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## Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances

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### Abstract

Brain injury in premature infants is of enormous public health importance because of the large number of such infants who survive with serious neurodevelopmental disability, including major cognitive deficits and motor disability. This type of brain injury is generally thought to consist primarily of periventricular leukomalacia (PVL), a distinctive form of cerebral white matter injury. Important new work shows that PVL is frequently accompanied by neuronal/axonal disease, affecting the cerebral white matter, thalamus, basal ganglia, cerebral cortex, brain stem, and cerebellum. This constellation of PVL and neuronal/axonal disease is sufficiently distinctive to be termed "encephalopathy of prematurity". The thesis of this Review is that the encephalopathy of prematurity is a complex amalgam of primary destructive disease and secondary maturational and trophic disturbances. This Review integrates the fascinating confluence of new insights into both brain injury and brain development during the human premature period.

### Introduction

The enormity of the problem of encephalopathy in premature infants relates in substantial part to the large number of affected infants. Every year in the USA, approximately 63000 infants are born with a very low birthweight (VLBW;  $\leq 1500$  g).<sup>1</sup> This group represents 1.5% of all livebirths, a proportion that has increased gradually over the past decade. The importance of encephalopathy in this large group is indicated by the subsequent occurrence of cognitive, behavioural, attentional, or socialisation deficits in 25–50%, and of major motor deficits (eg, cerebral palsy) in 5–10%.<sup>2–8</sup> Cognitive deficits without major motor deficits are by far the dominant neurodevelopmental sequelae in infants with VLBW. Particular note should be made of the increasingly important contribution to this burden of disability by the most premature infants. Because of sharply increased survival (50–70%) in recent years, these extremely premature infants comprise a substantial proportion of infants with VLBW in many centres. Disability in this subset exceeds 50% in most studies.<sup>8–12</sup>

The neuropathological correlates of this encephalopathy include various lesions, most notably periventricular leukomalacia (PVL; figure 1), and accompanying neuronal/axonal deficits that involve the cerebral white matter, thalamus, basal ganglia, cerebral cortex, brainstem, and cerebellum. Severe germinal matrix haemorrhage–intraventricular haemorrhage (GMH-IVH), particularly with periventricular haemorrhagic infarction (PHI; figure 1), is an important, albeit quantitatively less common, lesion in premature infants. Imaging studies indicate that 50% or

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#### Conflicts of interest

I have no conflicts of interest.

more of infants with VLBW show findings consistent with PVL and apparent neuronal/axonal disease, whereas severe GMH-IVH with PHI occurs in only approximately 5%.<sup>8</sup> (Importantly, the occurrence of PHI can rise to as much as 20–30% in infants below 750 g.) Thus, the emphasis of this Review is on PVL and neuronal/axonal disease, because quantitatively, this constellation seems to account for most of the brain injury and the resulting neurological sequelae. The term “encephalopathy of prematurity” is proposed for this combination. However, the emerging role for severe GMH-IVH with PHI, especially in the smallest infants, is discussed briefly.

The pathogenesis of PVL has been reviewed in detail elsewhere,<sup>8,13</sup> and will not be discussed here. The main initiating pathogenetic mechanisms are ischaemia and inflammation, the latter often due to maternal intrauterine infection or postnatal sepsis. These two upstream mechanisms often co-exist and can potentiate each other. The main downstream mechanisms are excitotoxicity and free-radical attack. Various maturation-dependent factors, including a propensity for premature infants to experience episodes of cerebral ischaemia and infection or inflammation, and an intrinsic susceptibility to excitotoxicity and free-radical accumulation, converge to accentuate vulnerability. The cellular targets of these pathogenetic mechanisms are discussed below.

The thesis of this Review is that the encephalopathy of prematurity is a complex amalgam of primary destructive disease and secondary maturational and trophic disturbances. Recent delineation of the extraordinary array of rapidly developing neurobiological processes that occur at 20–40 weeks of gestation in the human brain provides new insights into the bases for the likely maturational/trophic disturbances. I will first review the neuropathology of the encephalopathy of the premature infant, then describe the brain developmental events that occur in the premature period, and finally discuss the likely interrelations of destructive and developmental mechanisms in the genesis of the encephalopathy.

## Neuropathology

The main neuropathological processes in the premature infant—PVL and neuronal/axonal disease—have been defined in recent years both in vivo by MRI and post mortem by advanced histological and immunocytochemical techniques. The neuropathology of severe GMH-IVH with PHI, a venous infarction, has been well delineated by conventional histological approaches and by cranial ultrasonography and is described in standard sources.<sup>8</sup>

## PVL

PVL refers to injury to cerebral white matter, classically with two components: focal and diffuse (figure 1).<sup>8</sup> The focal component consists of localised necrosis deep in periventricular white matter, with loss of all cellular elements. These necroses can be macroscopic in size (several millimetres or more) and evolve over several weeks to multiple cystic lesions, readily visualised by cranial ultrasonography and known as “cystic PVL” (figure 1). In modern neonatal intensive care units, this severe lesion is observed in less than 5% of infants with VLBW and therefore accounts for a small minority of PVL.<sup>14–18</sup> Much more commonly, focal necroses are microscopic in size and evolve over several weeks to glial scars that are not readily seen by neuroimaging. This form of PVL, which accounts for the vast majority of cases, is termed “non-cystic PVL” (figure 1).<sup>8</sup>

The second component of PVL, which is more diffusely apparent in cerebral white matter, is characterised by marked astrogliosis and microgliosis, and initially by a decrease in premyelinating oligodendrocytes (pre-OLs).<sup>19–21</sup> Subsequently, the decrease in cells of the oligodendroglial lineage is counteracted by an increase in oligodendroglial progenitors.<sup>22</sup> This response of oligodendroglial progenitors to injury in the developing brain has been shown in

several animal models.<sup>23,24</sup> However, in PVL, these cells, which often lack processes, seem not to have the capacity for full differentiation to mature myelin-producing cells, and hypomyelination with ventriculomegaly is the later sequela.<sup>22,25–33</sup> The cause of the apparent disturbance of pre-OL maturation is currently unknown, but the failure of the regenerating oligodendroglial progenitors to mature has been well documented in a neonatal animal model of PVL.<sup>34</sup> In this animal model, these progenitors are exquisitely vulnerable to a subsequent hypoxic–ischaemic insult, a common feature in premature infants. Although precise imaging and anatomical correlations are lacking, the correlates of the diffuse component of PVL on MRI in the neonatal period seem to include diffuse signal abnormalities and disturbances in diffusion parameters.<sup>2,15,17,18,35–41</sup>

### Neuronal/axonal disease

Neuronal/axonal disease is a previously under-recognised accompaniment of PVL. The regions of involvement include the cerebral white matter (axons and subplate neurons), thalamus, basal ganglia, cerebral cortex, brainstem, and cerebellum (figure 2). The neuronal/axonal involvement has been delineated, particularly in vivo, by volumetric MRI analyses, which show in infants with VLBW a decreased volume of neuronal structures such as the thalamus, basal ganglia, cerebral cortex, and cerebellum, as early as term-equivalent age, as well as later in childhood, adolescence, and adulthood. Diffusion tensor MRI studies have similarly suggested the possibility of axonal disturbance at these various maturational times. Recent neuropathological studies have provided further insight into the neuronal/axonal involvement.

**Cerebral white matter: axons**—Cerebral white matter axons (ie, projection, commissural, and association fibres) are in a phase of rapid growth during the premature period, the peak period of vulnerability for PVL. Earlier neuropathological evidence for axonal injury in PVL was derived from studies of the necrotic foci and, expectedly, included findings of axonal spheroids and positive immunocytochemical staining for beta-amyloid precursor protein, both indicators of overt axonal damage.<sup>21,42–47</sup> Of particular interest, a recent report used the apoptotic marker fractin to show that widespread axonal degeneration is present in the diffuse component of PVL, separate from the focal necroses.<sup>48</sup> Although the latter finding does not allow distinction of a primary destructive lesion from a secondary disturbance, the observation suggests that axonal abnormality with PVL is more pervasive than previously thought.

Axonal disturbance is also suggested by MRI studies of premature infants, especially by diffusion tensor MRI (figure 2).<sup>33,39,49–59</sup> These reports show that the normal rapid increase in relative anisotropy in various fibre tracts of premature infants is blunted, especially (although not exclusively) in association with the MRI appearance of non-cystic PVL. The normal increase in this measure of preferred directionality of diffusion parallel to the fibre tract is likely to relate to increased axonal size or density, or axonal microstructural changes.<sup>60</sup> However, ensheathment of axons by pre-OLs, also an active process in the premature brain, might be involved in the increase in anisotropy.<sup>49,56,60–63</sup> Another potential indicator of cerebral axonal disturbance in premature infants, especially those with PVL, is the subsequent impairment of growth of the corpus callosum, as shown by MRI.<sup>64–70</sup>

**Cerebral white matter: subplate neurons**—The major neuronal type in cerebral white matter is the subplate neuron. This transient population of neurons reaches a maximum during the peak period for the occurrence of PVL in the premature infant and is central to both cortical and thalamic development. Subplate neurons contain excitatory amino acid receptors (both NMDA and calcium-permeable [GLUR2-deficient] AMPA receptors),<sup>71</sup> and have been shown in a developing animal model to be selectively vulnerable to hypoxia–ischaemia.<sup>72</sup>

Because hypoxia–ischaemia and excitotoxicity are important in the pathogenesis of PVL, and because PVL is associated with volumetric deficits of the cerebral cortex and thalamus, it is reasonable to suggest the occurrence of concomitant injury to subplate neurons. Initial work does show increased apoptosis (activated caspase 3 expression) in the subplate of premature infants with PVL versus those without PVL.<sup>21</sup> However, more detailed studies are needed.

**Thalamus**—Thalamic neurons are commonly affected in premature infants, especially those with PVL. The most detailed neuropathological analysis of 41 premature infants from a modern neonatal intensive care unit showed that, of supratentorial structures, neuronal loss (40%) and gliosis (60%) were most common in the thalamus (figure 2).<sup>73</sup> Neuronal loss was absent in those without PVL. In a later, more detailed study of the thalamus in 22 cases of PVL, thalamic pathology, consisting of neuronal loss, gliosis, and axonal abnormality (fractin expression), was noted in 60%.<sup>74</sup> The mediodorsal and reticular nuclei were especially involved, which is of particular relevance to the neurological sequelae in premature infants.

The neuropathological findings are consistent with the finding of diminished volume of the thalamus (often measured with basal ganglia) by MRI studies of premature infants at term-equivalent age and later in childhood and adolescence (figure 2).<sup>16,18,25–29,75–78</sup> For those studies that assessed the presence of PVL by MRI, the thalamic volumetric deficit was found to be particularly characteristic of (although not always confined to) infants with imaging features of white matter injury.<sup>16,18,25</sup> The MRI abnormalities correlated with subsequent cognitive deficits.<sup>18,25,77</sup> The mechanism that underlies the neuronal loss and gliosis observed neuropathologically and the volumetric deficits observed by MRI is not clear from these studies. The findings could indicate direct injury or a maturational/trophic disturbance, or both.

**Basal ganglia**—Basal ganglia neurons are affected only slightly less commonly than are thalamic neurons, again mainly in infants with PVL (figure 2). Thus, in the recent aforementioned post-mortem study of 41 premature infants, neuronal loss was observed in the caudate and putamen in approximately 15% of infants with PVL and in none of the infants without PVL.<sup>73</sup> In infants with PVL, gliosis occurred in these basal ganglia nuclei in 50–60%. As for the thalamus, the findings do not allow distinction of a primary destructive lesion from a secondary disturbance.

MRI volumetric studies of living premature infants at term-equivalent age or older show diminished basal ganglia volumes (figure 2).<sup>16,26,28,29,76–79</sup> Of the studies done with systematic MRI in the neonatal period to identify non-cystic PVL, a clear relation of the deep nuclear deficits with the presence of white matter injury has been apparent.<sup>16</sup>

**Cerebral cortex**—Neurons of the cerebral cortex are affected less than those of the thalamus and basal ganglia (figure 2). Earlier work showed that cortical neuronal injury might accompany particularly severe forms of cystic PVL.<sup>43,80–82</sup> The neuropathological study of the modern, less severe, noncystic PVL showed that neuronal loss or gliosis, or both, could be detected in several cortical regions in 13–30% of PVL cases, but only rarely in non-PVL controls.<sup>73</sup> Whether the cortical neuronal loss and gliosis indicated a primary destructive effect or a secondary maturational/trophic effect or both could not be determined.

MRI studies of living infants with VLBW also indicate a disturbance of the cerebral cortex, especially in the presence of PVL (figure 2). Decreased volume of the cerebral cortex in premature infants with non-cystic PVL has been documented as early as term-equivalent age.<sup>16,83,84</sup> Volumetric deficits occurred in multiple cortical regions, especially parieto-occipital cortex, which overlies the region of white matter most susceptible to PVL. Premature infants studied later in childhood, adolescence, and adulthood show persisting cerebral cortical

volumetric deficits.<sup>26–29,32,79</sup> The most pronounced decreases generally occur in parieto-occipital, sensorimotor, premotor, temporal, and hippocampal cortices.<sup>26,27,29,77,78,85,86</sup> These cortical neuronal deficits correlate with a wide variety of cognitive deficits observed at follow-up.<sup>26,32,77–79,84,86</sup>

**Cerebellum (and brainstem relay nuclei)**—Cerebellar abnormality is particularly characteristic of premature infants with VLBW. The aforementioned neuropathological analysis of 41 premature infants identified neuronal loss in the dentate nucleus and the cerebellar cortex in 25–30% of the infants (figure 2).<sup>73</sup> In the cerebellar relay nuclei, basis pontis, and inferior olive, neuronal loss was found in 15–20%. However, gliosis was more common, identified in the cerebellar cortex and dentate in 30–45%, and in the pons and olive in 90–100% (figure 2). The abnormalities were generally more likely to occur in the presence of PVL than in non-PVL, but in non-PVL cases, gliosis in the pons and olive did occur in 80–90%. Of note, PVL involving cerebellar white matter was unusual, occurring in only 8% of the infants. More detailed neuropathological study of the cytological characteristics of the cerebellar abnormality is needed.

MRI studies have been particularly valuable in the identification of cerebellar disease in premature infants. Cerebellar involvement has consisted most often of bilateral, generally symmetric, decreases in cerebellar hemispheric volumes at term-equivalent age or later in childhood or adolescence (figure 2).<sup>26,87–99</sup> A strong correlation with supratentorial lesions, particularly PVL, but also haemorrhagic lesions (eg, PHI), has been documented.<sup>93–97</sup> In two MRI studies that analysed pontine size, both pontine diameter and cerebellar volume were reduced in premature infants with PVL (figure 2).<sup>92,98</sup> In unilateral cerebral lesions, decreased volume of the contralateral cerebellar hemisphere has been greater than the decrease in the ipsilateral cerebellar hemisphere, consistent with an element of crossed cerebellar diaschisis.<sup>93</sup>

### Clinico-pathological correlations

Definition of specific clinico-pathological correlations in premature infants has been difficult, mainly because of a relative paucity of (1) detailed high-resolution, regional neuroimaging, (2) careful correlative neuropsychological studies, and (3) co-occurrence of white matter and neuronal/axonal disease. However, some important general conclusions seem warranted. With regard to PVL, cystic PVL probably accounts for the small group of infants who show spastic diplegia.<sup>8</sup> Non-cystic PVL correlates with the cognitive deficits observed later, usually in the absence of major motor deficits.<sup>18,41</sup> However, the full spectrum of cognitive, attentional, behavioural, and socialisation deficits is likely to relate in major part to neuronal/axonal disease. Such deficits include impairments in overall intelligence, object working memory, various executive functions, impulse control, and some characteristics of autistic spectrum disorders.<sup>2,3,7,77,78,85,87,100–102</sup> Initial correlations with deficits in volumetric development of the cerebral cortex, thalamus, basal ganglia, and cerebellum have been made. The correlations are consistent with studies in older children that concern the roles not only of the cerebral cortex, but also of the dorsomedial and reticular nuclei of the thalamus, the basal ganglia, and the cerebellum in this cognitive spectrum.<sup>103–107</sup>

### Brain development during the premature period

The neuropathology of brain injury in the premature infant as described above occurs against a background of multiple active developmental events that take place at 24–40 weeks of gestation and involve pre-OLs, microglia, axons, subplate neurons, the proliferative cerebral dorsal subventricular zone (SVZ) and ventral germinative epithelium of the ganglionic eminence (GE), thalamus, cortex, and cerebellum. Because of the very active and complex characteristics of these events, they are likely to be vulnerable to exogenous and endogenous



insults, such as ischaemia, inflammation, excitotoxicity, and free-radical attack. The possibilities of vulnerability to certain drugs, hormones, undernutrition, or other facets of neonatal intensive care deserve further study. This concept of enhanced vulnerability of rapidly developing events during brain maturation was postulated and corroborated by the classic studies of the effects of infantile undernutrition by Dobbing and colleagues nearly 40 years ago.<sup>108,109</sup>

### pre-OLs

pre-OLs, which have been shown to be a key cellular target in PVL, are in a phase of active development during weeks 24–40 of gestation.<sup>8,110–114</sup> The four sequential stages of oligodendroglial maturation include the oligodendroglial progenitor, the pre-oligodendrocyte (or late oligodendroglial progenitor; positive for monoclonal antibody O4), the immature oligodendrocyte (positive for monoclonal antibodies O4 and O1), and the mature myelin-producing oligodendrocyte (positive for myelin basic protein). Pre-oligodendrocytes and the immature oligodendrocytes are referred here together as pre-OLs. These differentiating forms (especially the O4/O1-positive immature oligodendrocytes) ensheath axons in preparation for full differentiation to myelin-producing oligodendrocytes (figure 3). Mature, myelin-basic-protein-expressing and ultimately myelin-producing oligodendrocytes do not become abundant in cerebral white matter until after term.

During the peak period of PVL, the O4-positive late oligodendroglial progenitors predominate in cerebral white matter and at 28 weeks account for 90% of the total oligodendroglial population.<sup>114</sup> At 28–40 weeks of gestation, the O4-positive cells begin differentiation to O1-positive immature oligodendrocytes, which account for approximately 30% of the total oligodendrocyte population during the later premature period and about 50% by term. These two early differentiating forms show maturation-dependent characteristics that render them especially vulnerable to injurious insults, such as ischaemia and inflammation, which lead to excitotoxicity and generation of free radicals. These pre-OL characteristics include enhanced vulnerability to the following factors: (1) reactive oxygen and nitrogen species, because of impaired antioxidant defences; (2) excitotoxicity, because of exuberant expression of calcium-permeable glutamate receptors (ie, GLUR2-deficient AMPA receptors, preferentially on cell bodies, and NMDA receptors, preferentially on cell processes), and enhanced expression of the main glutamate transporter, which can become a source of injurious glutamate; and (3) cytokine injury, because of both expression of the interferon-gamma receptor on the pre-OL in the context of pronounced availability of interferon gamma in the abundant astrocytes of PVL, and sensitivity to injury by tumour necrosis factor, which is secreted by the abundant activated microglia.<sup>8,13,19,20,71,115–125</sup>

### Microglia

Microglia have key roles during brain development, involving apoptosis, vascularisation, axonal development, and myelination.<sup>126–129</sup> Accordingly, these cells become prominent in the forebrain at 16–22 weeks of gestation,<sup>130–133</sup> and reach a peak abundance in cerebral white matter in the third trimester.<sup>133</sup> Microglia seem to be key effectors of cellular injury with both ischaemia and inflammation.<sup>8</sup> These cells generate free radicals, secrete injurious cytokines, and enhance excitotoxicity.<sup>117–121,131,134–136</sup> Because microglia are particularly abundant in normal cerebral white matter, they are in the right place at the right time in large numbers to lead to injury to white matter constituents (ie, pre-OLs), but also to axons and subplate neurons.<sup>133</sup> Not surprisingly, many activated microglia are present diffusely in cerebral white matter in association with preOL injury in PVL.<sup>19</sup>

## Axons

Axonal development is remarkably exuberant in the developing cerebrum over the last trimester of gestation and in the early postnatal period (figure 3). Haynes and colleagues<sup>126</sup> used an immunocytochemical approach, with staining for growth-associated protein-43, which is expressed on growing axons, to show pronounced expression in the cerebral white matter to the region of the subplate at 20 weeks, to the subplate and cortex at 27 weeks, and abundantly within the cortex at 37 weeks. These striking findings are consistent with detailed studies by Kostovic and coworkers<sup>137–140</sup> of the axonal connections to and from the subplate during the last half of gestation (panel 1).

## Subplate neurons

This crucial transient population of neurons reaches its peak size and maximum developmental impact at 24–32 weeks of gestation, the peak period for occurrence of PVL (figure 3).<sup>137–141</sup> These neurons are largely glutamatergic, originating in the dorsal telencephalic ventricular zone, and fewer are GABAergic, originating in the ventral telencephalic GE. Development of the subplate is inter-twined closely with the cerebral cortex, subcortical structures (especially thalamus), and axons (projection, commissural, and association; panel 1). The crucial main roles of subplate neurons are to serve as sites of synaptic contacts for so-called “waiting” thalamocortical and commissural/association cortico-cortical afferents before differentiation of the cortical plate, to serve as a functional link between these waiting afferents and their cortical targets, to provide axonal guidance into the cerebral cortex for the ascending afferents, to facilitate cerebral cortical organisation and synaptic development, and to provide pioneering axonal guidance for projections from the cortex to subcortical targets (eg, the thalamus).<sup>8, 137, 142–149</sup>

## SVZ (and cortical GABAergic neurons)

The SVZ is derived mainly from radial progenitors (radial glial cells) and consists of so-called intermediate progenitors (figure 3, panel 2).<sup>141</sup> Although some studies had suggested that the SVZ gives rise primarily to glia, recent studies show that early SVZ progenitors are largely neurogenic.<sup>141, 150–153</sup> These early intermediate precursors produce neurons, particularly for deeper cortical layers. These cells reach the cortex by radial migration before the premature period. Moreover, the previous notion that the SVZ is gliogenic after the early phases of neuronal proliferation is not accurate for the more complex brains of primates.<sup>141</sup> Indeed, after the 20th gestational week and extending into at least weeks 25–27, the SVZ actively generates neurons, mainly GABAergic interneurons for the upper cortical layers, the hallmark of the human cortex. Bystron and colleagues thus concluded that the SVZ becomes the main source of cortical neurons in the expanded human cerebrum.<sup>141</sup> These later arriving neurons are generated largely (65%) from the dorsal telencephalic SVZ and migrate radially, although approximately 35% are generated from the ventral GE and migrate first tangentially, parallel to the cortical plate, to the region of the dorsal SVZ, from which they migrate radially to the cortex (figure 3).<sup>141, 152, 154</sup> The origin of 65% of cortical GABAergic interneurons from the dorsal telencephalic SVZ is characteristic of the human brain (unlike the rodent brain), and seems to be critical for the development of the expanded upper cortical layers. When the generation of GABAergic neurons in the SVZ ceases after 27 weeks is unknown, but the SVZ per se is clearly a prominent structure during the entire premature period.<sup>155</sup>

## Thalamus

The thalamus receives its initial neurons early in the second trimester from the diencephalic ventricular zone.<sup>141</sup> However, recent data show that there is a second, later wave of neurons that are generated in the ventral telencephalic GE and migrate to the dorsal thalamus (figure 3).<sup>141, 154, 156</sup> These neurons are mainly GABAergic and migrate by homotypic–neurophilic

interactions. In the primate brain, approximately 30% of the neurons in every thalamic nucleus are GABAergic.<sup>157,158</sup> This population of telencephalon-derived dorsal thalamic neurons are unique to the human brain, and might lead to a specific increase in the population of GABAergic neurons in the large association nuclei (ie, the mediodorsal and pulvinar nuclei).<sup>141</sup> As mentioned previously, the mediodorsal nucleus in particular shows neuronal loss and gliosis in premature infants with PVL. In human beings, these unique telencephalon-derived neurons are probably linked to the expansion of the thalamic association nuclei, which are in turn anatomically related to the enlargement of association cortices involved in multiple higher cognitive functions.<sup>156</sup> The timing of this critical later development of the thalamus is not entirely known, but probably occurs during a long period from 15 weeks to approximately 34 weeks of gestation.<sup>141,156</sup>

**Panel 1: Development of human subplate and cerebral axons**

**<20 weeks**

- Subplate layer apparent at approximately 10 weeks
- Invasion of subplate by thalamic afferents (waiting afferents)
- Increase in size of subplate from 10 to 20 weeks

**20–24 weeks**

- Thalamic afferents abundant in subplate, with glutamatergic and GABAergic synapses on subplate neurons
- Axons (projection, commissural, and association) grow actively, especially in periventricular regions

**24–32 weeks**

- Thalamocortical afferents enter cortex
- Callosal (commissural) and association (cortico-cortical) axons enter subplate
- Subplate reaches maximum size (4–5 times thicker than cortical plate at 27–30 weeks)

**32–36 weeks**

- Callosal and cortico-cortical fibres enter cortex
- Subplate layer gradually decreases

Data based primarily on studies of Kostovic and co-workers.<sup>137–140</sup>

**Panel 2: Human SVZ—recent concepts**

- SVZ previously thought to appear late in gestation and to produce mainly glia
- SVZ in human beings appears early in gestation and early SVZ is mainly neurogenic
- Early SVZ progenitors give rise to neurons of deeper cortical layers
- Later SVZ progenitors give rise to neurons (especially GABAergic interneurons) of the expanded upper cortical layers, a hallmark of human cortex
- SVZ is still proliferating at 25–27 weeks' gestation (and probably later)



Summary from Bystron and co-workers.<sup>141</sup> SVZ=subventricular zone.

### Cerebral cortex

Most, but not all, of the neurons of the cerebral cortex have migrated from the proliferative dorsal telencephalic ventricular/subventricular zones before 24 weeks of gestation.<sup>8,141</sup> Subsequent events include development of regional, laminar, and cytological complexity. During weeks 24–32 of gestation, synapses become apparent in the deep cortical plate as thalamocortical axons exit the subplate and enter the cortex (panel 1).<sup>139</sup> The permanent sensory-driven circuitry of specific cortical areas also begins to evolve at this time.<sup>139,140</sup> Parallel acceleration of dendritic differentiation becomes prominent. Indeed, this dendritic development and the extensive elaboration in the cortex of afferent axonal terminals from thalamic, associative, and commissural fibres that enter the cortex after synapsing on subplate neurons leads to the striking four-times increase in cerebral cortical volume documented from 28 to 40 weeks' post-conceptual age by volumetric MRI.<sup>137,159,160</sup> Thus, this critical phase of cortical development occurs simultaneously with the premature period.

A crucial feature of this development is the disproportionate increase in thickness of upper cortical layers.<sup>141</sup> This thickening results because of later-arriving GABAergic interneurons from the dorsal SVZ and the ventral GE, as described above (figure 3). The time of termination of this process of GABAergic cortical neuronal proliferation and migration is unknown, but probably extends well into the third trimester, as noted above with regard to the SVZ.<sup>141,152</sup> In association with the expansion of the superficial cortical layers, the increase in cortical surface area and rapid gyral development documented by MRI become apparent.<sup>8</sup>

### Cerebellum

The cerebellum develops especially rapidly in the last half of human gestation. This fundamental finding was emphasised particularly by the work of Dobbing and co-workers.<sup>109,161</sup> Volumetric MRI study of premature infants has documented an approximately three-times increase in cerebellar volume from 28 to 40 weeks' gestation.<sup>95</sup> Indeed, the rate of overall growth during this period exceeds that of the cerebral cortex, and both neuronal proliferation and migration are prominent (figure 4).<sup>162,163</sup>

Major developmental events in the period before 24 weeks of gestation include establishment of the two proliferative zones, the ventrally located ventricular zone and the more dorsal rhombic lip.<sup>162,164</sup> Analogous to the ventral and dorsal telencephalic proliferative zones, the former gives rise to GABAergic neurons and the latter to glutamatergic neurons. The GABAergic neurons originating in the ventricular zone migrate radially to form the roof nuclei, the Purkinje-cell layer, and the molecular layer. Of note, the late-migrating GABAergic neurons, destined to be basket and satellite cells of the molecular layer, also proliferate in cerebellar white matter during their migration, probably well into the premature period.<sup>164</sup> The glutamatergic neurons originating in the rhombic lip largely migrate tangentially along the cerebellar surface to form the granule precursor cells of the external granule cell layer (figure 4; some rhombic lip neurons also migrate to the roof nuclei and to the pontine and olivary nuclei).

By 25 weeks, a prominent external granule cell layer is apparent (figure 4).<sup>162,163</sup> The layer contains two discrete zones: inner and outer. The outer zone, contiguous with the subarachnoid space and cerebrospinal fluid (CSF), actively proliferates during the premature period, whereas the inner zone contains post-mitotic cells that will migrate inwards to form the internal granule cell layer. These neurons migrate radially along the processes of the Bergmann glia of the Purkinje-cell layer. The proliferative activity of the outer zone of the external granule cell layer is under the control of sonic hedgehog homologue, which is secreted by Purkinje cells.<sup>164</sup>

These events are remarkably active during the entire premature period. After 40 weeks, the external granule cell layer becomes less prominent, neuronal differentiation is active, and axonal outflow from the roof nuclei develops rapidly. Thus, the most dramatic events during the 25–40-week period occur at the surface of the cerebellum, especially the establishment of the external granule-cell layer by tangential migration and enlargement of this cell layer by proliferation, culminating finally in the inward (radial) migration of these neurons to form the densely packed internal granule cell layer (figure 4).

## Combination of destructive and developmental disturbances

The ultimate degree of brain abnormality in survivors of premature birth is likely to depend on a combination of destructive and impaired trophic/maturation mechanisms. The relative importance of these two mechanisms and the nature and extent of their interactions are central issues. The trophic/maturation mechanisms include cell–cell interactions that can involve intercellular trophic support, retrograde effects, and anterograde effects (eg, Wallerian degeneration, trans-synaptic degeneration), among others.

For the discussion of these processes, I will focus on PVL and its accompanying neuronal/axonal abnormalities (ie, the encephalopathy of prematurity). The emphasis will first be on the supratentorial abnormalities. The cerebellar disturbance, an important component of the encephalopathy and also most common with PVL, is then discussed.

### PVL and neuronal/axonal disease: encephalopathy of prematurity

In PVL, the primary event is most likely to be a destructive process (injury) and the subsequent trophic/maturation (ie, developmental) disturbances are secondary. The following discussion will focus on the quantitatively more prominent diffuse component of PVL. The focal component of modern PVL consists of microscopic areas of necrosis. These areas of necrosis involve all cellular elements, and thus focal loss of pre-OLs, axons, and perhaps late-migrating interneurons are to be expected. The consequences will be similar to, but quantitatively less than, the wider cellular effects of diffuse PVL. On the basis of available data, five potential scenarios concerning the primary and secondary events in supratentorial structures in diffuse PVL seem most likely (figure 5). Of these, the first is best supported by available data. However, because rigorous studies in human infants of the other four scenarios are relatively sparse, it is quite possible that all scenarios are operative and that the degree to which one or the other predominates in a given infant varies substantially.

**pre-OL injury**—The pre-OL seems to be the main cellular target in the diffuse component of PVL (figure 5 and figure 6). This vulnerability of pre-OLs has been shown not only in human PVL,<sup>19–21</sup> but also in many excellent animal models.<sup>8,20,165–168</sup> Injury of pre-OLs consists of cell loss or process loss (with intact soma).<sup>22</sup> The cell loss mainly relates to activation of calcium-permeable AMPA receptors on the cell soma,<sup>71,169–179</sup> and the process loss, with intact soma, to activation of NMDA receptors on pre-OL processes.<sup>8,124,180–183</sup> The ultimate result of either event would be a deficit of mature oligodendroglia, and a consequential impairment of myelination, the hallmark of PVL (figure 5).

However, pre-OL injury could also lead to failure of axonal development and ultimately axonal degeneration. The critical trophic role of oligodendrocytes for axonal development, survival, and function is well established in experimental models.<sup>62,184–194</sup> The remarkable exuberance of axonal growth during the premature period suggests a particular need for trophic support at this time. Impaired axonal development and axonal degeneration would be consistent with diffusion-based MRI studies of cerebral white matter in premature infants that show abnormalities consistent with axonal deficiency.<sup>33,39,49–55</sup> The consequences of axonal deficiency would be diminished cerebral cortical and thalamic/basal ganglia volumes

secondary to retrograde and anterograde (trans-synaptic) effects (ie, projection fibres to and from the cortex, the thalamus, and the basal ganglia; figure 5 and figure 6).

**Axonal injury**—Axonal injury has been recognised 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.<sup>48</sup> Axonal injury has been described in experimental models of hypoxic–ischaemic injury analogous to PVL.<sup>195–198</sup> 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 and thalamic/basal ganglia volumes (figure 5 and figure 6). The active axonal development in cerebral white matter in premature infants could make these fibres particularly vulnerable.

**Thalamic injury**—The recent observations that neuronal loss and gliosis are more common in the thalamus than in other brain regions in human PVL is consistent with either primary injury or secondary anterograde and retrograde trophic effects.<sup>73,74</sup> If primary neuronal injury occurs, the secondary effects would involve white matter axons, with subsequent hypomyelination and impaired development of cerebral cortex and thalamus/basal ganglia (figure 5 and figure 6). To date, no experimental studies have investigated the possibility of primary injury. However, human neuropathological data are of particular interest.

**Subplate neuronal injury**—The pivotal role of subplate neurons in the development of the cerebral cortex and deep nuclei in the human premature brain suggests that injury to these key transient cells could have far-reaching secondary trophic/maturational effects. As noted earlier, initial data show increased apoptosis in the subplate of infants with PVL.<sup>21</sup> If this were a primary destructive event, secondary retrograde effects on afferent white matter axons and their originating neurons in the cerebral cortex and thalamus and anterograde efferent effects on developing cerebral cortical neurons could be substantial (figure 5 and figure 6). These suggestions are supported by abundant experimental data.<sup>8,137,142–149</sup> The axonal degeneration would be accompanied by subsequent hypomyelination, as discussed for pre-OLs and axonal injury. In a neonatal rat model of hypoxic–ischaemic injury and PVL, selective subplate neuronal death was identified.<sup>72</sup>

**SVZ and late-migrating neurons**—Although it is possible that the dorsal telencephalic SVZ is affected in PVL, supporting data in human infants are lacking. Experimental studies suggest that progenitors in the dorsal telencephalic SVZ are vulnerable to major hypoxia–ischaemia,<sup>199</sup> but in models of selective white matter injury similar to PVL, the SVZ responds by generating oligodendroglial progenitors after the insult.<sup>23,24</sup>

Although it seems unlikely that the SVZ is injured in PVL, one report suggests that late-migrating GABAergic neurons are affected (figure 5 and figure 6).<sup>21</sup> This small neuropathological study of human premature infants with PVL showed a blunting of the normal increase in GABAergic neurons in the cerebral white matter after 28 weeks postconception and an overall diminution of white matter GABAergic neurons.<sup>21</sup> Whether this phenomenon indicates decreased generation in the SVZ or injury during the neurons' late migration is unknown (figure 5 and figure 6), although the latter seems to be more likely. Because these GABAergic interneurons contribute particularly to the thickness of upper cortical layers, a blunting or diminution of this migration could have important structural and functional consequences.

**Cerebellum**—The prominent disturbance in cerebellar growth in premature infants, which occurs particularly in association with PVL, is another vivid example in the human brain of a

rapidly developing process that shows vulnerability. This vulnerability of the cerebellum during its phase of rapid growth was documented several decades ago in experimental models of undernutrition, x-irradiation, and glucocorticoid exposure.<sup>108,109,200,201</sup> Diminished DNA content was the main outcome. More recent work has shown a similar vulnerability of the cerebellum in fetal sheep and neonatal rats subjected to hypoxia–ischaemia.<sup>202,203</sup> Apoptosis of neuronal elements was the main outcome.

The fundamental nature of the cerebellar disturbance in premature infants is unclear. Except for the minority of infants who have cerebellar haemorrhage, overt tissue destruction is absent. A strong relation of the cerebellar growth failure with supratentorial white matter lesions, especially PVL, suggests that trophic interactions between the cerebrum and cerebellum might be operative. The excitatory interaction between the cerebellum and cerebral cortex via corticopontine tracts and then pontocerebellar connections might be crucial for cerebellar development.<sup>93</sup> Of note, the brainstem cerebellar relay nuclei (pontine and inferior olivary nuclei) show gliosis in 90–100% of premature infants with PVL.<sup>73</sup> In addition, trophic interactions between the cerebellum and cerebrum, presumably via cerebello–rubro–thalamo–cortical connections, can also be shown in premature infants.<sup>93</sup> Because of the prominent thalamic disease in infants with PVL, negative retrograde effects on cerebellar growth might occur.

The occurrence of marked cerebellar growth failure in premature infants has been almost completely confined to infants of less than 32 weeks' gestation and most commonly of 24–28 weeks' gestation. The most striking developmental event in the cerebellum at this time, the proliferation and inward radial migration of external granule cells (figure 4), is centred on the surface of the cerebellum. Indeed, the outermost portion of the external granule layer contains the proliferating cells. Exposure of these cells to noxious compounds in the CSF (eg, free radicals,<sup>204</sup> blood products [non-haem iron, haemosiderin],<sup>94,205–207</sup> and proinflammatory cytokines<sup>208</sup>) could be deleterious.

Thus, the negative effects on cerebellar growth in the small premature infant could relate to direct effects on the cerebellum and to effects on cerebellar connections. Concerning the latter, the negative effects could relate both to loss of positive afferent effects from the cerebrum and brainstem cerebellar relay nuclei, and to development of negative retrograde effects from loss of efferent connections to the thalamus and cerebrum. The direct effects could be multiple, but a disturbance in the proliferation of granule precursor cells in the external granule-cell layer seems most likely.

### GMH-IVH with PHI

This severe form of GMH-IVH, associated with PHI, is unilateral or grossly asymmetric and accounts for most of the neurological disability related to the entire spectrum of GMH-IVH.<sup>8</sup> Although this type of lesion occurs in only 4–5% of all premature infants with VLBW, the incidence in the most immature infants is markedly higher at 20–30% in those born at 24–26 weeks' gestation or below 750 g birthweight.<sup>14,209,210</sup> With rapidly increasing survival for these immature infants, the problem of GMH-IVH with PHI has increased markedly.<sup>8</sup> The haemorrhage originates and destroys the germinal matrix, referred to earlier as the ventral telencephalic GE (figure 7 and figure 8). The associated venous infarction (the PHI) destroys the dorsal telencephalic SVZ and the overlying cerebral white matter, including preOLs and axons (figure 7 and figure 8). The consequences of this constellation are a combination of primary destructive and secondary maturational disturbances. The destruction of cerebral white matter axons and pre-OLs results in a large area of tissue loss (ie, a porencephalic cyst). Thalamocortical fibres are interrupted,<sup>55</sup> and overlying cortical development is impaired.<sup>81</sup> Other effects are likely to include disturbance of the late GABAergic neuronal proliferation and migration from the SVZ and the GE to upper layers of the cerebral cortex and from the

GE to the thalamus (figure 7 and figure 8). Some neuropathological data in human infants support the former contention.<sup>211</sup>

## Conclusions

Brain abnormality in the premature infant is unlikely to consist of a straightforward addition of destructive non-haemorrhagic and haemorrhagic lesions, such as PVL and, less commonly, GMH-IVH with PHI. Recent insights into the full spectrum of the encephalopathy of prematurity and into the remarkable series of developmental events that occur in the brain during this period indicate a complex amalgam of destructive and developmental mechanisms. Although further clarification of this amalgam is needed, the general principle that in the premature period brain abnormality involves destructive and developmental mechanisms seems established.

### Search strategy and selection criteria

References for this Review were obtained from personal reprint files, supplemented by PubMed searches, with varying search periods (from 1980 to November, 2008). PubMed searches were initiated with all the topical areas covered in the Review. The full list of search terms is available from the author on request and included "periventricular leukomalacia", "cerebral white matter injury", "subplate neurons", "cerebral cortex", "axonal development", "oligodendroglial development", "neuronal development", "glial-neuronal interactions", "glial-axonal interactions", "GABAergic neurons", and "subventricular zone" ("prematurity" and "human brain" were frequent modifiers). The bibliographies of the most recent articles were also screened to find other previously unidentified articles. Only English language articles were included.

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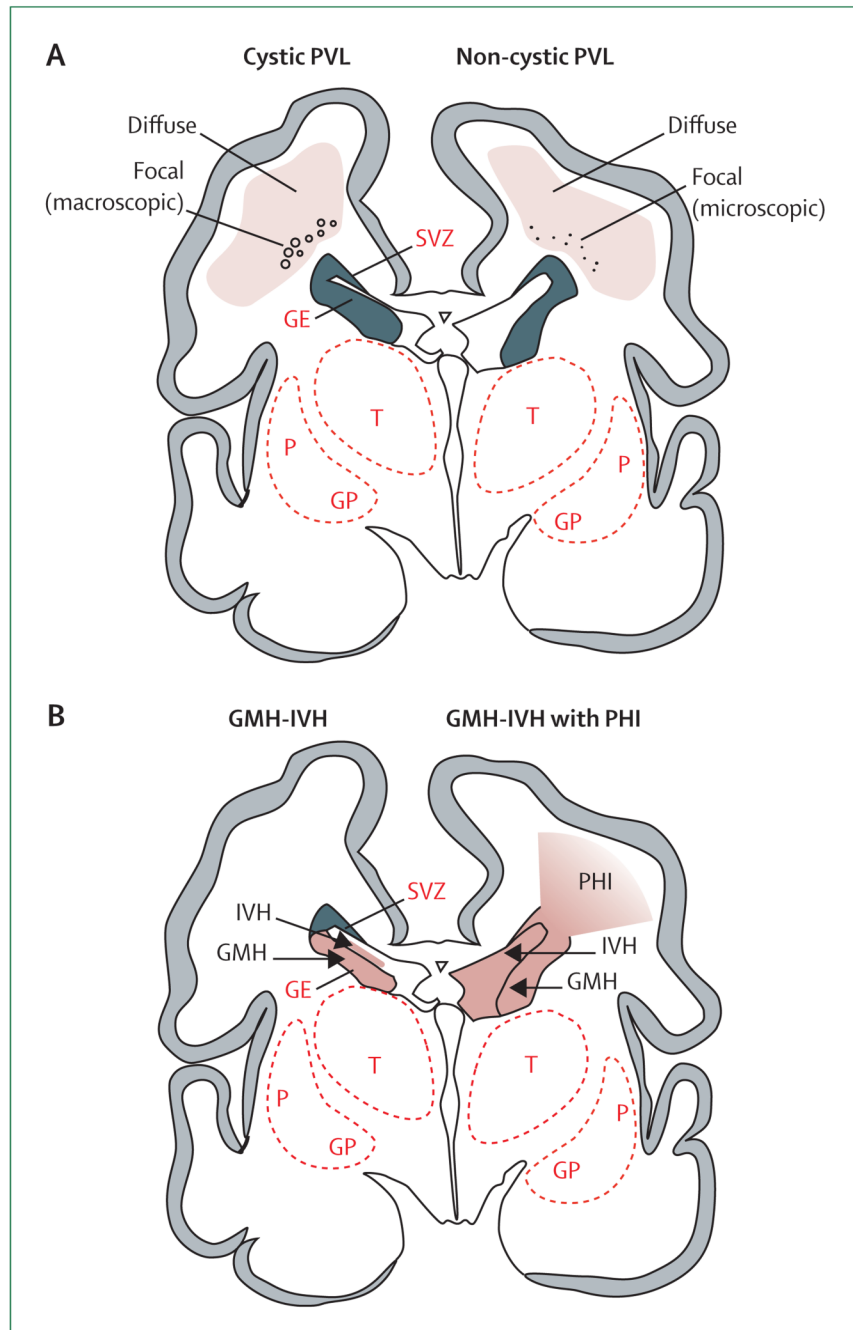
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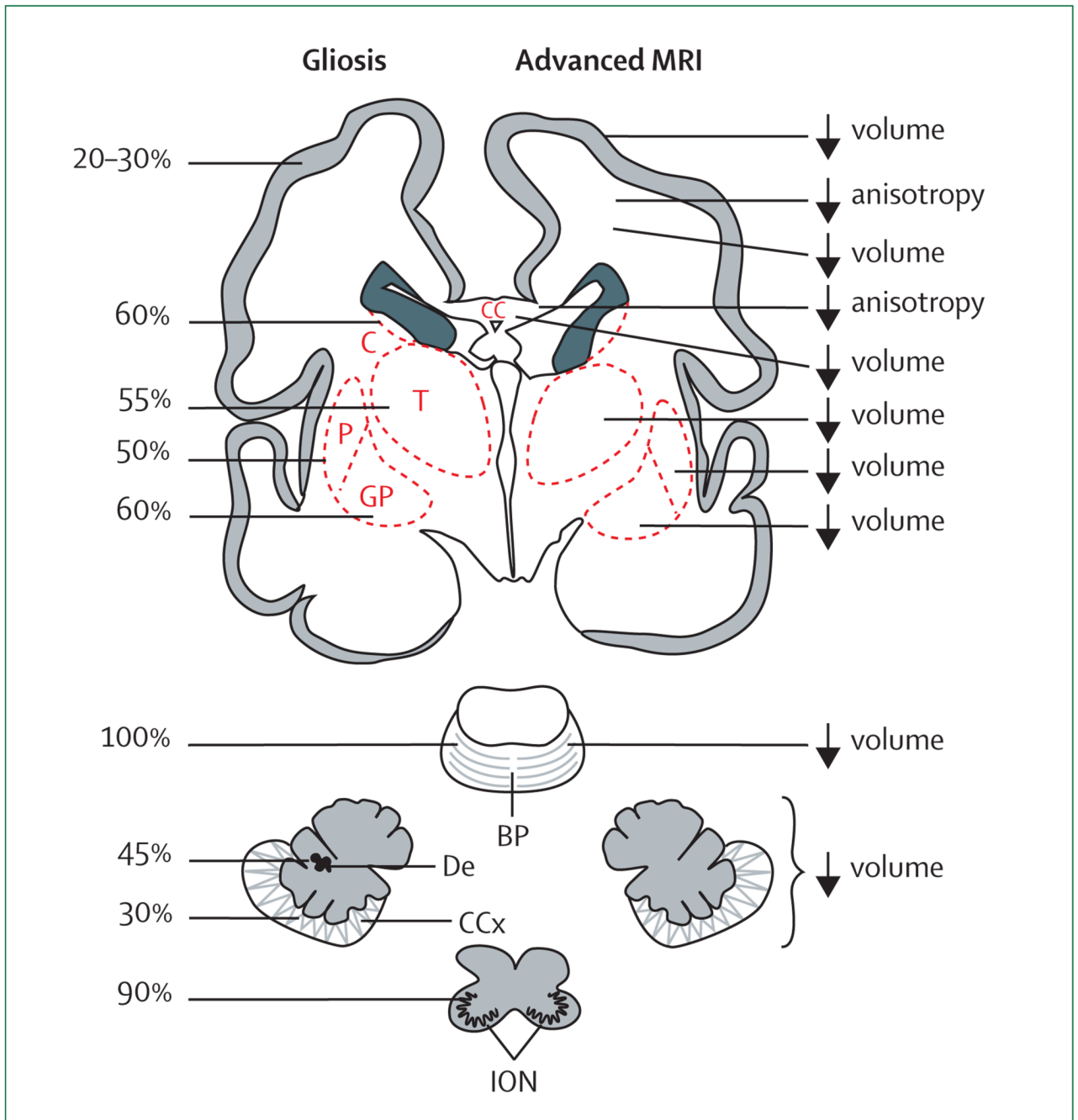
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**Figure 1. Cystic and non-cystic periventricular leukomalacia (PVL) and germinal matrix haemorrhage–intraventricular haemorrhage (GMH-IVH) and GMH-IVH with periventricular haemorrhagic infarction (PHI)**

Coronal sections from the brain of a 28-week-old premature infant. The dorsal cerebral subventricular zone (SVZ), the ventral germinative epithelium of the ganglionic eminence (GE), thalamus (T), and putamen (P)/globus pallidus (GP) are shown. (A) The focal necrotic lesions in cystic PVL (small circles) are macroscopic in size and evolve to cysts. The focal necrotic lesions in non-cystic PVL (black dots) are microscopic in size and evolve to glial scars. The diffuse component of both cystic and non-cystic PVL (pink) is characterised by the cellular changes, as described in the text. (B) Haemorrhage (red) into the GE results in GMH, which

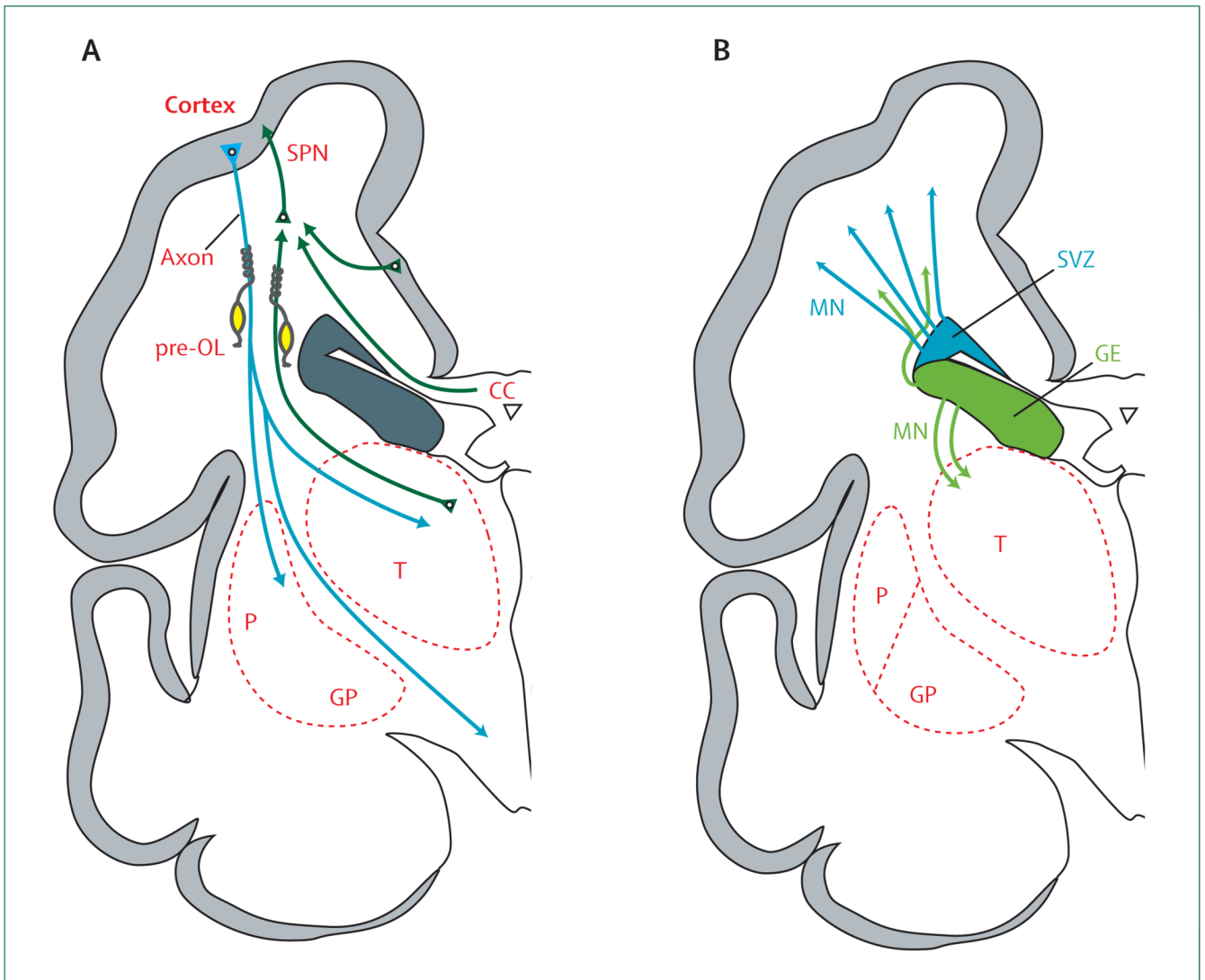
could burst through the ependyma to cause an IVH (left). When the GHM-IVH is large, PHI might result (right).



**Figure 2. Main neuronal/axonal structures affected in premature infants with periventricular leukomalacia**

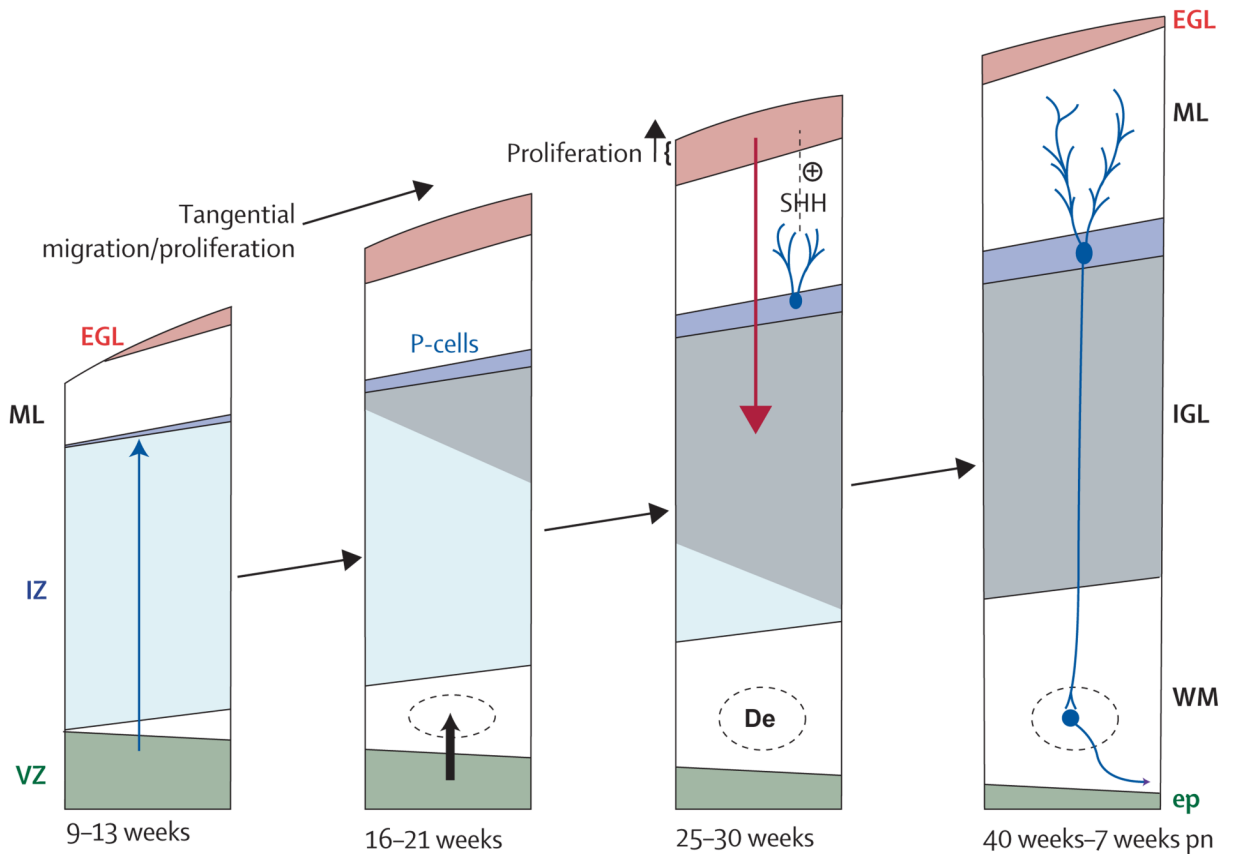
Coronal sections of the cerebrum, pons, cerebellum, and medulla (inferior olivary nuclei) are shown. The frequency of gliosis by neuropathological study and the major abnormalities detected by advanced MRI (volumetric and diffusion-based MRI) are shown. See text for details. BP=basis pontis. C=caudate. CC=corpus callosum. CCx=cerebellar cortex. De=dentate. GP=globus pallidus. ION=inferior olivary nuclei. P=putamen. T=thalamus.





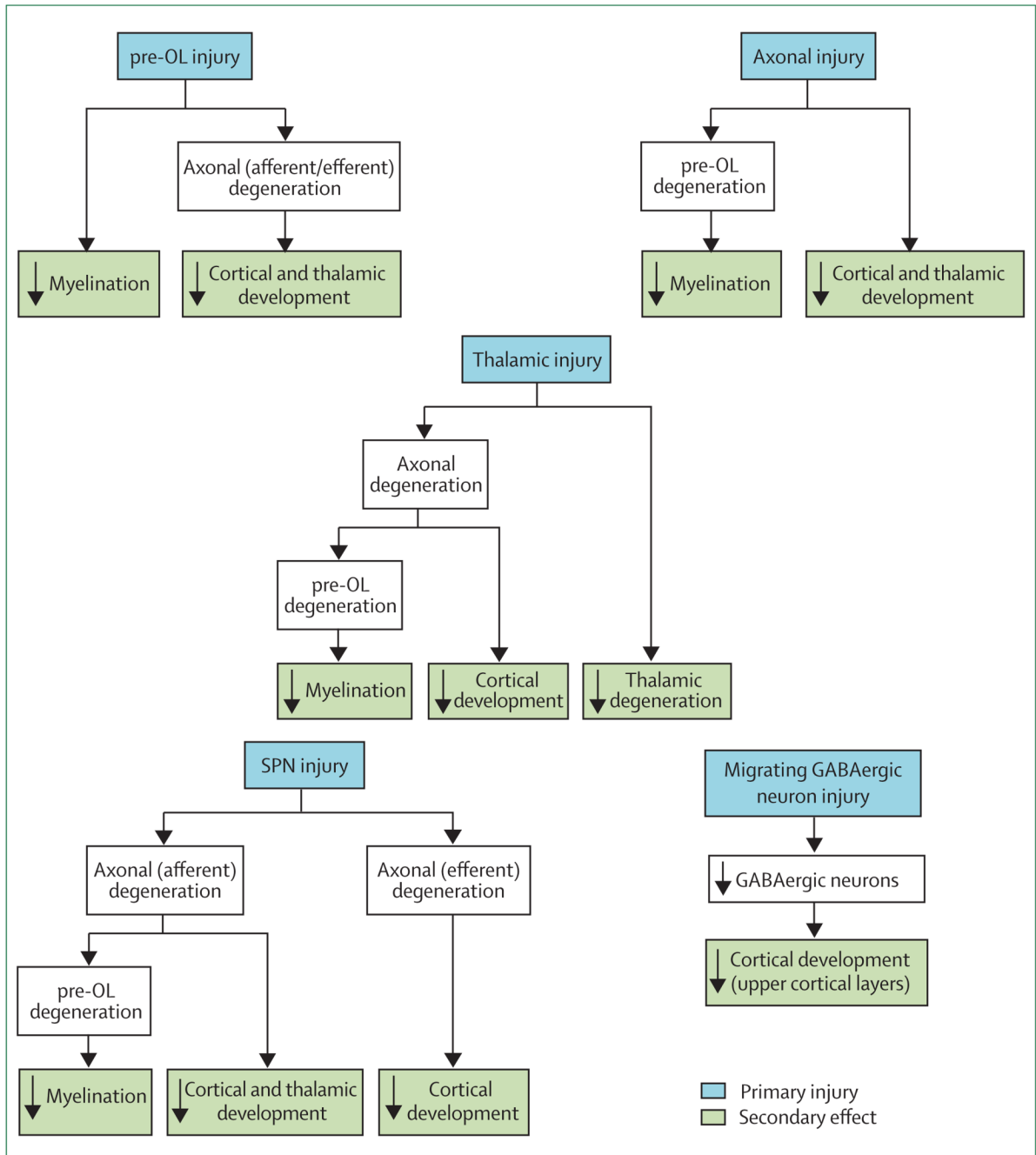
**Figure 3. Cerebrum in coronal section at 28 weeks' gestation showing critical events in cortical development**

(A) The axons (green) emanate from the thalamus (T; projection fibres), corpus callosum (CC; commissural fibres), and cortex (association fibres), which synapse initially on subplate neurons (SPNs). SPNs send axons to the cortex and promote cortical development before the thalamo-cortical and cortico-cortical fibres enter the cortex. From the cortex, axons (blue) descend to the thalamus, basal ganglia, and corticospinal (and corticopontine) tracts. Premyelinating oligodendrocytes (pre-OLs; yellow) ensheath axons before full differentiation to mature myelin-producing oligodendrocytes. (B) The proliferation and migration of GABAergic interneurons from the subventricular zone (SVZ) and ventral germinative epithelium of the ganglionic eminence (GE) are shown. Neurons from the SVZ (blue) migrate radially to the cortex and from the GE (green), tangentially and then radially, to the cortex. The migrating stream of interneurons from the GE to the dorsal thalamus is also shown. GP=globus pallidus. MN=migrating neurons. P=putamen.



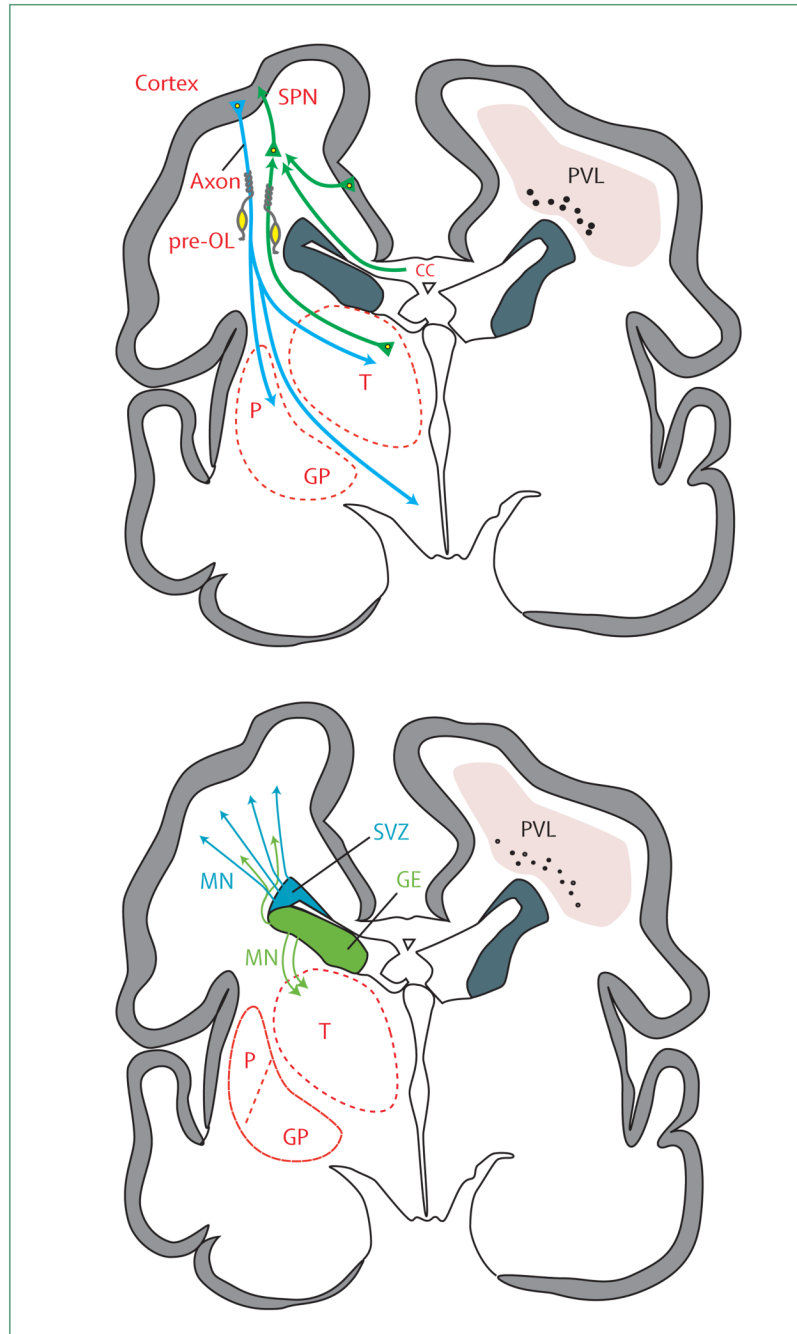
**Figure 4. The developing cerebellar cortex at four major time periods from 9 weeks' gestation to 7 weeks into the postnatal period**

The two proliferative zones are the ventricular zone (VZ) and the external granule cell layer (EGL) derived from the rhombic lip. The VZ gives rise to the Purkinje cells and the deep nuclei (dentate nucleus [De]). The granule precursor cells of the EGL migrate over the surface of the cerebellum. Proliferation in the EGL is activated by sonic hedgehog homologue (SHH), secreted by Purkinje cells (P-cells). The proliferating cells are concentrated in the outer half of the EGL. When post-mitotic, these cells migrate radially inwards (guided by Bergman glia [not shown]) to form the internal granule cell layer (IGL). Note the markedly active proliferation and migration of the granule precursor cells of the EGL during the premature period. ep=ependyma. ML=molecular layer. IZ=intermediate zone. pn=postnatal. WM=white matter. Adapted from ten-Donkelaar et al,<sup>162</sup> with permission from Springer.



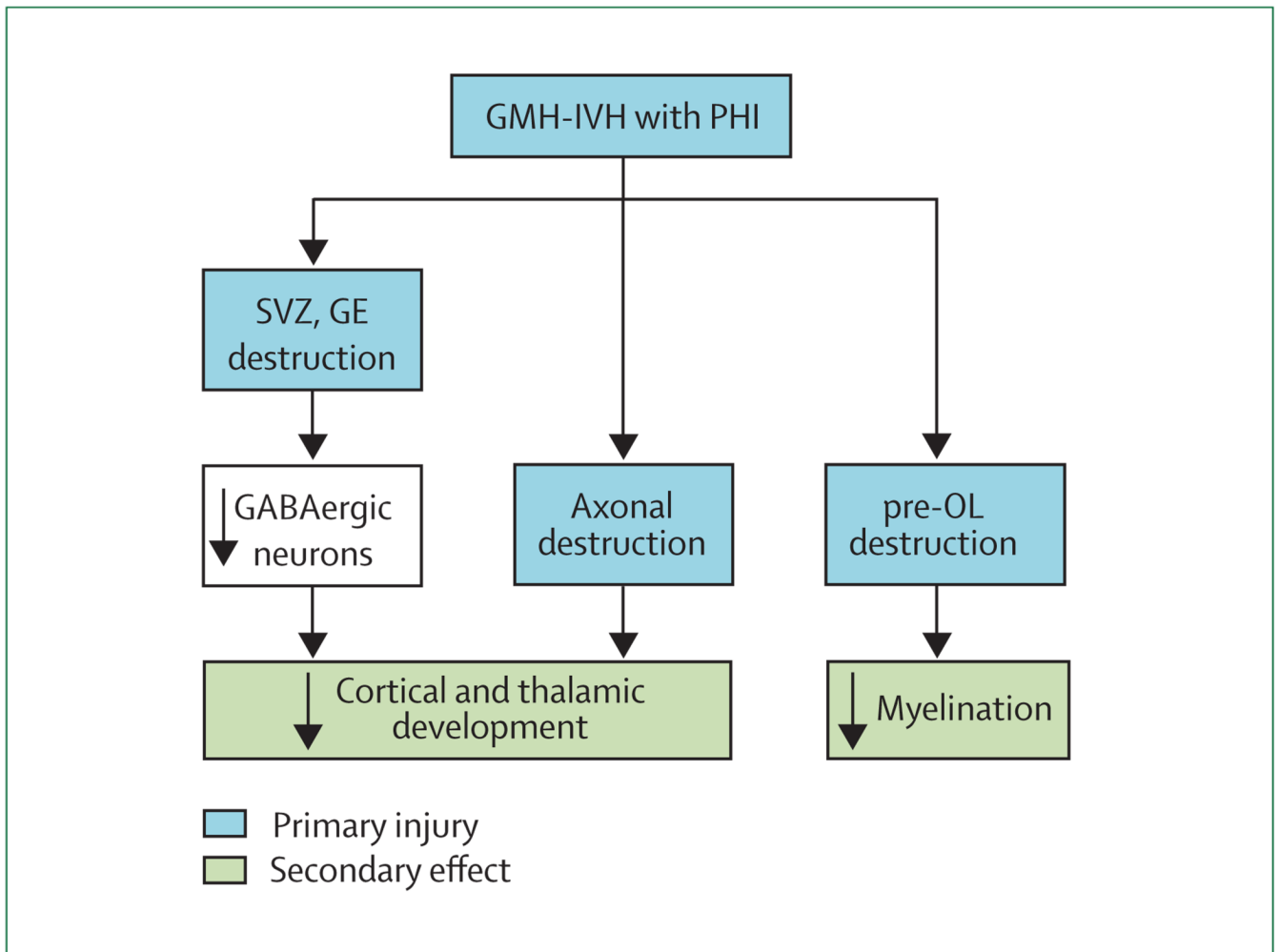
**Figure 5. Potential sequences of events leading to major brain sequelae observed with periventricular leukomalacia**

Potential events are hypomyelination, and impaired cortical and thalamic development (eg, seen on advanced MRI analysis by decreased volume). For each sequence, the initiating primary injury is shown, and the subsequent secondary effects are postulated to occur because of maturational/trophic disturbances, as described in the text. Pre-OL=premyelinating oligodendrocyte. SPN=subplate neuron.



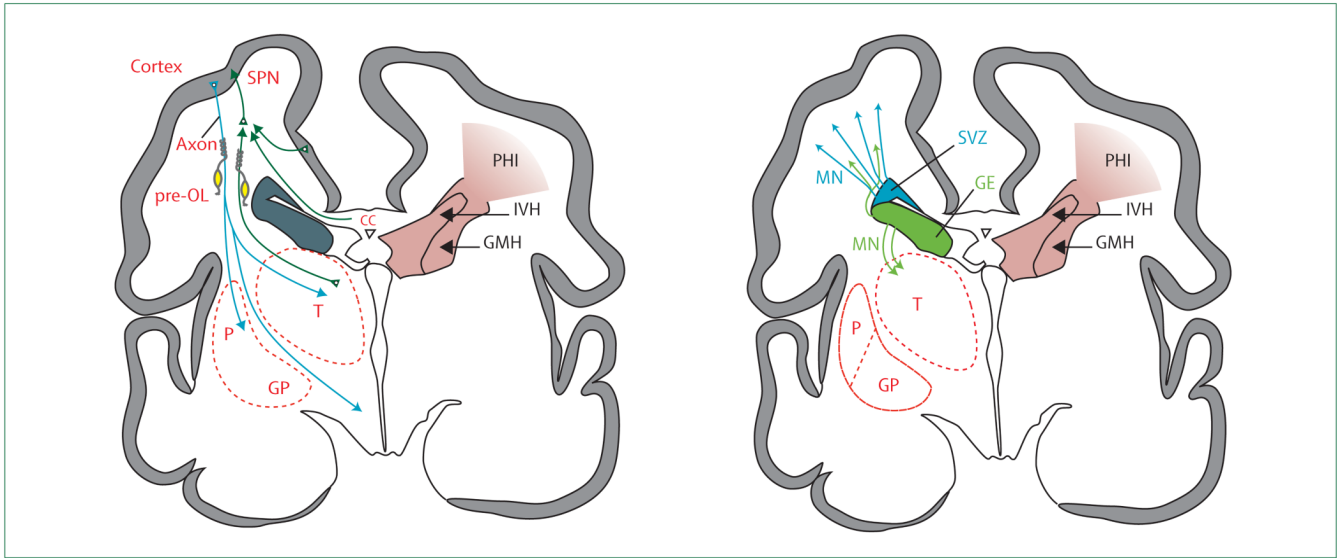
**Figure 6. Anatomical relationships between the major developmental events and the topography of non-cystic periventricular leukomalacia (PVL)**

For purposes of clarity, the developmental events are separated. CC=corpus callosum. GE=ganglionic eminence. GP=globus pallidus. MN=migrating neurons. P=putamen. Pre-OL=premyelinating oligodendrocytes. SPN=subplate neurons. SVZ=subventricular zone. T=thalamus.



**Figure 7. Likely sequence of events leading to the major neuropathological sequelae observed with germinal matrix haemorrhage (GMH)-intraventricular haemorrhage (IVH) with periventricular haemorrhagic infarction (PHI)**

The primary destructive events involve the GMH-IVH with PHI and the associated destruction of premyelinating oligodendrocytes (pre-OLs) and axons. The secondary consequence of the former is hypomyelination, and that of the latter is impaired thalamic and cortical development. In addition, because of destruction of the dorsal subventricular zone (SVZ) and ventral germinative epithelium of the ganglionic eminence (GE), impaired proliferation and late migration of GABAergic interneurons to upper cortical layers and the thalamus could contribute to defective cortical and thalamic development.



**Figure 8. The anatomical relationships between the major developmental events and the topography of germinal matrix haemorrhage (GMH)–intraventricular haemorrhage (IVH) with periventricular haemorrhagic infarction (PHI)**

For purposes of clarity, the developmental events are separated. CC=corpus callosum. GE=ganglionic eminence. GP=globus pallidus. MN=migrating neurons. P=putamen. Pre-OL=premyelinating oligodendrocytes. SPN=subplate neurons. SVZ=subventricular zone. T=thalamus.