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Developmental alterations of the prefrontal cerebral cortex in sudden unexplained perinatal and infant deaths

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Abstract

The aim of this study was to investigate the developmental patterns of the human prefrontal cortex involved in breathing control in a wide cohort of fetal and infant death victims, aged from the 22nd gestational week to 10 months of life, and to evaluate whether morpho-functional disorders are present in this specific cortical area in victims of sudden unexplained death. A further aim was to determine whether prenatal absorption of nicotine could also affect the maturational processes of the prefrontal cortex. A pronounced radial organization of the cerebral wall was evident from the 26th gestational week. By 36 gestational weeks this columnar structure disappeared, coinciding with the formation of a laminar cytoarchitecture. The mature cortex, observable from the 4th month of life, was organized horizontally into six laminae. In 33% of the sudden death victims the prefrontal cortex showed morphological alterations with anomalous laminar patterns and delayed neuronal maturation. A significant correlation with prenatal cigarette exposure was found.

Keywords: Fetal breathing; maternal smoking; prefrontal cortex; SIDS; unexplained perinatal death.

Introduction

Our studies on sudden unexpected perinatal and infant death revealed the presence of frequent neuromorphological and/or functional alterations of brainstem nuclei responsible for vital functions, primarily breathing, as well as cardiovascular control [10–13, 20, 23, 27].

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Recently, we reported developmental anomalies even in specific structures of the cerebellum that may play a critical role in compensatory responses to hypoxic insults occurring in pre- and/or post-natal life [16, 17, 19]. Currently, we also hypothesize that a suprapontine compensatory mechanism is involved in the control of vital functions. This suggestion is mostly based on several studies assessing the involvement of the pre-motor cerebral cortex in the behavior of the human respiratory muscles, and therefore, a cortical contribution to breathing [22, 31].

In this study we evaluate if the prefrontal cortex, that is specifically involved in the control of the pre-motor activity, could also be implicated, during its development, in the mechanism of sudden death.

We firstly investigated the developmental patterns of the human prefrontal cortex in a wide cohort of fetal and infant death victims, aged from the 22nd gestational week to 10 months of life, and then we evaluated whether morpho-functional disorders are present in this specific area of the cortex in victims of unexplained death. Finally, our previous observations of an increased incidence of structural and/or functional alterations of the central autonomic nervous system in victims born to mothers who smoke [9, 14, 15, 18] prompted us to investigate whether prenatal absorption of nicotine could also affect the developmental processes of the brain cortex.

Materials and methods

Our study focused on 63 brains from victims of sudden unexplained perinatal and infant death, aged from 22 gestational weeks to 10 postnatal months. The victims included 25 fresh fetuses (22–40 gestational weeks), 9 newborns (1–7 postnatal days) and 29 infants (1–10 months) and comprising 36 males and 27 females.

This was a selected set of cases collected on the basis of a specific decree of the Lombardy Region [21] following the guidelines provided by the Italian law n.31/2006 "*Regulations for Diagnostic Post Mortem Investigation in Victims of Sudden Infant Death Syndrome (SIDS) and Unexpected Fetal Death*". Both the regional decree and the national law impose that all suspected SIDS victims as well as fetuses deceased after the 22nd week of gestation without any apparent cause, after a complete autopsy performed according to the International Standardized Autopsy Protocol of the Global Strategy Task Force of SIDS International [8], must be sent to our Research Center for in-depth histopathological examination, particularly of the central autonomic nervous system and of the cardiac conduction system [25, 26, 28].

For every case, a complete clinical history, particularly related to mothers in perinatal deaths and including the death scene examination in infants' death, was collected. All the mothers were asked to complete a questionnaire on their smoking habit, detailing the number of cigarettes smoked before, during and after pregnancy. Characteristics of the mothers per smoking category were: 8% (n = 5) reported smoking only until pregnancy, 27% (n = 17) continued smoking during and after pregnancy (all more than 3 cigarettes/day) and 56% (n = 35) were non-smokers. In six cases (9%) no information about smoking was available.

After the in-depth histological examination, a diagnosis of effective "unexplained death" was established for 55 of the 63 victims, namely 22 stillbirths, 9 newborns and 24 infants, diagnosed as true SIDS.

The remaining eight cases (3 fetal and 5 infant deaths) (13%), were classified as "border-line", since it was difficult to establish whether the pathological findings were sufficiently severe to cause death. In the infant's death we observed encephalitic features (probably viral in etiology) in brainstem of two cases, a case with capillary hemangioendothelioma of the medullary area postrema and two cases with lung artery dysplasia. In fetal death cases we found dilated cardiomyopathy in one case and chorioamnionitis in two cases.

Hereafter we briefly describe the study protocol, provided by the guidelines of the Italian Law n.31/2006, for the in-depth histological examination of the central autonomic nervous system. These guidelines allow the reproducibility of the neuropathologic findings and can be carried out by all neuroscientists to produce homogeneous and comparable results.

Samples of 2–4 mm in thickness from the brainstem, cerebellum and cerebral cortex, were collected, processed and embedded in paraffin.

Examination of the brainstem includes sampling of three specimens, where the main vital centers are located, as shown in Figure 1. The first specimen, ponto-mesencephalic, includes the upper third of the pons and the adjacent portion of midbrain; the second extends from the rostral limit of the medulla oblongata to the adjacent caudal pons; the third specimen takes as reference point the obex and extends 2–3 mm above and below it.

In order to examine both the cortex and the deep nuclei of the cerebellum, a specimen of hemisphere extending along the major diameter must be cut. For the cerebral cortex examination, samples about 0.5 cm^3 of size, from the different brain areas and in particular from the anterior part of the frontal lobes (the prefrontal cortex-PFC), the main target of this study, were excised.

Transverse serial sections from all the samples were made at intervals of 30 μm. For each level, twelve 5 μm sections were obtained. Two of these sections were routinely stained for histological examination using alternately hematoxylin-eosin and Klüver-Barrera stains; additional sections were saved and stained as deemed necessary for further investigations.

Histological examination

In the brainstem the main nuclei controlling the vital functions were analyzed, namely the parafacial nucleus, the trapezoid nucleus, the locus coeruleus and the parabrachial/Kölliker-Fuse complex in the pons and mesencephalon; the hypoglossus, the dorsal motor vagal, the tractus solitarius, the ambiguus, the pre-Bötzinger, the inferior olivary and the arcuate nuclei in the medulla oblongata. In the cerebellum, the cortex layers (external granular layer, molecular layer, Purkinje cell layer and internal granular layer) and the medullary deep nuclei (the dentate nucleus, the fastigial nucleus, the globose nucleus and the emboliform nucleus) were examined.

In the cerebral cortex, we made in particular an in-depth analysis of the cytoarchitecture of the PFC and of the sequence of developmental steps from the 22nd gestational week.

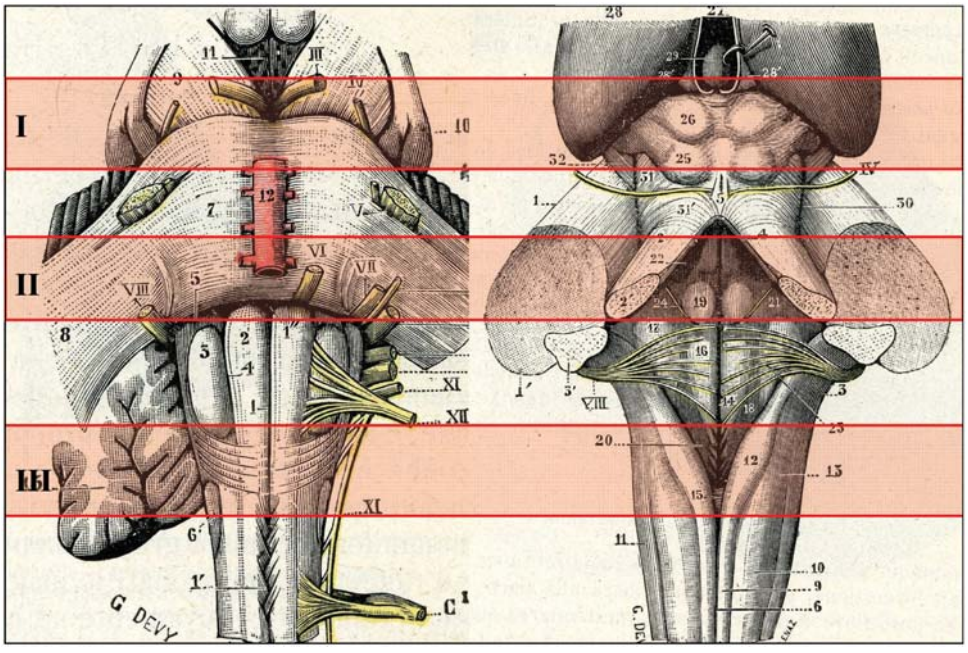

Figure 1 Sampling of the brainstem: ventral (left) and dorsal (right) surface.

Statistical analysis

The statistical significance of direct comparison between the two groups of victims with and without smoking mothers, respectively, was determined using analysis of variance (ANOVA). Statistical calculations were carried out with the SPSS 10.0 software (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). The selected threshold level for statistical significance was $P < 0.05$.

Results

Histological examination of the prefrontal cortex (PFC), the focus of this study, demonstrated that in most cases there were homogeneous, progressive developmental cortical steps. At the earliest observation (22 gestational weeks) the PFC shows high, diffuse cellularity. Only the molecular layer, with poor cell number, is outlined. Besides, a superficial cellular row can be observed at this stage (Figure 2).

At 25 weeks, the external granular layer is poorly delineated, below and bordering the molecular layer, as a dense row of small round cells. A general decrease of the neuronal numbers and an initial radial organization below this layer, perpendicular to the pial surface, can be recognized. The superficial line of cells is not visible (Figure 3).

The changes taking place in the cerebral wall between 26 and 35 weeks are characterized by a pronounced radial organization of the cortex below the external granular layer, showing parallel mini-columns of small neurons, and a progressive neuronal maturation, with increased body size and formation of cell processes.

By 36 weeks the radial organization begins to disappear, together with the appearance of the internal granular layer (Figure 4). At 37 weeks, large pyramidal cells, with evident axons projecting towards the pial surface, undergo maturation and organization in the internal layer (Figure 5).

During the last weeks of pregnancy (38–40 weeks) and the first month of life, a laminar structure of the cortex is discernible with scarcely defined borders between layers.

In the second postnatal month, a thick external pyramidal layer is well recognizable below the external granular layer, with numerous small polygonal neurons (Figure 6).

From the 4th month of life, the standard structure of the cortex, horizontally organized into six distinct laminae, is easily visible. From the outside inward these are: (I) the molecular layer; (II) the external granular layer, (III) the external pyramidal layer; (IV) the internal granular layer;

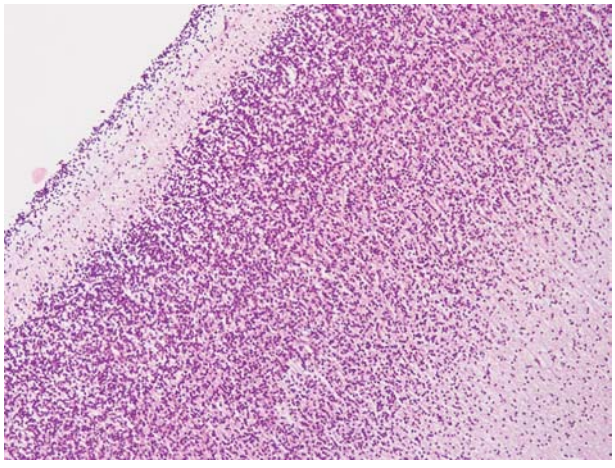

Figure 2 Diffuse cellularity in the prefrontal cortex of a fetus aged 22 gestational weeks. Only the molecular layer is discernible, below a thin external cell row.
Hematoxylin-Eosin stain, magnification 10×.

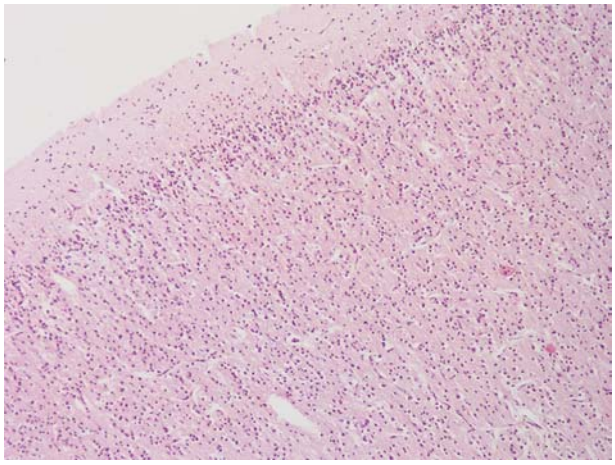

Figure 3 Formation of the external granular layer, below the molecular layer, in the prefrontal cortex of a fetus aged 25 gestational weeks. A sketch of radial organization can be remarked.
Hematoxylin-Eosin stain, magnification 10×.

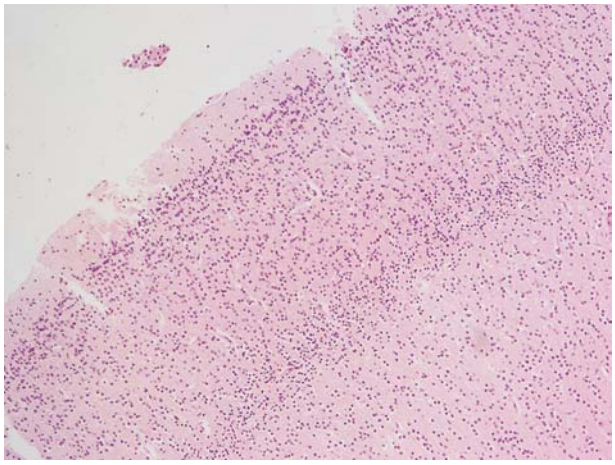

Figure 4 Initial organization of the internal granular layer in the prefrontal cortex of a fetus aged 36 gestational weeks.
Hematoxylin-Eosin stain, magnification 10×.

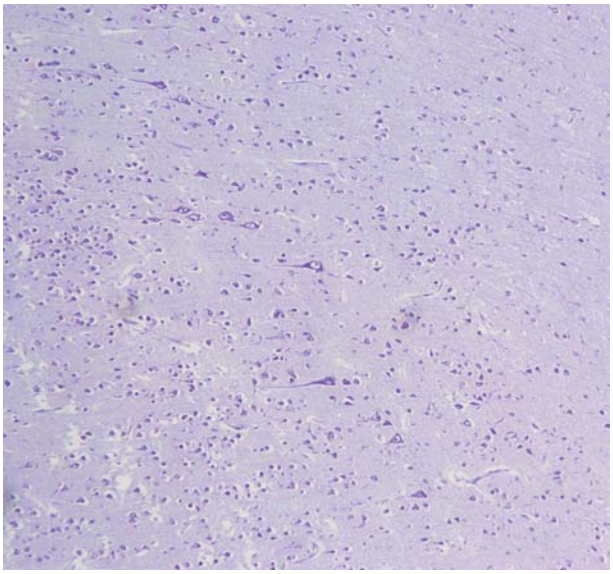

Figure 5 Formation of the internal pyramidal layer in the prefrontal cortex of a fetus aged 37 gestational weeks.
Klűver-Barrera stain, magnification 10×.

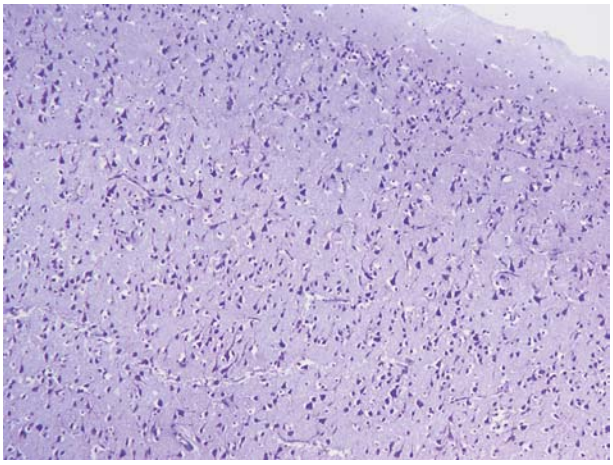

Figure 6 Formation of the external pyramidal layer in the prefrontal cortex of an infant aged 2 months.
Klűver-Barrera stain, magnification 10×.

(V) the internal pyramidal layer; (VI) the multiform layer (Figure 7).

The molecular layer (I) contains few scattered neurons and consists mainly of dendrites and horizontally oriented axons. Several stellate neurons can also be found. The external granular layer (II) is formed by densely packed small, round or polygonal neurons and numerous stellate neurons. The external pyramidal layer (III) shows small and medium sized pyramidal neurons, as well as non-pyramidal neurons with vertically oriented axons. The internal granular layer (IV) includes stellate and polygonal neurons. The internal pyramidal layer (V) contains large pyramidal neurons, as well as small interneurons. Pyramidal cells with smaller sized bodies tend to occupy the upper portion of layer V, whereas neurons with larger soma tend to reside within the lower sector. The multiform layer (VI) contains few large pyramidal neurons and many small polymorphous neurons, frequently spindle-like.

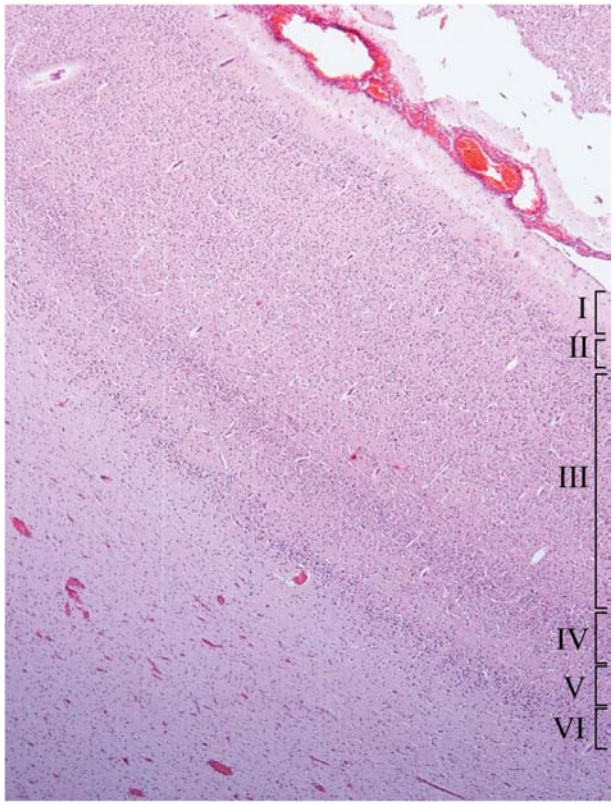

Figure 7 Organization in a six-laminar structure of the prefrontal cortex in an infant aged 4 months.
Klűver-Barrera stain, magnification 4×.

The above-described sequence of developmental steps has been found in the PFC of the vast majority of cases of this study. Nevertheless, in 21 victims of unexplained death, aged from 36 gestational weeks to 5 months of life, we observed different structural aspects: in four fetuses who died in the last weeks of pregnancy the PFC showed the same immature structure that may be found at 22–23 weeks' gestation, when only the molecular layer is outlined. In 17 postnatal deaths (1 early neonatal death and 16 infant deaths) the cortical cytoarchitecture was disorganized, with decreased thickness of the infragranular layers, delayed cytological maturation and few pyramidal neurons in layer V (Figure 8).

In the majority of both fetal and infant deaths with developmental abnormalities of the PFC, we also observed alterations of different brainstem and/or cerebellar structures, the most frequent being hypoplasia of the medullary arcuate nucleus.

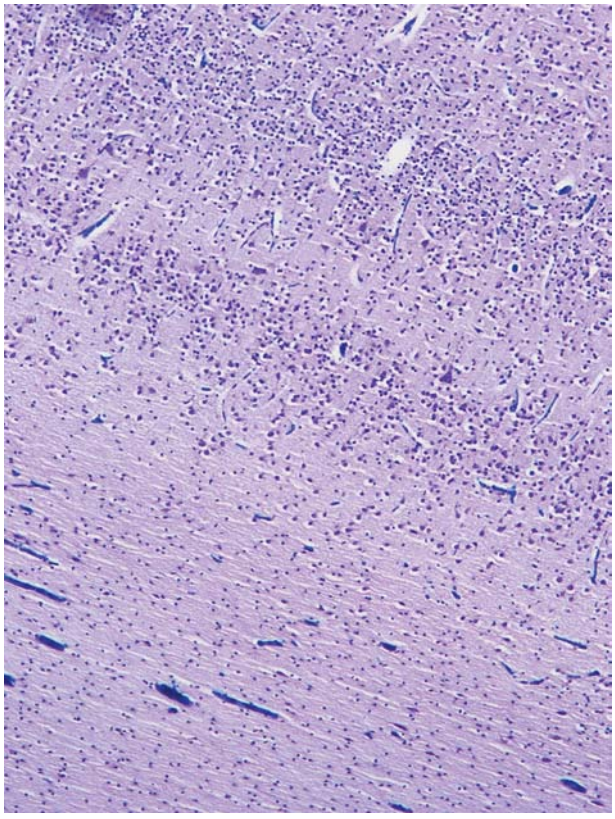

Figure 8 Decreased number of pyramidal cells in layer V of the prefrontal cortex in an infant aged 5 months.
Klűver-Barrera stain, magnification 10×.

Finally, we found a significant correlation between maternal smoking habit, and delayed PFC maturation. In fact, 16 of the 21 victims with cortical alterations had smoking mothers ($P < 0.05$).

Discussion

This study, performed on unexplained perinatal and infant deaths, focuses on cerebral cortex development, with special attention on the prefrontal cortex (PFC), lying in the anterior part of the cerebral frontal lobes, and on the means which the development of this structure can be influenced by environmental factors such as maternal smoking during pregnancy.

The frontal lobes have traditionally been associated with "higher cognitive functions" such as attention, memory, learning, personality and intellect [4, 29, 36, 37]. Nevertheless, much evidence exists that this cerebral area, and specifically the PFC, that in humans occupies the largest part of the frontal lobes, may have a larger role than previously thought as a modulator in the coordination of autonomic responses.

Even before 1900, several authors suggested additional functions of the frontal cortex besides intellectual capacity [33, 39], such as changes in respiration, heart rate and blood pressure, after stimulation of the frontal lobes. Subsequently, studies performed by tracing methods as well as stimulation techniques in rats, confirmed that the PFC is involved in the regulation of cardiovascular and respiratory activities [1, 5, 34, 35]. A role of the PFC in the autonomic control in humans was demonstrated through *in vivo* neuronal stimulation and/or magnetic resonance imaging (MRI) scanning techniques [32, 38].

The involvement of this high cortical structure in maintenance of ventilation could have important pathophysiological implications also in prenatal life, through the control of periodic fetal respiratory activity which is essential for lung development [3, 5]. Alterations of the PFC could reduce prenatal respiratory movements with consequently impaired expansion of the alveoli and pulmonary hypoplasia [2, 24].

The primary purpose of the present study was to investigate the developmental patterns of this specific cortex area in a large group of fetal and infant death victims.

We observed that the PFC develops through a series of stages, with complex histogenetic events.

A pronounced radial organization of the cerebral wall is clearly visible at 26 gestational weeks. The subsequent changes occur between 26 and 40 weeks in a stage that we defined as "cortical maturation". Early during this period, large pyramidal cells, generated in the cortical neuroepithelium and then migrating along radial glial cells to reach the cortex [30], begin their maturation. By 36 gestational weeks the radial organization disappears as the formation of a laminar structure begins. The mature cortex, already observed by the first months of life, is organized horizontally into six laminae.

In 21 (33%) of sudden death victims evaluated in this study, aged 36 gestational weeks to 5 months of life, the PFC appeared thinner, quite disorganized with aberrant laminar patterns and delayed neuronal maturation. These disorders could be ascribed to a failure in the radial glial processes and therefore, in cell migration [6, 30].

We believe that the developmental defects of the PFC may arise as a result of the negative effect of environmental factors and above all of maternal smoking during pregnancy, similar to changes in the brainstem and cerebellum that we previously reported [9, 14, 15, 18]. In fact, in the present study we found that 16 among the 21 victims of unexplained death with cortical delayed maturation had smoking mothers.

Therefore, on the basis of the results of this study and of our previous researches, we hypothesize that *in utero* exposure to maternal smoking could interfere with brain development, giving rise to widespread, delayed, maturation of all the neuronal structures involved in control of vital functions.

In addition, we suggest the possibility to successfully evaluate the laminar development of human cerebral cor-

tex within the limits of spatial resolution of MRI [7], likely to the maturation of the medullary arcuate nucleus and the lung [24].

The possibility of studying *in utero* the developmental stage of fetal specific structures by MRI, may provide a number of potential clinical applications and improve prenatal counseling and therapeutic planning.

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