotid blood flow, or arterial blood pressure, still markedly impaired the maturation of the cortical EEG (57). LPS infusion was associated with loss of the normal maturational shift to higher-frequency EEG activity, with reduced alpha and beta power and increased delta power, compared with saline controls, from 6 to 10 d (i.e., after the end of the LPS infusion). This impaired cortical maturation developed in the presence of low-grade inflammation, with a transient increase in serum interleukin-6, induction of microglia, and greater expression of tumor necrosis factor-α positive cells in the frontoparietal cortex, consistent with the hypothesis that exposure to proinflammatory cytokines directly contributed to the EEG dysmaturation.

In vitro studies show that proinflammatory cytokines mediate synaptic dysfunction, in part indirectly through adenosine and gamma aminobutyric acid (58). These effects appear to be dose dependent, with synaptic inhibition at lower concentrations (59). Furthermore, tumor necrosis factor-α was reported to inhibit growth and branching of neurons *in vitro*, which could be reversed by tumor necrosis factor-α receptor blockade (60). Similarly, exposure to interferon-γ, an inflammatory marker that is induced by injury and many inflammatory conditions, is associated with both inhibition of initial dendritic outgrowth in culture and retraction of existing dendrites (61). Thus, it is highly plausible that chronic low levels of cerebral cytokines associated with infection, or with traumatic events such as ventilation, can reduce neural excitability and impair connectivity in the cortex. Further long-term studies are essential to confirm this hypothesis.

SYNERGY WITH OTHER INSULTS: SENSITIZATION AND TOLERANCE

This apparent association between prenatal hypoxia and perinatal infection/inflammation may reflect a combined or even synergistic effect on neuronal development. There are parallels from experimental data in rodents that exposure to mild infection or inflammation can sensitize the brain so that short or milder periods of hypoxia–ischemia, which do not normally injure the developing brain, can in combination trigger severe damage (62). The effect is complex and time dependent. Nevertheless, recent data suggest that prenatal exposure to inflammation exacerbates ventilation-mediated brain injury (63), and thus it is highly plausible that multiple insults impair perinatal brain development.

OTHER FACTORS

Although this is not the focus of the present review, it is important to appreciate that neonatal care may also affect brain development. Likely factors include nutritional compromise (64), sedation (65), and the abnormal environment. For example, procedural pain has been associated with reduced subcortical gray matter *N*-acetylaspartate/choline ratio (a measure of neuronal viability) and white matter FA (66); however, other studies have reported that preemptive morphine infusions did not reduce the frequency of WMI and prolonged therapy may have had adverse effects (67). Glucocorticoids, whether exogenous or endogenously induced by stress, have profound effects on brain structure, including simplification of dendrites and shrinkage of dendritic spines (68). The relatively prolonged postnatal courses of glucocorticoid treatment used for lung maturation have been associated with greater risk of developmental delay and cerebral palsy (69). Furthermore, postnatal treatment with hydrocortisone (which has mixed glucocorticoid and mineralocorticoid effects) or dexamethasone was associated with impaired cerebellar growth, whereas antenatal maternal betamethasone was not associated with any change in infants' cerebellar volume (70). These findings may contribute to at least part of the complexity of clinical outcomes.

WHAT IS THE LINK BETWEEN CORTICAL MATURATION AND WMI?

Cells are "social" beings, and their survival is tightly linked to extrinsic signals from neighboring cells and synaptic activity (71). Disruption of early contact between the cortex and thalamus may also disrupt connectivity and lead to remodeling of cortical circuitry due to target deprivation-induced ("Wallerian") neurodegeneration (72). Nevertheless, although historical data demonstrate axonal injury within and around periventricular white matter necrosis (9), there does not appear to be significant axonal damage in nonnecrotic fetal ovine WMI (73). Similarly, in a postmortem cohort of preterm infants, axonal degeneration was only seen in necrotic foci, in association with microglial activation, but not in areas

of diffuse WMI (9). Although microscopic necrosis and axonopathy did occur, they affected less than 5% of the area of the periventricular white matter. Rather, WMI was characterized by diffuse astrogliosis with expansion in the total population of oligodendrocyte progenitors, but reduced myelination, consistent with arrest of the maturation of oligodendrocytes. Buser *et al.* (9) speculated that acute preoligodendrocyte death may trigger diffuse astrogliosis, as part of the neuroinflammatory responses, leading to an aberrant regenerative response that contributes to an impaired maturation of oligodendrocytes and consequent reduced myelination, as illustrated in **Figure 1**. This process may be mediated by inhibitory effects of astrogliosis and the induction of microglia through several mechanisms. These include inhibitory effects of hyaluronic acid and its digestion products on preoligodendrocyte maturation (74,75).

Hyaluronic acid digestion products likely act in concert with other signals in demyelinated lesions to prevent remyelination, many of which may be linked to reactive astrogliosis. For example, reactive astrocytes in spinal cord injuries increase their expression of bone morphogenetic proteins that inhibit oligodendrocyte progenitor cell differentiation, with concurrent promotion of astrocyte differentiation. Astrocyte-derived bone morphogenetic proteins inhibit oligodendrocyte progenitor differentiation with concurrent promotion of astrocyte differentiation (76). Similarly, the Notch ligand Jagged1 is elevated on reactive astrocytes in demyelinating lesions and activates Notch signaling on oligodendrocyte progenitors, preventing their maturation (77). Astrocytes also generate complement proteins that modulate synaptic pruning (78). Oligodendrocytes secrete trophic factors that influence the survival and function of neighboring neurons (79), enhance the number of functional synapses between neurons, and can directly regulate neuronal activity (80). Thus, impaired maturation of oligodendroglia may contribute to the impaired maturation of the developing gray matter and vice versa.

CONCLUSION

The most striking concept to emerge from recent clinical and experimental studies of the very immature brain has been that early WMI is associated with a reduction in cortical complexity and volume and, in turn, with neurodevelopmental impairment but that gray matter damage due to loss of connections—rather than cell loss—is also a major factor in long-term disability, as summarized in **Figure 1**. Focused experimental and clinical studies are essential to dissect the relative contributions of these mechanisms to establish whether they are independent or synergistic, in addition to finding ways to reverse them or stimulate normal development. Advanced neuroimaging techniques now offer the opportunity to track the long-term impact on the preterm brain and to assess neurodevelopmental outcomes and thereby provide novel opportunities for early interventions.

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