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OXFORD

INVITED REVIEW ARTICLE

Fundamentals of the Development of Connectivity in the Human Fetal Brain in Late Gestation: From 24 Weeks Gestational Age to Term

Ivica Kostović, MD, PhD, Milan Radoš, MD, PhD, Mirna Kostović-Srzentić, PhD, and Željka Krsnik, PhD

Abstract

During the second half of gestation, the human cerebrum undergoes pivotal histogenetic events that underlie functional connectivity. These include the growth, guidance, selection of axonal pathways, and their first engagement in neuronal networks. Here, we characterize the spatiotemporal patterns of cerebral connectivity in extremely preterm (EPT), very preterm (VPT), preterm and term babies, focusing on magnetic resonance imaging (MRI) and histological data. In the EPT and VPT babies, thalamocortical axons enter into the cortical plate creating the electrical synapses. Additionally, the subplate zone gradually resolves in the preterm and term brain in conjunction with the growth of associative pathways leading to the activation of large-scale neural networks. We demonstrate that specific classes of axonal pathways within cerebral compartments are selectively vulnerable to temporally nested pathogenic factors. In particular, the radial distribution of axonal lesions, that is, radial vulnerability, is a robust predictor of clinical outcome. Furthermore, the subplate tangential nexus that we can visualize using MRI could be an additional marker as pivotal in the development of cortical connectivity. We suggest to direct future research toward the identification of sensitive markers of earlier lesions, the elucidation of genetic mechanisms underlying pathogenesis, and better long-term follow-up using structural and functional MRI.

Key Words: Cortical connectivity, Growing axonal pathways, Human brain development, Neurodevelopmental disorders, Preterm infants, Transient lamination, White matter damage.

INTRODUCTION

The complex organization of the human brain results from histogenetic processes regulated by underlying genetic-molecular and cellular mechanisms (1–6). Despite the fact that histogenetic processes in human brain cannot be analyzed by direct, experimentally controlled conditions, prolonged development (from the embryonic period to young adulthood) and the size of the human brain contribute to proper spatiotemporal resolution of histogenetic events. Neuroanatomical, histological, immunocytochemical and electron microscopical techniques make it possible to delineate reliable spatial parameters, such as transient compartments, regions, areas, and cytoarchitectonic units (1, 2, 4, 7), which in turn exhibit a correlation with current MR imaging studies of the entire brain organization and large scale neural networks (8–10). This enables monitoring of histogenetic processes using in vivo and in vitro MR imaging as well as utilizing a postmortem material.

In terms of function, the most important neurogenetic event is connectivity development. Establishing proper connectivity of the cerebrum requires the interaction of all neurogenetic processes (proliferation, migration, axonal growth, dendritogenesis, synaptogenesis, myelination). In human basic and clinical research, the timing of neurogenetic events is a crucial parameter in distinguishing the development of human brains from other mammals, including experimental primates (4).

A prolonged occurrence and time overlapping of histogenetic processes does not obscure existing differences between developmental phases, with periods (developmental

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windows) characterized by increased intensity of events (1, 2). Researching connectivity development using postmortem material, spatial laminar compartments (Fig. 1), and other structural parameters for phases of growth and maturation requires a thorough comparison of available databases on fetal and infant collections (2) and in vitro postmortem imaging (11–13).

A precise definition of spatiotemporal patterns involving connectivity and underlying structural and cellular processes provides a better understanding of functional development in humans and a significantly better correlation with basic experimental research. In addition, current analysis of MR imaging-histology correlation along with a large-scale neural network approach and molecular-genetic analysis of the developing human cerebrum are crucial for identifying the vulnerability of specific circuits and a better understanding of neurodevelopmental outcomes (4, 8, 10, 14–19).

This review provides a structural overview of spatiotemporal patterns in connectivity development, focusing on the cerebral cortex and connected subcortical structure. Moreover, it discusses new concepts outlining vulnerability and identifies important circuits and neural pathways frequently damaged in the preterm brain. We will first delineate key phases of structural development of cerebral connectivity during the second half of gestation as revealed by histology and structural magnetic resonance imaging (MRI) and then discuss a neurobiological basis of vulnerability of specific neural systems.

DEVELOPMENTAL PHASES AND SPATIOTEMPORAL PARAMETERS OF CONNECTIVITY DEVELOPMENT IN THE LATE FETAL HUMAN BRAIN

Developmental phases of cerebral connectivity from 24 to 42 weeks of gestation (WG) are defined using spatiotemporal criteria: the timing of neurogenetic events, spatial delineation of transient radial, and tangential connectivity compartments where cellular elements interact during development. The additional criteria are maturation of white matter segments and growth and selection of trajectories of major axonal pathways and their engagement in neuronal networks. Two phenomena are essential for understanding the impact on development and reorganization of cerebral connectivity after perinatal injury in preterm infants. First, well-known histogenetic events, such as proliferation, migration, molecular specification, axonal growth, dendritic differentiation, and synaptogenesis occur throughout this period (1, 3, 20). Temporal overlapping, different intensities, and the rate of neurogenetic events have been documented in previous studies (1, 2). Second, changing laminar pattern of the organization of connectivity elements, such as axons, synapses, postsynaptic elements in major cerebral circuits indicates quite a dynamic development during late fetal phases (2). Despite continuous growth and developmental reorganization of human brain, protracted development provides insights into differences in circuitry organization at different gestational ages at least between extremely preterm (EPT) and late preterm in humans (21). Therefore, differences in vulnerability between early and late preterm ages can also be expected, despite the fact that the preterm brain is continuously vulnerable (22, 23). It is a generally accepted fact that the incidence of cerebral palsy and associated periventricular leukomalacia (PVL) increases with decreasing age and maturity of preterms (23–25). However, despite this gestational timeframe, prospective focal and specific lesions, predominant for a given spatiotemporal status of circuitry organization, require further investigation (21).

The developmental period between 24 and 27 WG (22–25 PCW) is important in clinical practice as it corresponds to prematurely born babies defined as EPT. During this period, the cerebral wall continues to show a fetal transient pattern of compartmental organization, but with significantly advanced sublaminar organization, axonal growth, neuronal maturation, gliogenesis, and intense proliferative and migratory histogenetic events. The cerebral wall consists of the following compartment visible on both histologically processed sections and MR images discernible from the ventricle to the pia (2, 11, 12, 23, 26): ventricular zone (VZ), inner subventricular zone (ISVZ), fiber rich callosal zone (inner fibrillary layer), outer subventricular zone (OSVZ) along with internal sagittal strata (SS) of the intermediate zone, subplate (SP), cortical plate (CP), and marginal zone (MZ) (Fig. 1A).

Conventional MR 3T images make it possible to distinguish the VZ-ISVZ complex from OSVZ, depending on the size of callosal periventricular fibers. Visualization of the marginal zone is beyond the resolution of 3T imaging, except in hippocampal formation where MZ is 3 times thicker than in the cortex (27). The most complex cytological and neurogenetic events take place in the OSVZ, which is characteristically expanded in the human brain due to an abundance of diverse progenitor cells (5, 28–31). Two classes of progenitor cells are important: intermediate progenitor cells, which show multipolar appearance and movement within the SVZ (5, 29), and a particular population of basal radial glia (also called truncated glia) (5, 7, 31). The term basal radial glia has been accepted given that these particular cells maintain contact with basal membrane but lose contact with the ventricular surface. Basal glia and intermediate progenitors produce neurons for the upper cortical layers. The intensive production of neurons in OSVZ results in late waves of migratory neurons propagating toward the cortical plate. This period is also important for the onset of intensive astrogenesis (7) and oligodendrogliogenesis (32). The early onset of astrogenesis is one of main characteristics of histogenesis of the human brain (7). Whereas early oligodendrogliogenesis originates in 3 successive ways from lateral (LGE) and caudal ganglionic eminence (CGE) (33), astrocytic precursors from OSVZ are generated by radial glia, which also migrate along radial glia and continue to divide locally (34). Cortical connectivity elements (terminal axons of cortical pathways), dendrites or the somata of postsynaptic neurons and synapses are distributed in the 3 most superficial compartments of the cerebral wall: SP, CP, and MZ. Importantly, what should be emphasized is that during the EPT period, lamination occurs within the cortical plate and appears as pale, poorly delineated lamina (Fig. 1). Thalamocortical axons in EPT babies enter the cortical plate creating electrical synapses (35–38). The EPT period can be described as sensory expectant or presensory connectivity (Supplemen-

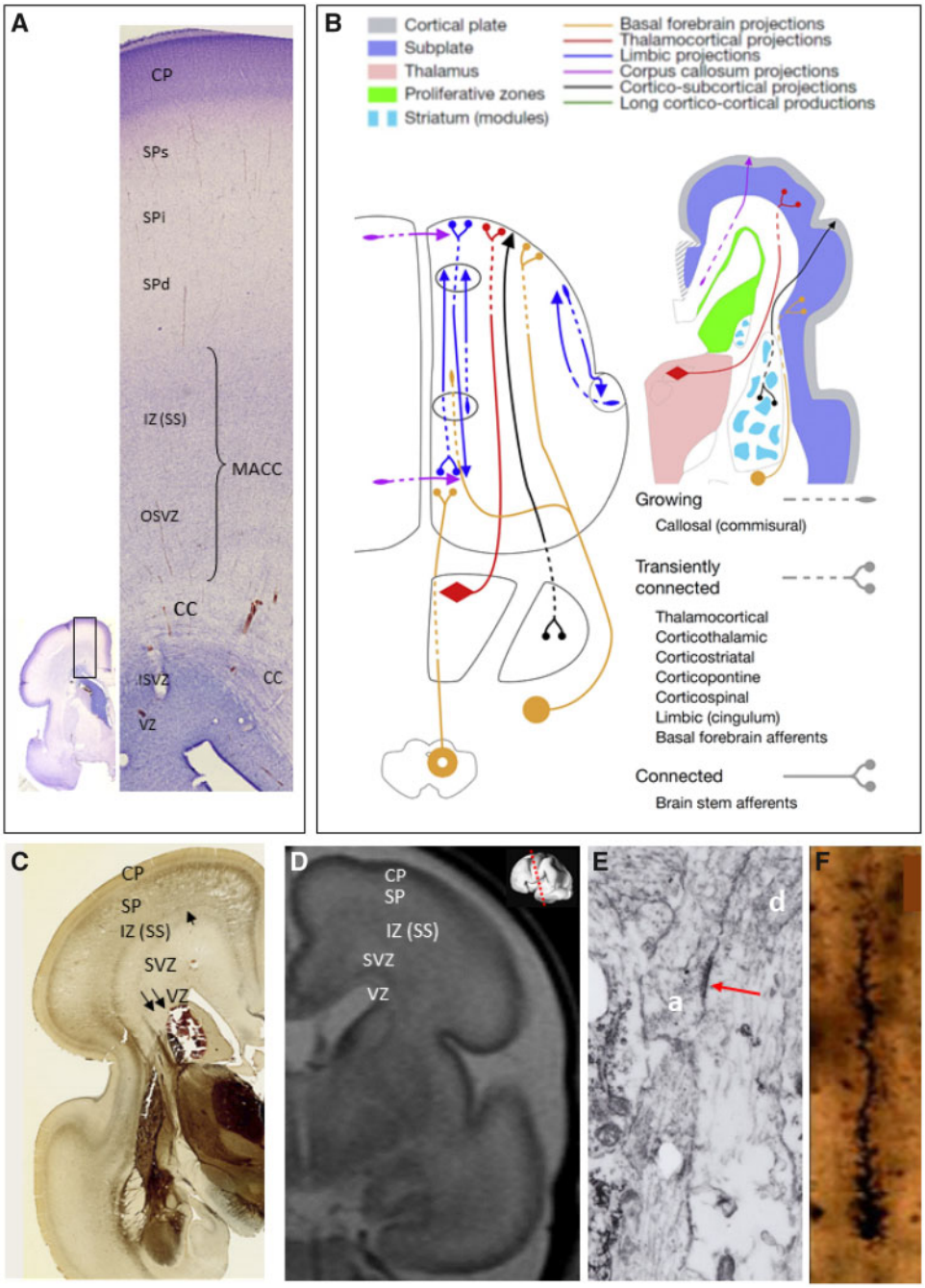

FIGURE 1. Neocortical laminar organization on Nissl-stained sections **(A)**, diagrammatic representation of growing circuitry **(B)**, AChE-stained sections **(C)**, and MR images **(D)** in extremely preterm (EPT). First synapses (arrow) in the CP between axon (a) and dendrite (d) are illustrated **(E)**. **(F)** Transforming radial glia shown by Stensaas modification of Del Rio-Hortega Golgi method. Note sublaminar organization of the SP (SPs, SPi, SPd) and multilaminar arrangement of proliferative outer SVZ (OSVZ) and sagittal strata of IZ (marked between curly brackets) as multilaminar axonal cellular compartment (MACC) in Figure 1*A*. Ventricular zone (VZ) is separated from ISVZ (inner subventricle zone) by callosal fibers (CC). The main laminar compartments (CP, SP, IZ, SVZ, VZ) are visible on both AChE **(C)** and MRI in vivo images **(D)**. AChE staining shows fiber delineation of external sagittal stratum (single arrow) and the SP. Double arrow indicates periventricular hemorrhage (PVH), which damages ganglionic eminence and caudate **(C)**. Reprinted from Dubois et al (130) with permission **(B)** and Kostović et al (7) with permission **(F).**

tary Data Fig. S1) and stimulation of periphery through the thalamic system can elicit electrical responses in the cortex (20, 37, 39–42).

In the next phase, very preterm (VPT), which is 28–31 WG (26–29 PCW), compartmental organization of the cerebral wall shows notable changes in all cerebral compartments.

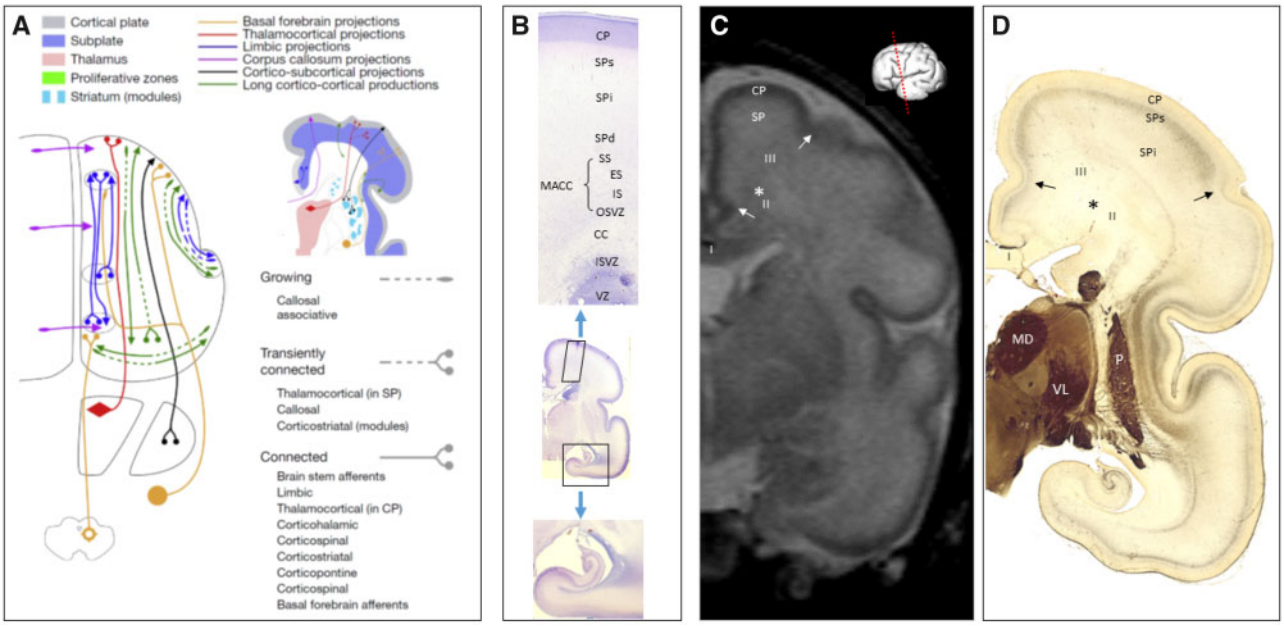

FIGURE 2. Diagrammatic representation of growing cortical circuitry **(A)**, neocortical and hippocampal lamination **(B)**, an in vivo MR T2 image **(C)** and matching AChE coronal sections **(D)** in very preterm telencephalon and diencephalon. Note that the associative and callosal pathways are still growing **(A)**, while some pathways are transiently connected. The Nissl stained section **(B)** shows proliferative zones (VZ, SVZ) and a multilaminar axonal cellular compartment (MACC), which includes sagittal strata and part of OSVZ. Segments of white matter are labeled with roman numbers (I, II, III) on in vivo MR **(C)** and AChE histological sections **(D)**. Sagittal strata and crossroads (asterisk) below segment II WF. Arrows indicate initial reduction of the SP bellow sulci. AChE stained sections show mosaic-like reactivity in the putamen (P) and differentiation of individual thalamic nuclei: mediodorsal (MD) and ventrolateral (VL), centrum semiovale, segment III is AChE negative. Reprinted from Dubois et al (130) with permission **(A).**

Due to the simultaneous growth of all major afferent and efferent projection and associative pathways (Figs. 2 and 3), the fibrillar intermediate zone is transformed into segmented fetal "white" matter. The following "white" matter segments (WMS) (43–45) are visible pale laminas on Nissl-stained (Fig. 2B) and acetylcholinesterase (AChE)-reacted (Fig. 2D) sections, as well as on structural MR images: WMS I is closest to the ventricles and consists of massive callosal fibers and periventricular fiber systems associated with ganglionic eminence (GE) (subcallosal fascicle, containing corticostriatal fibers and prominent fronto-occipital fascicle) (46). WMS II consists of a periventricular crossroad of pathways (43, 45, 47) and sagittal strata, which contain most of the cortical afferent and efferent projection and associative pathways (45, 48, 49).

From the point of neuropathology and neuroimaging, 2 PWCs are very important given that they are the most frequent sites of focal PVL (23, 43, 47). The first is the main frontal crossroad, located at a lateral angle to the lateral ventricles at the level of the interventricular foramen (47). The other clinically important site is the parietal crossroad located at the exit of the posterior limb of the internal capsule, which continues into the occipital crossroads situated dorsolaterally of the posterior horn of lateral ventricles and it incorporates important visual pathways from the lateral geniculate body and pulvinar (45, 50). WMS III is the centrum semiovale containing the bulk of radiating (corona radiata) and interdigitating cortical pathways, which in turn form a large transitional territory between the deeper "white" matter segments and more superficial cortical fiber system. WMS IV (gyral "white" matter) does not develop in this phase because cortical convolutions are still not adequately developed in this period. Instead, the voluminous extracellular matrix (ECM)-rich subplate compartment fills most of the content of primary gyri. In addition to prominent changes in fibrillar compartments (fetal "white" matter, intermediate zone) in VPTs and in cell-rich layers (i.e. deep cellular proliferative zones [VZ, SVZ] and superficial compartments comprising the cortical anlage [SP, CP, MZ]), there are measurable changes (51).

In deep proliferative zones, there is a characteristic shift of proliferative activity from VZ to OSVZ occurring in this period and is associated with the production of associative classes of neurons (28, 31, 52–54) and a new wave of gliogenetic events (33, 55). This is the last period of neuron production for superficial cortical layers, and gives rise to corticocortical pathways (in monkey E90) (55, 56). The period described in the article by Rash (E90 in monkey) corresponds approximately to human development at ~30 WG (36). Intensive gliogenesis is qualitatively a new trend of proliferative events within the OSVZ. Thus, although oligodendrogliogenesis (32, 33) starts early in GE and astrogenesis is also an early fetal event (7), the third wave of oligodendrogliogenesis (33)

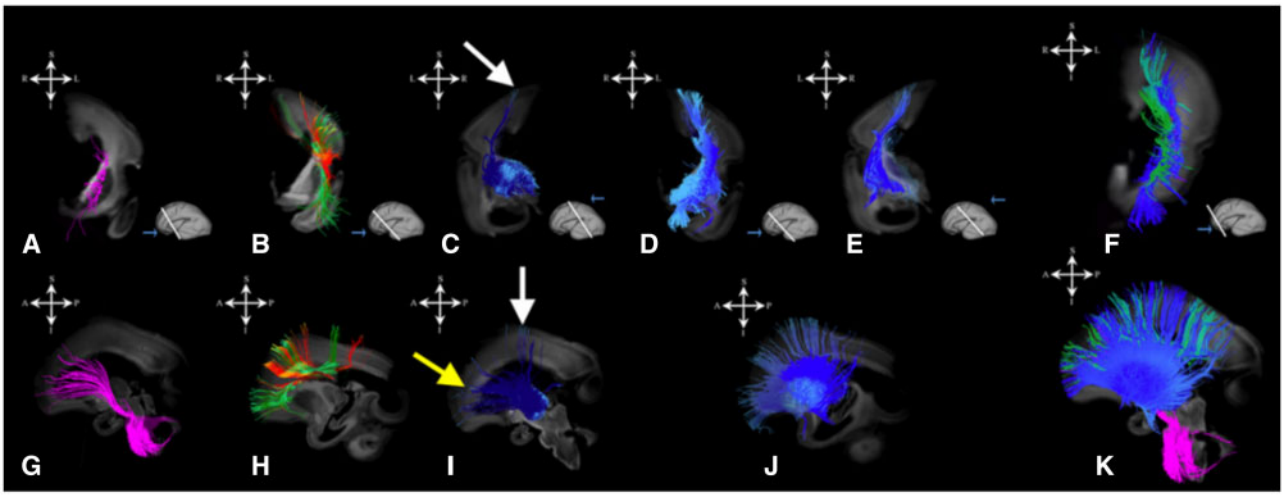

FIGURE 3. Reconstruction of projection corticopontine, ponto-cerebellar and fibers related to pons **(A, G)**, fasciculus subcallosus—Muratoff bundle **(B, H** in green**)**, thalamic **(C, I)**, and basal ganglia and basal forebrain fibers conveyed to the external capsule **(D, E, J)** in 26 WG (24 PCW) old brain. Reconstruction of association fronto-occipital fasciculus is shown in orange **(B, H)**. A composite image of the spatial arrangement of fibers is shown in **(F, K)**. Coronal sections of DWI images are in the upper row, sagittal sections are in the bottom row. Adjacent to each coronal slice is an illustration of the reference brain surface with an approximate level of the coronal slice (white line), and a view of the slice (anterior or posterior, marked with a blue arrow). Reference orientations: anterior (A), posterior (P), superior (S), inferior (I), left (L), and right (R), are placed in the left upper corner of each slice. White arrows indicate voluminous portion of thalamocortical fibers reaching the cortical plate in central regions. **(I)** The yellow arrow indicates thalamocortical fibers that reach the subplate (not the cortical plate) of the frontal regions. Reprinted from Vasung et al (48) with permission.

and intensive astrogliogenesis (7) most likely occurs in the VPT, after a reduction of neurogenetic events. Since the number of glia in primates outnumbers cerebral neurons, their production in the outer ventricular zone can also be an important factor in brain enlargement and development of cerebral convolutions (55). As a precursor of cell pool in the cerebral wall, radial glia continues to exist in the VPT. According to Nowakovski (54), the majority of proliferative radial glia at that stage is situated in the OSVZ and belongs to the basal radial glia, which maintains contacts to the basal membrane on the brain surface just below the pia (31, 54, 57).

With increasing age, robust projection fiber systems of sagittal axonal strata (45) disperse subventricular proliferative cell layers in the OSVZ (29) and together form a unique mixture of intermediate progenitors, basal radial glia, and growing fibers described as the multilaminar axonal-cellular compartment (MACC) (Fig. 2B). Close contacts of growth cones from developing afferent axons with cells in proliferative layers showing the Ki 67 marker (45) suggests histogenetic interaction in this important compartment of the human cerebrum (45).

The 3 superficial cellular compartments of the cerebral wall, which actually represent the anlage of the cortex (i.e. SP, CP, MZ) undergo changes resulting from the ingrowth of callosal and associative pathways in the subplate and cortical plate, including an enormous increase in dendritic branching of neurons in the cortical plate (58, 59). The differentiation of dendrites (58, 59) and changes in fetal columnar organization (2, 3, 53) of the cortical plate results in adult-like outlines of cortical layers (2–6), a process that begins in deep cortical layers (4–6). The loss of radial coherence is revealed using noninvasive water diffusion anisotropy MRI (60). Important cytoarchitectonic changes also take place in the most superficial cortical compartment, that is, the marginal zone, at ~27–30 GW (25–28 PCW) gradual resolution of the superficial granular layer occurs (2, 61). Marginal zone remains exceptionally cellular given that typical fetal type of large Cajal-Retzius cells continue to exist (58, 59) while new cells are continuously added (1, 56, 61). These new cells resembling Cajal-Retzius neurons are smaller and express Reelin, Calretinin, and NOS (61) and possibly replace "old" large Cajal-Retzius neurons during subsequent development. In the basal ganglia (caudate, putamen and amygdala), the VPT period is characterized by a peak transient modular pattern as described in P3. Amygdala shows transformation of barrel-like cytoarchitectonic units of the lateral nucleus and which have not been described in species other than humans (62). Another human-specific structure, the gangliothalamic body, comprises migratory streams of cells connecting GE and associative thalamic nuclei (63, 64). In analyzing selective vulnerability of different cortical types, it is important to note that the limbic cortex in general, and hippocampal formation in particular show advanced cytoarchitectonic (27), synaptic (27), and pathway (65) maturation, when compared with neocortex. The specifics involving early development of corticocortical connectivity in hippocampal formation was described by Hevner and Kinney (66) using DiI tracing.

In the preterm phase (32–36 WG, 30–34 PCW), cerebral organization is characterized by the coexistence of transient fetal compartments and a microstructural feature revealing a pediatric-like organization (Fig. 4). Development of cerebral convolutions occurs rapidly along with the appearance of a

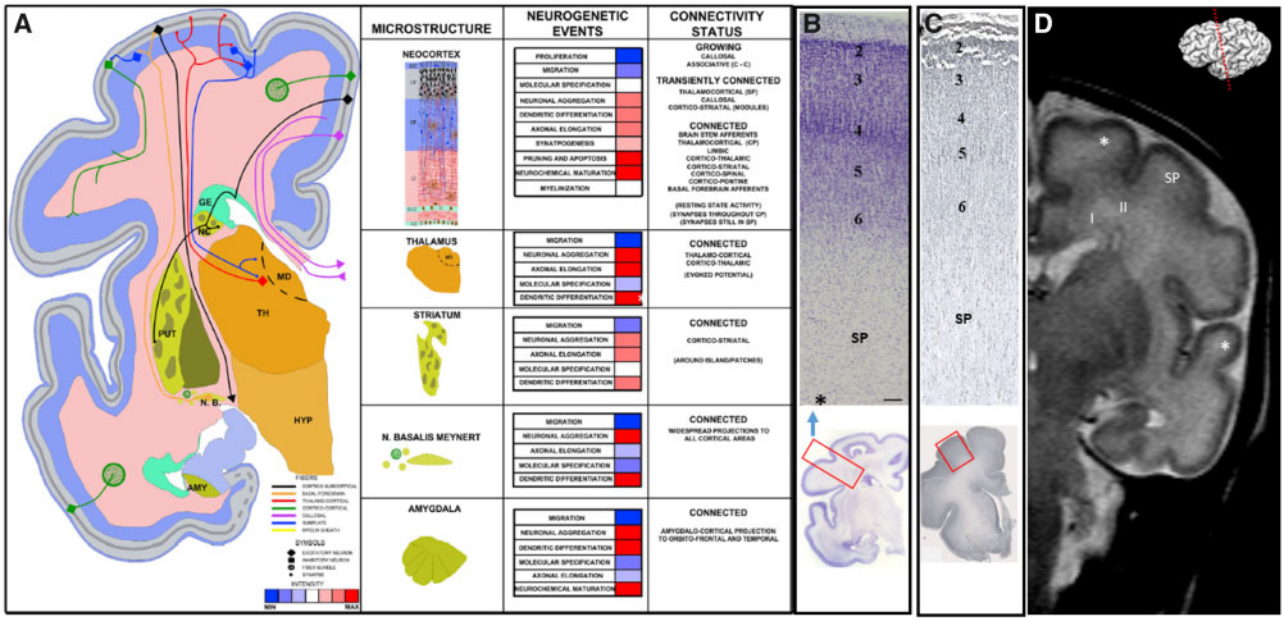

FIGURE 4. Shows a diagrammatic representation of cortical growing pathways and intensity of histogenetic events in the telencephalon and diencephalon **(A)**, laminar organization on Nissl stained sections **(B)**, MAP2 immunohistochemistry **(C)**, and an in vivo MR **(D)** for the preterm. Note that callosal pathways are still growing, while limbic and subcortical-cortical-subcortical pathways are already connected. The Nissl-stained section **(B)** shows for the first time a clear 6-layer pattern (Grundtypus of Brodmann, see Kostović and Judaš, 2002). The main sign of immaturity for the preterm brain is the presence of the SP compartment (SP). In vivo MR imaging shows higher signal intensity in cortical gyri with well-developed associative connectivity (frontal and temporal cortex) (asterisk). Segments of WM are marked with I, II. Reprinted from Kostovic et al (2) with permission (**A**), and Krsnik et al (95) with permission **(B).**

new "white" matter segment, that is, gyral white matter or WMS IV (44). The second WMS or so-called periventricular crossroad of pathways (43, 44), shows still higher T2 signal intensity (Fig. 5A, B). Sagittal strata are prominent in the occipital cortex, lateral to the posterior horn, showing "2-track" (on histological sections) and "triplet" (on MRI recording) (Fig. 5C, double arrow). It is important to emphasize that the normal "triple" appearance of sagittal strata at occipital levels (Fig. 5C) is a good marker of white matter integrity given that they also contain associative long corticocortical pathways. In contrast, the posterior limb of the internal capsule, a standard structure for evaluating lesions of cortical pathways, contains only projection pathways. Cerebral compartments that represent the cortical anlage (i.e. SP, CP, MZ) undergo significant changes. Commissural and associative pathways relocate in the CP and the volume of the SP gradually decreases, especially below the cortical sulci. The CP shows a definitive 6-layer lamination (Brodmanns's Grund typus) (67). Pyramidal neurons belonging to layer 5 in the primary motor cortex increase in size facilitating analysis of prospective hypoxic-ischemic damage at the cellular level on postmortem material using standard MAP2 immunostaining (Fig. 4C) or Nissl method. The establishment of initial corticocortical connectivity complemented with basic wiring of cortical circuitry initiates synchronized electrical activity of the preterm brain (39, 41, 68) and advanced maturation of behavioral states (69). However, deep synaptic circuitry of the voluminous subplate compartment is still a significant player in shaping cortical waves in the preterm brain (20). Transient circuitries and progressive maturation of permanent circuitry underline global brain networks (connectome) of preterm infants as revealed by recent diffusion tensor magnetic resonance imaging (DTI) and fetal resting-state functional magnetic resonance imaging (fMRI) (10, 70). At ~32 GW (30 PCW), network architecture exhibits a so-called small world modular organization similar to the adult pattern and cortical hubs (nodes) displaying rich-club organization (71).

In approaching term age (37–42 WG; 35–40 PCW), transient proliferation (VZ, OSVZ) and connectivity compartments (SP) gradually decrease in volume and complexity in terms of cellular organization. A gradual reduction in the thickness of proliferative bands in the OSVZ-MACC complex is a useful structural factor in conventional in vivo MR imaging when evaluating the maturational state of the vulnerable occipital component belonging to axonal sagittal strata. In "normal" term born babies, good delineation of "triple" track appearance on MR images shows the sagittal strata (Fig. 6A, C) due to the fact that associative and thalamocortical fibers are compact, while myelinated and remnants of proliferative cell bands are not damaged (Fig. 6C′). In the frontal lobe of the human infant (72), migratory streams of neurons were observed within the WMS II proving that in associative cortical areas, neurons can migrate after birth. This also suggests early postnatal neural production in some parts of the cerebrum. At approx. term age, transient connectivity compartments undergo significant reorganization. The subplate compartment is then reduced to the subplate remnant and situated between layer 6 and gyral "white" matter (44). The subplate rem-

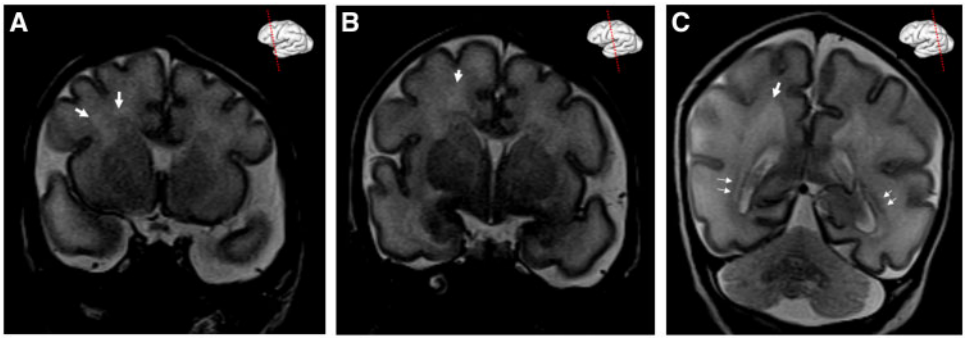

FIGURE 5. Periventricular crossroad of pathways at 33 WG (preterm) shown on the T2 images on the frontal **(A)**, midlateral **(B)**, and occipital **(C)** planes. Note the moderate signal intensity (arrows), which is still stronger than in surrounding areas. Pathways are rich in "watery" ECM and are an important sign of normal maturation **(B)**. See also in Judas et al (43). Double arrow marks the triple "appearance" of occipital sagittal strata.

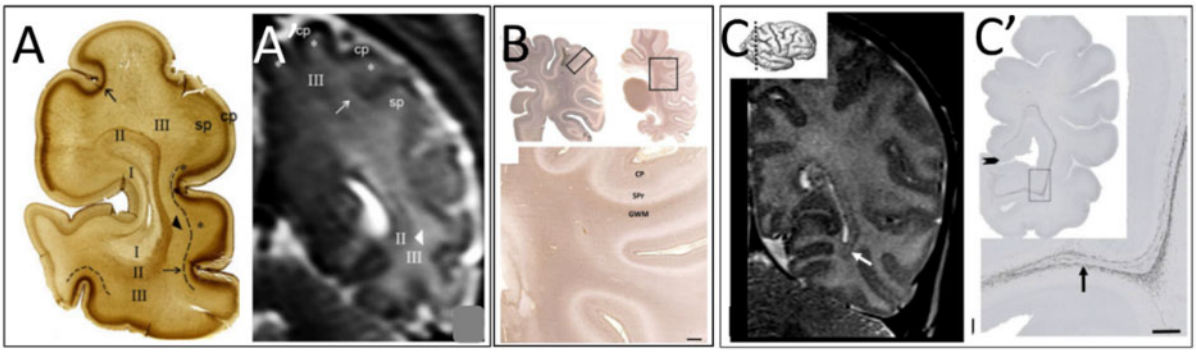

FIGURE 6. (A) Reduction of the subplate at the bottom of cortical sulci (arrows) in a 35 PCW human fetus and preterm infant revealed by AChE-histochemistry **(A)** and in vivo, in utero T2-weighted MRI coronal sections through the occipital region **(A′)**. The subplate is clearly recognized as an AChE-reactive zone underlying the cortical plate (sp and asterisks), as well as the undulating hyperintense zone below the hypointense cortical plate in MRI scan (sp and asterisks). Note that arrowheads mark the position of the external capsule and thus the border between developing white matter segments II (sagittal strata) and III (centrum semiovale), while dashed lines mark the border between the centrum semiovale and the subplate. The posterior part of callosal radiation (I) is also clearly delineated. Reprinted from Kostović et al (44) with permission. **(B)** Characteristic distribution of extracellular matrix marker chondroitin sulfate at the newborn age. Subplate remnant (SPr), seen as wavy unstained line, along the hemisphere is in contrast to moderately stained underlying gyral white matter (GWM) and cortical plate (CP). Scale bar: 1 mm. Reprinted from Kostović et al (7) with permission. **(C)** Visibility of occipital sagittal strata (SS) on coronal plane T2 MR images and corresponding histological preparations from newborn brains. Sagittal strata are visible as a "triplet structure" (white arrow) in newborn brains at T2 coronal MR image **(C)**. In the newborn brain, histological coronal sections through the occipital lobe at the level of the calcarine fissure (arrowhead) show immunoreactivity for myelin basic protein (SMI 99) in the axonal SS **(**black arrow in **C′)**. Rectangle in **B** is shown at higher magnification (scale bar = 1 mm). Reprinted from Žunić Išasegi et al (45) with permission.

nant is identified using MR due to the abundant ECM componence (44). The gradual resolution of SP may be explained by the fact that a majority of growing "fronts" of projection and associative fiber systems leave this compartment and enter the CP (20) or retract their "exuberant" fibers (73). Concomitantly neurons from the former transient tangential nexus (20, 74) are now incorporated in gyral white matter. The significance of the remaining SP neurons is discussed in paragraph 3 (P3). Parallel to the ongoing resolution and reorganization of transient patterns in the cerebral cortex, cytoarchitectonic modular reorganization of striatum, amygdala, and thalamus takes place. These changes occur during dendritic differentiation (38, 59), incipient myelination (75), terminal arborization of presynaptic axons and reduction of ECM (44), and gliogenesis (32). The most important quantitative indicator of connectivity development in this period is explosive synaptogenesis in all cortical areas (76, 77).

DEVELOPMENTAL VULNERABILITY: SELECTIVE, RADIAL, AND TANGENTIAL VULNERABILITY. PATHOGENESIS AND SPATIOTEMPORAL FACTORS INFLUENCING PATHOGENESIS OF DIFFERENT TYPES OF LESIONS: WHEN, WHERE, WHAT, AND HOW

The main difference between the vulnerability and pathology of an adult compared with a brain still developing is that developmental lesions affect neurogenetic processes, alter

developmental events and impair the establishment of proper neuronal circuitry. It is generally accepted that there is increased vulnerability during periods of increased growth rates and intensive occurrence of neurogenetic processes. Therefore, the question of "when" a pathogenetic factor was active is essential for studying of origin of neurodevelopmental disorders. The process of proliferation, migration, molecular specification, and their spatiotemporal regulation by genes and transcription factors is the focus of neuropathological analysis during events that occurred during the first half of gestation (6). In contrast, the period between 22 and 40 gestational weeks, which corresponds from the EPT to term period, is dominated by growth of axonal pathways from their origin, path-finding, sorting, accumulation, target-selection, and synaptic address finding (20, 65). Therefore, vulnerability of growing axonal pathways (fetal WM) is a key problem in preterm infants (15, 21–23, 78). This leads to the question of "where", because different classes of axonal pathways form a large contingence deep toward superficial cerebral compartments (as defined in P1), that is, WMS the subplate, CP, and marginal zone (7, 21, 43, 45). As pointed out by Volpe (23) and Edwards (79), the pathogenetic effect of focal PVL may be different than diffuse type of PVL, especially because involvement of PVC (segment 2 of fetal "white" matter). The problem of radial vulnerability was elaborated recently by Kostović et al (21). Moreover, the concept of radial vulnerability was recently complemented with the concept of tangential organization and widespread effect of tangential subplate nexus (TSN) impairment (cf. para. 3 and the review by Kostović [20]). The question of "what" is also essential for understanding vulnerability and selective vulnerability. For example, subplate neurons (SPNs) are selectively vulnerable during the developmental window when they have synaptic contacts and are therefore exposed to glutamatergic neuroexcitoxicity. On other hand, CP neurons, which do not have synapses until 23 PCW, show less vulnerability even if exposed to hypoxia-ischemia. The concept of SS, recently introduced by Žunić Išasegi et al (45), is relevant for the problem associated with radial extent of lesion (relates to the question of "when" and "where"), and identification of injured pathways (the question "what") based on classical papers by Sachs, Dejerine, and Von Monakow (for a review of the literature, see in Žunić Išasegi et al [45]). These classical and recent studies have shown that different classes of projection associative and commissural pathways occupy specific trajectories within internal, intermediate, or external sagittal stratum and interact with proliferative and synaptic zones of the cerebral wall during development. The radial extent of the hypoxic/ischemic (H/I) lesion may determine, especially in diffuse PVL, which one of the specific motor, sensory or associative pathways will be damaged in a given sagittal stratum (21). As mentioned earlier, the appearance of SS in the occipital lobe is an important marker of normal maturational status of major projection and associative pathways in preterm lesion (45). The "normal" (Figs. 5C and 6A′) or "abnormal" appearance of SS can be analyzed on both histological and conventional MR images in vitro and in vivo (45).

The question of "how" is also essential for understanding the pathogenesis of developmental lesions. The best example is the growth of axons, which depends on precise guidance toward target areas. Disturbances of the ECM, guidance molecules or SPNs will result in abnormal functional organization as well as impaired genetic and environmental interactions (20, 21). The other example of how lesion of neurons can disturb the formation of ocular dominance columns has been presented in different experimental studies (38, 80). Finally, it has been shown that even deprivation of input during critical period may change cortical functional organization (80).

To identify human characteristic lesions in a late preterm brain, focus should be directed not only on classical projection pathways but also on damage to associative circuits. Greatly assisting in these tasks is MR in vivo monitoring and follow-up, the lesion of white matter segments containing associative pathways, especially in the frontal, parietal, and occipital lobe where it may explain neurodevelopmental outcome (Fig. 7).

DEVELOPMENT AND DISTURBANCES OF SPECIFIC NEURONAL CIRCUITRIES

CORTICOSTRIATAL PATHWAYS

The cortex-striatum-pallidum-thalamus-cortex circuit is one of the most basic neuronal circuits for cortical regulation of complex sensory, motor, limbic and associative functions (49, 81).

It is modulated by pathways from other cerebral (basal) ganglia, such as amygdala and subthalamus, as well as robust input from mesencephalic tegmental nuclei. Given the central position of corpus striatum (caudate and putamen) in this circuitry, its early fetal development (81, 82) and periventricular trajectory (Fig. 8) of the corticostriatal pathway (46, 49), surprisingly, very few studies have described the lesions on this important cortico-subcortical pathway, and possibly vulnerable system in preterm infants. However, hypoxic-ischemic lesions on the major target of corticostriatal pathways, striatum, in the term-neonatal brain have been described in terms of the complex picture of hypoxic-ischemic injuries of basal ganglia (23). A general indicator of abnormalities in the striatum is its diminished volume found in living prematurely born infants at term age (14) or older ages (83, 84). Circuitry abnormalities (afferent and efferent axons), postsynaptic neurons, synapses and compartmental modular organization were not systematically studied. Afferent corticostriatal pathways running within the fasciculus subcallosus of Muratoff (49) run through the most vulnerable periventricular point just lateral to the angle of the anterior horn of lateral ventricles. This pathway is a component of WMS1 (43, 44, 46) and can be damaged in focal PVL and PVHs (21).

The most likely developmental window of vulnerability for corticostriatal pathways is in EPT and VPT when the pathways grow and transfer the area of PCP (21, 43, 46). During the EPT periods 24–27 WG, corticostriatal terminal fibers surround the striatal cell islands. They reach their maximal ingrowth phase during the VPT period (28–31 GW). A transient modular organization of the striatum is particularly prominent

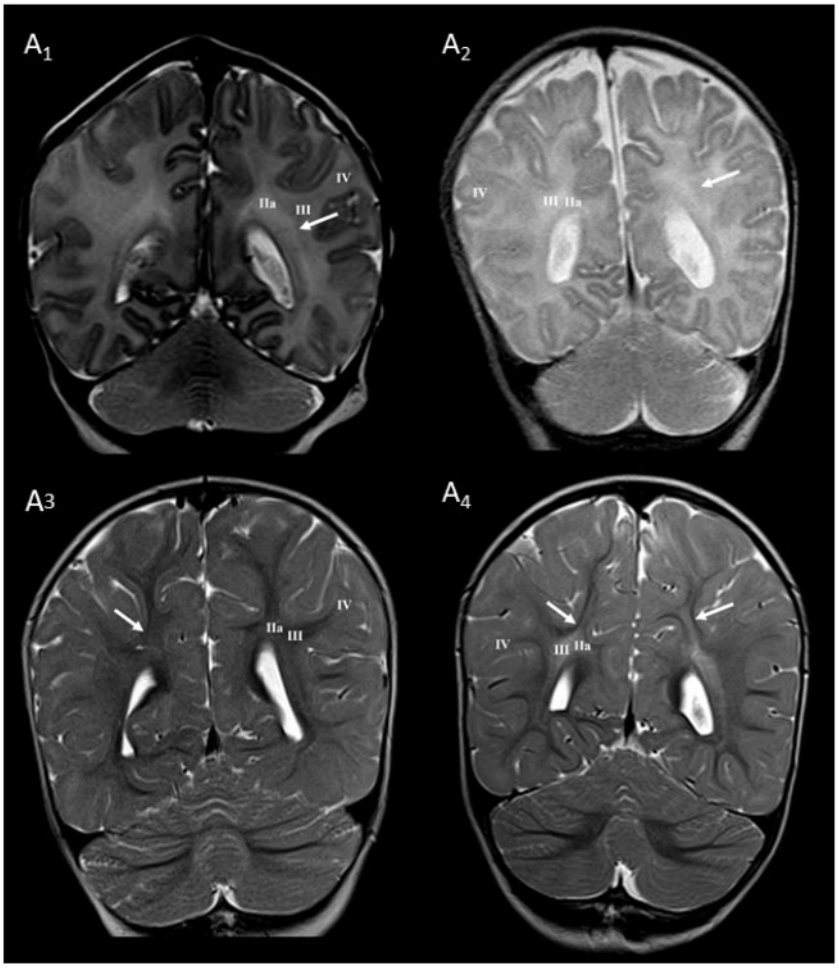

FIGURE 7. Longitudinal MRI follow-up of the centrum semiovale perinatal lesion on coronal T2 images. Normal findings of the centrum semiovale at term age **(A1)** with a visible border between the parietal crossroad and centrum semiovale **(**arrow in **A1)**. Normal findings at the age of 13 months with a barely visible border between U-fibers and the centrum semiovale **(**arrow in **A3)**. Term born child with perinatal asphyxia and diffuse hyperintensity of white matter with a diminished border between parietal crossroad and centrum semiovale at term equivalent age **(**arrow in **A2)** but with enhanced visibility of a border between U-fibers and the hyper-intensive centrum semiovale at the age of 13 months **(**arrows in **A4)**. Numbers I–IV represent segments of white matter as previously described. Reprinted from Kostović et al (21) with permission.

when sections are prepared for AChE histochemistry (Fig. 9B, B′, C) (82, 85). Importantly, at term, the basal ganglia circuit (cortex-striatum-pallidum-thalamus-cortex) is connected. This modular organization is gradually transformed in a "pediatric" type of less prominent cellular and histochemical compartments indicating that afferent fibers are more evenly distributed within the putamen and caudate and changed concomitantly of changes of histochemical properties of mosaic-like organization.

The question remains as to what extent damage to corticostriatal pathways change the organization of striatal circuitry. Namely, corticostriatal pathways form a main input to the so-called matrix component of the striatum (86). The diminished innervation of matrix neurons by corticostriatal pathways may cause alternations in compartmental island-matrix organization (86). In corticostriatal lesions, a major player in reorganization of striatal modular compartmental organization may be the nigrostriatal dopaminergic input, which innervates islands; its distribution roughly matches histochemically with AChE-reactive patches (Fig. 9B, B′, C) in the developing brain (82, 85). The possible postlesional structural plasticity of AChE-reactive patch compartments was reported by Vukšić et al (85) in infants who survived radiologically identified lesions of the striatum for the period covering 4 postnatal months (Fig. 9D).

The most striking evidence that corticostriatal pathways in primate brains show vigorous structural plasticity is derived from the pioneering experimental work of Patricia Goldman-Rakic in developing nonhuman primates (86). A series of papers demonstrated that in animals that had undergone prenatal or early postnatal unilateral prefrontal ablations, not only the normal ipsilateral projection but an enhanced contralateral projection, becomes more prominent (for a review see Goldman-Rakic [86]). The evidence for this type of connectiv-

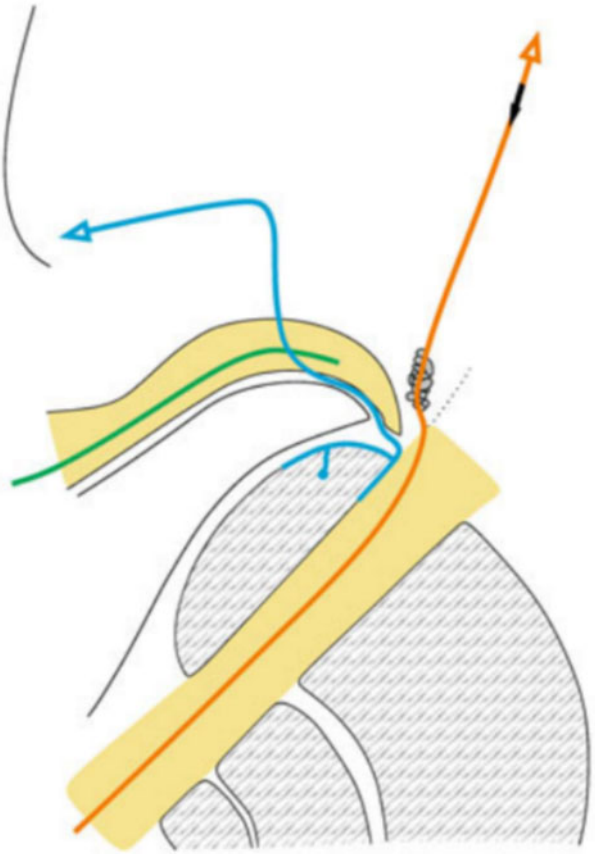

FIGURE 8. The schematic representation of the PVP system during the preterm and term (32–37 WG) period. Note the fiber arrangement of the fronto-occipital fascicle (FOF as a group of gray circles), curved course of the subcallosal (cortico-caudate) fascicle of Muratoff (SFM in blue) and spatially limited course of fronto-pontine pathways (FPP in orange) through the territory of the FOF. The corpus callosum fibers are marked in green; the internal capsule in yellow band is between basal ganglia and thalamus (marked as stippled gray areas). Reprinted from Vasung et al (46) with permission.

ity reorganization in the human brain is lacking, which can be explained by difficulties in tracing contralateral corticostriatal projections using existing DTI techniques. The conclusion indicates that little is known about effect of hypoxic-ischemic developmental lesions on corticostriatal reorganization and modular striatal organization in preterm infants. Consequently, the effect of striatal lesions on the neurological and cognitive outcome within the spectrum of cerebral palsy abnormalities after damage in preterm infants is poorly understood. One of the most characteristic pathologies of the striatum is seen in abnormally myelinated scars of the basal ganglia (87), described as status marmoratus. It is more associated with complicated delivery of full-term infants (in 50% of cases) and exists in <5-percentage preterm births (87). The histological analysis of the status marmoratus indicates a focal loss of neurons and dispersed myelinated fibers that are not grouped in characteristic bundles (radial spoke wheel pattern in characteristic bundles, Wilson's pencil). In fact, microinfarcts caused by perinatal hypoxia result in disappearance of neurons, random dispersion of myelinated fibers with predominantly preserved fine structure of myelin sheets (88).

Based on the fact that compartmental organization of striatum does not mature at birth (2, 82, 85) the authors of this article suggest that status marmoratus may involve in some extent a pathological structural reorganization stemming from selective lesions on some components of the striatal mosaic.

CORTICOPONTINE PATHWAYS/CORTICO-PONTO-CEREBERAL CIRCUITRY

The cortico-ponto-cerebello-thalamo-cortical neuronal system as an essential component of connectivity is responsible for complex supramodal executive processing in primates. Accordingly, the reasonable expectation is that it plays an important role in development of motor behavior and motor learning. During perinatal and early postnatal development, perinatal lesions have a significant effect on neurodevelopmental outcomes in prematurely born children. Therefore, it comes as a surprise that very little is known about development of corticopontine pathways, as a crucial component of this circuitry (46).

On the other hand, normal and abnormal cerebellum development has received considerable attention in pediatric neurological literature (89). A consideration of corticopontine cerebellar circuitry importantly requires noting that it begins to develop in the relatively early during fetal period and shows prolonged maturation during the first and second year of life. Importantly, in terms of anatomy, corticopontine pathways grow through vulnerable periventricular crossroads of pathways in close contact with the prominent periventricular fiber system (46). This site shows a high frequency of periventricular lesions corresponding to focal PVL (23). In fact, corticopontine pathways from the frontal cortex run closer to the periventricular vulnerable area (Fig. 8)—at the level of then interventricular foramen—than corticospinal pathways (46), which are considered the main target pathway in cerebral palsy. According to experimental studies on rodents, corticopontine pathways show a remarkable developmental reorganization in terms of sites of origin and termination (90). Although data on primates and humans are not available, the authors of this article expect that corticopontine pathways will show significant structural plasticity due to lesion of corticopontocerebellar circuitry. The corticopontocerebellar system achieves in primates some evolutionary new components, becoming an exceptionally robust system with many collaterals and strong interaction with evolutionary new portions of the neocerebellum (91). The "plastic" changes in this new part of the corticopontocerebellar circuitry may be involved in functional and structural recovery after periventricular focal lesions.

CALLOSAL (COMMISSURAL) PATHWAYS

The developmental abnormalities of the CC in terms of size, morphology, and regional fiber loss, are a frequent structural finding in clinical ultrasounds and MR imaging of the

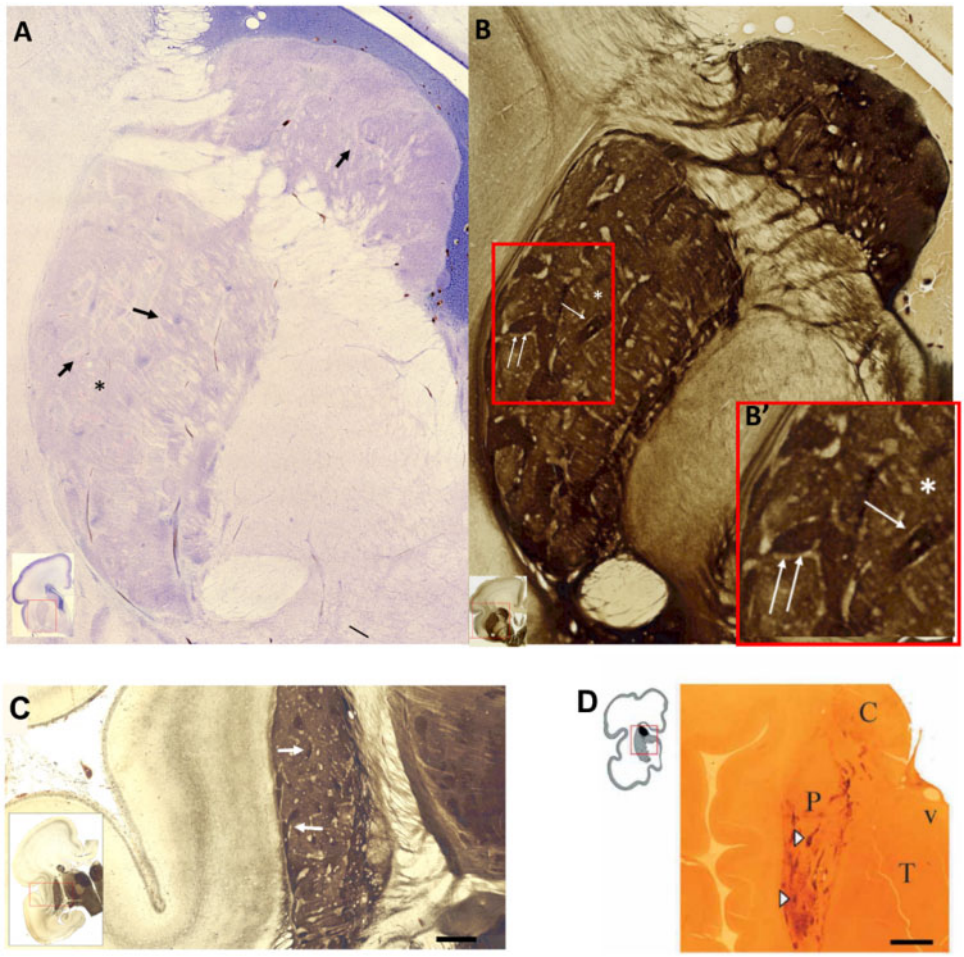

FIGURE 9. Modular organization of striatum and structural plasticity after lesion. **(A)** Nissl-stained coronal section at the anterior levels showing a cytoarchitectonic organization of striatum (putamen and caudate) in a 28 WG human fetus. Note the cytoarchitectonic units: cell islands (single arrows) and matrix (asterisk). **(B)** AChE patches (single arrows) are surrounded by AChE-negative perimeters (double arrows) and embedded in moderately stained matrix (asterisk), as shown enlarged on **B′**. Appearance of AChE negative zones around AChE positive patches (30 WG). Arrowheads indicate cell islands, arrows point to cell-poor zones. A normal specimen **(C)** and a case of periventricular hemorrhagic lesion with a premortem survival period of 4 postnatal months **(D)**. Abbreviations: *P*, putamen; *C*, caudatus; *T*, thalamus, *v*, vena thalamostriata. Scale bar: 1 mm **(C, D).**

brain and found in different pediatric neurological disorders of various etiologies (84, 89, 92). Recent studies of WM lesions in preterm infants confirm that the CC is the most frequently damaged fiber structure in perinatal hypoxic-ischemic brain lesions (18, 84). The results of measurements of the CC in "mild" H/I vary a lot and changes in the CC size without other white matter abnormalities cannot be a reliable predictor of cognitive outcome. However, frequent changes in preterm infants certainly moderate the vulnerability of this largest cerebral fiber system. There are 2 complementary explanations for frequent abnormalities and variability of CC size and shape. First is the growth of CC fibers through complex guidance zones and "decision points" (Fig. 10). The outgrowth of axons starts from pyramidal layer 3 neurons that find their path through the deep periventricular corridors in the ipsilateral hemisphere (93) and where hemispheres are fused by midline "zipper" glia. Subsequently, axons are attracted toward the other hemisphere and simultaneously prevented from growing back by the Slit and Robo system (94) during early fetal period. The first axons follow axons from cingulate cortex ("pioneering" callosal neurons [94]). After crossing the midline callosal, axons find their path within the contralateral periventricular crossroads of pathways (43).

From these crossroads, the fibers are directed anteriorly and posteriorly, running through a periventricular fiber-rich zone (inner fibrillary layer), situated between the proliferative ventricular-ISVZ and SVZ of the frontal and occipital lobe (2, 45, 67). In the midlateral cortex, fibers of the CC interdigitate with thalamocortical radiation (95). Finally, some fibers reach the subplate compartment where they branch profusely and after a prolonged period of waiting (86), penetrate the CP and contact their layer 3 pyramidal partner neurons on other side. The second factor causing variability of the callosal side is phenomena involving exuberance as described by Innocenti and Price (73, 96). Accordingly, the exuberant super numerous callosal axons in white matter of the visual cortex of cats are

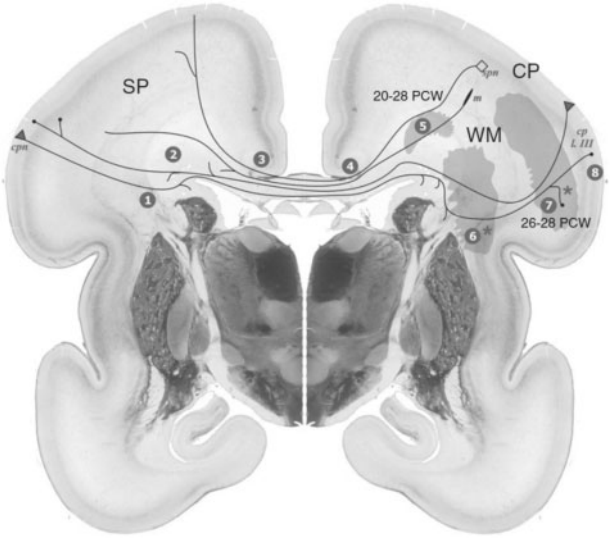

FIGURE 10. Corpus callosum fibers growth through the guidance zones and decision points (marked 1–8). Asterisks mark areas of intermingling with thalamocortical fibers. Abbreviations: *cp*, cortical plate; *cpn*, cortical plate neuron; *l. III*, developing layer III; *m*, migrating neuron; SP, subplate; *spn*, subplate neuron. Reprinted from Kostović and Judaš (93) with permission.

later eliminated. The pronounced retraction and reduction of axons in the primate's visual cortex is consistent with the fact that area 17 does not have interhemispheric connections during development (50). We propose that a thinning of the CC in H/I of preterm infants is related to the disturbance of the complex growth, guidance, and path-finding of the CC through vulnerable periventricular compartments (44, 45, 47), where requirement for ECM substrate and guidance molecules is increased during the preterm period.

Namely, both focal PVL and PVH affect the periventricular zone of callosal growth as reflected in diagnostic work using conventional MR and it therefore becomes important that the callosal zone is visible on MR scans both in vitro (26) and in vivo images (45). This spatially (topographically) delineable lamina has been neglected in previous studies on the effect of H/I on the CC and shows that the CC can be analyzed within the distinct compartment as a characteristic fiber system. This fact should be considered in future studies on the pathogenesis of cognitive impairment of prematurely born infants (84). After a lesion of growing CC fibers, a major reorganization of connectivity in ipsilateral and contralateral hemisphere is anticipated as shown by Schwartz et al (97) and Goldman-Rakic (86). This developmental reorganization may compensate the effect of lesions and promote functional repair resulting in a relatively mild outcome.

CORTICOSPINAL AND CORTICOBULBAR PATHWAYS (NUCLEAR CORTICOMOTONEURONAL PROJECTIONS)

The main efferent pathways for voluntary control (corticospinal and corticonuclear) of muscles in body extremities, neck, and head are composed of axons that originate predominantly from giant pyramidal neurons of layer 5 in the primary contralateral motor cortex of the frontal lobe. The long growth trajectory of corticospinal pathways and developmentally demanding process of myelination are factors that may explain prolonged maturation of these pathways. Layer 5 pyramidal neurons that give rise to corticospinal and corticonuclear (corticomotoneuronal) pathways are formed in the monkey brain after layer 6 and SPNs are established, after the 40th embryonic day (E 40) (98), corresponding approximately to the human age of 11 PCW. In the classical paper authored by Rakić (56), it is documented that layer 5 neurons of the frontal cortex are established at approx. E 50. Moreover, Rakić (53) has found that the time of origin of neurons destined for layer 5 in motor regions subserving the head, neck, hand, and trunk areas in the rhesus monkey are generated simultaneously, although they project 2 different levels of spinal cord (53). After migration through the intermediate and presubplate zone, future layer 5 neurons take up position in the deep third of the CP in the human fetal brain at around 15 PCW. Two months after completing migration of layer 5 neurons their axons reach the cervical spinal cord, that is, by 24 PCW (99). Interestingly, similar timing also occurs with the thalamocortical axons (2, 20, 37, 50, 95, 100). Namely, thalamocortical axons start to grow very early in the wide subplate between 13 and 15 PCW but penetrate the CP after 24 PCW. The process of fate selection and efferent identity of layer 5 neurons requires early genetically regulated molecular specification (5, 6).

The mechanisms of axon direction and guidance toward the internal capsule are explored (4), but the guidance of corticospinal axons in the brain stem remains unexplored. The pyramidal neurons of layer 5 and their numerous collaterals, including collateral in the pons, may become pruned (90). The crucial question is when do corticospinal pathways exhibit first functional monosynaptic activity on motoneurons in the spinal cord. The answer to this question comes from neurophysiological studies on humans at term (99). The authors of this article propose that functional monosynaptic corticomotoneuronal projections (Fig. 11) are likely to be present from as early as 26 PCW (99). The timing of events associated with the growth of corticospinal pathways is important for interpreting developmental lesions of corticospinal pathways after the onset of H/I lesions that seem to be the most common substrate for PVL and cerebral palsy. Lesions of corticospinal neurons in the cortex or axons running through periventricular WM occur most frequently during the ingrowth process in the spinal cord, and impairing the establishment of corticospinal circuitry, altering electrophysiological maturation that may result in the structural reorganization of injured cells, axons, and collaterals. Variability associated with developmental lesions of corticospinal pathways, corticopontine, thalamocortical, and corticocortical along radial axes is probably the main reason for extremely complex and variable motor-sensory and cognitive deficit in the cerebral palsy (21).

THALAMOCORTICOTHALAMIC SYSTEM

Thalamocortical fibers are among the earliest afferent systems to grow toward the cortical anlage and reach the presubplate layer, below the CP (3, 35), and subsequently interact

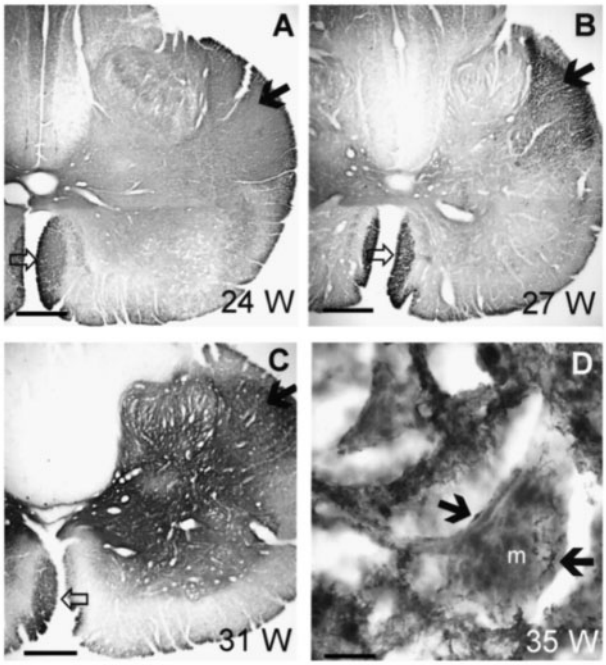

FIGURE 11. Human spinal cord C5–6: **(A)** at 24 weeks PCA, shown by GAP43 immunoreactivity in white and gray matter; **(B)** at 27 weeks PCA, corticospinal tracts represent the only major axon tracts GAP43 positive and weaker immunoreactivity in the intermediate gray matter; **(C)** at 31 weeks PCA, immunoreactivity is intense in the intermediate gray matter and motoneuronal pools and dorsal horn; **(D)** at 35 weeks PCA, motoneuron cell bodies are opposed by GAP43 immunoreactive varicose axons. The solid arrows **(A–C)** mark the lateral, open arrows the anterior corticospinal tracts; in **D**, the solid arrows mark GAP43 positive varicose axons. Abbreviation: *M*, motoneuronal cell body. Scale bars: **A–C** = 500 μm; in **D** = 20 μm. Reprinted from Eyre et al (99) with permission.

with the deepest portion of the CP at around 13 PCW (98), creating the first synapses with deep cells which form together with presubplate neurons in a new expanding compartment—the subplate. For a review of the relevant literature, see the recent paper by Kostović (20). The early arrival of thalamocortical axons in the expanding subplate is probably important for the earliest modulation of spontaneous activity of the fetal cortex. However, in addition to spontaneous activity, thalamocorticals may activate the cortex after peripheral stimulation as shown in experiments on large gyrencephalic animals (38). During the critical period of axonal growth in EPT infants, the subplate is still a major recipient of transient thalamic input (Fig. 12) and we can expect a high degree of vulnerability, abnormal development after the lesion, and in case of lesion a permanent sensorimotor deficit (Fig. 9). Topographically, thalamocortical pathways also run through vulnerable periventricular zones through the PCP (17, 21, 43, 47). The primary visual projection from the lateral geniculate body, (which is directed toward the calcarine cortex) and the associative projection from the pulvinar (which is destined for peristriate area 18) exit from the posterior portion of the internal capsule and run through the periventricular crossroads of pathways and SS (45, 50), which is one of the most vulnerable topographical areas in focal PVL. These vulnerable spatial conditions may explain the frequent visual impairment in preterm children (18). After exiting from the internal capsule, the massive projection from the mediodorsal nucleus (which is, together with pulvinar, the largest thalamic nucleus), enters the main PCP, which is the second most vulnerable topographic point in focal PVL, situated externally at a lateral angle to the frontal horn of lateral ventricles (100). The projection of the VPL nucleus shows early fast ingrowth into the somatosensory cortex during the EPT period (95) and is engaged in rapid synaptogenesis (35, 36) establishing cortical circuitry within layer 4 of the cortex. The early outgrowth of thalamic axons for the auditory cortex was reported by Krmpotić-Nemanić et al (101) and strong cholinesterase activity within the primary auditory cortex was demonstrated during the same EPT period (101). The authors of this article are not aware if other important thalamic nuclei with direct connection to the cortex, such as the reticular thalamic nucleus, show similar vulnerability and participate in poor visual sensorimotor and cognitive outcome after hypoxic-ischemic lesions in preterm infants. On the basis of these growth events during the vulnerable preterm period, the prediction is that hypoxic-ischemic lesions in preterm infants affect thalamocortical circuitry and that alternation of thalamocortical connectivity predicts cognition impairment in children born prematurely (14, 71, 102–104). Thalamocortical pathways described by histochemical and histological approaches in preterm infants (37, 50, 95, 100) are today made accessible using modern diffusion MR techniques (12, 48, 105). In future research, more attention should be placed on the functional correlation of normal and abnormal thalamocortical activity in interaction with the environment (40), pain-activated activity (42), and the role of thalamocortical connectivity in development of consciousness (37).

SUBPLATE AND WHITE MATTER NEURONS—TSN

As described in P1, the transient, synaptic and nonsynaptic subplate network exists throughout the last trimester of gestation (7, 11, 20, 26, 36, 37, 106, 107). During the EPT 24–27 WG period, the transient subplate compartment presents the most voluminous component of the cerebral wall (1, 7, 20, 36, 51). Furthermore, the SP compartment is the major site of synaptogenesis, neuron to neuron to glia interaction, axonal ingrowth, pathfinding, waiting, accumulation, and the target finding zone (7, 20, 37, 38, 50, 80, 100, 106). It has unique SP nexus (TSN) stretching out as a continuum of 3 dimensions (Fig. 12), predominantly in the tangential plane, but extending further through all aspects of the neocortical cerebral mantle, reaching the basal and limbic portions of both hemispheres and exhibiting interareal differences. Thalamocortical fibers are synaptically engaged on SPNs and simultaneously produce the first synapses in the CP, presumably on layer 4 neurons (7, 20).

In both EPT and VPT, the transient subplate nexus is prominent across the hemispheres, but in VPT the subplate

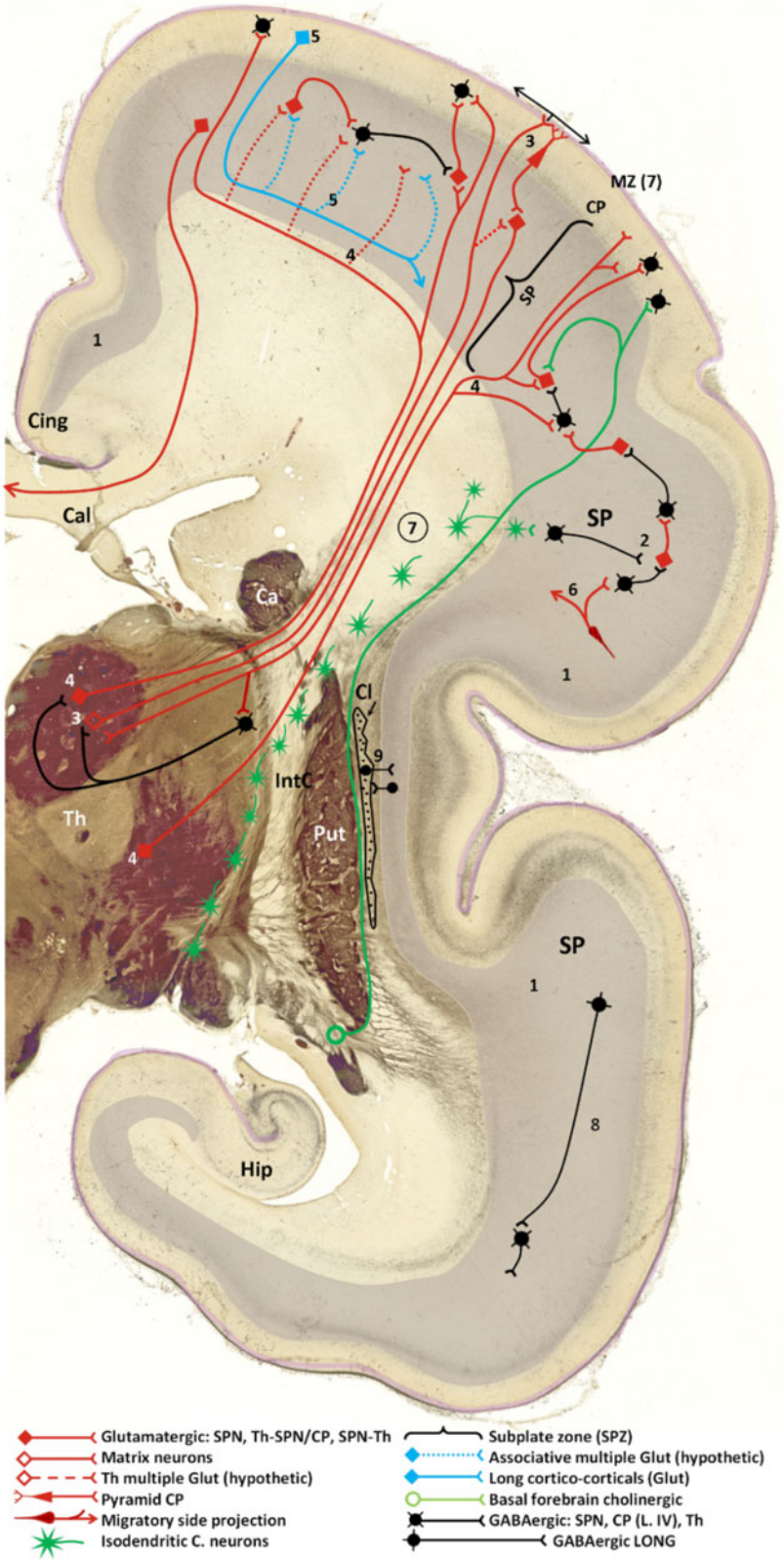

FIGURE 12. Diagram of subplate circuitry that involves the SPC and subplate neurons, basal forebrain, thalamus, CP and extends tangentially across the hemispheres. Numbers mark different circuits, that form tangential connectivity nexus of the subplate. 1. Extracellular matrix, ECM (gray, extends from the cingulate cortex [cing] to hippocampus [hip]). 2. Network of glutamatergic (red) and GABAergic (black) neurons. 3. Nonspecific thalamic matrix neuron circuit that connects thalamus-apical dendrites in the MZ, belonging to CP neurons and backwards projection to subplate neuron, then forwarded to the reticular nucleus of the thalamus and to thalamic matrix neurons. The arrows on the surface of the MZ mark possible tangential spread of innervation of this system. 4. Specific thalamic circuit from thalamic nucleus projects to subplate neurons and CP neurons, with multiple branches to the subplate along the course in the segment of white matter, sagittal strata. 5. Late developing corticocortical long pathways that run at the interface of the subplate and sagittal strata. 6. Migratory neuron with side collaterals. Abbreviations: *SP,* subplate; *CP*, cortical plate; *MZ,* marginal zone; *Cal,* corpus callosum; *th,* thalamus; *put,* putamen; *ca,* caudate; *int c,* internal capsule. The legend shows neurons and their transmitter profiles. 7. Interstitial neurons in the major WM telencenphalic bundle. 8. GABAergic neurons with long range projections in the SPC. Reprinted from Kostović (20) with permission.

becomes gradually smaller below the sulci, while remaining well developed in the wall and crown of gyri. In preterm period, 32–36 WG, the future adult-type 6 layers coexists with the transient subplate zone. At term, the subplate is reduced to the subplate remnant, a thin plexiform band situated between layer 6 and gyral white matter. Thus, SPNs become incorporated into gyral white matter and form a significant population of white matter interstitial neurons (WMIN), however, its functioning is poorly understood. Most of the ingrowing fibers of the preterm subplate (thalamocortical basal forebrain and long corticocortical fibers) are now in their terminal targets within the CP and this is another mechanism of subplate resolution. Some axons, that is, exuberant axons, simply retract as described by Innocenti and Price (73). Finally, a decrease in the synthesis of ECM is another significant factor in resolution of the subplate. The TSN, which contains local and projection SPN, glia cells, growing projection, and associative pathways, is a unique vulnerable connectivity system (Fig. 12) that can be damaged in the diffuse type of PVL (21, 108). Within the voluminous subplate compartment, which can be visualized on both histological and MR images, there is deep sublamina (7) where long afferent associative axons actively grow into the cortex after 28 PCW, that is, during the preterm period. The subplate is important for normal development of cerebral circuitry and lesions of the subplate, where its neurons represent the main substrate of cognitive deficit in encephalopathy due to prematurity (23, 109).

The lesion of the deep subplate at the border with SS are prone to different motor (14, 16) and cognitive deficit (17, 18, 109). Hypoxic-ischemic and other neurotoxic factors, genetic mechanisms involved in SP neuron damage (21, 23, 109) cause substantial changes in the number of WMIN. An increase or decrease in the number of WMIN has been attributed as an important cause of hyper or hypo connectivity in different developmental disorders, such as schizophrenia and autism (110, 111).

The exact pathogenetic mechanism of WMIN lesions is not known, but these neurons called "gate keepers" may modulate afferent inputs to the cortex at the very entrance point in the interface between gyral white matter and the cortex. The authors of this article emphasize that prospective hypoxic-ischemic lesions reveal an important tangential component of diffuse type cerebral lesions given that TSN spreads from the neocortex to limbic cortex, and may cause poor cognitive outcome without obvious transparent structural changes.

The lesions are not necessarily expressed in cell death and a decreased number of neurons. Hypoxic-ischemic incidents may be fine functional (112) or impaired regulators of protein synthesis. Experimental studies have shown that subplate neuronal circuits are uniquely susceptible to hypoxic-ischemic encephalopathy (112, 113). The finding of abnormal functional connectivity in subplate circuits without obvious structural changes on a macroscopic level or without cell loss in mild cases of hypoxia-ischemia (112) is important for interpreting possible subplate lesions in preterm infants based on normal MRI findings as it shows that abnormalities in subplate connectivity may lead to poor functional outcomes but "normal" structural findings. Similar abnormalities may be expected from fine lesions of the ECM, which is important for nonsynaptic communication of small molecules within the subplate nexus and guidance of axons (7, 20). The important role in hypoxic-ischemic lesions in preterm infants can also be attributed to astroglia.

The origin and developmental history of diverse types of astrocytes in the subplate seem to be very complex. The common mechanisms of early astrogliogenesis in SP may be equal to astrogliogenesis of the cortical plate: origin in proliferative zones, migration along with glia and local division after migration (34). Indeed, Rash et al (55) have shown tritiated thymidine positive cells in the subplate of the E90 monkey (55). However, the most specific mechanism seems to be transformation of the basal radial glia with cell bodies located in subplate into astrocytes (7, 114) (Fig. 1F). In their Golgi study, Schmechel and Rakic (1979) describe this process in fetal monkey at the subpial depths, which correspond to the subplate (115), but they did not specifically mention this compartment. Kostović et al speculated that basal glia in subplate may produce not only astrocytes but also some late born SPNs and this may be hypothetically one of the characteristics of the primate brain with special prominence in the voluminous human subplate (20). In this respect, it is interesting to find neural stem cells marker SOX2 in the subplate (unpublished data) (116). Although, this factor may be just remnant of previous molecular identity.

Astrogliogenesis starts in the human fetal cortex during early fetal life (7) and by 22–23 PCW ~40% of GFAP reactive astroglia displays relatively mature forms and probably participates in ECM production and synaptogenesis (7). The evidence of changes in the number of astroglia and their morphology in the subplate compartment were documented in cases on noncystic diffuse WM injury of the cerebral wall in preterm infants (108). Thus, the enigmatic subplate compartment may be a crucial playground for lesions of cortical circuitry underlying cognitive deficit in cerebral palsy (21, 23, 109). Lesion of TSN relates to poor cognitive outcome in preterm infants for other neurodevelopmental disorders, which may have a strong cognitive component in abnormality, such as schizophrenia and autism (20, 111). On the basis of recent evidence in both human and experimental animals, the enigmatic subplate compartment and its neurons may be the missing link for the cause of cognitive deficit in encephalopathy of prematurity (109) as well as other neurodevelopmental disorders (20).

LONG AND SHORT CORTICOCORTICAL PATHWAYS AND LOCAL CIRCUITRY NEURONS

The description of histogenetic events described earlier indicates that long corticocortical associative pathways begin to develop during the EPT and VPT period. Long corticocortical associative pathways grow just below the voluminous synaptic subplate compartment within the external sagittal stratum. The long trajectories of long corticocortical associative pathways (LCC) make them vulnerable to lesions in different locations along the cerebral mantle. As described earlier, an emphasis was placed on the VPT period in which these LCC are functionally immature because axons of this associative pathway, which originate in layer 3 pyramidal neu-

rons, do not yet establish synaptic contacts with their layer 3 pyramidal "partner" neurons in remote cortical areas. At birth, all major LCC are in their final position below the cortical target area (12, 48). However, the size (thickness) and shape of LCC pathways may change during the perinatal and postnatal period (70). Results of DTI tractography in a postmortem human preterm brain have shown that associative corticocortical pathways that connect the medial "limbic" cortex, such as the cingulum bundle, develop much earlier than LCC pathways connecting the lateral cortex. The early development of limbic associative pathways connecting the medial prefrontal cortex with the posterior cingulate cortex is present as early as 18 WG (65). The main associative pathways connecting "language" areas, such as arcuate fascicle are also revealed using in vitro imaging of the preterm brain (12). DTI studies using HARDI imaging have confirmed the existence of main LCC pathways in the preterm brain (Fig. 3), such as the superior longitudinal fasciculus (arcuate), middle longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus (sup) (48, 65). In vivo studies have confirmed the presence of major LCC in preterm infants (13).

Short corticocortical pathways that connect 2 adjacent gyri develop predominantly postnatally (44). It has been proposed that the subplate remnant compartment (44), which is situated in the interface between gyral white matter and layer 6, forms a growth substrate ("mini" waiting compartment). Since short corticocortical fibers grow predominantly after birth (44, 117), they are not damaged during preterm period. In some cases of H/I occurring during the preterm period, short corticocortical fibers are preserved and are visible on MR images due to their enhanced intensity when compared with underlying "damaged abnormal" centrum semiovale (Fig. 7); short fibers are not seen in the so-called normal brain.

Development of local circuitry and synaptology is the most difficult subject to analyze in a developing preterm brain. Even more difficult is investigating abnormalities of local circuitry neurons. The vast majority of data on local circuitry neurons refers to development of GABAergic neurons, which represent key local circuitry neurons. In a mature brain, GABAergic neurons can be divided in several classes according to their Golgi type of morphology and coexistence with different peptides and transmission modulators. In the cerebral cortex of adult brains (118), several types of basket neurons are distinguishable, coexisting with calbindin and parvalbumin, axon targeting chandelier neurons coexist with calbindin and parvalbumin and double-bouquet neurons coexist with calretinin, VIP, and calbindin. The most numerous are basket cells (118). There are also other types of GABAergic interneurons, such as neuroglia form cells. Besides the mentioned combination of transmitters and modulators, there are also other coexisting combinations of GABA and peptides, including Somatostatin, NPY, and CCK. All these GABAergic neurons belong to nonpyramidal Golgi 2-type neurons. The main classes of nonpyramidal neurons were described in the fetal human cortex, using Golgi impregnation methods (58, 59).

Nowadays, the main classes of nonpyramidal neurons are easily identified using immunocytochemistry: GABA, calretinin, somatostatin, NPY, CCK, which work well on postmortem tissue. As regards GABAergic interneurons and their origin, they represent ~20% of cortical neurons in the human cortex (119) and derive from progenitor cells located in the ventricular and subventricular zones of the ventral telencephalon including medial (MGE), lateral (LGE), and caudal (CGE) GE, as well as from the basal-preoptic and septal areas. In humans, their origin has been a matter of debate for a long time, and over the last 20 years, some authors have reported that contrary to rodents a proportion of interneurons in humans could arise from the proliferative zones in the dorsal telencephalon. It is now well accepted that the vast majority of interneurons in primates including humans originate in the GEs (120–123). In humans, DLX2-positive cells progressively increase in number between 8 and 12 PCW across the cortical wall and the majority co-express LHX6 indicating that they originate either in the MGE and migrate to the lateral cortex, or from the septal area of mediobasal telencephalon and populate the medial wall. A minority of cells co-express COUP-TFII, which identifies cells from CGE. From recent studies, VZ and SVZ of the dorsal telencephalon, as well as LGE, appear to house temporarily interneuronal precursors during their journey over long distances (121). Injury of GABAergic neurons or their progenitors may occur at the site of their origin by hemorrhagic lesions, which are most frequent between 24 and 28 GW. Even small hemorrhagic lesions can "split" GE from the telencephalic wall (see double arrow on Fig 1C).

The origin of cortical GABAergic neurons in the rodent brain is GE (1). In primates, including humans, GABAergic interneurons originate not only in GE but also in proliferative zones of the lateral telencephalon (120). GABAergic neurons first appear in the synaptic subplate compartment (20, 120, 124). The exact proportion of GABAergic neurons formed in GE and other proliferative zones of the human cerebral cortex is not known. Injury to GABAergic neurons may occur at the site of origin of massive PVH, which can destroy the proliferative zone and also impair the migration route. H/I events can also damage GABAergic neurons and result in the loss of γ-aminobutyric acid pathway expression.

REORGANIZATION AND STRUCTURAL PLASTICIY OF CONNECTIVITY AFTER DEVELOPMENTAL LESIONS

The generally accepted view is that every lesion of a developing cerebrum results in subsequent developmental reorganization of neuronal connectivity (23, 44, 86, 92). However, it is extremely difficult in the human perinatal neurology to correlate nature, location, and extent of lesion of neurogenetic cellular processes with the abnormalities, reorganization, and plasticity of connectivity and specific deficit in neurodevelopmental outcome (21). This review lists key steps of connectivity development in answering the questions of when, where, what, and how specific circuitry components develop. There is a strong evidence in both experimental (86) and clinical literature that long motor and sensory projection pathways may be structurally rerouted during growth, provided that they maintain growth potential before entering their final gray matter target and before finding postsynaptic address. It is important to emphasize that when preserved axons form new pathways, there are no new neurons formed. The reorganization process

of major pathways occurs before myelination. When axons are in their final targets, there is a limited but important possibility of local "sprouting" to compensate loss of innervation by deprived axons. It is well known that the strongest potential for local sprouting reveals monoaminergic pathways that innervate subcortical and cortical structures from early fetal life (2, 20). However, reorganization of monoaminergic pathways currently remains largely unexplored. Due to massive dopaminergic innervation of the striatum, it is very likely that their terminals play a significant role in reorganization of the striatum after the perinatal lesion (85). One of the most intriguing questions is whether neuronal loss due to cell death induces reorganization of remaining circuitry and preserved normal circuitry by simply "occupying" empty synaptic targets (86). Alternatively, healthy circuitry can simply compensate loss of neurons by increasing functional activity. It is also not clear whether common H/I lesions cause massive cell death (22). It seems that in the preterm brain, SPN exhibit increased vulnerability (22, 23, 113) compared with CP neurons (layers 2–6). The most likely reason for this phenomenon is early synaptic engagement of SPN, which facilitates development of neuroexcitotoxicity. Many SPN receive glutamatergic input or use glutamate as their transmitter (20, 38, 80, 125). A toxic effect of synaptically released glutamate results in the well-known toxic metabolic cascade after H/I injury (23).

CORRELATION OF DEVELOPMENTAL CONNECTIVITY LESION WITH NEURODEVELOPMENTAL OUTCOME

In explaining the rationale behind this review (see Introduction), the authors emphasize that the study of perinatal lesions requires defining focal and/or specific lesions that are predominant for a given spatiotemporal pattern of circuitry organization (44). However, common perinatal lesion of connectivity, which occur as a consequence of H/I, PVH, migration disorders, and different genetic syndromes, show a spatiotemporal large-scale pathogenetic effect. Therefore, finding a correlation between developmental structural, functional disturbance and neurodevelopmental outcome is extremely difficult, even with modern precision MR imaging and functional diagnostic tests (Fig. 13). Thus, confidence in prediction of outcome is limited (19). All studies have shown that massive structural lesions of the cerebrum in preterm infants have generally poor motor, sensory and cognitive outcomes (14, 15, 24, 25). The problem is prediction of neurodevelopmental outcome when MR scans are normal or show only mild structural changes (15, 19, 21, 25, 83). There are only a few studies providing systematic evaluation of neurodevelopmental outcome based on findings of fine anatomical abnormalities of cerebral pathways (17, 103), WM microstructure (14), and abnormalities of neuronal networks (71, 102). However, initial anatomically oriented approaches seem to be promising in evaluating mild lesions (17). The precise anatomical delineation of lesions is also helpful in determining specific cognitive deficit in children born prematurely with significantly abnormal MRI findings (18). Despite numerous original and meta-analysis studies, the question remains: does neurodevelopmental outcome and cerebral palsy deficit show age-characteristic abnormalities for different preterm age groups (EPT, VP, P). Of course, there is general agreement that less mature preterm infants have overall poorer neurodevelopmental outcome (15, 24). One of the reasons why comparison between the EPT, VP, and P periods is still a matter of controversy is that the number of cases in the EPT group is usually lower than in older groups. This review presents the concept of spatiotemporal parameters for every neurogenetic and histogenetic event and developmental phase, and hopes that some of these precise developmental criteria will be applied in future structural MR scoring systems in order to define better developmental windows and the radial/tangential extent of vulnerability of major axonal pathways and neuronal circuits. There are many examples to support the concept of developmental windows (critical periods), radial, and tangential vulnerability. For example, impaired migration of neurons in the cerebral cortex does not occur after 32 WG because this histogenetic process has already ceased for most cerebral neuronal systems. The most striking example is the PVH lesion of GE, frequently occurring during EPT period. This proliferative structure and its vulnerable vessels are resolved when approaching term (2, 23, 51). Therefore, GE cannot be locus minoris resistentiae of PVH during this period. This example shows that transient fetal structure, which is important for proliferation, migration, and synaptogenesis, disappears at term age and does not play a significant role in pathogenetic events during later development. However, all structural and functional indicators of neurogenetic and gliogenetic processes should be longitudinally studied in every child (15, 17, 25, 83, 126, 127). For some processes that extend from fetal to childhood, such as synaptogenesis, dendritogenesis, myelination, and transmitter maturation, there is a prolonged period of vulnerability (called prolonged vulnerability) throughout childhood and adolescence. The most striking example is myelination where precursors of oligodendrocytes appear early in fetal life and can be damaged by H/I (32, 128), whereas myelination of cerebral pathways is predominantly a postnatal process (75). Thus, a prenatal lesion of preoligodendrocytes is considered a precursor of late myelination abnormalities. These examples show that in clinical practice, individual longitudinal follow-up must be the gold standard and include at least 3 consecutive MR exams (following preterm birth, at term, and at ~2 years of age when the myelination process is in an advanced stage). Structural analysis should be complemented with age appropriate functional tests (14). The significance of long-life longitudinal follow-up (68, 83, 84, 127) is required due to the prolonged occurrence of histogenetic events in the human brain, such as changes in number of synapses throughout adolescence and early adulthood (76, 77) and the myelination process that finishes at ~28 years of age (129).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The period between 24 WG and term is characterized by intensive histogenetic events, the presence of transient laminar compartments, and their dynamic reorganization, all of which are predominantly regulated by genetic mechanisms (2). The most complex histogenetic event is the growth of axonal path-

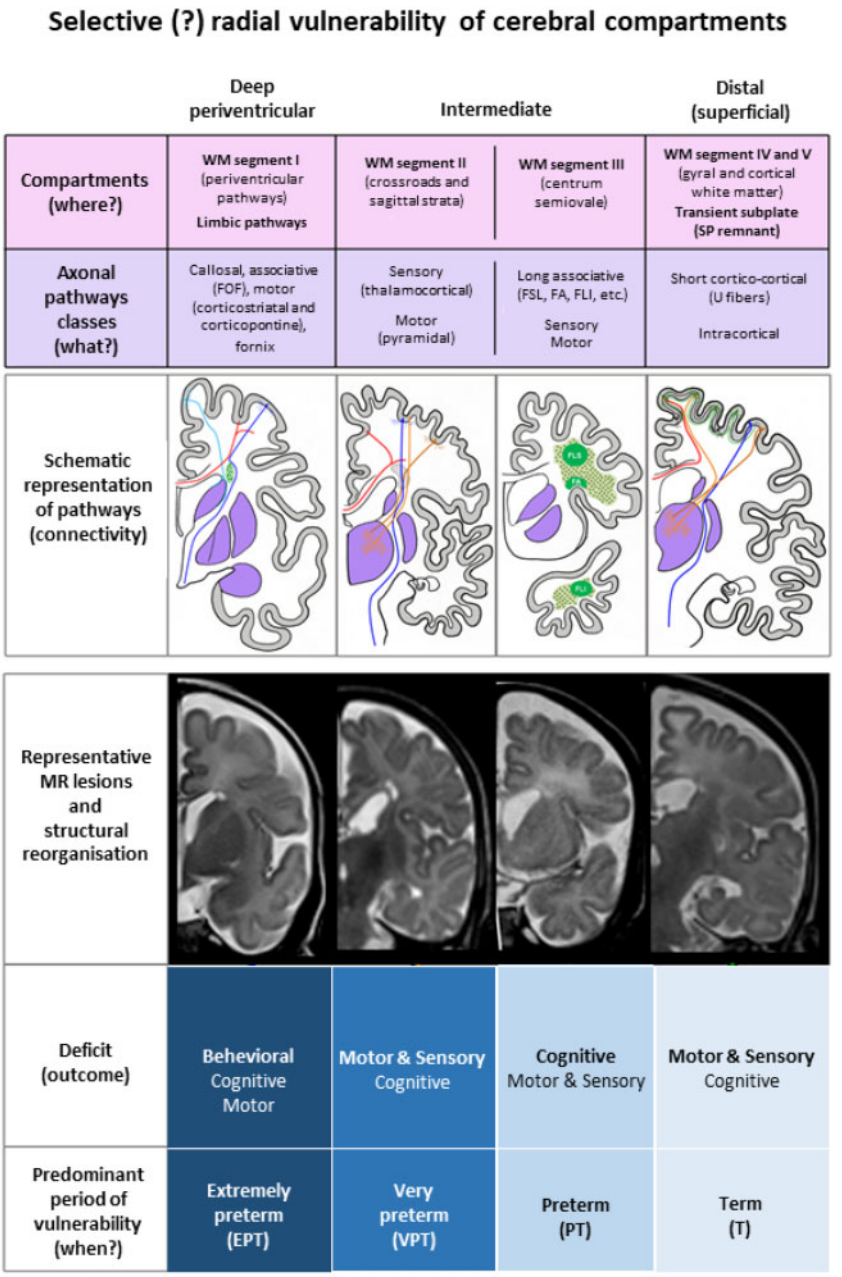

FIGURE 13. Diagram showing radial vulnerability of cerebral compartments from the ventricle (left) to pia (right). Cerebral compartments and schematic representation of the main pathways are outlined in the first 3 row of the diagram. Fourth row display MR representative lesions of different compartments (from left to right): small cystic periventricular lesion in area of fronto-occipital and subcallosal fascicles, large cystic lesion at the crossroads area, diffuse T2 hyperintense lesion of the centrum semiovale, focal T2 hyperintense lesion of gyral white matter. Fifth and sixth row display predicted clinical deficits and predominant period of vulnerability for lesions of specific classes of axonal pathways. Abbreviations: *FA,* arcuate fascicle; *FLI,* longitudinal inferior fascicle; *FLS,* longitudinal superior fascicle; *FOF,* fronto-occipital fascicle. The centrum semiovale is marked in light green. Reprinted from Kostović et al (21) with permission.

ways and other connectivity elements (dendritogenesis and synaptogenesis).

Etiological pathogenetic factors and environmental influences may cause disturbances to programmed structural-functional development and alter further development of connectivity through developmental interactions of endogenous and external factors, including the reorganization of circuitry and "plastic" response. Thanks to the advancement of MR imaging methods, immunocytochemical techniques and large-scale genomic analysis, human connectivity can be analyzed using new spatiotemporal parameters and finding answers to the question of when and where based on integration data

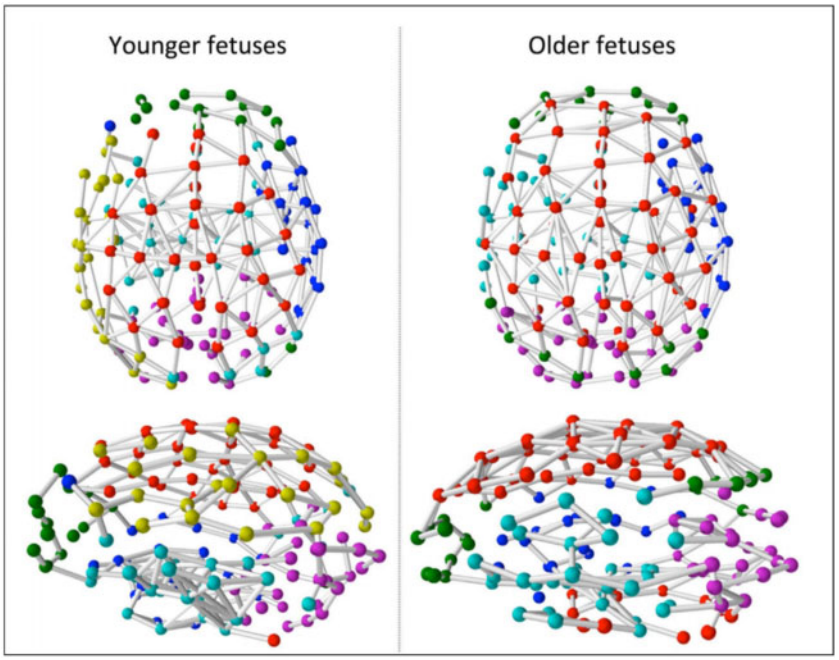

FIGURE 14. Macroscale functional brain network modules for younger (27.6 weeks; n = 17) and older (34.4 weeks; n = 16) fetuses. Nodes are shown as colored spheres and edges are shown as the connections between them. Colors are assigned to maximally similar modules in the 2 groups; color assignments are arbitrary. Reprinted from Thomason et al (70) with permission.

from in vivo and in vitro structural MR imaging, fMRI and neuroanatomically oriented postmortem analysis. The main problem are fine lesions of circuitry showing normal MR findings, where such lesions cause poor cognitive outcome and are a possible cause of developmental disorders, such as autism, schizophrenia and cognitive impairment in cerebral palsy.

Several new concepts can be helpful in analyzing fine brain lesions in preterm infants and predicting neurodevelopmental outcome. These include data on large-scale structural and functional neural networks (Fig. 14), large-scale data on gene expression, fine cellular and histological analysis of radial vulnerability within the cerebral wall, abnormalities of the transient tangential neuronal networks and disturbed modular cytoarchitectonics. Identification of location and extent of frequently affected specific neural connections (corticostriatal pathways, corticopontine pathways, callosal pathways, corticospinal pathways, thalamocorticothalamic circuitry, subplate and WM neurons and long and short associative pathways, local circuitry neurons) is important for proper prediction, targeted habilitation and longitudinal follow-up of children who have been exposed to pathogenetic factors after preterm birth. The spatiotemporal pattern of connectivity development indicates that sensorimotor and behavioral disturbances develop during earlier preterm periods due to the fact that thalamocortical and limbic pathways develop earlier, their window of vulnerability occurs during earlier preterm ages. On the other hand, significant cognitive impairment may predominantly be a result of damage of associative corticocortical connectivity that develops during later preterm ages. Importantly, future research should integrate MR imaging, neuroanatomical parameters, and genomic data for early diagnosis, prediction of deficit, proper time for early habilitation and longitudinal follow-up of children born prematurely. These integrated large-scale approaches will advance the concept of "connectivity" disorders, such as cerebral palsy, schizophrenia, and autism.

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