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Research article

Development of the human oculomotor nuclear complex: Somatic nuclei

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ABSTRACT

Background: Precise anatomical data on the development of human oculomotor somatic nuclei (OSN) remain rare.
Design/subjects: This study describes the histology of human OSN in 11 preterm and full-term infants aged 20–43 postmenstrual weeks who died of various causes. Celloidin-embedded serial sections were stained with the Klüver–Barrera and other conventional methods including silver impregnation. To evaluate the growth of OSN quantitatively, the author estimated the nuclear volume and the average neuronal area on morphometry.
Results: Four subnuclei were identified at 20–21 weeks: the fascicular, principal, dorsal median, and ventral median nucleus. Early tigroid Nissl bodies appeared in presumed motoneurons by 27–28 weeks, then resembled adult Nissl bodies at birth. On silver impregnation, the oculomotor nerve roots, crossed or uncrossed fibers at the midline, and a plexus of efferent or afferent axons in the neuropil were observed at 20–21 weeks. Then, the plexus was elaborated to form a perineuronal net of thin axon terminals by 28–29 weeks. The nuclear volume of OSN exponentially increased with age over 20–43 weeks, while the average of neuronal profile areas linearly increased in each subnucleus; the coefficient of regression was largest in the principal nucleus, and the regression lines nearly overlapped among the other subnuclei. Statistical analysis confirmed that the average neuronal area was largest in the principal nucleus in older cases.
Conclusion: This study suggests that four subnuclei can be distinguished in human OSN by mid gestation, and that the principal nucleus may be different in neuronal cytoarchitecture from the others.

1. Introduction

The oculomotor nuclear complex (nIII) is a unique brainstem nucleus because it is a compound of somatic and visceral nuclei comprising functionally different types of neurons. The oculomotor somatic nuclei (OSN) contain motoneurons innervating four extraocular muscles (the superior rectus, inferior rectus, medial rectus, and inferior oblique muscles) and the levator palpebrae muscle in mammals (Parent, 1996). The anatomy of the OSN has been extensively investigated in various species of mammals, and it is generally accepted that it is made up of two parts: the lateral and median (or central) nuclei. The lateral nucleus (LN) is highly conserved throughout the phylogeny of vertebrates (Ramón and Cajal, 1911), while the median nucleus (MN) may be present in some species of mammals and birds. The visceral nuclei contain densely packed smaller, non-motor type neurons, and are traditionally divided into the Edinger–Westphal nucleus (EW) and the anterior median nucleus (AM). Recent immunohistochemical studies have revealed that the majority of EW neurons express immunoreactivity against urocortin 1, but not choline acetyltransferase, in humans (Ryabinin et al., 2005).

With advances in obstetric medicine, fetal eye movements can be visualized by means of real time ultrasonography, and the first slow changes in eye position have been documented at about 16 weeks (Prechtl and Nijhuis, 1983). Failure of development in the oculomotor system has been reported in rare inherited diseases such as congenital fibrosis of the extraocular muscles (Engle, 2007). To appreciate radiological, physiological, or pathological data accurately, precise knowledge of the developmental anatomy of human nIII may be necessary.

Descriptive studies on the histogenesis of human nIII have been reported by some authors (Ramón and Cajal, 1911; Mann, 1927; Cooper, 1946; Hogg, 1966; Szyszka-Mróz, 1999a,b; O'Rahilly and Müller, 2006). Their works have been limited to earlier events of

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Table 1
Details of the material.

Case no.	Age[†]	Sex	Brain weight (g)	Clinical diagnosis
1	20	Male	48	Medical termination
2	21	Male	70	Medical termination
3	27	Male	130	Septicemia
4	28	Female	160	Asphyxia
5	29	Male	178	Asphyxia
6	30	NR[‡]	170	Hydrops fetalis
7	35	Female	250	Esophageal atresia
8	38	Male	NR[‡]	Diaphragmatic hernia
9	39	Female	390	Meconium aspiration
10	39	Female	380	Diaphragmatic hernia
11	43	Female	430	External anomaly

[†] Age, expressed in postmenstrual weeks.
[‡] NR, not recorded.

Table 2
Classification of nuclei in the human oculomotor nuclear complex.

	Synonyms
I. Somatic nuclei	
Lateral nucleus (LN)	
Principal nucleus (PN)	Chief nucleus
Fascicular nucleus (FN)	Intrafascicular part of lateral cell column, lateral group
Median nucleus (MN)	Central nucleus
Dorsal median nucleus (DM)	Caudal central nucleus (Tsuchida)
Ventral median nucleus (VM)	Nucleus of Perlia
II. Visceral nuclei	
Edinger–Westphal nucleus (EW)	
Anterior median nucleus (AM)	

histogenesis in embryos or younger fetuses. According to their results, the anlage of human LN can be identified as early as 4–6 weeks, and the basic pattern of cellular arrangement may be accomplished by the end of the embryonic stage. Few reports, however, are available on the histology during the second half of gestation. Pearson (1944) only stated that the nIII resembled adult features in 5- to 6-month-old fetuses. Moreover, to my knowledge, quantitative data on the histogenesis of human nIII have not yet been published.

This study is a continuation of our previous works on the trochlear (nIV) and abducens (nVI) nuclei (Yamaguchi and Honma, 2011, 2012). The aim was to provide qualitative and quantitative anatomical basis on the development of human OSN during the second half of gestation.

2. Material and methods

2.1. Subjects

Eleven brains were obtained from preterm and full-term infants aged 20–43 weeks, who had died of various causes (Table 1). The brains of Cases 1 and 2, the youngest cases, were through medical termination due to the sibling's chromosomal anomaly and the mother's rubella virus infection, respectively. Removal of the brain was approved at autopsy by the board of ethics of the Dokkyo University School of Medicine, and by the parents who were informed that the brain would be used for both diagnostic and scientific studies. 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. Unfortunately, no pathological data was available on intraorbital structures including the extraocular muscles and their innervating nerves.

2.2. Histology

After initial fixation in a 10% formalin solution, the brain was immersed in a secondary fixative, a 4:1 mixed solution of 5% potassium chromate and 5% potassium dichromate, for several weeks. Then, it was washed in running water, dehydrated in graded ethanol solutions, and finally embedded *en bloc* in celloidin. The tissue block was transversely sliced into 30-μm-thick serial sections. After numbering with India ink, every fifth or tenth section was stained with the Klüver–Barrera (K–B) method (Klüver and Barrera, 1953). The remaining sections were stored in a 70% ethanol solution, and some were stained with other conventional methods including silver impregnation, a modified Bielschowsky method for celloidin sections (Starega, 1985).

2.3. Nuclear volume

The total volume of the OSN, the sum of the volumes of LN and MN, was estimated using Cavalieri's point-counting method. Attaching a square ocular grid to the eyepiece, the author counted the intersections hitting the nuclear area on the K–B sections with the aid of an Optiphot microscope (Nikon, Tokyo, Japan) at magnification of 40× or 100×. The volume (V) was calculated with the following formula: $V\,(\mathrm{mm}^3) = a \times \Sigma P \times d$, where 'a' was the area of a unit square (0.04 or 0.0064 mm^2); 'ΣP' was the sum of the numbers of intersections counted on both sides; 'd' was the distance between two adjacent sections measured, which was obtained from multiplication of the true section thickness and the number of intervening sections. The true section thickness was estimated microscopically with a 40× objective lens. Measurements were repeated on several sections at a constant interval, and the mean measured section thickness was calculated. Then, the true section thickness was yielded by multiplication of this mean value and the refractive index of the cover glass (1.5). In general, strong intra- and inter-rater reliability of Cavalieri's point-counting method is shown for estimating 3-D reference volumes from tissue sections (Mouton, 2002).

2.4. Neuronal profile area

To quantitatively assess the growth of neuronal somata, the author measured the neuronal profile areas in each subnucleus of OSN. In this study, the subnuclei were classified as follows (Table 2): the fascicular nucleus (FN), principal nucleus (PN), dorsal median nucleus (DM), and ventral median nucleus (VM). The first two nuclei comprised the LN, and the second two, the MN. The FN was the author's own nomenclature (see below). Measurements were carried out independently for these four nuclei. Only the left pair of nuclei was measured for the LN (PN and FN). To minimize sampling bias, the author systematically selected the K–B sections at constant intervals, and evenly placed several sampling areas along the dorsoventral axis in the nuclear territory. The total number of sampling areas varied case to case: 2–4 for the FN, 4–10 for the PN, 1–4 for the DM, and 1–5 for the VM. Then, using a light microscope (Optiphot; Nikon, Tokyo, Japan) equipped with a drawing tube, the author traced the outline of a neuronal profile onto white paper at a final magnification of 500×. A neuron was histologically defined as a cell with a droplet-like clear nucleus containing single or multiple distinct nucleoli and surrounded by a basophilic perikaryon.