Brain Development and Perinatal Vulnerability to Cerebral Damage

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Abstract

The recent exponential rise in detailed magnetic resonance (MR) imaging studies has emphasized the concept of gestationally determined regional vulnerability in the brain. The site and nature of the injury is determined by a combination of the characteristics of the insult, the specific tissue and cell vulnerability, and the gestational age. Acute perinatal hypoxic ischemic events, previously considered characteristic for the term newborn presenting with hypoxic-ischemic encephalopathy, may occur at earlier points in gestation. White matter lesions, which are considered the hallmark of injury to the preterm brain, may also occur in a small percentage of term neonates. The regional tissue vulnerability at a given gestational age will be determined by the local metabolic requirements in combination with specific cell characteristics, such as the expression of different glutamatergic receptor subtypes and endogenous antioxidant mechanisms. In addition, neonatal neurons are programmed for cell death to allow for essential pruning and optimal connectivity, but this characteristic increases the vulnerability of such cells to injury. The nature of the insult is also important in dictating lesion site. In this chapter we will discuss the vulnerability of tissue and cell types in relation to gestational age and examine how these relate to patterns of injury seen on brain MR imaging and the clinical history and presentation of the infant.

117.1 Salient Points

- Gestational age is an important determinant of the lesion site, making white matter (WM) more vulnerable in preterm infants and gray matter more vulnerable in term infants, but the nature of the insult is also an important factor.
- The first half of gestation is characterized by neuronal proliferation and migration. During the second half, glial cell proliferation and programmed cell death predominate.
- Neuronal migration continues during a period when an infant born preterm may be exposed to a variety of potentially damaging factors. Acquired injuries to the immature brain may target replicating neuroblasts and disrupt neuronal migration.
- The subplate is a structure present at all phases of intrauterine brain development; it reaches its maximum width between 25 and 29 weeks, when WM is highly vulnerable to injury, before gradually decreasing in size.
- There are many factors responsible for WM vulnerability in preterms, ranging from the anatomy of the cerebrovasculature and low baseline blood flow to the inherent susceptibility of pre-oligodentrocytes to injury.
- Microglia are the main cell type implicated in injuries to the developing WM.
- Early migrating neurons and pre-oligodentrocytes are vulnerable to injury as well as the germinal matrix in the preterm brain.
- Cortex, central gray matter, and brainstem are common targets for perinatal injury complicating an acute hypoxic ischemic event.
- A predisposition to programmed cell death may be induced after injury, resulting in an increase in cellular apoptosis in injured regions and consequent disturbed and disrupted connectivity.

117.2 Introduction

The recent exponential rise in detailed magnetic resonance (MR) imaging studies has emphasized the concept of gestationally determined regional vulnerability in the brain: the site and nature of the injury sustained being determined by a combination of the characteristics of the insult, the specific

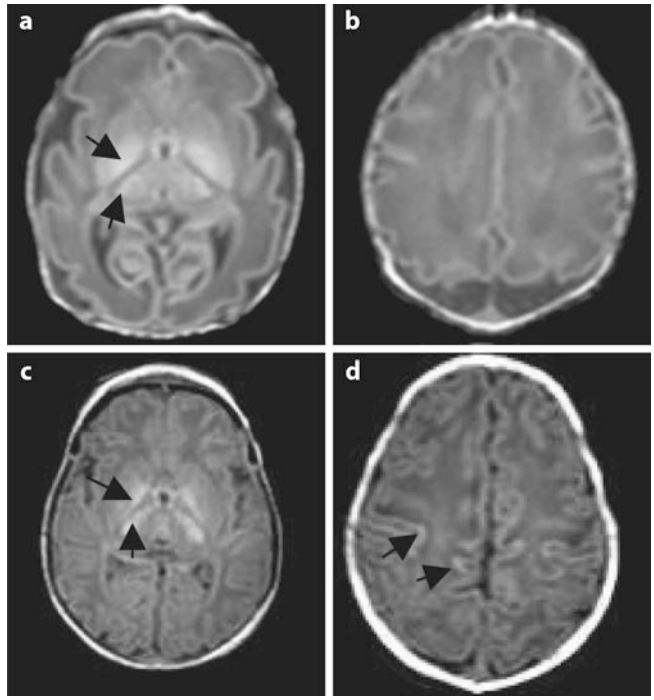

Fig. 1 Basal ganglia and thalamic (BGT) injury. BGT lesions are the hallmark of an acute hypoxic-ischemic event regardless of gestational age. (**a**, **b**) Preterm infant born at 32 weeks gestation by emergency cesarean section (EMCS) for abnormal fetal heart rate. There is abnormal high signal intensity (SI) either side (*arrows*) of the low SI posterior limb of the internal capsule (PLIC) (unmyelinated at this age). There is no obvious abnormality in or adjacent to the developing central sulcus or interhemispheric fissure (**b**). (**c**, **d**) Term born neonate born by EMCS for fetal distress. There is bilateral abnormal high signal intensity in the globus pallidus and thalamus (*arrows*). There is no myelin in the PLIC, which is abnormal at this age (**c**). Superiorly there is some increased SI in the central sulci and cortex along the interhemispheric fissure (*arrows*). The subcortical white matter adjacent to these regions is abnormally low SI (**d**)

tissue and cell vulnerability and the gestation of the infant. The type of insult may also be partly dependent on gestation. However, it is now known that acute perinatal hypoxic ischemic events, previously considered characteristic for the term born neonate presenting with hypoxic-ischemic encephalopathy (HIE), may occur at earlier points in gestation (Barkovich and Sargent 1995; Logitharajah et al. 2009). Nevertheless, such events occur less often in the infant born preterm where lesions develop in similar brain regions and in other areas characteristically more vulnerable in more premature babies (Fig. 1). Similarly, white matter (WM) lesions, which are considered the hallmark of injury to the preterm brain because they are characteristic of perinatal injury relating to inflammation, infection or hypoglycemia in the term brain, may also occur in a small percentage of neonates with an encephalopathy (Fig. 2) (Rutherford et al. 2010).

The regional tissue vulnerability at a given gestation will be determined by the local metabolic requirements in combination with specific cell characteristics, e.g., density of glutamate receptors (Patel et al. 2005). Regional tissue vulnerability at different gestational ages has been demonstrated by numerous *in vivo* brain imaging studies, as well as by conventional postmortem examinations. However, animal studies have highlighted the concept of a specific vulnerability by certain cell types, e.g. the subplate neurons (McQuillen et al. 2003) and oligodendrocyte precursors (Volpe 2009) are most vulnerable in the preterm brain. In the term brain, excitatory pyramidal projection neurons especially in the deep grey nuclei are at greatest risk during ischemic insults (Fig. 1). The vulnerability of a

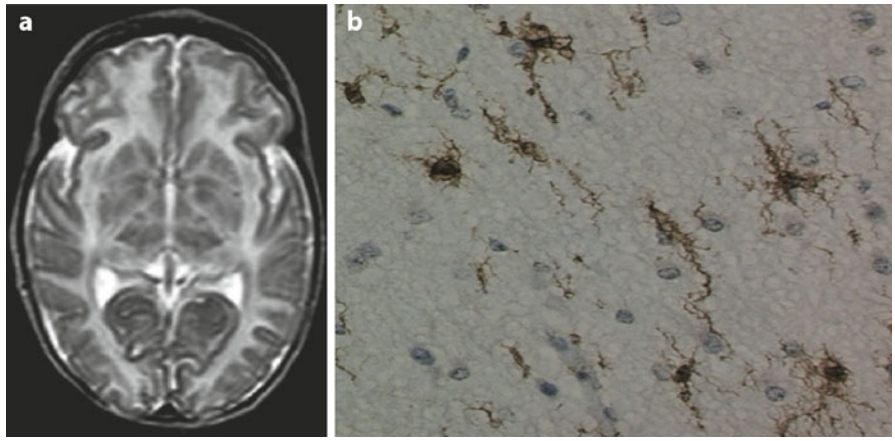

Fig. 2 T2 weighted scan (**a**) obtained at 9 days of age in a neonate with severe perinatal basal ganglia and thalamic injury showing diffuse high signal intensity in the white matter. Marked microglial activation (**b**) was seen in the white matter at post mortem ten weeks later

specific cell type at a given gestation relates to characteristics such as the expression of different glutamatergic receptor subtypes that favor calcium entry and excitability, and endogenous antioxidant mechanisms (Kjellmer 1991). In addition, the neonatal neuron is programmed for cell death to allow for essential pruning and optimal connectivity, but this characteristic increases the vulnerability of such cells to injury. However, while research has focussed on WM vulnerability in preterm infants and gray matter vulnerability in term infants, the nature of the insult is also important in dictating lesion site.

In this chapter we will discuss the vulnerability of tissue and cell types in relation to gestational age and examine how these relate to patterns of injury seen on brain MR imaging and the clinical history and presentation of the infant.

117.3 Brain Development

In order to understand the vulnerability of the immature brain to injury, it is essential to consider the stages of brain development from early fetal life until term at around 40 weeks' postmenstrual age (PMA). A full description of brain development is beyond the scope of this chapter so we will concentrate on regions of the preterm brain that are known to be particularly vulnerable; the immature white matter and the germinal matrix. In the more mature term brain we will focus on the deep gray matter and myelinating white matter with different cell types that may contribute to this changing vulnerability.

The first half of gestation is characterized by neuronal proliferation and migration. During the second half, glial cell proliferation and programmed cell death predominate (Rakic 1978; Skoff 1980; Rakic and Zecevic 2000). The last trimester of gestation is characterized by developing connectivity. There is axonal and dendritic sprouting and synapse formation. These processes, which involve programmed cell death, continue throughout childhood and into adolescence. Whilst early myelination in central brain structures, such as the brainstem and thalami, is present by 26 weeks, further myelination in the posterior limb of the internal capsule is then not detected until the end of gestation (Counsell et al. 2002). The majority of brain myelination occurs in the first 2 years after birth (Fig. 3). There is, however, extensive premyelination occurring within hemispheric white matter during late gestation and the abundance of oligodendrocyte precursors are particularly vulnerable to damage (Volpe 2009). This pre-myelination is in part responsible for the anisotropy of WM tracts documented in diffusion tensor images of the preterm brain before the appearance of myelination. Measures of anisotropy can therefore be used as an indirect measure of oligodendrocyte injury in

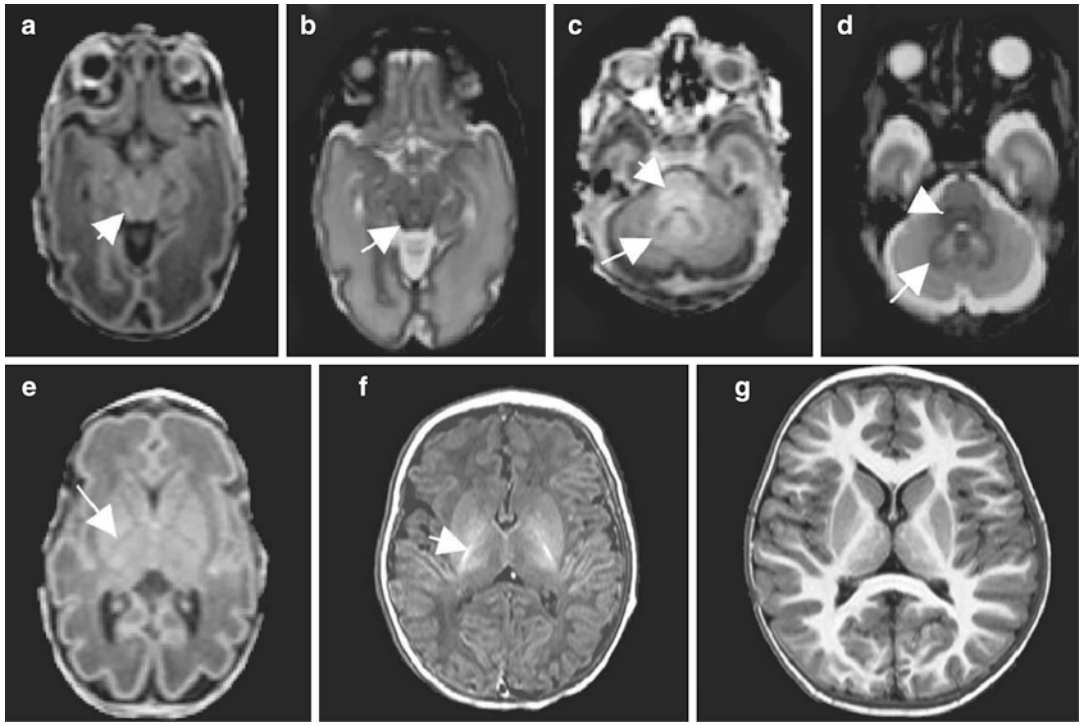

Fig. 3 Myelination. Preterm infant born at 26 + 5 weeks gestation and imaged at 29 weeks (**a**–**e**). Term born neonate (**f**). Two year old child (**g**). Myelination is seen as high signal intensity on T1 weighted images (**a**, **c**, **e**, **f**, **g**) and low signal intensity on T2 weighted images (**b**, **d**). At 29 weeks postmenstrual age myelination is detected in many central brain areas such as in brainstem regions e.g., lateral lemnisci (*arrows*) (**a**, **b**) and the medial lemnisci (*short arrows*) (**c**, **d**) and in the dentate nuclei (*long arrow*) (**c**, **d**). Myelination is easier to detect on T2 weighted images in the preterm brain. Myelination in the PLIC is visible from around 37 weeks postmenstrual age (*arrow*) (**f**). Prior to myelination the PLIC may look low signal intensity on T1 weighted images (*arrow*) (**e**). The majority of brain myelination occurs during the first 2 years of postnatal life (**g**)

the immature brain prior to myelination (Counsell et al. 2002; Hüppi and Dubois 2006).

Knowledge of the timing of events, such as myelination, allows us to understand the vulnerability of tissue types. *In vivo* methodology to assess such injuring processes provides an insight into the neurological sequelae that may result from lesions sustained at a given gestation.

117.4 Regional Vulnerability

117.4.1 Neuronal Migration

From the fifth week PMA, the neural tube starts to develop and differentiate. Specific proliferation areas can be identified in the ventricular and subventricular zones from where excitatory pyramidal neurons migrate to their final destination, mostly following specialized radial glial fibers. This radial migration process is closely regulated by complex molecular interactions between neurons and glial cells mediated by glycoproteins, membrane lipids and neurotransmitters (Métin et al. 2008).

More recently it has been appreciated that, in addition to this radial migration, there are two further modes of migration, tangential and multipolar migration (Nadarajah and Parnavelas 2002; Tabata and Nakajima 2003). The latter occurs independently of radial glia. It was originally thought that all neuronal migration ceased by 20 weeks PMA, but this referred to radially migrating pyramidal neurons. Gabaminergic

inhibitory interneurons generated in the ganglionic eminences migrate in a tangential manner initially and then along the radial glia into the cortex in an inside-out manner. This process continues until past term (Rakic 1978; Zhang et al. 2009). Later, the medial and lateral ganglionic eminences appear and give rise to different populations of interneuron; those from the lateral or caudal eminence migrate and occupy superficial layers of the cortex (Miyoshi et al. 2010). Thus neuronal migration continues during a period when an infant born preterm may be exposed to a variety of potentially damaging factors. Many recognized disturbances in neuronal migration result from genetic defects (Marcorelles et al. 2010), giving rise to profound abnormalities of cortical development such as the lissencephalies. Acquired injuries to the immature brain such as ischemia, viral infection (Barkovich and Lindan 1994; Luo et al. 2010) or maternal drug abuse (Lee et al. 2010) may target replicating neuroblasts and disrupt neuronal migration. Acquired injuries result in more focal cortical dysplasias such as polymicrogyria, which is frequently associated with cytomegalovirus infection (Fig. 4) (Luo et al. 2010; Barkovich and Lindan 1994). Earlier insults (Fig. 4) cause more severe disruption of cortical development, and later insults may result in subtle abnormalities in cortical development that may not be visually obvious but that could be investigated using more advanced imaging techniques such as diffusion tensor imaging.

117.4.2 The Cortical Subplate

The cortical subplate is an important anatomical and functional structure, which is present at all phases of intrauterine brain development from early gestation to near term. The first populations of migrating neurons occupy the transient cortical subplate from approximately 7–8 weeks post conception. The subplate is essential for normal cortical development and subplate neurons develop both intracortical and subcortical projections (Allendoerfer and Shatz 1994). The subplate zone becomes visible in the human brain at around 14–15 post-conceptional weeks (PCW),

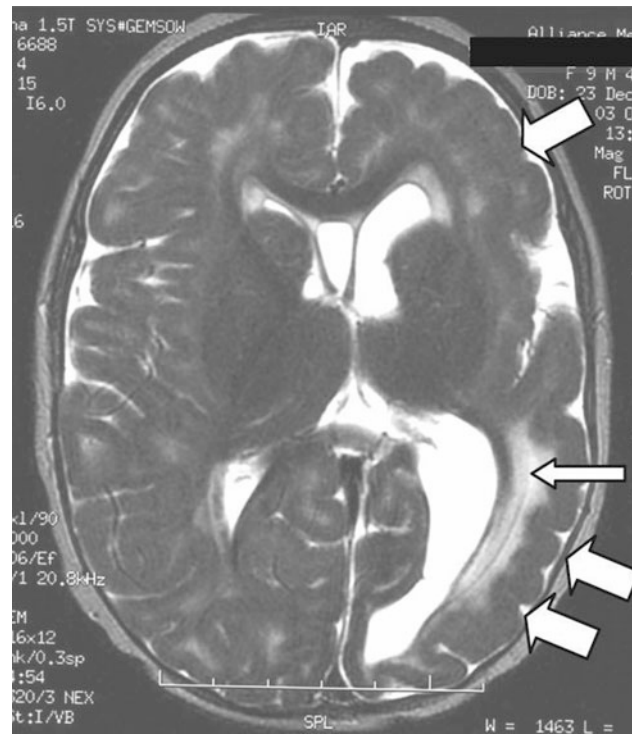

Fig. 4 Congenital CMV. Axial T2 scan of a term baby with CMV infection acquired around the 12th week of intrauterine life. The early fetal infection has determined cortical malformations such as polymicrogyria (*white arrows*) more evident in the right hemisphere and diffuse and severe white matter abnormalities (*thin arrow*)

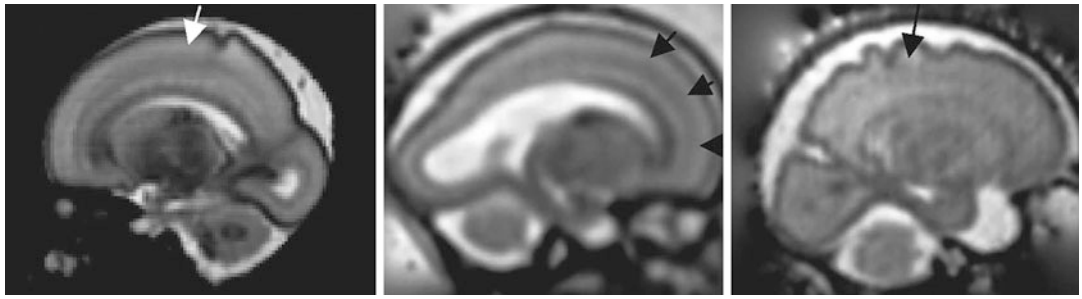

Fig. 5 Subplate visualization. T2 weighted images. Preterm infant 26 + 2 imaged at 28 + 1 weeks post-menstrual age. The subplate can be seen as a band of high signal intensity adjacent to the cortex (*arrows*)

the beginning of the second trimester of human gestation. This coincides with the invasion of the subplate region by thalamocortical afferents as well as basal forebrain afferents, leading to rapid expansion of the subplate so that it comprises 35% of the cerebral wall by 16 PCW. From 14 to 25 PCW in the human, a large number neurons are continuously added to the subplate compartment, which increases in width concurrent with the growth of the cortical plate. The highest density of cells is always found at the border between cortical plate and subplate. At its peak of development, the human subplate zone is four times the width of the cortical plate (Kostovic and Rakic 1990), reaching its maximum thickness in the late second and early third trimester, more or less between 25 and 29 weeks. This is a period of high vulnerability for WM injury. Thereafter it gradually decreases in size and becomes unrecognizable by the sixth month post-term (Kostovic and Rakic 1990).

Early thalamo-cortical afferents remain within the subplate until approximately 28 weeks when they progress into the cortex to form the earliest functional neurocircuits of the neocortex. The subplate layer contains a hydrophilic extracellular matrix that enables visualization of the subplate *in vivo* using MR imaging of the fetal or preterm infant brain. It can be quantified using volumetric scanning or diffusion tensor imaging (Dudink et al. 2010; Widjaja et al. 2010). On MR imaging the subplate layer is thicker than the cortex until approximately 24 weeks, equivalent to the cortex until about 28 weeks, and then starts to involute, being visible only at the tops of gyri throughout the third trimester (Figs. 5 and 6). A normal term infant usually only displays a few regions of residual subplate on imaging, and, in infants with overt lesions, the subplate may disappear prematurely. In contrast, in preterm infants imaged at term equivalence, the subplate may persist abnormally when compared to term born controls. This alteration in subplate involution associated with preterm birth may be due to more subtle forms of injury. The consequences of these alterations in subplate evolution on cortical development are unknown. However cortical gyration is reduced in the preterm brain at term equivalence, in comparison with normal term born control brains. However, MR cortical maturation at term corrected age is delayed in preterm babies with mild white matter abnormalities compared to those without (Fig. 7) (Ajayi-Obe et al. 2000; Kapellou et al. 2006; Ramenghi et al. 2007).

In a rodent animal model of perinatal HI, the subplate neurons appear to be selectively vulnerable and their loss corresponds to the severity of the insult (McQuillen et al. 2003). Investigators speculated that the glutamate receptor played a role in the pathogenesis, although the problem seems further complicated by more recent observations, suggesting a novel mechanism for subplate vulnerability.

It is, however, well known that selective ablation of subplate neurons at critical time periods in development may cause altered cortical organisation (McQuillen et al. 2003; Ghosh and Shatz 1992). Thus, isolated injury to the subplate neurons, which play a fundamental role in axonal

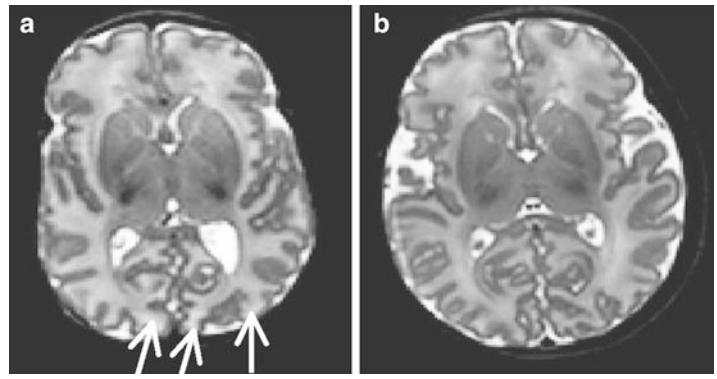

Fig. 6 Subplate involution. Preterm infant born at 26 + 3 and imaged at term (**a**). Term born control infant (**b**). Preterm infants at term have more subplate remnants, seen as regions of high SI adjacent to the gyral cortex (*arrows*) than term born controls, suggesting abnormal or delayed involution of the subplate layer

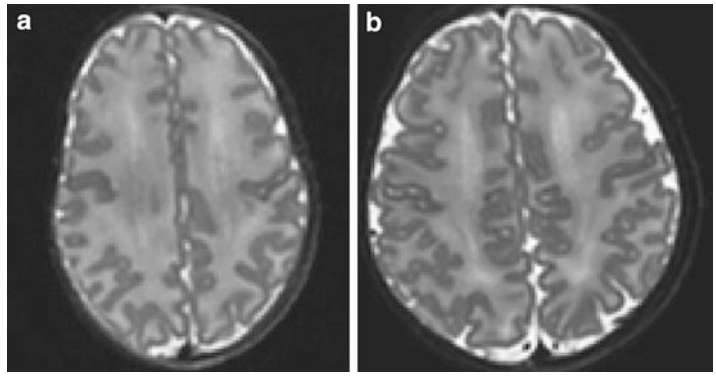

Fig. 7 Cortical development in the preterm. (**a**) Preterm infant born at 26 + 3 weeks gestation and imaged at term age. (**b**) Term born control infant T2 weighted images acquired in the transverse plane, at the level of the centrum semiovale. Preterm infants imaged at term age have less complex cortical folding than the term born controls (Ajayi-Obe et al. 2000; Kapellou et al. 2006)

routing to and from the developing cortex, may result in abnormal thalamocortical connectivity. It has been speculated that this may explain the visual and somatosensory impairment seen in prematurely born humans (Kostovic and Judas 2006). Seventy-one percent of premature infants with moderate periventricular leukomalacia (PVL) during the neonatal period were found to have at least one abnormality of visual testing at 1 year of age, and yet 66% of these children had normal optic radiations, and all had a visual cortex that appeared normal (Cioni et al. 1997). Primary abnormalities within the visual cortex of subcortical white matter have not generally been demonstrated by imaging studies of infants with PVL, although the accelerated disappearance of the subplate may be a sign of injury. Cortical abnormalities in terms of loss of volume have been documented following PVL (Inder et al. 1999), but no studies to date have looked specifically at the visual cortex. The neurobiological basis for their visual impairment is therefore not well understood and abnormalities in the visual cortical function may occur as a secondary phenomenon due to injury elsewhere. In more recent studies, Ricci (Ricci et al. 2006) and colleagues reported an association with poor visual function and thalamic atrophy. However, others (Bassi et al. 2008) have suggested a plausible explanation for failing visual test during the neonatal age period is that an unspecific parenchymal lesion may damage the complex connectivity network as (Ricci et al. 2006; Bassi et al. 2008; Ramenghi et al. 2010).

The cortical subplate continues to be related to brain development even late in gestation. In

newborn babies at term with HIE, potential WM involvement may occur due to subcortical laminar necrosis in the subcortical subplate, as shown by highlighting in areas close to the cortex, which is particularly evident at the depths of sulci. The predilection of cortical necrosis at the bottom of sulci remains unexplained, although the fact that the subplate first disappears at the depths of sulci as the cortex folds may indicate an increasing vulnerability of the local resident neurons as they are more mature than those at the tops of gyri.

The role of subplate neuronal injury as a neurological basis for later impairments needs to be further investigated. A more sophisticated *in vivo* imaging approach such as serial diffusion tensor imaging studies is needed (Dudink et al. 2010; Widjaja et al. 2010).

117.4.3 The Developing White Matter

The association of preterm delivery and white matter injury in the form of periventricular leukomalacia (PVL) has been recognised since the 1960s (Banker and Larroche 1962). Imaging studies have emphasised that injury to the developing white matter is associated with secondary abnormalities involving the entire thalamo-cortical circuit, giving rise to thalamic atrophy and a decrease in cortical volume (Inder et al. 1999). Recent MR studies have also identified a spectrum of WM abnormalities in the preterm brain. PVL represents the most severe, with new findings of punctuate WM lesions and the diffuse appearance of DEHSI being reported more recently (Rutherford et al. 2010).

117.4.4 Oligodendrocytes

The vulnerability of preterm white matter appears to be multifactorial, relating to vessel anatomy with arterial end and border zone regions of relatively poor perfusion, low baseline blood flow, impaired regulation of blood flow in the sick infant and inherent susceptibilities of pre-oligodendrocytes (preOLs) to injury (Volpe 2009; Khwaja and Volpe 2008). The current theory is that both hypoxia-ischemia and infection lead to microglia activation that in turn leads to cytokine release, production of free radicals with both reactive oxygen and reactive nitrogen species and glutamate release, all of which contribute to death of the immature oligodendrocyte which demonstrate a vulnerability to oxidative stress (Haynes et al. 2003) and glutamate receptor immaturity (Deng et al. 2004).

The ultimate result of either event would be a deficit of mature oligodendroglia, and a consequent impairment of myelination, the hallmark of PVL. Early white matter injury may result in a combination of delayed preOL degeneration and preOL maturation arrest. The persistence of susceptible populations of preOLs renders chronic white matter lesions markedly more vulnerable to recurrent hypoxia-ischemia. PreOL maturation arrest may predispose to more severe white matter injury in preterm survivors that sustain recurrent hypoxia- ischemia (Segovia et al. 2008).

117.4.5 Axons

Axonal injury has been recognized for many years to be a feature of the focal necrotic component of PVL. Perhaps more important quantitatively, axonal degeneration, detected by the apoptotic marker fractin, has been recently found to be a feature of the diffuse component of human PVL. Whether the axonal degeneration observed in diffuse PVL is a primary injury or a secondary effect remains unclear. However, if primary axonal injury did occur, the expected results would be hypomyelination via failure of axonal-oligodendroglial interactions and decreased cortical (Inder et al. 1999) and thalamic/basal ganglia volumes (Boardman et al. 2006; Srinivasan et al. 2006).

117.4.6 Thalami

We have included thalami in this section due to direct links with white matter. Recent human neuropathological data have demonstrated a high incidence of damage to specifically mediodorsal and reticular nuclei of the thalami in infants with PVL. Four patterns may be considered: diffuse gliosis, status marmoratus, micro-infarcts, and

macro-infarcts. Irrespective of the pattern and/or mechanism, it has been suggested that the reduced thalamic volumes revealed by neuroimaging are caused by neuronal loss (Ligam et al. 2009). Thalamic neuronal loss would be consistent with either primary injury and/or secondary anterograde and retrograde trophic effects. If there is primary neuronal injury, secondary effects would involve white matter axons, with subsequent hypomyelination and impaired development of the cerebral cortex, and such changes would be expected to compound the effects of the initial WM injury characteristic of PVL.

The role of the cerebral cortex in the cognitive deficits in preterm survivors is poorly understood. Periventricular leukomalacia (PVL), the key feature of the encephalopathy of prematurity, is characterized by periventricular necrotic foci and diffuse gliosis in the surrounding cerebral white matter. A recent study tested the hypothesis that reductions in the density of layer I neurons and/or pyramidal neurons in layers III and/or V are associated with PVL, indicating cortical pathology potentially associated with cognitive deficits in long-term survivors. In 15 controls (23 gestational weeks to 18 postnatal months), there was no difference in pyramidal density among incipient Brodmann areas, suggesting that cytoarchitectonic differences across functional areas are not fully mature during the fetal and infant periods. There was a marked reduction (38%) in the density of layer V neurons in all areas sampled in children with PVL cases compared with controls, in whom the six-layer cortex was visually distinct ($P < 0.024$). This may reflect a dying-back loss of somata complicating transection of layer V axons projecting through the necrosis in the underlying white matter. This study emphasised the potential role of secondary cortical injury in the encephalopathy of prematurity (Andiman et al. 2010).

117.4.7 The Role of Microglia in WM Injury

Microglia are the main cell type implicated in injuries to the developing WM. Microglia are hemopoietic in origin and migrate into the brain from approximately 6 weeks' gestation. There are at least three morphological forms (amoeboidal, activated and ramified), which are distinguished by staining with markers such as tomato lectin and Iba. In the normal adult brain, the majority of microglia are ramified or resting, and activated microglia are assumed to be pathological. The normal immature brain contains all recognized morphological forms of microglia (ramified, activated and amoeboidal), although the exact site and numbers of the different forms alters with increasing gestation. Normal developmental or resident microglia perform many essential roles. These include the phagocytosis of unwanted tissue from programmed cell death as shown by labelling of microglia and apoptotic cells in regions of synaptogenesis and neuronal differentiation (Rezaie and Male 1999). Resident activated microglia are also involved in brain modelling by secreting growth factors such as nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and neurotrophin-3 (Elkabes et al. 1998), which provide trophic support for neurons and glia cells.

There is a maturation dependent concentration of microglia in apparently normal cerebral white matter during the third trimester of human gestation (Billiard et al. 2006). A few of these microglia are MHC II immunopositive cells, further evidence that they are developmentally activated resident cells and not immune induced. It has been suggested that the presence of numerous resident microglia in the immature brain increases its susceptibility to WM injury. Microglia co-exist during this developmental period with pre-oligodendrocytes which, as discussed, are also particularly susceptible to ischemic and inflammatory injury. Microglia are known to play a pivotal role in diffuse white matter injuries in the immature brain such as PVL.

Microglia are a potent source of both inflammatory cytokines and free radicals. Reactive oxygen and nitrogen species (ROS/RNS) release is mediated via Toll-like receptors known to be present on microglia. Interferon γ expression has been demonstrated in astrocytes in diffuse PVL. Interferon g toxicity is greater for pre-OLs than for mature cells and is potentiated by tumor necrosis factor alpha (TNFα), which is produced by microglia. Evidence

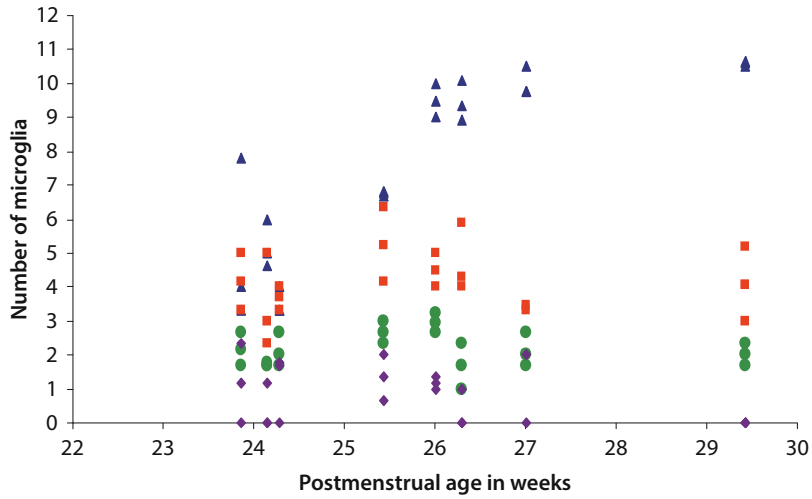

Fig. 8 Distribution of Iba 1 immunopositive microglia in the frontal, occipital and parietal lobes of the extremely preterm normal brain. Microglia numbers at the PVWM (*blue*) were significantly higher than deep white matter ($p = 0.0251$; *red*), subplate ($p < 0.0001$; *green*) and cortex ($p < 0.0001$; *purple*). This difference was most marked over the gestational age range in which PVL is most common

for the contribution of inflammation to preterm white matter injury emanates from studies of pregnant and neonatal animal models, where responses to exogenous lipopolysaccharide (LPS) are particularly involved. These studies have identified striking upregulation of inflammatory cytokines and microglial activation. LPS activates the innate immune system through an interaction with a specific toll-like receptor (TLR4) on immune cells, including microglia. Further evidence supporting the role of microglia in WM injury is provided by many studies using animal models of WM injury which demonstrate a decrease in injury in the presence of inhibitors of microglial activation such as chloroquine, minocycline and melatonin. Strategies for decreasing immune activated microglial activity in response to injurious processes should ideally not interfere with the function of normal developmentally active resident microglia.

In vivo and *in vitro* imaging studies of the immature brain have attributed regions of altered signal intensity within the developing white matter to the presence of dense clusters of microglia (Judas et al. 2005). An increase in activated microglia in all periventricular white matter areas (sometimes called WM crossroads) compared to the deep white matter and subplate has been demonstrated in a cohort of 23 human brains with gestational ages of 22–40 weeks (Fig. 8). In addition, these authors demonstrated increased numbers of periventricular microglia in association with germinal matrix/intraventricular hemorrhage (Supramaniam et al. 2010). Although the morphological appearances of Iba positive cells do not differentiate normal developmentally activated microglia from abnormal immunoactivated microglia, some differentiation between developmentally activated microglia and immune/injury activated microglia may be made based on their site and number and the presence of overt tissue injury. However, functional differentiation may require more specific immunological markers. For example, mouse models have shown that in response to ibotenate, early microglial activation was CD45 negative, i.e., these cells are likely to have originated from the normal resident population (Dommergues et al. 2003).

In vitro studies of animal models have shown that the LPS-induced inflammatory response initiated by microglia was mediated via the TLR4 *My*D88-dependent pathway (Dean et al. 2009; Wang et al. 2009). This activation of microglia causes neuronal death, whilst *My*D88-deficient microglia do not. Manipulation of this activation may provide an opportunity for neuroprotective intervention. In a separate rodent model, LPS preconditioning was shown to have an altered protective response to subsequent ischemic injury via the TRIF-IRF3-IFNβ TLR4 cascade (Marsh et al. 2009), which may have a beneficial effect on microglia. Investigation of these TLR4 activation pathways would enable functional

characterization of different microglial phenotypes in the immature human brain, especially in association with lesions detected by MR imaging. In addition, co-labelling for apoptosis and for axonal injury would determine any deleterious effect by immune activated microglia.

117.4.8 The Germinal Matrix

The term germinal matrix is used by neonatologists to describe dense regions of tissue seen primarily within the caudothalamic notch on cranial ultrasound. On MR imaging scans of younger babies similar areas are also seen in the roof of the temporal horn and in the frontal areas externally adjacent to the frontal horns. These areas correspond to the ganglionic eminences referred to by neurobiologists. The germinal layer lining the entire ventricle may be identified on MR imaging of the fetal or very preterm brain but is not seen on ultrasound. In the fetal brain this layer is very prominent, but in infants born preterm, involution is already evident. On MR imaging of postmortem specimens, the germinal layer increases exponentially; it reaches a maximum at around 23 weeks' PMA and then decreases dramatically (Kinoshita et al. 2001).

The germinal and subventricular layers are sites of neuronal and glial cell proliferation and migration. The characteristic vulnerabilities of early migrating neurons and preoligodendrocytes to injury have already been discussed, but their site of origin, the germinal matrix itself, is a common site of injury in the preterm brain. It is vulnerable to both hemorrhagic injury, as a result of the dense vascular network, and to injury from infection, e.g. by cytomegalovirus, which results in necrosis and cyst formation.

Germinal matrix intraventricular hemorrhage (GMH/IVH) has decreased in incidence because of improvements in intensive care, specifically in respiratory morbidity with the introduction of antenatal steroids for threatened preterm labor and artificial surfactant at the time of delivery. However, with the survival of the most extremely preterm infants who remain very vulnerable, germinal matrix hemorrhage remains a major cause of morbidity and mortality. The incidence in those born at 24–26 weeks' gestation or below 750 g birthweight is approximately 20–30%. The inverse relationship with gestational age indicates that the larger the matrix, the more susceptible it is to hemorrhage. In the term born neonate in contrast, intraventricular hemorrhage is thought to originate from the choroid plexus and is recognized as being a complication of sinus thrombosis particularly when associated with thalamic hemorrhage (Wu et al. 2003). A germinal matrix hemorrhage can also derive from sinus thrombosis in latepreterm babies (Ramenghi et al. 2002). The site of hemorrhage in the preterm is usually from the ganglionic eminence of the caudothalamic notch or less frequently in the temporal horn (Hambleton and Wigglesworth 1976). These regions have a rich capillary network, which is vulnerable to rupture and hemorrhage because of the poor vascular integrity of involuting immature vessels and inadequate connective tissue support with a deficiency in mesenchymal and glial elements. Furthermore, a pressure passive circulation, compliant skull and disturbances in coagulation in the sick preterm exacerbate the fragility of the matrix. Hemorrhagic lesions are thought to be secondary to venous congestion with distortion and tearing of local small venous tributaries by the presence of blood in the perivenous space (Ghazi-Birry et al. 1997; Towbin 1968; Leech and Kohnen 1974). The hemorrhage originates and destroys the germinal matrix and this may impair proliferation and late migration of GABAergic interneurons to upper cortical layers, and the thalamus could contribute to defective cortical and thalamic development. There is some neuropathological data to support this (Marin Padilla 1999), although it is difficult to determine the independent effect of progenitor cell loss on subsequent brain development because infants that die are likely to have additional brain lesions. Such complications of GMH/IVH include venous infarction and ventricular dilation, both of which will additionally injure developing white matter and possibly thalamic tissue and therefore disrupt thalamo-cortical connectivity and cortical development. So, whilst there is likely to be a disturbance of the late GABAergic neuronal

proliferation and migration from the subventricular zone (SVZ) and the ganglionic eminence (GE) to upper layers of the cerebral cortex and from the GE to the thalamus, venous infarction will cause additional destruction of both axons and pre-OLs, resulting in the formation of a porencephalic cyst. Thalamocortical connections will be disrupted, resulting in thalamic atrophy and overlying cortical development may be impaired. Thalamo-cortical disconnection may be demonstrated at a distance from the site of infarction.

The neurodevelopmental consequences of this are evident in follow-up studies such as the DRIFT trial, which enrolled neonates with marked ventricular dilation complicating GMH/IVH. The majority also had evidence of venous infarction. While the trial demonstrated some improvement in neurodevelopmental outcome following specific intervention, as a study group these infants with complications showed severe neurodevelopmental impairments at 2 years corrected age (Whitelaw et al. 2010). A secondary imaging study of total brain tissue volume and cerebellar volume highlighted the need to preserve supratentorial tissue and avoid cerebellar compression by a dilated fourth ventricle (Carli et al. 2010). Reduced cerebellar size following GMH/IVH may be multifactorial and related to a primary cerebellar hemorrhage or to secondary atrophy as a consequence of supratentorial lesions (Boardman et al. 2006; Srinivasan et al. 2006).

117.4.9 The Cerebellum in Extremely Preterm Babies

Primary cerebellar hemorrhage arising from the external granular layer and associated tissue infarction is relatively common in very preterm infants. There is a well documented association between intraventricular and cerebellar hemorrhage. In one large ultrasound study of 1242 preterm infants with intraventricular hemorrhage, 77% of infants also had cerebellar hemorrhages (Limperopoulos et al. 2005). MR imaging studies seem to confirm this high vulnerability of the most premature babies to develop cerebellar hemorrhage (Fumagalli et al. 2009).

A study using the mastoid window for better visualization of the posterior fossa demonstrated an incidence of cerebellar hemorrhage in 3% of preterm infants <1500 g, with nearly 60% of these occurring in infants less than 750 g (Limperopoulos et al. 2005). In the very preterm infant, the hemorrhage originates within the hemisphere with involvement initially of the subpial and subependymal layers sites of the germinal matrices and subventricular zones respectively. The more extensive lesions extend and involve both the cerebellar cortex and white matter. In contrast, in the term infant the site of origin is more frequently the vermis. Pathogenesis in the preterm infant shares many similarities with the causes of intraventricular hemorrhage. In the term infant, trauma is a more important factor. Injury to the developing cerebellum will result in disruption of neuronal migration, which continues into infancy in the cerebellum with consequent cerebellar disconnectivity.

The role of the cerebellum in cognitive function is being increasingly recognized. It is not surprising therefore that 40% survivors of preterm cerebellar hemorrhage demonstrate cognitive deficits and 37% have autistic spectrum disorders (Limperopoulos et al. 2007). In term infants, subsequent motor impairments are described more frequently, particularly in association with larger lesions (Limperopoulos et al. 2009; Takashima 1982).

117.4.10 Effects of Germinal Matrix Intraventricular Hemorrhage

There is evidence to suggest that less severe forms of GMH/IVH may be associated with periventricular WM injury. This may result in milder degrees of ventricular dilation and studies have reported an association with later cognitive function in such infants (Ment et al. 2005; Miller et al. 2005). In addition, two studies have reported worse outcomes in survivors of preterm birth where subsequent ventricular dilation was associated with an original GMH/IVH (Vollmer et al. 2006; Dyet et al. 2006). In the rabbit kit, IVH was associated with an increase in periventricular white matter (PVWM) microglial activation and axonal disruption and apoptosis (Dommergues

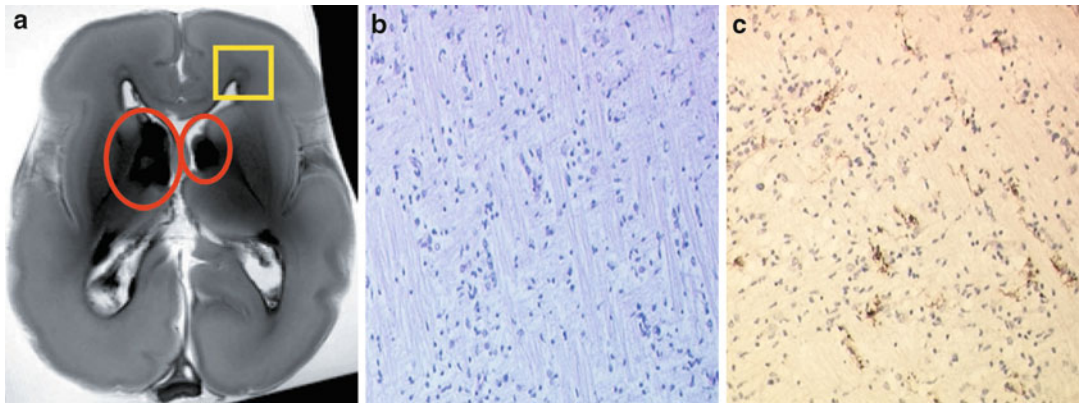

Fig. 9 T2 weighted MRI image (**a**) of a preterm infant born at 26 weeks and imaged postmortem at 28 weeks. There is bilateral germinal matrix/intraventricular hemorrhage seen as foci of low signal intensity (red circles). PAS (**b**) and Iba (**c**) staining through anterior periventricular white matter, (in the region of the yellow rectangle) showing intersecting axons, so called WM crossroads (**b**). Many of the cells in this region are microglia (**c**), which have roles associated with axonal guidance and WM modeling. Their presence may enhance the susceptibility of these regions to injury

et al. 2003). An increase in activated microglia has also been demonstrated in human PVWM in the presence of uncomplicated IVH (Fig. 9). Tissue injury may occur secondary to free radical release, secondary to the presence of free iron from the blood and the presence of resident activated microglia may exacerbate this process.

It is unclear whether as the germinal matrix involutes it becomes less susceptible to the effects of infection. The development of subependymal cysts in the persisting germinal matrix of the caudothalamic groove postnatally suggests that the tissue remains vulnerable to viral infection in more mature infants, but the consequences for progenitor cell destruction may be less severe as the infant matures.

117.5 The Late Preterm Infant

In recent years the vulnerability of late preterm infants of gestation between 34 and 37 weeks has become recognized. Reports demonstrate increased short-term neonatal morbidity in low risk late preterm pregnancies (Mateus et al. 2010; Melamed et al. 2009) with a 12-fold increase in mortality compared with term born controls and a mortality rate of 0.8% (Kitsommart et al. 2009). Studies of surviving late preterm infants have conflicting results, with no significant differences in childhood outcome (Gurka et al. 2010). A study at school entry showed that the risk for developmental delay or disability was 36% higher among late preterm infants compared with term infants (Morse et al. 2009). However, outcome studies at 12 and 18 months stress the importance of correcting for gestational age when interpreting results (Romeo et al. 2010).

Neuromorbidity in this group has been attributed to many factors. The cellular pathology of PVL and the developmental characteristics of oligodendrocytes and neurons put the late preterm brain at risk of injury. The cortical volume in the very preterm infant at 28 gestational weeks is 13% of term volume (Counsell et al. 2002). The cortical volume in the late preterm infant is only 53% of the term volume, with approximately half the volume being attained in the last 6 weeks before 40 gestational weeks. In addition, minimal myelinated white matter is present in the very preterm infant (around 29 weeks), but increases dramatically in volume as term is approached, with a fivefold increase between 35 and 41 weeks (Hüppi et al. 1996).

Studies of HIE in the preterm usually only include mature preterms and demonstrate that whilst mature preterms sustain injury to the central gray matter, the sites may differ to the more mature term brain. Mature preterm infants are

more likely to show thalamic and globus pallidum lesions. In addition, they are more likely to sustain brainstem lesions (Logitharajah et al. 2009). This may because of more severe injury or indicate a particular susceptibility of the brain stem at these slightly younger gestations (Jiang et al. 2009).

It is of interest that the late preterm infant does not demonstrate cortical abnormalities around the central sulcus, which is a frequent abnormality n the term infant. This region is probably vulnerable due to the presence of myelination, which does not occur until approximately term (Logitharajah et al. 2009).

117.6 The Term Brain

Studies of injury to the more mature term brain have concentrated on the vulnerability of the neuronal population. Imaging studies of neonates with perinatal injury complicating an acute hypoxic ischemic event demonstrate involvement of the cortex, central gray matter and brainstem. Vulnerability to specific neuronal populations in the term brain is due to multiple factors but, like white matter vulnerability in the preterm brain, excitotoxicity and oxidative stress play major roles in term injury. Vulnerability may be related to the maturational state of the neurons. There is over-expression of certain glutamate receptors in selective regions like the basal ganglia.

The N-methyl-D-aspartate (NMDA) glutamate receptor subtype is the predominant mediator of this type of injury because of its coupling to neuronal nitric oxide synthase-containing neurons in the postsynaptic density complex. The NMDA receptor can change subunit compositions with development of the NR2B subunit predominating early, followed by increasing expression of NR2A. NMDA receptors with NR2B seem to have a slower deactivation and higher conductance. Following an hypoxic insult, there are differential effects on NMDA receptor subunit composition and these effects differ by age. This interaction may result in the production of both nitrogen and oxygen free radicals that in turn injure nearby cells (McQuillen and Ferriero 2004). The vulnerability to neurons in the basal nuclei also appears to be related also to the local environment as neuronal nitric oxide synthase-containing neurons make neurons relatively resistant to severe hypoxic ischemia (Ferriero et al. 1988). There is also an overabundance of NMDA receptors in this region at term allowing for the robust glutamatergic synapses necessary for long-term potentiation and connectivity but also allowing the neuron to be more vulnerable to glutamate attack. It has been suggested that these neurons may be protected either pharmacologically or by gene knockout to render the region less vulnerable and prevent neuronal loss (Ferriero et al. 1995, 1996).

Regions of vulnerability following acute hypoxic ischemia at term are recognized as being those that are metabolically active with increased energy requirements such as the basal ganglia and thalami (Chugani et al. 1993). Regions that are actively myelinating also appear to be more vulnerable. In the term infant, regions around the myelinating corticospinal tracts arising from the cortex at the central sulcus are frequently involved. However, in less mature infants these areas are often spared the metabolic demands of myelination, which may compound the additional vulnerability of cortical neurons, which express a high number of Ca^{2+} permeable glutamate receptors at term. This contrasts with the peak period for overexpression of these receptors in oligodendrocytes and subplate neurons, which is in the late second and early third trimesters.

117.7 Focal Infarction

White matter injury is often seen in the mature term brain, presumed either to be due to a vascular event relating to vessel obstruction by an embolus or by vessel spasm. Affected areas are as middle cerebral artery infarction or more global hypoperfusion such as in parasagittal or watershed injury. Bilateral injury is often associated with a history that suggests chronic hypoxia, e.g. decreased fetal movements, infection or hypoglycemia. Injury usually involves both the

WM and the cortex. Thalamic involvement is common but may occur as a secondary phenomenon. Posterior WM may be involved more frequently than anterior WM regions, particularly in association with hypoglycemia. This may be explained in terms of watershed injury, with posterior regions representing the watershed areas for all three cerebral arteries. However posterior involvement with hypoglycemia does not always selectively involve borderzones between arterial territories.

The hypothesis of a chronic or repeated event priming the WM suggests that the WM is made progressively more vulnerable so that it can be injured by a relatively mild hypoxicischemic event. The additional role for infection in such term infants may again relate to a cascade of injurious events precipitated by a fetal inflammatory response. The underlying mechanism for this acquired increased vulnerability remains unclear, but it may involve altered mitochondrial function. When mature oligodendrocytes die after exposure to kainate, (1) AMPA receptors are the most important mediators, (2) kainate receptors play a smaller role, and (3) death occurs predominantly by necrosis, not apoptosis (Leuchtmann et al. 2003).

117.8 Programmed Cell Death

Apoptosis is a critical component of normal brain development but, as the brain is poised to initiate programmed cell death around human birth, it becomes more susceptible to the initiation of a cell death pathway. Although necrosis plays a major role in early neuronal death in both the immature and mature brains after injury, there is a spectrum of cell death that includes apoptosis within the first 24 h following perinatal hypoxic ischemia (Northington et al. 2005). Programmed cell death is a crucial mechanism in the control of the final number of neurons and glial cells. This process, commonly referred to as apoptosis, can be observed from the earliest gestational ages with different times of cell death peaks in different brain regions (Graaf-Peters and Hadders-Algra 2006). In addition to refining normal brain development and connectivity, the plasticity it provides may be important in repair mechanisms following injury. However, a predisposition to programmed cell death may be exploited following injury and result in an increase in cellular apoptosis in injured regions as a consequence of disturbances disrupted connectivity.

The concept of priming of a brain region to injury is an important one, although a region such as the white matter is not traditionally considered to be vulnerable. There is, however, animal evidence to suggest that such priming occurs with preinjury insults (i.e., delivery related events) such as hypoglycemia, intrauterine growth restriction (IUGR) and infection. The biological mechanisms by which such events increase the susceptibility of WM to injury are poorly understood but investigations of preconditioning may explain the acceleration of such events due to birth.

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