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Supratentorial Parenchyma in the Developing Fetal Brain: In Vitro MR Study with Histologic Comparison

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PURPOSE: To assess the in vitro MR signal of the developing brain through histologic comparisons. **METHODS:** Five healthy fetal specimens aged 16, 19, 22, 27, and 34 gestational weeks were studied in vitro using T1- and T2-weighted sequences in frontal and axial planes. Neuropathologic studies included sections in the same frontal plane. Comparison of histologic sections with measurements of the relative widths of the layers of different signal intensities enabled us to assign cellular correspondence to each MR layer. **RESULTS:** In the cerebral mantle, a layered pattern was observed on both T1- and T2-weighted images. In the basal ganglia, signal from the pallidum and thalamus was isointense with white matter from 16 to 22 weeks' gestation; then, from 27 and 34 weeks' gestation, the signal was relatively high on T1-weighted images and low on T2-weighted images. The neostriatum had a relatively low signal on T1-weighted images and a high signal on T2-weighted images from 16 to 27 weeks' gestation; then, at 34 weeks' gestation, the signal was relatively high on T1-weighted images and low on T2-weighted images. **CONCLUSION:** MR imaging can clearly show specific patterns of growing fetal brain in vitro.

Index terms: Brain, growth and development; Fetus, magnetic resonance

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Owing to progressive improvement in technology, magnetic resonance (MR) imaging now provides a means for diagnosing diseases of the fetal brain parenchyma during pregnancy. In France, since decisions regarding termination of pregnancy can be made independent of gestational age, fetal MR examinations can be performed in vivo from 16 to 40 weeks' gestation. Consequently, knowledge of the normal maturational process of the fetal brain is a prerequisite to the optimal interpretation of these in utero examinations.

Descriptions of the normal appearance of the fetal brain in utero have previously been provided primarily by T1-weighted MR sequences (1–6). Chong et al (7) have recently reported the results of MR and histologic comparisons made at 18 weeks' gestation, but, more often, MR images have been compared with age-matched pathologic specimens (8–10) or with anatomic atlases. Our aim was to compare MR images obtained in vitro of healthy fetal brains of 16 to 34 weeks' gestation with their histologic counterparts in order to understand better the maturational process of the brain's mantle and basal ganglia.

Materials and Methods

Five fetuses were selected for study. Inclusion criterion was the absence of histologic abnormalities. Exclusion criteria were pronounced maceration (which would prevent adequate histologic examination) and chromosomal abnormalities or polymalformative syndrome (known to be associated with brain malformations). The menstrual ages of the fetuses were 16, 19, 22, 27, and 34 gestational weeks, respectively, as determined on the basis of the usual criteria (fetal measurements, kidney and pulmonary histologic maturation, and skeletal maturation). Causes of death included miscarriage in the case of the youngest fetus, twin-twin syndrome, and abortion (for anamnios, or premature membrane rupture, in one case, and for heart malformations in two, left ventricular hypoplasia in one and univentricular heart in the other).

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Brains were extracted and fixed in 10% formalin solution within 6 hours after expulsion. Time from fixation to MR imaging was 10 to 90 days. MR studies were performed on a 0.5-T imager with circular receptive (14- or 17-cm-diameter) surface coils. All brains were studied in vitro in the formalin solution. To achieve homogeneous signal, brains were positioned in such way that sections were acquired parallel with the coil plane. Frontal and axial planes were defined with respect to the brain stem axis (parallel or perpendicular to it). Inversion-recovery T1-weighted sequences were obtained with parameters of 1500/25/3 (repetition time/echo time/excitations) and an inversion time of 600; spin-echo T2-weighted sequences were obtained with parameters of 4000/140/2 with a 12 × 12-cm field of view, a 256 × 256 matrix, and a 4- to 5-mm section width.

Neuropathologic examinations included macroscopic study of frontal sections (in the same plane as the MR examinations) of paraffin-embedded brain tissue cut in 8- to 10-mμ thicknesses with gaps of 4 to 5 mm, deparaffinized, and stained with hemalum-phloxine, cresyl violet, and cresyl violet–Luxol. Cerebral mantle comparisons were made on frontal sections by measuring the relative width of the layers of different signal intensities on T1- and T2-weighted images secondarily transposed on the histologic sections. Measurements were taken on a line drawn through the foramen of Monro and the external point of the frontal horn. For the basal ganglia, the MR signal was evaluated by comparing it with the signal of the internal capsule and then correlating it to the histologic sections to confirm anatomic location. All signal intensities were graded on the basis of visual evaluation.

Results

Cerebral Mantel

The MR-histologic comparisons are summarized in Table 1.

At 16 weeks' gestation, three layers were differentiated in the cerebral mantle. The inner layer had a relatively high signal on T1-weighted images and a low signal on T2-weighted images, and corresponded to the germinal zone, containing two concentric waves of neuroglial migrating cells (one medial with sparse cells and one external with higher cellularity). The intermediate layer showed a relatively low signal on T1-weighted images and a high signal on T2-weighted images, and corresponded to the intermediate zone, containing a few sparse neuroglial cells. The outer layer had a relatively high signal on T1-weighted images and a low signal on T2-weighted images, and corresponded to the immature cortex, containing a molecular layer and the subplate zone.

At 19 weeks' gestation (Fig 1), four layers were differentiated. The first was a thin inner

TABLE 1: MR-histologic comparison of the cerebral mantle in fetal brain specimens

Gestational age, wk	Thickness, mm	No. of Layers on MR Images	Signal Intensity (%)*		Histologic findings
			T1-Weighted Images	T2-Weighted Images	
16	4	3	High (45)	Low (47)	Matrix and two concentric areas of neuroglial migrating cells
			Low (25)	High (34)	Intermediate zone with a few sparse neuroglial cells
			High (30)	Low (19)	Cortex
19	6	4	High (10)	Low (9)	Matrix
			Interm† (41)	Interm (38)	Neuroglial migrating cells
			Low (34)	High (38)	Intermediate zone containing a few sparse cells
			High (15)	Low (15)	Cortex
22	9	4	High (21)	Low (19)	Matrix and a thin layer of migrating cells
			Interm (33)	Interm (22)	Dense layer of migrating glial cells
			Low (35)	High (48)	A few sparse glial cells and axonodendritic prolongations
			High (11)	Low (11)	Cortex
27	13	3	High (21)	Low (24)	Matrix and few migrating cells
			Low (68)	High (63)	White matter
			High (11)	Low (13)	Mature cortex
34	22	2	Low (88)	High (90)	White matter
			High (12)	Low (10)	Cortex

* % means the relative size of the layer in the cerebral mantle.

† Interm means intermediate signal, lower than that of the matrix and higher than that of the intermediate zone.

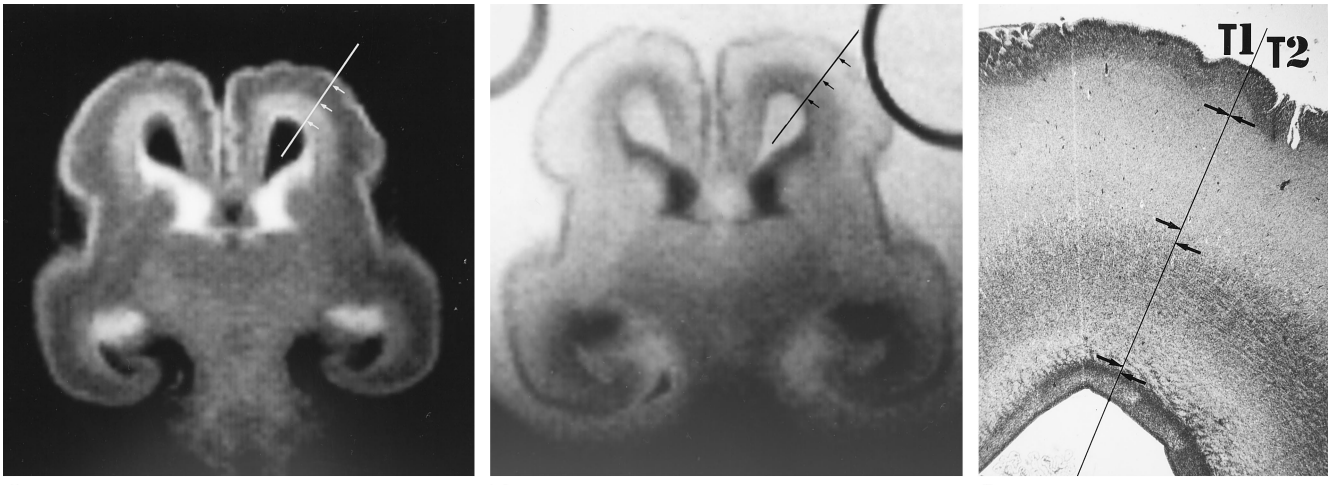


Fig 1. Fetal specimen (frontal section) at 19 weeks' gestation. T1-weighted MR image (1500/25/3; inversion time, 600) (*A*); T2-weighted MR image (4000/140/2) (*B*); and cresyl violet–stained histologic section at same level (*C*). A four-layer pattern is seen (*arrows* represent the boundaries between the MR layers): a thin medial layer with a relatively high signal on the T1-weighted image and a low signal on the T2-weighted image corresponds to the germinal matrix; a layer with intermediate signal on the T1- and T2-weighted images corresponds to the neuroglial migrating cells; a layer with a relatively low signal on the T1-weighted image and a high signal on the T2-weighted image corresponds to the intermediate zone, containing a few sparse cells; and a lateral layer with a relatively high signal on the T1-weighted image and a low signal on the T2-weighted image corresponds to the immature cortex.

layer with a relatively high signal on T1-weighted images and a low signal on T2-weighted images, corresponding to the matrix. The second layer had intermediate signal on T1- and T2-weighted images, corresponding to neuroglial migrating cells. The third layer had a relatively low signal on T1-weighted images and a high signal on T2-weighted images, corresponding to the external area of the intermediate zone, containing a few sparse cells. The outer layer showed a relatively high signal on T1-weighted images and a low signal on T2-weighted images, corresponding to the cortex.

At 22 weeks' gestation, four layers were differentiated. The first was an inner layer with a relatively high signal on T1-weighted images and a low signal on T2-weighted images, corresponding to the matrix, containing a thin layer of migrating cells close to the matrix. The second layer had an intermediate signal on T1- and T2-weighted images, corresponding to a dense layer of migrating glial cells. The third layer had a relatively low signal on T1-weighted images and high signal on T2-weighted images, corresponding to the external intermediate zone, containing a few sparse glial cells and axonodendritic prolongations. The outer layer had a relatively high signal on T1-weighted images, and a low signal on T2-weighted images corresponding to the cortex (containing pyramidal cells).

At 27 weeks' gestation (Fig 2), three layers were differentiated. The first was an inner layer with a relatively high signal on T1-weighted images and a low signal on T2-weighted images, corresponding to the matrix, containing a few migrating cells. The intermediate layer showed a relative low signal on T1-weighted images and a high signal on T2-weighted images, corresponding to the white matter. The outer layer showed a relatively high signal on T1-weighted images and a low signal on T2-weighted images, corresponding to the mature cortex, with characteristic lamination.

At 34 weeks' gestation, only two layers were observed: the internal layer had a relatively low signal on T1-weighted images and a high signal on T2-weighted images, corresponding to the white matter; the outer layer had a relatively high signal on T1-weighted images and a low signal on T2-weighted images, corresponding to the cortex.

The differences in thickness between the layers on T1- and T2-weighted images were minimal and considered nonsignificant.

Basal Ganglia

The MR-histologic comparisons are summarized in Table 2.

From 16 to 22 weeks' gestation, the thalamus was isointense with the internal capsule on both T1- and T2-weighted images. At 27 and 34

weeks' gestation, it had an increased signal on T1-weighted images and a decreased signal on T2-weighted images, more pronounced in the ventrolateral aspect.

The pallidum was also isointense with the internal capsule at 16, 19, and 22 weeks' gestation, and was therefore not identified. It appeared isointense with the thalamus on both T1- and T2-weighted images at 27 and 34 weeks' gestation.

At 16 weeks' gestation, the putamen was isointense with the internal capsule. Compared with the internal and external capsules, it had decreased signal on T1-weighted images and increased signal on T2-weighted images at 19, 22 (Fig 3), and 27 weeks' gestation. At 34 weeks' gestation, the putamen showed an increased signal on T1-weighted images and a decreased signal on T2-weighted images relative to white matter. Nevertheless, it could be differentiated from the pallidum by the relatively low signal of its medial aspect on T1-weighted images and high signal on T2-weighted images (Fig 4).

The caudate nucleus was visible as early as 16 weeks' gestation, by virtue of its signal relative to the germinal zone. Compared with the matrix, the signal appeared decreased in intensity on T1-weighted images and increased in intensity on T2-weighted images. From 19 weeks' gestation, the caudate nucleus and the putamen had the same signal on both T1- and T2-weighted images.

No myelination was depicted within the basal ganglia with conventional Luxol staining.

TABLE 2: MR-histologic correlations of the basal ganglia in fetal brain specimens

Gestational Age, wk	Basal ganglia	Signal Intensity*	
		T1-Weighted Images	T2-Weighted Images
16	Thalamus	Isointense	Isointense
	Pallidum	Isointense	Isointense
	Putamen	Isointense	Isointense
	Caudate nucleus	Low	High
19	Thalamus	Isointense	Isointense
	Pallidum	Isointense	Isointense
	Putamen	Low	High
	Caudate nucleus	Low	High
22	Thalamus	Isointense	Isointense
	Pallidum	Isointense	Isointense
	Putamen	Low	High
	Caudate nucleus	Low	High
27	Thalamus	High	Low
	Pallidum	High	Low
	Putamen	Low	High
	Caudate nucleus	Low	High
34	Thalamus	High	Low
	Pallidum	High	Low
	Putamen	High	Low
	Caudate nucleus	High	Low

Note.—No myelination was depicted within the basal ganglia with conventional Luxol staining.

* Signal intensity relative to the internal capsule.

Discussion

Cerebral Mantel

Embryological data show that by 10 weeks the cortex is highly individualized (11–13). The subcortical layer, the future white matter, is ex-

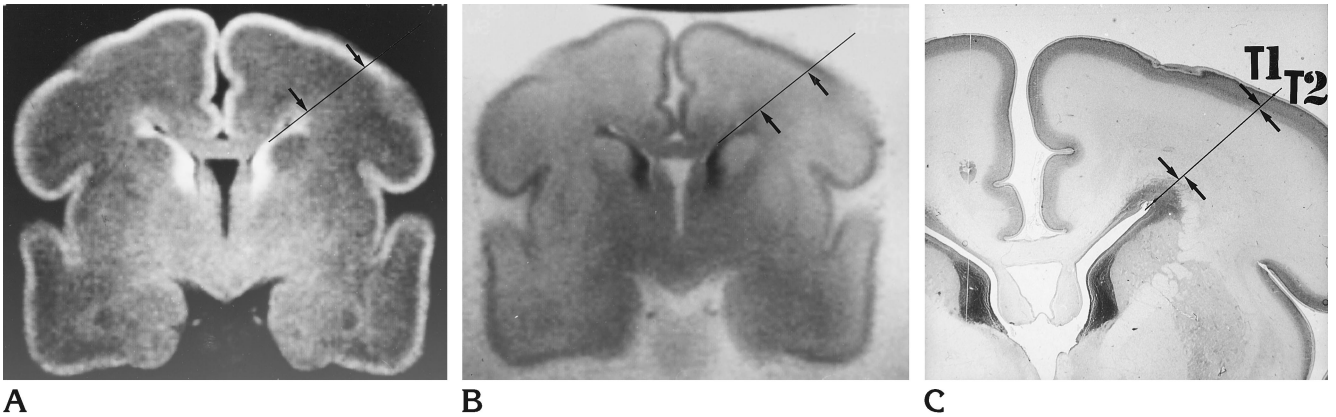
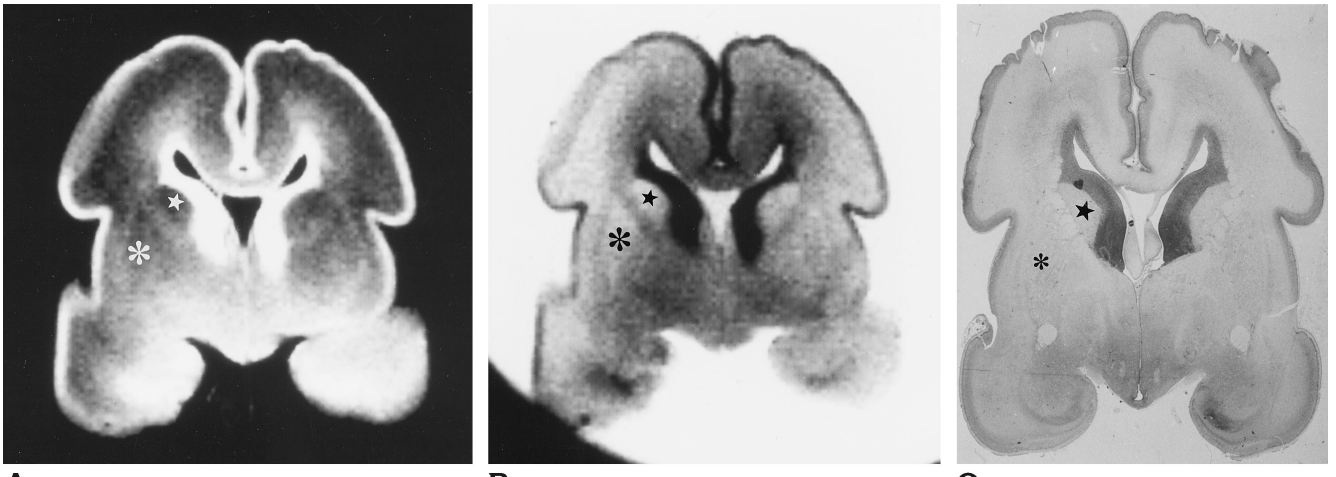
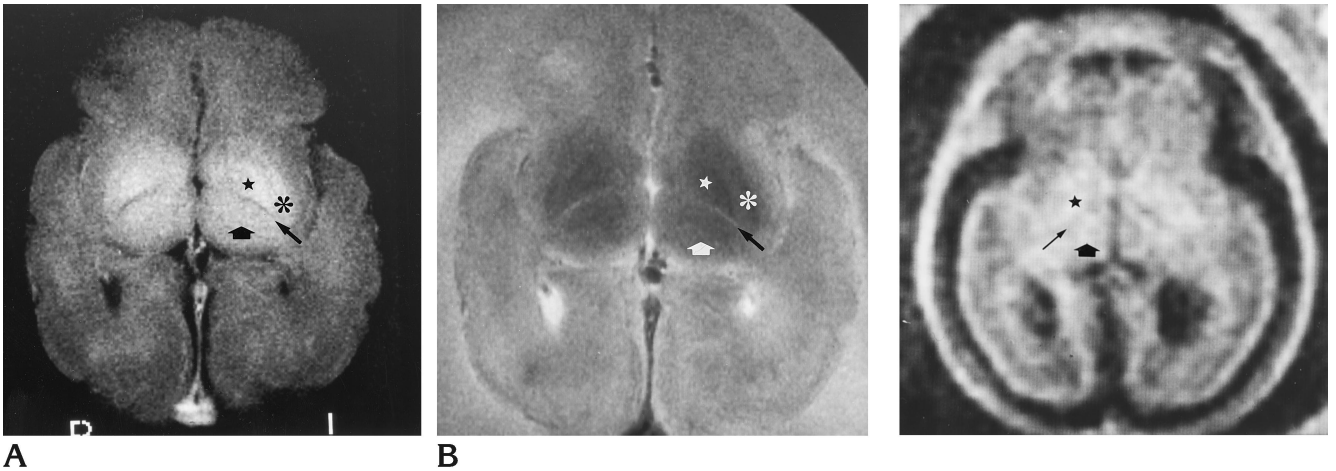


Fig 2. Fetal specimen (frontal section) at 27 weeks' gestation. T1-weighted MR image (1500/25/3; inversion time, 600) (*A*); T2-weighted MR image (4000/140/2) (*B*); and cresyl violet–stained histologic section at same level (*C*). A three-layer pattern is observed (*arrows* represent the boundaries between the MR layers): a medial layer with a relatively high signal on the T1-weighted image and a low signal on the T2-weighted image corresponds to the matrix and a few migrating cells; an intermediate layer with a relatively low signal on the T1-weighted image and a high signal on the T2-weighted image corresponds to the white matter; and a lateral layer with a relatively high signal on the T1-weighted image and a low signal on the T2-weighted image corresponds to the mature cortex.



A B C

Fig 3. Fetal specimen (frontal section) at 22 weeks' gestation. T1-weighted MR image (1500/25/3; inversion time, 600) (*A*); T2-weighted MR image (4000/140/2) (*B*); and cresyl violet–stained histologic section at same level (*C*). The caudate nucleus (*star*) and the putamen (*asterisk*) show a low signal on the T1-weighted image and a high signal on the T2-weighted image. The pallidum is not differentiated from the internal capsule.



A B

Fig 4. Fetal specimen (axial section) at 34 weeks' gestation. T1-weighted MR image (1500/25/3; inversion time, 600) (*A*) and T2-weighted MR image (4000/140/2) (*B*). Compared with the internal capsule (*long arrow*), the pallidum (*star*) and the thalamus (*short arrow*), especially the ventrolateral part, display a high signal on the T1-weighted image and a low signal on the T2-weighted image. Apart from its medial aspect, which allows its differentiation from the pallidum, the putamen (*asterisk*) also has a high signal on the T1-weighted image and a low signal on the T2-weighted image.

Fig 5. In vivo T1-weighted axial MR image (300/15/4; flip angle, 75°) of healthy fetal brain at 27 weeks' gestation. The pallidum (*star*) and the thalamus (*short arrow*) have a relatively high signal. The posterior limb of the internal capsule (*long arrow*) has a relatively low signal.

tremely cellular. Deeper still, beneath the ependyma, a large dense band of immature cells surrounds the ventricles, called the germinal zone (or matrix). After 10 weeks, the matrix is concentrated over the ganglionic eminence (the groove between the caudate nucleus and the thalamus) from the frontal to the temporal horn of the ventricle. Many cells migrate from this zone, which thins considerably during gestation. From 8 to 15 weeks, concentric waves or parallel rows of migrating cells are visible in the intermediate zone. These cells will populate the cortex in an "inside-out" fashion. Neuron-generating mitoses in the juxtaventricular area probably stop at about 15 to 16 weeks. During weeks 16 to 24, fewer mitoses are seen in the matrix, and the migratory process slows down, but the migration of daughter cells and glial cells continues until the matrix is exhausted. By 26 to 28 weeks, few immature cells remain in the periventricular zone except in the ganglionic eminence, in the external wall of the occipital

horns, and in the roof of the temporal horns. The six-layered pattern of the motor cortex is already suggested at about 28 weeks.

In our in vitro study, we were able to define each MR layer by measuring the extent of the different signals and then transferring those measurements onto corresponding histologic sections. When the size of the brain was sufficient to allow good spatial discrimination on MR images (starting from 19 weeks' gestation), we found good correlation between the MR layers and the histologic cell populations.

In vivo T2-weighted MR images are of special interest, as fast spin-echo sequences with a short acquisition time are unaffected by fetal motion, afford numerous sections per acquisition, and provide better signal-to-noise ratio than do spin-echo or gradient-echo T1-weighted sequences.

At most, we saw four layers of the cerebral mantle on MR images at 19 and 22 weeks. At 16 weeks' gestation, the spatial resolution did not permit us to separate the matrix from the migrating cells. At 19 weeks' gestation, the layer of migrating cells was probably so close to the matrix that the deep intermediate zone could not be differentiated. At 22 weeks' gestation, the migrating cells were also adjacent to the matrix; thus, the inner layer on MR sections corresponded to the matrix and to the deeper part of the layer of migrating cells. At 27 weeks' gestation, some migrating cells were also included in the inner layer, with a high signal on T1-weighted images.

Our results agree with those of Mintz et al (8) and Hansen et al (10), who described a three-layer pattern at 17, 18, and 24 weeks' gestation. Unfortunately, those studies were done with aged-matched fetuses from various origins, notably the Yakovlev collection, the NEPE collection, or the Feess-Higgins and Larroche atlas (14). Our findings also are in conformity with those of Resta et al (6), who showed the physiological high signal of the matrix on T1-weighted images at 21 weeks' gestation (6).

Girard et al (4, 5) and Chong et al (7) described a five-layer pattern. Girard et al showed this pattern in vivo between 23 and 28 weeks' gestation. In a series of fetal specimens that ranged from 9 to 24 weeks' gestational age, Chong et al observed in vitro the same aspect between 16 and 18 weeks. These investigators all interpreted the layers as representing the germinal matrix, the deep white matter, the intermediate migrant cells zone, the subcortical white matter, and the cortical plate, respectively. Chong et al made only one histologic comparison at 18 weeks' gestation, but did not obtain precise measurements.

Neuroglial migration is not a continuous process. Anatomic data show several migrating waves with marked temporal and individual variation (12). Accordingly, the observed MR pattern is variable, depending on whether the migrating wave is close to or far from the matrix. According to the histologic data, the migration can also be observed as a pattern of parallel rows of cells instead of as waves of migrating cells (12). In such cases, one can observe on MR sections a progressive decrease of signal intensity on T1-weighted images, as in our 27-week specimen. In other cases, the highly cellular wave of migrating cells can be clearly separated from the matrix, giving a five-layer pattern. However, the term *deep white matter* is probably not appropriate, considering that this layer is not yet mature white matter but rather a zone of neuroglial migration. To clarify our description, we propose that during the migration process the area between the cortex and the matrix be called the *intermediate zone*, as the neuropathologists refer to it.

We have no definitive explanation to account for the signal intensities of the different layers. Histologic studies exclude the hypothesis of a difference in myelination state (11). Like Girard et al (4, 5), we found a good correspondence between signal intensity and cellular density. The germinal matrix and the cortical plate had the higher cellular density, exhibiting higher signal on T1-weighted images and lower signal on T2-weighted images. However, the relationship between the relaxation times and the cellular density remains unclear. McArdle et al (15) suggested that the higher interstitial water content in immature brain could explain the long T1 and long T2 of white matter.

Basal Ganglia

Previous investigators reported high signal intensity within the basal ganglia only during the third trimester of pregnancy (2, 3, 6). More recently, Girard et al (5) depicted increased signal on T1-weighted images in the basal ganglia as early as 21 weeks' gestation, and suggested that it represented an increased cellular density.

In our study, we noted that each basal ganglia

shared a common embryological origin and MR signal: the caudate nucleus and the putamen (neostriatum, from telencephalic origin) on one hand, and the ventrolateral thalamus and the pallidum (paleothalamus and paleostriatum, from diencephalic origin) on the other hand.

Myelination of the basal ganglia, when studied with a conventional Luxol stain (which displays a phospholipid component in the myelin), shows that the internal medullary lamina of the pallidum is myelinated at about 26 weeks' gestation, the external medullary lamina at 32 weeks' gestation, and the ventral thalamus and putamen at 38 weeks' gestation (11). We did not find in our sample any myelination pattern on histologic sections, although we observed significant signal variation among our specimens. In 1992, Hasegawa et al (16) studied the development of early myelination as indicated by the myelin basic protein (the protein component of myelin). Using this highly sensitive immunohistochemical technique, these researchers observed a myelination process earlier than seen with Luxol staining: from 25 weeks' gestation for the pallidum and ventrolateral thalamus, and from 35 weeks' gestation for the neostriatum. This timing corresponds very well with our observations, and indicates that MR imaging is more sensitive than conventional Luxol staining for early detection of myelination.

We are aware of the small size of our series, due in part to our decision to include only histologically proved healthy specimens. In abnormal specimens, not included in this study, healthy areas of brain parenchyma showed the same signal patterns as described in the material presented.

The question arises as to whether this in vitro material can be used as a reference for in vivo studies. The following arguments can be given for such a use: first, our results agree with the published or observed findings in the few healthy cases studied in utero in our department (Fig 5); second, the in vitro signal differences are similar to those reported in healthy premature newborns studied at 31 (15, 17) weeks' gestation; and third, in seven other cases (not included in this study) in which the fetal brain was studied both in utero and after abortion, no significant variation of qualitative signal contrast was observed in either normal or abnormal areas of the parenchyma. Nevertheless, further in vivo MR studies are necessary to confirm our in vitro data.

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