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Morphological study of the perireticular nucleus in human fetal brains

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Abstract

The perireticular nucleus consists of scattered neurons that are located in the internal capsule. The presence of perireticular neurons in the rat, ferret, cat and human has been described previously. Evidence suggests that the perireticular neurons in various species decrease in number with increasing gestation, but in humans this finding has not been supported by quantitative data. This study aimed to investigate (1) the morphology of the human fetal perireticular neurons, (2) the average number of perireticular neurons within the anterior and posterior crus of the internal capsule per unit area, and (3) the magnitude and the stage of neuronal loss in the human perireticular nucleus subsequent to maturation. Nissl-stained sections of the internal capsule of human fetal brains of 24, 26.5, 32, 35, 37 and 39 weeks of gestation showed a number of clearly distinguishable large perireticular and small microglia cells. A regular increase of both perireticular and microglial cells was observed up to 32 weeks of gestation, after which a dramatic reduction in the number of both perireticular and microglia cells was observed. The average number of perireticular and the microglia cells per unit area, located within the posterior crus, was more than in the anterior crus of the internal capsule. In the adult, no perireticular neurons were detected within the internal capsule. The results show that perireticular neurons are not restricted to the region lateral to the thalamus and medial to the globus pallidus (posterior crus) but are also present at the region lateral to the caudate nucleus and medial to the globus pallidus (anterior crus).

Key words human fetal brain; internal capsule; perireticular.

Introduction

The perireticular nucleus is a newly described group of neurons that are scattered within the internal capsule. The perireticular nucleus and the reticular nucleus of the thalamus are derived developmentally from the ventral thalamic mass (Mitrofanis, 1992a, 1994a,b). Previous studies have shown that cells in the lateral and ventral regions of the diencephalon are generated before those lying in more medial and dorsal regions (Altman & Bayer, 1988). The reticular and the perireticular neurons are among the first neurons generated in the thalamus (Clemence & Mitrofanis, 1992).

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The presence of the perireticular nucleus has been described in the rat, cat, rabbit, ferret and human (Clemence & Mitrofanis, 1992; Mitrofanis, 1992a, 1994a,b; Letinic & Kostovic, 1996; Ulfig et al. 1998). Studies have shown that during early development the perireticular nucleus is very large, but subsequent to maturity the size of the perireticular nucleus reduces dramatically. The perireticular nucleus almost completely disappears in the adult rat, but in the cat and ferret many more perireticular cells are apparent (Clemence & Mitrofanis, 1992; Mitrofanis, 1992b; Mitrofanis & Baker, 1993; Earle & Mitrofanis, 1996). The factors causing the reduction in perireticular neuron number are not known, although several have been suggested: (1) a period of heavy cell death, (2) migration of cells into the adjacent globus pallidus and (3) the growth of an internal capsule spreading the cells further apart, thus giving the impression, from the sections, that there has been a dramatic cell loss due to death or to migration.

Neuroanatomical studies have reported the existence of connections of the rat perireticular nucleus to the dorsal thalamus (embryonic day 14, E14) and the cerebral cortex (E16, E17) in the early prenatal stage (Mitrofanis & Baker, 1993). However, the few remaining perireticular neurons in the adult rat were labelled after tracer injection into the dorsal thalamus, but not into the cerebral cortex (Adam & Baker, 1995; Mitrofanis et al. 1995). The existence of these connections, together with the strategic position of the perireticular nucleus in the internal capsule and its large size in early development, have led to the suggestion that the perireticular nucleus might play a role in the reorganization of thalamocortical and corticothalamic connections and in guiding and separating fibres towards their appropriate targets (Mitrofanis & Guillery, 1993; Adams et al. 1997).

The interstitial neurons are the earliest generated neurons in the telencephalon. The interstitial neurons are in developmental and spatial continuation with neurons of the transient subplate zone. The subplate zone contains neurons that are the earliest generated cells of the cortical anlage and largely disappear during later development. Perireticular neurons closely resemble these neurons. Although the function of the perireticular nucleus remains unknown, morphological studies together with experimental evidence have led to the suggestion that their main function is developmental.

Very little is known about the human fetal perireticular nucleus: is the crucial role of the perireticular nucleus during development as described for the rat and cat brain? It is also unclear whether the human perireticular nucleus shows neuronal reduction subsequent to maturation.

This study aimed to investigate the perireticular nucleus in the developing human fetal brain with regard to: (1) the morphology and the neuronal types of the human fetal perireticular neurons, (2) the average number of perireticular neurons within the anterior and posterior crura of the internal capsule per unit area and (3) the magnitude of the neuronal loss in the human perireticular nucleus subsequent to maturation.

Materials and methods

Seven human fetal and two adult brains were used in this study. Fetal brains were obtained from premature stillbirths and adult brains were obtained from routine autopsies. All procedures were approved by the ethics committee of Marmara University.

The fetuses were at 20, 24, 26.5, 32, 35, 37 and 39 weeks of gestation and the adults were 23 and 67 years old. Only normal fetal and adult brains were included in this study. Brains were fixed as soon as possible to avoid extended degeneration of brain tissue.

The calvaria of the fetuses were removed via routine dissections, and the brains were removed from the skull. Horizontal sections passing through the right Sylvian sulcus and the insular cortex were made in order to expose the right anterior and posterior crus of the internal capsule. The right anterior and posterior crura of the internal capsule were resected. Specimens were post-fixed with 7% paraformaldehyde solution. Horizontal sections (30–50 μm thick) of the internal capsule were cut using a cryostat (Microtom, Germany). Sections were laid on gelatinized slides and Nissl staining was applied to the sections. Serial sections of the internal capsule were evaluated for perireticular and glial cells. From the beginning of the internal capsule, every 10th section (2nd, 12th, 22nd, 32nd, 42nd, etc.) from both the anterior and the posterior part of internal capsule were chosen and cells were counted. From each section, 15 areas were chosen by systematic randomized sampling. The perireticular neurons and the microglia cells within the anterior and posterior crura of the internal capsule were defined and counted by the same researcher at 400$\times$ magnification without knowing the age of fetuses or adults. An eyepiece graticule (covering 0.07850 mm^2) was used to avoid overlapping counting areas. Areas selected in each region were surveyed for perireticular and microglia cells, and the density was expressed as cell numbers per unit area. The results were statistically analysed using Wilcoxon's signed rank test.

Results

The results of the present study showed that the anatomical organization of the internal capsule of the fetus was different from that in the adult. Horizontal sections of the fetal brains showed irregular organization of anterior and posterior crura of the internal capsule. As age of gestation increased, the internal capsule became more apparent and thicker.

Nissl-stained sections from the fetus of 20 weeks gestation showed no axons within the region of the internal capsule. The internal capsule of the 20-week

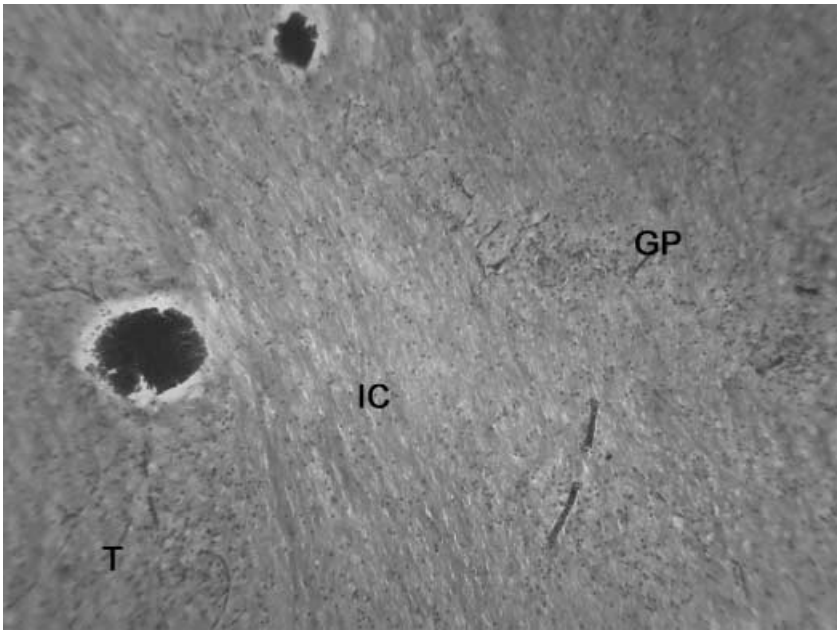

Fig. 1 Low-magnification micrograph of the internal capsule (IC) fibres with adjacent thalamus (T) and globus pallidus (GP) (black arrow, perireticular cell; open arrow, glial cell) (×40). The highlighted area is shown at high magnification in Fig. 2.

fetus consisted of undifferentiated blastic cells of different cytoplasmic content compared with the thalamus, caudate and the cerebral cortex. The blastic cells within the internal capsule were loosely orientated compared with the thalamic, caudate and cortical cells. The blastic cells within the region of the internal capsule were organized parallel to the anteroposterior axis of both crura whereas the blastic cells of the caudate and the thalamic cells were vertically organized.

The perireticular neurons within the internal capsule of the human fetal brains at early gestation (24 and 26.5 weeks) showed a close topographical relationship with the neurons of the reticular nucleus, and indeed were so close that it was extremely difficult to define the boundaries. The Nissl-stained sections showed that the perireticular neurons were closely packed at this stage compared with later stages of gestation.

The sections of the internal capsule of the human fetal brains of 24, 26.5, 32, 35, 37 and 39 weeks gestation showed clearly distinguishable perireticular and microglia cells within the internal capsule. The perireticular neurons were multipolar, fusiform shaped or polymorphous and consisted of dendritic arborizations extending from the neuron bodies (Figs 1 and 2). The neuronal axis of the perireticular cells was parallel to the reticular nucleus axis and parallel to the fibres of the internal capsule. The microglia cells were regular, circular and were scattered within the internal capsule with no specific topographical distribution (Figs 1 and 2).

The average number of perireticular and microglia cells per unit area within the anterior (between the caudate nucleus and the globus pallidus) and posterior (between the reticular nucleus of the thalamus and the globus pallidus) crura of the internal capsule was calculated (Fig. 3). The average number of perireticular and microglia cells per unit area showed a gradual increase up to 32 weeks of gestation, followed by a dramatic reduction in the number of both (Fig. 3). The average number of perireticular and microglia cells per unit area was higher in the posterior crus than in the anterior crus. The numbers of perireticular ($P = 0.0313$, $T = -21.00$) and microglial ($P = 0.0313$, $T = -21.00$) cells in the posterior crus of the internal capsule were significantly different from the anterior crus. The number of microglial cells was almost three times higher than the number of perireticular neurons (Fig. 4). In the adult, no perireticular neurons were detected within either the anterior or the posterior crura of the internal capsule.

Despite the fixation procedures being implemented as early as possible, histological examination of the perireticular nucleus revealed some degeneration of the fetal brain tissue.

Discussion

The results of present study indicate that the perireticular nucleus in the developing human fetal brain

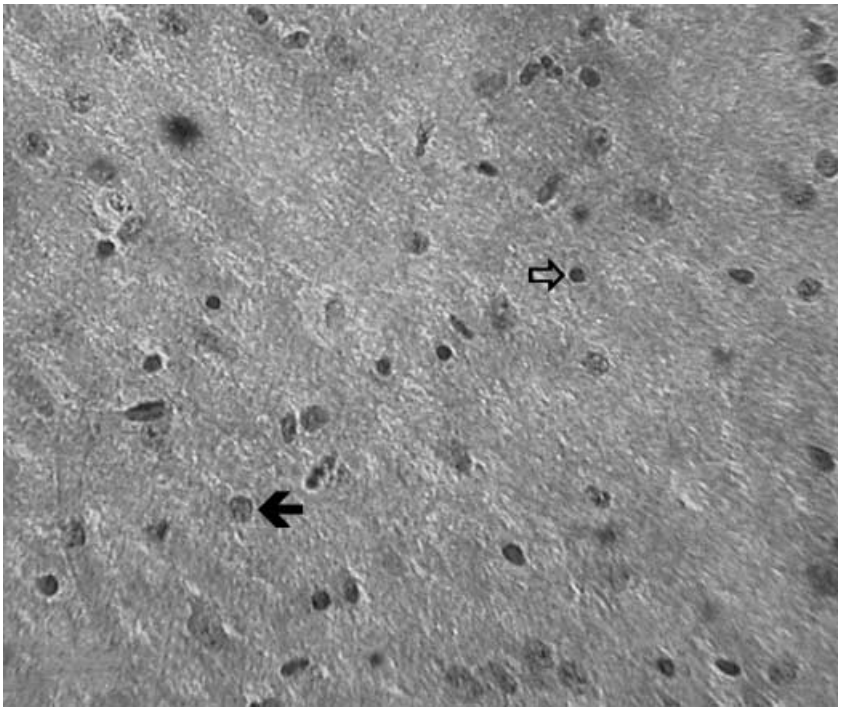

Fig. 2 Micrograph of perireticular and glial cells in the posterior crus of the internal capsule (black arrow, perireticular cell; open arrow, glial cell) (×400).

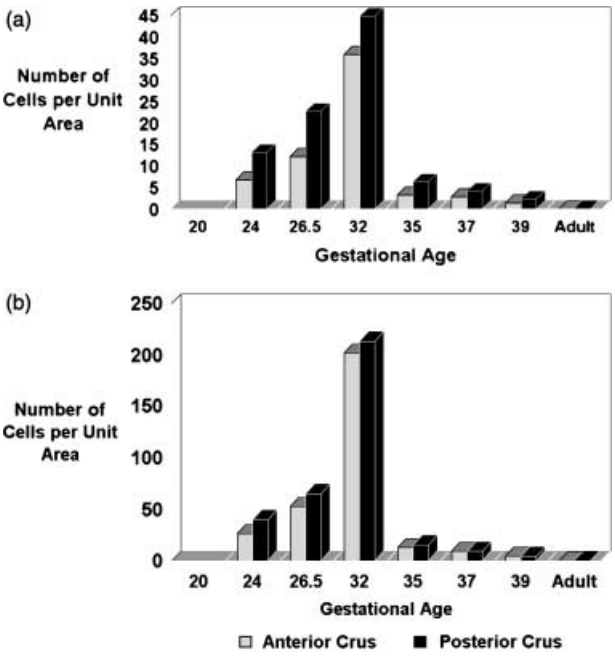

Fig. 3 Average number of perireticular and microglial cells per unit area within the anterior and posterior crus of the internal capsule.

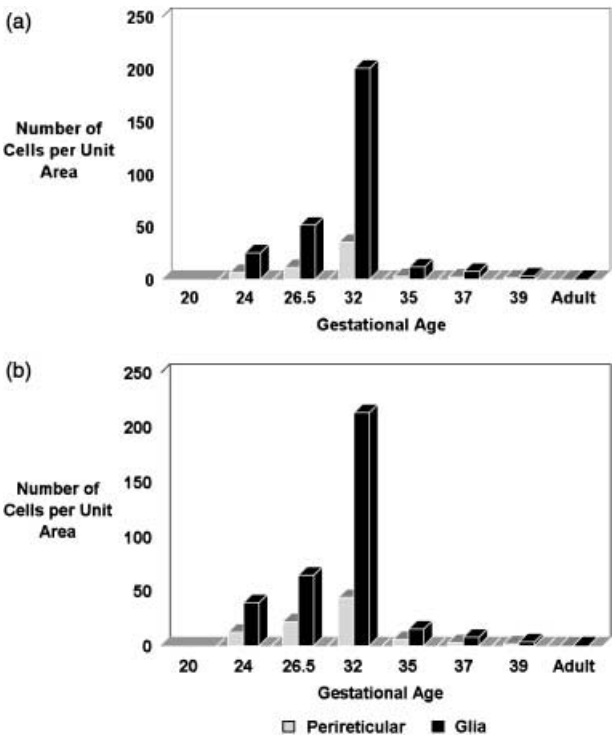

Fig. 4 The ratio of perireticular and microglial cells per unit area within the anterior and posterior crus of the internal capsule.

(1) has similar morphology to the rat, cat and ferret but not a similar pattern of cell distribution; (2) that the distibution of the number of perireticular and microglial cells within the anterior and posterior crus was variable, being greater in the posterior crus of the internal capsule; and (3) that the relationship between the number of neurons in the perireticular nucleus compared with increasing gestational stages showed a gradual increase up to 32 weeks of gestation – subsequently a

dramatic reduction in the number of both perireticular and microglia cells was observed.

The present study on the human fetal brain showed that within the fibres of the internal capsule large multipolar, fusiform or polymorphous perireticular neurons and numerous small microglial cells were clearly distinguishable at all gestational ages examined, except at 20 weeks. Mitrofanis (1994a,b) also reported two types of neurons in the perireticular nucleus of ferret: small perireticular neurons were located near the reticular nucleus of the thalamus, whereas large perireticular neurons were scarce over the entire internal capsule. Contreras-Rodriguez et al. (2002) reported two types of neurons in the rabbit: the cell population close to the reticular nucleus consisted of large neurons, whereas those scattered in the internal capsule were small. Our results obtained from human fetuses showed that small microglial cells were scattered within the internal capsule with no specific topographical distribution. The large perireticular neurons were closely localized to the reticular nucleus of the thalamus during early gestation. The variable distribution of the perireticular and microglial cells could be the result of differences between the species.

Recent studies have reported differences among species; in the adult rat, only very few perireticular neurons were seen among the fibres of the internal capsule, in contrast to distinct perireticular neurons in the adult cat and ferret (Clemence & Mitrofanis, 1992; Mitrofanis & Baker, 1993). Earle & Mitrofanis (1997) reported a large transient population of phagocytic amoeboid microglial cells with round cell body and no or very fine and short processes; these disappear from about 2 weeks after birth until none is left in the adult rat (Earle & Mitrofanis, 1997). Our study on human fetal brains showed a gradual increase of perireticular neurons up to 32 weeks and thereafter dramatic decline. Furthermore, in the present study microglia cells were one of the dominant cell types of the perireticular nucleus at all gestational ages, and these also reduced with maturation as with the perireticular neurons.

Additionally, the axis of the perireticular neurons showed considerable across-species differences. The perireticular neurons were described as perpendicular to the regional fibres of the internal capsule in the rat (Ramcharan & Guillery, 1997), and orientated in all directions in the rabbit (Contreras-Rodriguez et al. 2002); in the present study on human fetal brain they were mainly parallel to the fibres of the internal capsule. The difference in the orientation of the perireticular neurons may be related to the possible role played by the perireticular neurons in reorganization of corticothalamic and thalamocortical fibres.

Mitrofanis & Guillery (1993) showed that the perireticular nucleus was ventrocaudally continuous with the reticular nucleus in rat, cat and ferret. Letinic & Kostovic (1996) studied the transient neuronal population of the internal capsule in the human fetus and showed that these neurons were particularly numerous in areas of the internal capsule near the ventral parts of the reticular nucleus. In the present study the perireticular neurons were closely localized to the reticular thalamic neurons, especially at 26.5 weeks of gestation. The average number of perireticular and microglial cells was greatest at the posterior crus of the internal capsul of the human fetus. This could be due to its function in guiding and separating the corticothalamic fibre from the corticofugal fibres.

In accordance with the study of Ulfig et al. (1998), we have not observed perireticular neurons in the human adult. Therefore, the presence of perireticular neurons in the fetus but not in the adult can be attributed to the fact that perireticular neurons are transient neurons that function developmentally.

The perireticular nucleus in the rat has projections to the dorsal thalamus and the cerebral cortex (Adams & Baker, 1995). In contrast to the findings of Adams & Baker (1995), Coleman & Mitrofanis (1999) showed that no perireticular neurons project to the cerebral cortex. Furthermore, they concluded that patterning of the thalamocortical axons in the developing neocortex is not the function of perireticular nucleus because perireticular projections to the cerebral cortex do not exist. Mitrofanis & Baker (1993) studied the connections of the reticular and perireticular nucleus with the dorsal thalamus in the rat and found that the perireticular nucleus was connected to the dorsal thalamus 1 week after the neurons of the reticular nucleus. They suggested that there could be two distinct populations of perireticular cells. One population may be born early, enter the internal capsule during prenatal development and lack a projection into the dorsal thalamus. A second group of perireticular cells may be born later, enter the internal capsule during postnatal development and have axons that extend into the thalamus. Crabtree (1996) further suggested that perireticular cells project diffusely to dorsal thalamic structures rather than to ventral thalamic structures, and similar

to the reticular nucleus the perireticular nucleus can be divided into sectors, which are directly related to specific connections. The somatosensory sector of the perireticular nucleus has been defined as lying opposite to the somatosensory sector of the reticular nucleus of the thalamus (Crabtree, 1996).

Developmental studies of the perireticular nucleus in relation to the growing corticofugal and corticopetal axons showed that the perireticular nucleus is very large at a stage when the first thalamocortical axons leave and when the first corticothalamic axons approach the thalamus (Mitrofanis & Baker, 1993). These axons seem to change course in the region of the internal capsule, where there are many perireticular cells. Corticothalamic axons turn towards the reticular nucleus, and thalamocortical axons turn towards the cortical subplate. The corticospinal and corticobulbar axons, by contrast, pass directly through the perireticular region to their caudal targets. After these axons have reached their targets, the perireticular nucleus reduces dramatically in size.

It has been shown that a number of perireticular neurons migrate and settle within the globus pallidus (Earle & Mitrofanis, 1996). These authors injected biotinylated dextran in the rat dorsal thalamus and showed that none (or very few) of the perireticular neurons that migrated into the globus pallidus survive into more mature postnatal stages. The developmental function of the migrated neurons into the globus pallidus is still unknown.

The perireticular nucleus has been described as a sheath of cells lying among the fibres of the internal capsule, external to the reticular nucleus of the thalamus. In the present study we have described perireticular neurons within the anterior crus of the internal capsule, located between the caudate nucleus and globus pallidus as well. Therefore, the perireticular neurons are not restricted to the region between the reticular nucleus and globus pallidus (posterior crus of the internal capsule) but also exist between the caudate and globus pallidus (anterior crus of the internal capsule). The presence of perireticular neurons within the internal capsule suggests that the function of the perireticular nucleus is not only to reorganize the pathways passing through the posterior crus (corticospinal, sensory, temporopontine, and visual and auditory pathway), but that it is also involved in organizing the fibres passing through the anterior crus (frontopontine and frontothalamic) of the internal capsule.

The fetus of 20 weeks of gestation showed that the axons of the internal capsule were not present and that this was filled with undifferentiated blastic cells. Neurogenesis begins at the ventricular zone, the cells undergo multiple divisions and migration of neurons then occurs. The migrated neurons in the superficial layers (1, 2 and 3) project to other cortical targets, whereas deep layers (4, 5 and 6) project to subcortical targets. In the single case of the fetus of 20 weeks gestation, no projection of migrated neurons was observed. The reticular and perireticular neurons share a number of common properties with the cortical plate. These neurons are all generated relatively early in development, and show heavy neuronal loss during later development. The neurons of the perireticular nucleus at early embryonal stages express the same antigens (parvalbumin, pro-α-thyrotropin releasing hormone calbindin and GABA+) as the neurons in the reticular nucleus (Mitrofanis, 1992a; Letinic & Kostovic, 1996; Amadeo et al. 1998; Contreras-Rodriguez et al. 2002). Our sections from 26.5 weeks of gestation showed that the perireticular nucleus had a close anatomical relationship or even a continuity with the reticular nucleus of the thalamus. With increasing gestational age the separation of the two nuclei became more apparent. The similarities of the reticular neurons with the perireticular neurons may suggest a similar origin with a separate role during development. Different roles have been proposed for the function of the perireticular neurons in organizing and guiding the connections between cerebral cortex and thalamus. The data from the present study and from the literature regarding the functional role of the perireticular nucleus also suggest that the human fetal perireticular nucleus fulfils important developmental functions.

Acknowledgement

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