Laminar Organization of the Human Fetal Cerebrum Revealed by Histochemical Markers and Magnetic Resonance Imaging

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The developing human cerebrum displays age-specific changes in its patterns of lamination. Among these, the subplate zone is the most prominent transient compartment because growing major afferent systems temporarily reside in this zone, establish synapses and take part in cellular interactions that are crucial for subsequent cortical development. We explored the potential of magnetic resonance imaging (MRI) for tracing the developmental history of the most prominent cortical layer (the subplate zone) and other laminar compartments of the fetal cerebral wall between 15 and 36 weeks post-ovulation. We found that changes in the MRI lamination pattern of the human fetal cerebral wall are predominantly caused by changes in the subplate zone. Histochemical staining of the extracellular matrix (ECM) enables selective visualization of the subplate zone and correlation with an increase in MRI signal intensity in the subplate zone and ingrowth and accumulation of thalamocortical and corticocortical afferents and their subsequent relocation to the cortical plate. Thus, dynamic changes in the MRI appearance of the subplate zone and histochemical staining of its ECM can be used as indirect parameters for an assessment of normal versus disturbed unfolding of crucial histogenetic events that are involved in prenatal shaping of the human cerebral cortex.

Introduction

The human cerebral cortex develops through a series of stages during which complex histogenetic events occur in transient embryonic and fetal zones of the cerebral wall (Rakic, 1982; Sidman and Rakic, 1982; Kostović 1990a,b; Kostović and Rakic, 1990; Rakic, 1995a; Meyer *et al.*, 2000). Our current understanding of these events is largely based on findings obtained through experiments in non-human primates, carnivora and rodents (Caviness, 1982; Rakic, 1982, 1988, 1995b; Crandall and Caviness, 1984a,b; Allendoerfer and Shatz, 1994; O'Leary *et al.*, 1994; Caviness *et al.*, 1995; Levitt *et al.*, 1997; Del Rio *et al.*, 2000). Comparative studies in monkeys and humans have yielded a detailed description of region-specific development, peak and dissolution of the prominent compartment of the neocortical anlage, the subplate zone (Kostović and Rakic, 1980, 1990), as well as the development of major cortical afferent fibre systems (Kostović and Goldman-Rakic, 1983; Kostović and Rakic, 1984; Kostović, 1986; Verney, 1999). Recent studies have indicated that the developing human brain can be successfully analysed within the limits of spatial resolution of magnetic resonance imaging (MRI) devices. Indeed, recent advances in MRI technology have opened new vistas for *in vivo* studies of human brain development (Barkovich, 2000; Inder and Hüppi, 2000; Rivkin, 2000). In order to explore the potential of MRI for *in vivo* studies of laminar development fully we need a correlation with lamina-specific cellular events observed in the developing human cerebral wall. In addition, the data obtained from series of static MR images have to be interpreted as reflecting the spatio-temporal occurrence of histogenetic events.

In order to study the laminar development of the human cerebral wall, we analysed high-resolution (2.0 T) MR images in a series of formalin-fixed human fetal brains, as well as variously stained histological sections, which are part of the Zagreb Neuroembryological Collection (Kostović *et al.*, 1991). This collection contains specimens of various prenatal ages, processed with a variety of histological and histochemical techniques, that were used for the study of normal human brain development (Kostović, 1990a,b; Mrzljak *et al.*, 1990; Kostović *et al.*, 1995). The specific aim was to analyse laminar organization of the cerebral wall between 15 and 36 post-ovulatory weeks and to examine how changes in the composition of the subplate zone correlate with changes in developing cerebral fibre systems and axon strata.

Materials and Methods

The present observations are based on data from 13 brains of human fetuses (15, 16, 18, 19, 22, 23, 25 and 26 post-ovulatory weeks). The specimens were obtained from medically indicated abortions or spontaneous abortions at the School of Medicine, University of Zagreb. The ages of the fetuses were estimated on the basis of their crown–rump lengths (CRL) (120–270 mm) and/or pregnancy records and expressed as weeks from ovulation (Olivier and Pineau, 1961). In addition, we analysed the brains of four prematurely born infants aged 28, 29, 30 and 36 post-ovulatory weeks whose deaths were attributed to respiratory disease or sudden infant death syndrome. The procedure for the human autopsy material was approved and controlled by the Internal Review Board of the Ethical Committee at the School of Medicine.

Whole brains were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 and first used to obtain MR images by using the high-field 2.0 T MRI device (Gyrex Prestige, GEMS/Elscint) and the three-dimensional spoiled gradient-echo (3-D GRE) sequence that accentuates T_1 weighting. The parameters used were as follows: repetition time (T_R) 23 ms, echo time (T_E) 8.3 ms, number of excitations (N_{EX}) 1, flip angle of 20° and section thickness of 1.1 mm. The matrix size and the field of view were adjusted to obtain a spatial resolution of at least 0.416×0.416 mm^2.

After completion of MR imaging, the specimens were embedded in paraffin, serially sectioned at 15–20 mm and alternately stained by cresyl violet and Periodic Acid Schiff–Alcian Blue (PAS-AB) histochemical staining for the visualization of acid-sulphated glycoconjugates (Vacca, 1985). We also used Nissl-stained serial celloidin sections and acetylcholinesterase histochemistry-stained sections from other age-matched specimens that are part of the Zagreb Neuroembryological Collection. Images of selected MRI and histological sections were captured through a charge-coupled device (CCD) camera or Nikon scanner and processed using Adobe Photoshop.

Results

The terminology, topographic criteria and cytoarchitectonic definitions of the transient compartments of the human fetal cerebral wall as well as criteria for distinguishing several stages of cortical development were used according to the description given in previous studies (Sidman and Rakic, 1982; Kostović, 1990a,b; Kostović and Rakic, 1990). The Nissl-stained sections were used for determining the cytoarchitectonic boundaries and

assessing laminar differences in cell-packing density. The acetylcholinesterase histochemistry was used for direct demonstration of a subset of growing thalamocortical afferents and certain sagittally oriented axon strata (such as the external capsule). The differences in the intensity of diffuse background acetylcholinesterase staining were also useful in visualization of different laminar compartments and/or their borders. The PAS-AB histochemistry was used for examining the laminar location and relative regional abundance of the extracellular matrix (ECM) at different stages of cortical development.

Transient Fetal Compartments

At 15–18 post-ovulatory weeks the Nissl-stained sections enabled the delineation of seven laminar compartments within the cerebral wall (Fig. 1*A*,*D*): (i) the ventricular zone, i.e. a germinal matrix with high cell-packing density, (ii) the periventricular zone with a lower cell-packing density and an abundance of tangentially oriented bundles of tightly packed parallel fibres, (iii) the subventricular cellular zone, (iv) the intermediate zone, with cells arranged in tangential stripes dispersed among the bundles of tangentially running fibres, (v) the subplate zone of moderate cell-packing density and randomly scattered cells, (vi) the cortical plate, composed of tightly packed columns of radially arranged cells and (vii) the cell-sparse and narrow marginal zone – prospective layer I. These compartments are particularly clear in the parieto-occipital region (Fig. 1*A*) and they are also clearly discernible in older fetuses aged 19–29 post-ovulatory weeks (Fig. 2*A*–*E*). The thick subplate zone is sharply delineated from the cortical plate above it and the intermediate zone below. The basic arrangement of the axon strata is indirectly visualized by the arrangement of cells that surround fibre bundles (Fig. 1*D*). From 30 post-ovulatory weeks onwards, both the germinal matrix and the subplate zone gradually disappear. Therefore, the transient laminar pattern transforms into the mature-like structure of the cerebral wall, consisting of the cortex, underlying gyral white matter and corona radiata–centrum semi-ovale.

The sections stained with PAS-AB histochemistry show that the border between the subplate and the cortical plate can be clearly delineated from 15 to 20 post-ovulatory weeks (Fig. 2*F*,*G*) and from 29 to 36 post-ovulatory weeks (Fig. 3*E*), because at these ages the subplate zone is AB positive (blue) while the cortical plate is AB negative and PAS positive (pink) as are the fibre tracts of the intermediate zone (Fig. 2*F*,*G*). However, the border is blurred between 21 and 28 post-ovulatory weeks when the AB-positive blue staining is most intense in the upper subplate (single arrow in Fig. 2*H*), but also appears in the cortical plate in an inside-out manner (double arrows in Fig. 2*H*). This suggests that, in the human fetal cortex, the expression of acid-sulphated glycoconjugates in the cortical plate occurs concomitantly with the relocation of thalamocortical afferents from the subplate into the cortical plate, as already described in rodents (Bicknese *et al*., 1994).

At 27–30 post-ovulatory weeks, AB-positive blue staining progressively disappears from the cortical plate but remains present in the subplate zone and most pronounced in its upper part, indicating the heaviest accumulation of acid-sulphated glycoconjugates below the cortical plate. At 31–36 post-ovulatory weeks, the subplate zone disappears from primary cortical areas and becomes very thin around the bottom of the cortical sulci. However, it remains well developed within the gyral crests, particularly in the frontal and temporo-parietal regions, where its presence is clearly revealed by the strong AB-positive blue staining of the ECM (Fig. 3*E*).

From 15 to 29 post-ovulatory weeks, differences in diffuse background acetylcholinesterase staining enable the delineation of the cortical plate, the subplate zone and the intermediate zone (Figs 1*C*,*G* and 2*B*,*C*). The border between the intermediate and the subplate zone is indicated by the acetylcholinesterase-reactive sagittal fibre system of the external capsule (arrowheads in Figs 1*C*,*G* and 2*B*,*C*,*E*). The intermediate zone, i.e. the fetal 'white' matter, contains three major axon strata (Figs 1*C* and 2*B*,*C*): (i) an outermost sagittal stratum represented by the acetylcholinesterase-reactive external capsule radiation, (ii) an intermediate stratum occupied by proximal parts of transiently acetylcholinesterase-reactive thalamocortical afferent fibres and deeply located acetylcholinesterase-negative cortical efferent fibres and (iii) the deepest, subventricular stratum, which contains growing acetylcholinesterase-negative fibres of the callosal radiation. While these fibre systems are well delineated in the parieto-occipital and prefrontal region, they intermingle in the territory lateral to the angle of the lateral ventricle at the level of the interventricular foramen (the zone behind the asterisk in Fig. 1*B*).

The cortical plate is at first acetylcholinesterase negative (Fig. 1*C*) and later begins to display acetylcholinesterase staining in a region-specific manner (Fig. 2*C*,*E*). Thalamocortical afferents emanate from intensely acetylcholinesterase-reactive thalamic nuclei (dorsomedial, ventrolateral and pulvinar), pass through the internal capsule and enter the external capsule on their way to the subplate zone (Fig. 1*C*). These fibres initially reside in the deep part of the subplate zone, just above the external capsule (Figs 1*C*,*G* and 2*B*) and subsequently accumulate in the upper part of the subplate zone, just below the cortical plate (Fig. 2*B*). Finally, they gradually relocate into the cortical plate (Fig. 2*E*). The thickness of the subplate zone between 27 and 30 post-ovulatory weeks, particularly in associative cortical areas, can be explained by the presence of massive contingents of acetylcholinesterase-negative ipsilateral and commissural cortico-cortical fibres which still 'wait' in the subplate zone.

MRI Reveals the Transient Fetal Lamination Pattern and its Transformation in Pre-term Infants

At 15–18 post-ovulatory weeks, a number of structures can be easily recognized on 3D-GRE T_1-weighted MR images (Fig. 1*B*,*E*,*F*,*H*,*I*). For example, nuclear structures such as the thalamus, caudate nucleus and putamen, which all display a similar MRI signal of moderate intensity, are separated by the fibre-rich internal capsule, which displays an MRI signal of lower intensity (Fig. 1*B*,*F*). The external capsule is clearly visible as a thin, dark and sagittally oriented stripe of low MRI signal intensity, which is situated along the lateral border of the putamen (double arrows in Fig. 1*B*,*F*). The cerebral wall displays five laminar compartments of varying MRI signal intensity, which partly correspond to laminar compartments delineated on Nissl-stained and histochemical sections and are most sharply delineated on coronal MRI slices through the parietal (Fig. 1*H*) and occipital region (Fig. 1*I*). Starting from the ventricular surface, these laminar compartments are as follows. (i) The ventricular zone (germinal matrix) of high MRI signal intensity, which corresponds to the highly cellular ventricular zone in Nissl-stained sections and, therefore, is marked with number 1 (Fig. 1*H*); note that the ganglionic eminence (G in Fig. 1*A*,*B* and 1 in Fig. 1*H*) represents a localized thickening of the germinal matrix. (ii) The periventricular zone of low MRI signal intensity, which largely corresponds to the periventricular fibre-rich zone (2 in Fig. 1*D*), but cannot be clearly visualized in all cerebral regions; therefore it is marked with an asterisk on MR images

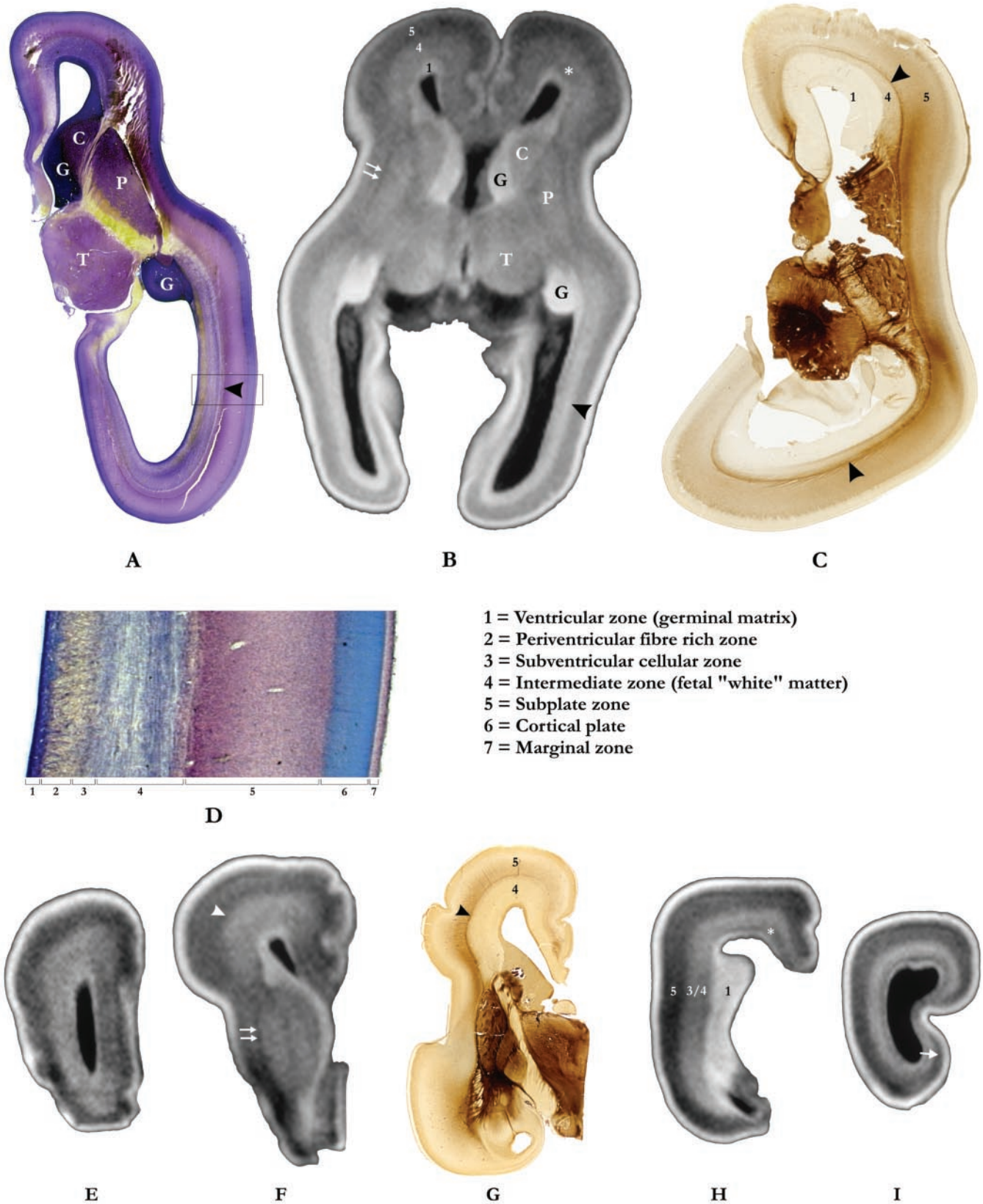

Figure 1. At 18 post-ovulatory weeks the histological, histochemical and MRI sections reveal a transient pattern of lamination in the cerebral wall. Low-power views of brains sectioned (*A–C*) horizontally and (*E–I*) coronally and stained with (*A,D*) cresyl violet, (*C,G*) acetylcholinesterase histochemistry or (*B,E,F,H,I*) displayed on 3-D GRE T_1-weighted MRI sections. For the horizontal sections anterior is to the top and for the coronal sections medial is to the right and dorsal is to the top. The box in (*A*) corresponds to the higher power view in (*D*). For details, see the text. Abbreviations (see also the legend to panel *D*): C, caudate nucleus; G, ganglionic eminence; P, putamen; T, thalamus. The asterisk in (*B,H*) indicates the periventricular fibre-rich zone as seen on T_1-weighted images, the arrowheads in (*A–C,F,G*) indicate the external capsule or its position, the arrow in (*I*) indicates the wedge-shaped narrowing of the subplate zone in the prospective primary visual cortex and the double arrows in (*B,F*) indicate the position of the external capsule in the MRI sections.

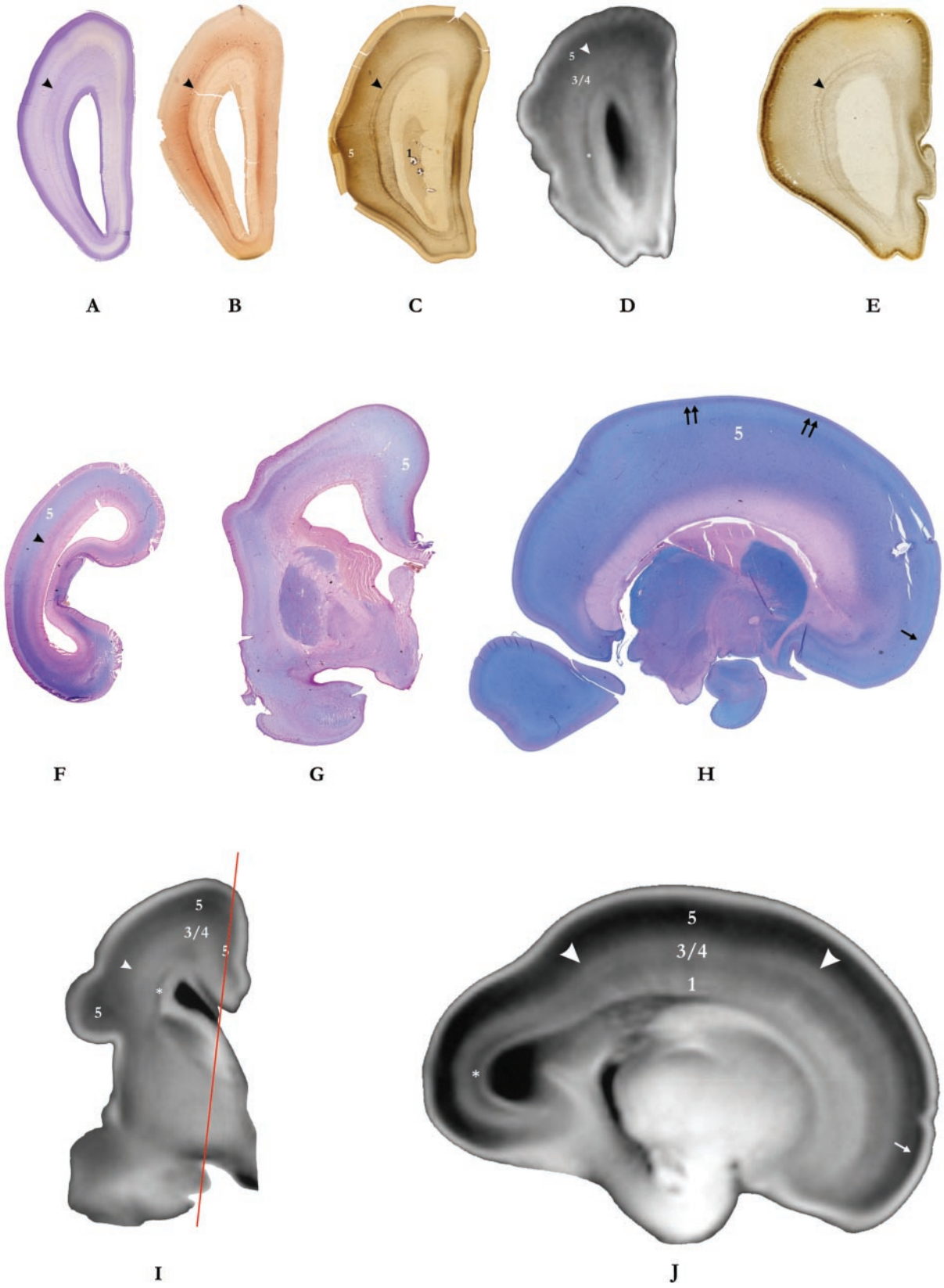

Figure 2. Transient laminar compartments of the cerebral wall in fetuses aged 18–19 post-ovulatory weeks (*A*,*B*,*F*,*G*), 22 post-ovulatory weeks (*H*,*J*), 23 post-ovulatory weeks (*C*,*D*), 26 post-ovulatory weeks (*I*) and 29 post-ovulatory weeks (*E*). Low-power views of brains sectioned (*A–G*,*I*) coronally and (*H*,*J*) parasagitally and stained with (*A*) cresyl violet, (*B*,*C*,*E*) acetylcholinestrase histochemistry, (*F–H*) PAS-AB histochemistry or (*D*,*I*,*J*) displayed on T_1-weighted MRI sections. Histochemical markers reveal the accumulation of (*B*,*C*) growing axons and (*F–H*) an extensive hydrophilic ECM in the subplate zone which at these ages also displays a low-intensity MRI signal (*D*,*I*,*J*). From 19 to 29 post-ovulatory weeks, acetylcholinesterase-reactive thalamocortical fibres first reside in (*B*) the deep part of the subplate zone, then accumulate in (*C*) its upper part just below the cortical plate and finally relocate into (*E*) the cortical plate. However, at 29 post-ovulatory weeks the subplate zone is at the peak of its development (*E*) because it contains a massive accumulation of acetylcholinesterase-negative corticocortical fibres. Note that the subplate zone in panel (*H*) appears very thick because it was sectioned parasagitally, as indicated with a red line on panel (*I*). For details, see the text. All other abbreviations are given in the legend to Figure 1.

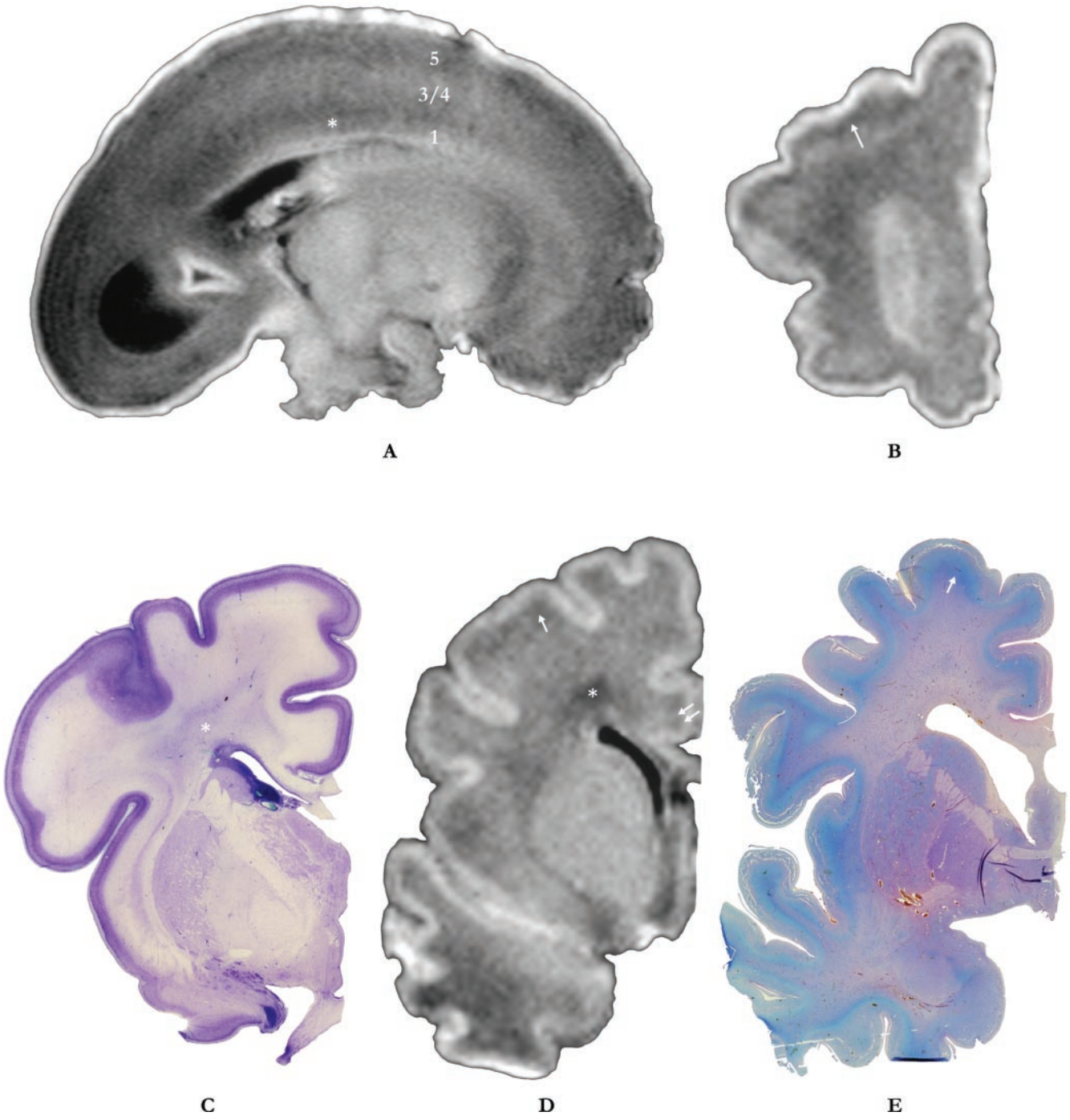

Figure 3. The lamination pattern of the cerebral wall during the developmental peak (27–30 post-ovulatory weeks) and subsequent dissolution (31–36 post-ovulatory weeks) of the subplate zone. Low-power views of (*A*,*B*,*D*) T_1-weighted, (*C*) Nissl-stained and (*E*) PAS-AB-stained (*A*) parasagittal and (*B–E*) coronal sections through the brains of premature newborns at (*A*) 29 post-ovulatory weeks, (*B*) 31 post-ovulatory weeks and (*C–E*) 36 post-ovulatory weeks. After 30 post-ovulatory weeks the subplate zone is visible just below the cortical plate and within gyral crests in the T_1-weighted and PAS-AB sections (arrows in *B*,*D*,*E*). The double arrows in (*D*) indicate the cingulum bundle. The central part of the white matter has a non-homogeneous appearance (asterisk in *C*,*D*), probably because it contains late-developing longitudinal association tracts. For details, see the text. Other abbreviations are given in the legend to Figure 1.

(Fig. 1*B*,*H*). (iii) The intermediate zone of moderate MRI signal intensity, which encompasses both the subventricular cellular zone and the fetal white matter (3 and 4 in Fig. 1*D*); therefore it is described as three-quarters on MR images (Fig. 1*H*). (iv) The subplate zone of low MRI signal intensity, which closely corresponds to the compartment marked with 5 in Nissl-stained (Fig. 1*A*,*D*) and acetylcholinesterase-stained (Fig. 1*C*,*G*) sections; therefore it is marked with 5 on MR images (Fig. 1*B*,*H*). (v) The cortical plate of high MRI signal intensity, which closely corresponds to the compartment marked with 6 on Nissl-stained sections (Fig. 1*D*), but on MR images cannot be separated from the marginal zone (7 in Fig. 1*D*); therefore, it is always described on MR images as a band of high signal intensity situated above the subplate zone.

While the external capsule is clearly delineated on MRI slices through the middle part of the cerebral hemisphere (arrowheads in Fig. 1*B*,*F*), it cannot be delimited in the frontal or parieto-occipital parts of the hemisphere. However, even in these parts of the cerebrum its position (as an outermost sagittal stratum of the fetal white matter) is clearly marked by a sharp decline in MRI signal intensity at the transition from the intermediate to the subplate zone (Fig. 1*E*,*H*,*I*).

From 19 to 26 post-ovulatory weeks, five layers described in the previous stage remain well delineated in all cortical regions (Fig. 2*D*,*I*,*J*). The subplate zone stands out as a wide and sharply delineated band of low MRI signal intensity, in particular on parasagittal MRI slices (Fig. 2*J*). However, the increase in MRI signal intensity of the subplate on T_1-weighted images begins already during its developmental peak (27–30 post-ovulatory weeks). Therefore, the contrast resolution of the subplate zone in comparison to the subjacent white matter decreases and the subplate/white matter border becomes less distinct on coronal MRI sections, although it is still recognizable on parasagittal MRI sections (Fig. 3*A*). Nevertheless, the upper part of the subplate zone remains clearly visible as a narrow band of low MRI signal intensity situated just below the cortical plate (arrow in Fig. 3*B*). During the subplate dissolution stage (31–36 post-ovulatory weeks), the intensity of the MRI signal of the subplate zone increases further. Therefore, the MRI signals of the subplate zone and the white matter become almost isointense on T_1-weighted images (Fig. 3*D*). At these ages, the subplate zone is no longer visible at the bottom of the sulci in any cortical region and in the gyri of primary cortical regions. However, it is still recognizable below the cortex of gyral crests in associative cortical areas (arrow in Fig. 3*D*).

Comparison of Histological, Histochemical and MRI Features of the Fetal Cerebrum

From 15 to 26 post-ovulatory weeks there is a close correspondence in the delineation of the germinal matrix, periventricular fibre-rich zone and the cortical plate on Nissl-stained and MRI sections, as well as in the delineation of the subplate zone in Nissl-stained, acetylcholinesterase histochemistry, PAS-AB stained and MRI sections. While the subventricular and intermediate zones are separate compartments on Nissl-stained sections, they appear as a single zone on acetylcholinesterase-stained, PAS-AB stained and MRI sections. The cell- or fibre-packing density alone does not correlate well with the intensity of the MRI signal. For example, the germinal matrix and the cortical plate are composed of densely packed cells and both have a high MRI signal intensity. However, the subplate zone has a higher cell-packing density than the intermediate zone (the fetal white matter), but has a much lower MRI signal intensity. The periventricular fibre-rich zone, external and internal capsule and corpus callosum are all composed of tightly packed parallel bundles of fibres, but have a low or very low MRI signal intensity. In fact, the lowest MRI signal intensity is displayed by the subplate zone, which represents a unique mixture of three major tissue components: numerous cells dispersed within the loose plexiform network of fibres and embedded in an abundant and hydrophilic ECM.

Regional differences in the development and thickness of the subplate zone are also apparent on both histological and MRI sections, although it could not be discerned in the supracommissural archicortex and basal paleocortex. The subplate zone is also diminished in size in regions between the neocortex and mesocortex: it is thick in the dorsal (neocortical) cingulate cortex, but displays a wedge-shaped narrowing in the ventral (mesocortical) cingulate cortex, tapering towards the cortical limbus (Fig. 1*A–C*,*F–H*). Similar wedge-shaped narrowing of the subplate zone is present along the neocortical–mesocortical transitional region in the temporal lobe (Fig. 1*G*). In addition, regional differences in the thickness of the subplate zone are present within the neocortex itself. For example, the subplate zone is thick below the lateral occipital cortex (prospective associative visual area 18) but becomes much thinner below the medial occipital calcarine cortex (prospective primary visual area 17) (Fig. 1*A*,*B*,*J*). In general, areas of the dorsolateral cerebral convexity, which receive a massive thalamocortical as well as corticocortical input, have a thicker subplate zone than the medial and limbic cortical areas.

The upper part of the subplate zone displays a band of increased acetylcholinesterase reactivity, reflecting the accumulation of growing thalamocortical fibres, displays the most intense Alcian Blue staining and corresponds to the thin band of low MRI signal intensity (Fig. 2*C*,*H*,*J*).

During the developmental peak of the subplate zone (27–30 post-ovulatory weeks), the border between the subplate zone and subjacent intermediate zone becomes less distinct in both Nissl and MRI sections. However, it is still visible on PAS-AB-stained sections and clearly demarcated on acetylcholinesterase-stained sections. Only the upper part of the subplate zone is clearly visible on MRI sections at these ages.

During the subplate dissolution stage (31–36 post-ovulatory weeks), the border between the subplate zone and underlying white matter becomes obscured on Nissl-stained, acetylcholinesterase histochemistry and MRI sections. In addition, the dissolution of the subplate zone around the bottom of rapidly developing cortical sulci occurs irrespective of regional and areal borders. However, in associative cortical areas and in the crest of gyri the subplate zone can be clearly identified on PAS-AB-stained sections and in these cortical areas it is also visible on MRI sections. After 30 post-ovulatory weeks, there is a rapid onset of secondary gyrification. In combination with the progressive dissolution of the subplate zone, this leads to the development of the centrum semi-ovale and corona radiata in the subcortical position formerly occupied by the transient subplate zone. The local inhomogeneities within the white matter on MR images at least partly correspond to developing association fibre tracts, such as the cingulum (double arrows in Fig. 3*D*) and fronto-occipital bundle (asterisk in Fig. 3*D*). Another circumscript area of the decreased signal intensity is present near the lateral wall of the lateral ventricle (asterisk in Fig. 3*D*). This area corresponds to the crossroads of afferent, efferent and association fibres and probably encompasses sagitally oriented long association fibres, such as the fronto-occipital bundle of Von Monakow (Von Monakow, 1905). Due to its topographic location and MRI appearance, that area could be easily misinterpreted as a circumscript zone of periventricular leukomalacia (Banker and Larroche, 1962; Volpe, 1987).

Discussion

In this study, we present a correlation between the cytological, histological and histochemical appearance and MRI images of the transient laminar pattern in the human fetal cerebral wall during the mid-fetal and late-fetal period. We also show that the presence of an abundant and hydrophilic ECM can be explored in order to visualize the subplate zone selectively and delineate the border between the neocortical anlage and the intermediate zone.

We find that changes in the MRI lamination pattern of the human fetal cerebral wall are predominantly caused by changes

in the appearance of the subplate zone. In addition, the changes in the MRI appearance of the subplate zone occur in parallel with dissolution of its ECM. Finally, developmental changes in both the histochemical and MRI appearances of the subplate zone display a close spatio-temporal correlation with ingrowth and accumulation of thalamocortical and corticocortical afferents in the subplate zone and their subsequent relocation to the cortical plate. These findings confirm and extend our previous description of the developmental history of the subplate zone, which is related to the accumulation and subsequent relocation of major classes of cortical afferents and laminar shifts of synaptogenesis (Kostović and Rakic, 1990).

Developmental Changes of Histochemical Markers in the Subplate Zone Occur in Parallel with the Ingrowth of Axons and Synaptogenesis

The tissue elements of the subplate zone are embedded in an abundant, very hydrophilic and transient ECM (Pearlman and Sheppard, 1996; Margolis and Margolis, 1997). It was shown in both primates and humans that thalamocortical afferents accumulate and 'wait' in the subplate zone before entering the cortical plate (Rakic, 1977; Kostović and Goldman-Rakic, 1983; Kostović and Rakic, 1984, 1990). Moreover, together with the marginal zone, the subplate zone is the site of the earliest synaptogenesis in the developing cortex (Molliver *et al.*, 1973; Kostović and Rakic, 1980, 1990; Allendoerfer and Shatz, 1994). These synapses are transient and disappear from the subplate zone when afferent systems relocate from the subplate zone into the cortical plate (Kostović and Rakic, 1990; Allendoerfer and Shatz, 1994). The transient presence of the ECM in the subplate zone may be closely connected with above described events that are involved in the formation and maintenance of transient fetal circuitry because the ECM contains a number of permissive, attractive or repellent guidance cues for growing axons (Sheppard *et al.*, 1991; Bicknese *et al.*, 1994; Miller *et al.*, 1995; Emerling and Lander, 1996; Pearlman and Sheppard 1996). The transient presence of that ECM in the subplate zone further suggests that the extracellular milieu of the subplate zone mediates a unique set of cellular interactions that are required for cortical development. Several lines of evidence support that conclusion about the role of the subplate ECM.

First, the subplate zone contains a large extracellular space through which neurons and growth cones migrate (Kostović and Rakic, 1980, 1990; Allendoerfer and Shatz, 1994; Letourneau *et al.*, 1994). Second, that extracellular space is filled with an abundant and dynamic ECM that contains a rich mixture of transiently expressed polymeric carbohydrates and glycoconjugates: glycosaminoglycans (Nakanishi, 1983; Hankin and Silver, 1988; Lander, 1993), chondroitin sulphate proteoglycans (CSPGs) such as neurocan (Bicknese *et al.*, 1994; Miller *et al.*, 1995; Emerling and Lander, 1996), hyaluronic acid (Bignami and Delpech, 1985), polysialic acid in the form of polysialylated neural cell adhesion molecule (Seki and Arai, 1991), laminin and *s*-laminin (Hunter *et al.*, 1992) and fibronectin (Stewart and Pearlman, 1987; Chun and Shatz, 1988; Sheppard *et al.*, 1991). Third, many glycoconjugates of the subplate ECM can be selectively stained by PAS-AB histochemistry as described previously (Nakanishi, 1983) and confirmed in our study. Fourth, polymeric carbohydrate moieties of these glycoconjugates have in common certain physicochemical properties: a large size, space-filling properties, high viscoelasticity and a polyanionic character (Comper and Laurent, 1978). Their high charge density renders them extremely hygroscopic (Fryer and Hockfield, 1996; Rutishauser and Landmesser, 1996) and enables them to enhance the retention of water and the establishment of diffusion gradients in the extracellular space (Lehmenkühler *et al.*, 1993) and, thus, to create a permissive or promotive environment for the guided growth of axons, the defasciculation of axons that arrived in bundles, axonal branching and synaptogenesis in the subplate zone (Letourneau *et al.*, 1994; Fryer and Hockfield, 1996; Pearlman and Sheppard, 1996).

It should be noted that the dissolution of the abundant and hydrophilic ECM of the subplate zone begins after 28 post-ovulatory weeks (when thalamocortical axons have completed their relocation to the cortical plate), but that the ECM remains present below the cortical plate at least until 36 post-ovulatory weeks, i.e. during the period when corticocortical fibres still reside in the subplate zone (Kostović and Rakic, 1990; Rakic, 1995a).

Developmental Changes in the MRI Appearance of the Cerebral Wall are Predominantly Caused by Changes in the Subplate Zone and its ECM

Our MRI study shows that, between 15 and 26 post-ovulatory weeks in humans, the subplate zone can be sharply delineated by a low MRI signal intensity on T_1-weighted images. In addition, we found that, from approximately 28 post-ovulatory weeks onwards, the MRI signal intensity of the subplate zone progressively increases along with its contrast resolution with respect to the intermediate zone. Therefore, on T_1-weighted images of late premature brain, the subplate zone is visible only as a narrow band of lower signal intensity situated just below the cortex and present mainly in the gyral crests of associative cortical areas.

These findings are in agreement with the results of previous studies of the layered appearance of the fetal cerebral wall on T_1-weighted MR images (Girard and Raybaud, 1992; Girard *et al.*, 1995; Chong *et al.*, 1996; Brisse *et al.*, 1997; Childs *et al.*, 1998; Sbarbati *et al.*, 1998; Felderhoff-Mueser *et al.*, 1999; Lan *et al.*, 2000). For example, we found that, from 15 to 26 post-ovulatory weeks, the cerebral wall displays five layers of alternating MRI signal intensity on T_1-weighted images, as generally described previously (Girard and Raybaud, 1992; Childs *et al.*, 1998; Felderhoff-Mueser *et al.*, 1999). We also found that a high MRI signal intensity of the cortical plate and the germinal matrix closely correlate with their pronounced cell-packing density.

However, with respect to the laminar appearance of the wide zone situated between the germinal matrix and the cortical plate, our findings cast doubt on the interpretations presented in the previous publications. In previous studies, that part of the cerebral wall was interpreted as a fetal white matter consisting of three sublayers: a deep and superficial layer with a low T_1-weighted MRI signal intensity, separated by an intermediate compartment with moderate MRI signal intensity (Girard *et al.*, 1995; Brisse *et al.*, 1997; Felderhoff-Mueser *et al.*, 1999). These studies were limited to correlation of T_1-weighted MR images with Nissl-stained histological sections and, thus, interpreted differences in the MRI signal intensity as differences in the cell-packing density, suggesting that an increased MRI signal intensity of the putative intermediate layer of the fetal white matter reflects the presence of migrating cells (Girard and Raybaud, 1992; Girard *et al.*, 1995; Brisse *et al.*, 1997; Felderhoff-Mueser *et al.*, 1999).

We observed three compartments of alternating MRI signal intensity between the germinal matrix and the cortical plate, i.e. in the wide fetal intermediate zone. However, two of them (deep and intermediate) represent the intermediate zone or fetal white matter, whereas the third (superficial) one corresponds to the

subplate zone, a separate fetal compartment transiently present between the developing white matter and the cortical plate. The distinction between two deep (white matter) and one superficial (the subplate zone) compartments of the fetal intermediate zone is particularly prominent in histochemical preparations.

In addition, we found that differences in the packing density of any single tissue element are not sufficient for realistic interpretation of the layered appearance of the cerebral wall as seen in T_1-weighted MR images. For example, from 15 to 26 post-ovulatory weeks both the subplate zone and several bundles of tightly packed and orderly arranged axons (e.g. the corpus callosum, external and internal capsule and periventricular fibre-rich zone) display a MRI signal of low intensity. However, in addition to a loose plexiform network of fibres the subplate zone also contains numerous cells, synapses and an abundant hydrophilic ECM.

In an important recent study, a line scan diffusion-weighted MRI sequence with diffusion tensor analysis was applied to measuring the apparent diffusion coefficient (ADC), to calculating the relative anisotropy (RA) and to analysing the microstructural development of the cerebral white matter in living newborns aged 28 to 40 post-ovulatory weeks (Hüppi *et al*., 1998). It was found that the RA in the central cerebral white matter increased ~2-fold between 28 and 40 post-ovulatory weeks, whereas the overall ADCs decreased markedly (Hüppi *et al*., 1998). Because the RA measures the directionality of diffusion, these findings of pre-myelination anisotropy suggest a change in the microstructural arrangement of fibre tracts, particularly in the outer part of the fetal cerebral white matter. Interestingly, the superficial cerebral white matter as described in the above mentioned study, largely corresponds to the subplate zone as defined in our study. Therefore, from approximately 28 post-ovulatory weeks to term, one should expect a significant reduction in the ADC, i.e. an increasing restriction of the free diffusion of water within the subplate zone. As already noted (Hüppi *et al*., 1998; Inder and Hüppi, 2000),these changes occur concomitantly with three-dimensional rearrangement of the axonal plexus within the subplate zone. On the other hand, changes in the directionality of water diffusion cannot convincingly explain changes in the intensity of the MRI signal in T_1-weighted images. Namely, it is well known that the T_1 relaxation time primarily depends on the free water content and not on the free diffusion of water. This suggests that changes in the MRI signal in T_1-weighted images correlate predominantly with histological changes leading to an increase or decrease in the amount of free water and the hydrophilic ECM of the subplate zone in the extracellular space.

Notes

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