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Research article

Development of the human trochlear nucleus: A morphometric study

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ABSTRACT

Background: The trochlear nucleus, the smallest of the extraoculomotor nuclei, is unique or even curious, because the nerve roots emerge dorsally from the superior medullary velum after decussation. Little information is available on the developmental anatomy of this nucleus in humans.
Design/subjects: We examined serial brain sections from 10 premature infants aged 20–39 weeks of gestation to document the histology and morphometry.
Results: The trochlear nucleus was composed of three parts: the rostral tip, the main body, and the caudal division. The rostral tip was a rostral continuation of the main body, being closely related to the oculomotor nucleus; the main body was enveloped by a fibrous capsule; the caudal division was a small separate cluster of neurons in the medial longitudinal fasciculus or the root fibers with individual variations. Tigroid Nissl bodies first appeared at 28 weeks in presumed motoneurons. Various sizes of motoneurons were recognized; medium-sized to small motoneurons were preferentially accumulated in the rostral tip. Among the motoneurons, presumed non-motor neurons were infrequently scattered. Morphometric analysis showed that the nuclear volume exponentially increased with age, about 15 fold over 20–39 weeks, while the average profile area of the neurons linearly increased. Statistical analysis confirmed that cell area was smallest in the rostral tip among the three parts.
Conclusion: Although the sample number is small in this study, it suggests that the human trochlear nucleus can be divided into three parts, and that the overall growth may be accelerated at about 30 weeks of gestation.

1. Introduction

Eye movements are important for catching the clearest image of an object. With recent advances in obstetric medicine, fetal eye movements can be now visualized by means of real time ultrasonography, and the first slow changes in eye position have been documented at about 16 postmenstrual weeks (Prechtl and Nijhuis, 1983). Clinically, failure of the oculomotor system to develop has been reported in rare inherited diseases such as congenital dysinnervation disorders (Engle, 2007). To appreciate physiological, pathological, or clinical developmental data, it is necessary to have a precise knowledge of the developmental anatomy of extraoculomotor nuclei.

The trochlear nucleus (nIV) innervating the superior oblique muscle, the smallest of these nuclei, is unique or even curious, because the nerve roots emerge dorsally from the superior medullary velum after decussation. In experimental animals, the neuronal cytoarchitecture of the nIV has been vigorously investigated across various classes of the vertebrates, such as lampreys (Meléndez-Ferro et al., 2000), amphibians (Naujoks-Manteuffel et al., 1986; Muñoz and González, 1995), reptiles (El Hassni et al., 2000), birds (Sohal et al., 1985), and mammals (Reis and Machado, 1981; Murphy et al., 1986; Sturrock, 1991; Büttner-Ennever et al., 2001; Eberhorn et al., 2006). Developmental anatomy of this nucleus was also reported by Sohal et al. (1985) and Sonntag and Fritzsch (1987).

For humans, reports on the morphology of the nIV are largely limited to adults (Zaki, 1960; Vijayashankar and Brody, 1977; Olszewski and Baxter, 1982) or embryos (Pearson, 1943; Cooper, 1946; Cooper, 1947; Müller and O'Rahilly, 1988; Szyszka-Mróz, 1999), and developmental events have been less intensively studied during the later stages of fetal period. Here we report on the histology and morphometry of the nIV in preterm infants at 20–39 weeks of gestation (WG).

2. Material and methods

2.1. Tissues

Ten human brains were examined (Table 1). They were from premature infants at 20–39 WG. The infants died of various causes

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Table 1
Details of the material.

Case no.	Age (weeks[a])	Brain weight (g)	Clinical diagnosis
1	20	48	Medical termination
2	21	70	Medical termination
3	27	130	Septicemia
4	28	160	Asphyxia
5	29	178	Asphyxia
6	30	NR[b]	Hydrops fetalis
7	35	250	Esophageal atresia
8	38	NR[b]	Diaphragmatic hernia
9	39	390	Meconium aspiration
10	39	380	Diaphragmatic hernia

[a] Gestational weeks.
[b] Not recorded.

shortly after birth, and their gestational ages were estimated from the first day of the mother's last menstrual period. Removal of the brains at autopsy was approved by our institute's board of ethics. Gross and microscopic examinations of the brain revealed that serious pathological changes including severe ischemic–hypoxic changes, massive hemorrhages, structural anomalies and injuries were absent.

2.2. Histology

After fixation in 10% formalin, each brain was post-fixed in a 1:4 mixture of 5% potassium dichromate and 5% potassium chromate solutions for three weeks (Goto and Seki, 1980), embedded in celloidin and 30-μm serial sections made. Every fifth or tenth section was stained according to the Klüver–Barrera (K–B) method (Klüver and Barrera, 1953). The remaining sections were stored in 70% ethanol; later some of them were stained with other staining methods, such as hematoxylin and eosin, Kultschitzky's stain (a modified Weigert's myelin stain), or silver impregnation. Immunohistochemical staining is desirable for definitive classification of neurons, but in this study, it was not possible to apply it to long-stored celloidin sections.

2.3. Volumetry

The nuclear volume was estimated using Cavalieri's point-counting method (Mouton, 2002). After attaching a square ocular grid to the eyepiece, we counted the intersections hitting the nuclear area on the K–B specimens. The volume (V) was calculated by the following formula: $V\,(\mathrm{mm}^3) = a \times \Sigma P \times d$, where '$a$' was the area of a unit square (0.0064 or 0.04 mm^2), 'ΣP' was the sum of the numbers of intersections for one side of the nucleus and 'd' was the distance between two adjacent slides (0.15 or 0.3 mm).

2.4. Neuronal profile area

Neuronal profile areas were measured to make a quantitative assessment of the size of neurons. Because it is generally difficult to estimate cell volume accurately with conventional light microscopes, we decided to measure the profile (or sectional) area on the left side. Cell diameters have long been applied to morphometry, but it is laborious to determine and handle two variables (long and short axes) at the same time in a comparative analysis. Thus we felt a two-dimensional parameter (profile area) would produce the most accurate and efficient measurement for analysis. For the study, we selected three K–B specimens to compare the data from the following three parts of this nucleus: the rostral tip, the main body, and the caudal division (see below). For each specimen, all the neuronal profiles observed were drawn with a light microscope (Optiphot, Nikon, Tokyo, Japan) equipped with a drawing tube at a final magnification of 500×. The line drawings were digitized and entered into a personal computer (HP Compaq DX 2000 ST, Hewlett Packard, Palo Alto, CA, USA) using a tablet (UD 1212R, Wacom, Saitama, Japan). Quantitative data was obtained from a program for image analysis (VM32, Rise Corporation, Sendai, Japan). Morphological determination of a neuron was made using the following criteria: (1) a relatively large cell body, (2) prominent and distinct nucleus surrounded by a basophilic perikaryon and (3) presence of single or multiple distinct nucleoli. Tissue shrinkage is generally inevitable during processing of histological preparations. However, because an accurate estimation of the extent of shrinkage was difficult in these specimens, we did not correct for shrinkage. After examining normality of distribution and equality of variance, we used the unpaired Welch's (or Student's) t-tests to compare the averages, and took $P < 0.05$ as significant.

3. Results

3.1. Three parts of the trochlear nucleus

The nIV was identified as a compact mass of relatively large cells ventral to the central grey substance of the midbrain as early as 20 WG. Observations of serial sections revealed that the nucleus is comprised of two small parts, as well as the main body: the rostral tip and the caudal division. The rostral tip was a rostral continuation of the main body, being closely related to the most caudal part of the oculomotor nucleus (nIII) with a narrow cell-sparse zone (Fig. 1A). This part was constantly seen in our sample brains, and was mainly occupied by medium-sized to small neurons. The main body, the largest part of the nIV, was enveloped by a thick fibrous capsule, where relatively large neurons predominated (Fig. 1B). The caudal division was a small separate cluster of neurons in the medial longitudinal fasciculus, caudal to the main body. In two cases (Cases 4–5), it lay more laterally in the trajectory of root fibers (Fig. 1C). It showed some individual variations, as it appeared unilaterally or bilaterally, or was even absent in Case 2. No distinct differences were seen in appearance of the neuronal cytoarchitecture between the main body and the caudal division, except that the large neurons were somewhat elongated in the latter.

3.2. Neuronal types of trochlear nucleus

Before 28 WG, the neurons were only divided into two types (large and small neurons), because their Nissl bodies were immature and rather fine even in the larger neurons. It was first possible to distinguish presumed motoneurons from other types of neurons (non-motor neurons) at 28 WG. Various sizes of motoneurons were recognized: from small to large motoneurons. The larger subtypes had a plump or ovoid cell body, and were generally conspicuous over the nucleus, except the rostral tip (Fig. 2A). The smaller subtypes had a relatively poor cytoplasm, but coarse Nissl bodies of tigroid type were visible (Fig. 2B). Among these motoneurons, presumed non-motor neurons were infrequently encountered. These presumed non-motor neurons were generally small, round or oval-shaped, and their Nissl bodies were fine and located peripherally (Fig. 2C).

3.3. Nuclear volume

The caudal division was not included for the volume measurement, because this part was too small and variable. The nuclear volume increased about 15 fold with age during the examined period (20–39 WG). We independently measured the two sides as an in-specimen control. Measurements of the left trochlear nucleus (0.029–0.348 mm^3) and right trochlear nucleus (0.02–0.336 mm^3) showed very similar sizes and rates of increase. This dramatic