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Research article

Development of the human lateral geniculate nucleus: A morphometric and computerized 3D-reconstruction study

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ABSTRACT

Introduction: The lateral geniculate nucleus (LGN) is the major relay center of the visual pathway in humans. There are few quantitative data on the morphology of LGN in prenatal infants. In this study, using serial brain sections, the author investigated the morphology of this nucleus during the second half of fetal period.
Material and methods: Eleven human brains were obtained at routine autopsy from preterm infants aged 20–39 postmenstrual weeks. After fixation, the brain was embedded *en bloc* in celloidin and cut serially at 30 μm in the horizontal plane. The sections were stained at regular intervals using the Klüver–Barrera method.
Results: At 20–21 weeks, the long axis of LGN declined obliquely from the vertical to horizontal plane, while a deep groove was noted on the ventro-lateral surface of the superior half. At this time, an arcuate cell-sparse zone appeared in the dorso-medial region, indicating the beginning of lamination. From 25 weeks onwards, the magnocellular and parvocellular layers were distinguishable, and the characteristic six-layered structure was recognized. The magnocellular layer covered most of the dorsal surface, and parts of the medial, lateral, and inferior surfaces but not the ventral and superior surfaces. Nuclear volume increased exponentially with age during 20–39 weeks, while the mean neuronal profile area increased linearly during 25–39 weeks.
Conclusion: Human LGN develops a deep groove on the ventro-lateral surface at around mid-gestation, when the initial lamination is recognized in the prospective magnocellular layer. Thereafter, the nuclear volume increases with age in an exponential function.

1. Introduction

Development of visual function has been long a matter of interest for researchers working in various disciplines. The lateral geniculate nucleus (LGN) is the major relay center of the visual pathway. It appears as a characteristic six-layered structure on a cross-section of the human adult brain. The ontogenesis of the LGN has been investigated in several mammals, such as monkeys [1–3], cats [4], and rats [5]. However, human studies on this subject are limited to descriptive reports [6–16]; morphometric or quantitative data in the context of human brain are relatively scarce [17–21]. The author used serial brain sections in a morphometric and computerized 3D-reconstruction study to explore the morphology of the human LGN in prenatal infants.

2. Material and methods

2.1. Material

A total of 11 brains were examined in this study (Table 1). All brain specimens were obtained at routine autopsy from preterm infants aged 20–39 postmenstrual weeks. Cases 1 and 2 were terminated medically due to abnormality of chromosome 11 (details unknown) and intrauterine rubella virus infection, respectively. Case 6 was stillborn. The other preterm infants had died of various causes, such as prematurity (Cases 3–5), esophageal atresia (Case 7), umbilical hernia (Case 8), diaphragmatic hernia (Cases 9 and 11), and meconium aspiration (Case 10), after a short duration of postnatal life. Removal of the brain was approved by the institutional board of ethics; informed consent was obtained from all parents for use of the brain of their offspring for diagnostic and scientific studies. Physiological data pertaining to visual responses, such as ocular movements or pupillary light reflex, were not available for every case.

2.2. Histology

After fixation in 10% formalin solution, the brain was successively immersed in a 4:1 (v/v) mixed solution of 5% $KCrO_4$ and 5% $K_2Cr_2O_7$ for several months, washed in running water, dehydrated by passage

Abbreviations: K–B, Klüver–Barrera; LGN, lateral geniculate nucleus; ML, magnocellular layer; PL, parvocellular layer

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Table 1
Details of the material.

Case No	Age[a]	Sex	Brain weight (g)	Clinical diagnosis
1	20	male	48	abnormality of chromosome 11
2	21	male	70	intrauterine rubella virus infection
3	25	male	130	prematurity
4	28	female	160	prematurity
5	29	male	178	prematurity
6	30	NR[b]	170	hydrops fetalis
7	35	female	250	esophageal atresia
8	35	male	330	umbilical hernia
9	38	male	NR[b]	diaphragmatic hernia
10	39	female	390	meconium aspiration
11	39	female	380	diaphragmatic hernia, twin

[a] Age, expressed in postmenstrual weeks.
[b] NR, not recorded.

through a graded ethanol series, and finally embedded *en bloc* in celloidin [22]. Tissue blocks were serially sliced in the horizontal plane using a microtome setting at 30 µm. Every fifth or tenth section was stained using the Klüver–Barrera (K–B) method, which is of great benefit to simultaneously observe neuronal cell bodies and myelinating fibers [23]. The remaining sections were stored in a 70% alcohol solution, and some were later stained using other histological methods as required. Gross and microscopic examination revealed no severe pathological changes, such as infarction, massive hemorrhage, structural anomalies, or acquired injuries.

2.3. Computerized 3D-reconstruction

Since the LGN is a symmetric structure, only the right pair was chosen for reconstruction. Low-power (4.2–14.2×) photographs were taken of the K–B sections containing the right LGN, which were selected at regular intervals. Subsequently, the cross-sectional outlines were traced on a tablet (Intuos; Wacom, Saitama, Japan) connected to a personal computer (FMV-D5220; Fujitsu, Tokyo, Japan). Digitized data were stacked slice by slice to make a 3D-reconstruction figure aided by software (TRI; Ratoc, Tokyo, Japan). To adjust the precise length along the *z*-axis, the corrected section thickness was estimated microscopically with a 40× objective lens. Measurements were repeated on several K–B sections selected at regular intervals, and the mean was calculated. The corrected section thickness was obtained by multiplying the mean value with the refractive index (n) of the cover glass (n = 1.5).

2.4. Nuclear volume

Nuclear volume was estimated using the Cavalieri point-counting method. Low-power (42–43×) photographs were taken of the K–B sections obtained at regular intervals and superimposed with a transparent plastic lattice (unit length: 10 mm). Subsequently, the author counted the intersections hitting the nuclear area of LGN. Volume (V) was calculated with the following formula: $V\ (mm^3) = a \times \Sigma P \times d$, where "$a$" was the area ($mm^2$) of a unit square (0.054–0.056), "ΣP" was the sum of intersections, and "d" was the length (mm) of an interval obtained by multiplication of the corrected section thickness (see 2.3) and the difference of the section number between two adjacent slides. Tissue shrinkage is inevitable during histological procedures and may depend on fixatives, embedding media, staining methods, or individual variations among subjects. In this study, the extent of shrinkage (s) during the staining procedure was estimated by the following formula: $s = ls/lu$, where "ls" was the length of tissue on the K–B section and "lu" was that on the adjacent untreated section. Corrected volume (Vc) was calculated by the following formula: $Vc = V/s^3$ [19].

2.5. Neuronal profile area

Measurements were conducted for the right LGN (see 2.3). To minimize sampling bias, the following systematic-random sampling method was adopted. Three photographs were selected from those used in the previous section (see 2.4) at three different levels of horizontal serial sections: 1) inferior one-sixth, 2) middle, and 3) superior one-sixth levels. The nuclear area was superimposed with a transparent plastic lattice (unit length: 10 mm) to mark the sampling sites of neurons at the intersections selected at regular intervals. Then, using a light microscope (Optiphot; Nikon, Tokyo, Japan) equipped with a drawing tube and a square ocular grid, the author traced the outlines of neuronal profiles, of which nucleoli were located within a square (one side length: 80 µm) or across the left or lower line, at a magnification of 500×. Here, a neuron was defined morphologically as a cell, which is relatively large and has a clear, droplet-like nucleus with single or multiple nucleoli and a basophilic cytoplasm. Line drawings were digitized on a tablet connected to a personal computer (see 2.3) and were analyzed to obtain morphometric parameters (area, perimeter, and diameter) with the aid of an image analysis program (VM32; Rise Corporation, Sendai, Japan).

2.6. Statistical analysis

Data were transferred to a spreadsheet (Microsoft Excel 2016) and analyzed using Statcel3 statistical software (OMS Press, Saitama, Japan). Normality of the histogram of neuronal profile areas was tested using the chi-square test for goodness of fit. Scatter plots of morphometric data and gestational age were created, and regression analysis was performed. Mean neuronal profile areas were compared between the magnocellular and parvocellular layers with Student's or Welch's *t*-test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. General microscopic findings

3.1.1. 20–21 postmenstrual weeks (Cases 1, 2)

The LGN was identified as a round or ovoid nuclear mass situated lateral to the medial geniculate nucleus and the pulvinar, and dorsal to the crus cerebri (Fig. 1A). The dorsal margin was rather unclear, because it was closely related to a subdivision of the pulvinar, presumably the suprageniculate nucleus [24]. An arcuate, cell-sparse zone was observed in the dorsomedial region. This was considered as an interlaminar zone between two layers of the magnocellular layer (ML). In Case 2, the superior part of this nucleus was flexed ventro-laterally to hold massive fiber bundles and clusters of relatively large neurons belonging to the pregeniculate nucleus (Fig. 1B). On neuronal morphology, in Case 1, most neurons of the LGN had scant cytoplasm, and their nuclei were darkly stained with no prominent nucleoli; thus, these neurons were hardly distinguishable from glial cells. In Case 2, the LGN neurons had acquired the characteristic features of the neuron described above (see 2.5) and were readily discernible, although the cytoplasm was still poor (Fig. 1C, D); they were larger in the ML than in the prospective parvocellular layer (PL). Pyknotic cells were rarely encountered.

3.1.2. 25–39 postmenstrual weeks (Cases 3–11)

A distinctive laminar structure was noted in the LGN (Fig. 2A); two laminar groups were identified, i.e., the ML and PL. As seen in Case 2, the superior part of this nucleus was flexed ventro-laterally, holding fiber bundles and clusters of pregeniculate neurons (Fig. 2B). The LGN neurons varied in shape (round, fusiform, triangular, or multipolar) in both two groups, while they were generally larger in the ML than the PL (Fig. 2C, D). Most neurons appeared to be arranged perpendicular to the long axis of a layer. The layers showed a serpentine appearance with an