Journal of Chemical Neuroanatomy xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Morphological and morphometrical maturation of ventral cochlear nucleus in human foetus

Sabita Mishra*, T.S. Roy, Shashi Wadhwa[1]

All India Institute of Medical Sciences, New Delhi, India

ARTICLE INFO

Article history:
Received 21 December 2016
Received in revised form 6 March 2017
Accepted 16 March 2017
Available online xxx

Keywords:
Human ventral cochlear nucleus
Development
Stereology
Parvalbumin
Synaptophysin

ABSTRACT

Auditory impulses perceived by the hair cells of the organ of corti are relayed in the cochlear nucleus, the first relay station in the brainstem, by the cochlear nerve. The human foetus is well known to respond to sound during the last trimester of gestation. On the contrary, studies conducted in rat, cat and mouse have shown that these mammals have an immature auditory system at the time of birth. There are very few reports available regarding the morphological and functional maturation of the cochlear nucleus in human. Although the human cochlear nucleus neurons attain adult morphological characters by mid-gestation, there are hardly any studies discussing the functional maturation of the cochlear nucleus. Hence the present study was aimed at observing the morphological as well as functional maturation of the human foetal cochlear nuclei at various gestational ages. Morphological maturation was observed qualitatively while stereological estimation of the volume of well defined ventral cochlear nucleus (VCN) was calculated by the Cavalieri principle; neuronal count and density was estimated by dissector principle. The functional maturation was assessed by observing the expression of synaptophysin, a synaptic marker, at different gestational ages and by the presence of parvalbumin, a calcium binding functional neuronal marker by immunohistochemistry. Neurons showed coarse Nissl's substance and well developed cell processes and gradual increase in cell size by the 24th–30th gestational week. Synaptophysin labeling in the complete cochlear nucleus was observed at 20 weeks of gestation. Adult pattern of synaptophysin labeling was observed finally at37weeks of gestation. Earliest presence of parvalbumin expression was detected at 16 weeks of gestation and a distinct adult pattern was seen at 37 weeks of gestation. This study concluded that morphological and functional maturation of the human cochlear nuclei occurs simultaneously during mid-gestation which represents the critical period of development and continues up to term.

1. Introduction

The cochlear nucleus is the first relay station of the brain stem to receive auditory impulses and is situated at the lateral part of the pontomedullary junction medial to the inferior cerebellar peduncle. The nucleus is divided into a ventral cochlear nucleus (VCN) and dorsal cochlear nucleus (DCN). The cochlear nerve further divides the ventral cochlear nucleus into an anteroventral cochlear nucleus (AVCN) and posteroventral cochlear nucleus (PVCN). Each division of the nucleus is characterised by different sets of neurons, based on structure. Each cell type encodes specific sound information in a characteristic way and makes contact with the higher centers in the brainstem to form a link to a highly specific neuronal circuit that helps in auditory processing (Moore and Osen, 1979; Morest et al., 1990; Moore et al, 1998; Moore and Linthicum, 2007). It is interesting to observe that throughout the auditory pathway topography and tonotopy are maintained. Sound waves of low frequency from the basal cochlea relay in the VCN, while the high frequency sounds from the apical part of the cochlea relay in the DCN (Friauf and Kandler, 1993; Moore et al., 1995; Iyengar, 2012). Adult cochlear nuclear morphology and organisation have been studied in human (Adams, 1986; Terr and Edgerton, 1985; Richter, 1983; Seldon and Clark, 1991; Sharma et al., 2014a,b).

Human foetus responds to acoustic stimuli before birth, even when the stimuli are different before and after birth (Moore et al., 1997; Pujol and Lavgine-Rebillard, 1992; Birnholtz, 1980; Wakai

Abbreviations: GW, gestational week; CN, cochlear nucleus; VCN, ventral cochlear nucleus; DCN, dorsal cochlear nucleus; AVCN, anteroventral cochlear nucleus; PVCN, posteroventral cochlear nucleus; PV, parvalbumin; DAB, diammi-nobenzidine; CRL, crown rump length; BPD, biparietal diameter; FL, foot length.

* Corresponding author at: Maulana Azad Medical College, New Delhi, India.
E-mail address: sabitamishra12@gmail.com (S. Mishra).
[1] [1]NDMC Medical College, Delhi, India.

http://dx.doi.org/10.1016/j.jchemneu.2017.03.002
0891-0618/

et al., 1996; Hykin et al., 1999; Moore and Linthicum, 2007; Iyengar, 2012). On the contrary lower animals like rat, mice, cats and gerbils have an immature auditory system at the time of birth. In these animals anatomic maturation of the auditory system is attained by two to three weeks postnatal. Physiological maturation corresponds with the anatomic maturation of the auditory structures. Following the onset of physiological hearing in lower mammals; there is a critical period during which appropriate auditory stimuli are required for the normal development of the higher auditory nuclei and fine tuning of acoustic characteristics present in adults. Thus lower mammals have been used as an excellent experimental tool in research to understand the critical period of auditory development (Friauf and Kandler, 1993). There are still lacunae in our knowledge regarding the appearance of the neuronal groups in the developing human cochlear nuclei. There are a few morphometric studies on the developing cochlear nucleus (Nara et al., 1993), and the cochlear nerve (Ray et al., 2005). The cochlear ganglion development has also been studied in human (Sethi et al., 2013, 2015). Cochlear nucleus has been studied by 3D reconstruction of the nucleus at various ages from infancy to old age (Sharma et al., 2014b).

The differentiation of pre and postsynaptic elements during synaptogenesis has been studied in the developing nervous system of different species using ultrastructural, immunological and biochemical methods. Synaptophysin, an integral membrane protein of the small synaptic vesicles has been used to study synapse formation during development of the nervous system of various animal species and is a reliable marker for nerve terminal differentiation and synaptogenesis (Knaus et al., 1986; Jahn et al., 1985; Becher et al., 1999; Saranat and Born, 1999 Ulfig et al., 2000). Synaptohysin expression in cochlear ganglion has also been observed (Sethi et al., 2013). Parvalbumin, calbindin and calretinin are three-calcium binding proteins of the EF hand family expressed in the nervous tissue at different phases of development (Baimbridge et al., 1992). Parvalbumin is known to be expressed in a subpopulation of GABAergic neurons and during the later part of development in fast firing neurons (Schlosser et al., 1999).

The present study aimed at observing the morphological maturation of the cochlear nucleus qualitatively. Neuronal count and density were estimated by the physical dissector method and the volume of the cochlear nucleus was calculated by the Cavaliere method (Wadhwa et al., 1997; Gundersen et al., 1999). The morphological maturation was further correlated with the functional maturation of the cochlear nuclei neurons by observing the appearance of synaptophysin, a membrane protein of the synaptic vesicles; and expression of parvalbumin, a calcium binding protein, a neuronal marker for neuronal activity at different ages of gestation. This study aimed to provide a normal baseline data on the morphological and functional development of the cochlear nuclei, and determine the critical period of human development. This study may also help in explaining the influence of prenatal sensory stimuli, diseases and other noxious insults on the auditory pathway.

2. Material and methods

Human aborted fetuses of various gestational ages ranging from 12 to 37 weeks of gestation, were procured from the Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India, with prior approval of the Institute Ethics Committee. The foetuses were obtained within 4 h of abortion to minimize the postmortem changes and were immediately immersion fixed in 4% buffered (0.1 M phosphate buffer, pH 7.4) paraformaldehyde solution. A midline craniotomy incision along the sagittal suture was made prior to immersion to ensure proper fixation of the brain in the fixative and was preserved at 4 °C for further processing. Foetuses showing any evidence of autolysis were excluded. The foetuses below 20 weeks of gestation were obtained from cases of medical termination of pregnancy (MTP), performed for family planning, legalised in India (under MTP-Act Government of India, 1971). Foetuses older than 20 weeks of gestation were still-births. Informed consent from the mothers or legal representatives was taken. None of the mothers suffered from any medical illness during pregnancy and none of the fetuses had any congenital abnormalities. Various morphometric parameters such as weight, crown-rump length (CRL), biparietal diameter (BPD) and foot length(FL) of the aborted fetuses were recorded immediately on arrival (Table 1). The fetal age was determined from the above-mentioned parameters, especially the bi parietal diameter and foot length (Mandarim-de-Lacerda, 1990; Sailaja et al., 1996) and also from the history of last menstrual period of the mother. The fixative was changed after 24 h and the pontomedullary junction lodging the cochlear nucleus was dissected out. The pontomedullary junction was further preserved in paraformaldehyde for a period of 1–2 weeks, with a change of fresh fixative every third day. The tissue was washed in 0.1 M phosphate buffer (PB, pH 7.4) and cryoprotected. The brain was removed within 24 h and preserved in fresh fixative at 4 °C.

2.1. Morphological study

For morphological study, pontomedullary junction was dissected and processed for paraffin embedding. Serial sections of seven-micron thickness were stained with cresyl violet. Entire series was examined and the cochlear nuclear complex was identified. The dorsal and ventral cochlear nuclei were studied

Table 1
Estimated ages and various parameters of foetal specimens. Morphometric parameters of foetuses used in the study.

Age (weeks)	CRL(cm) (mean+/−SD)	BPD(cm) (mean+/−SD)	Foot length(cm) (mean+/−SD)	Body Wt. (gms) (mean+/−SD)
10 ≥ 12	6.5 ± 0.92	1.6 ± 0.43	0.88 ± 0.18	29 ± 6.51
12 ≥ 14	11.428 ± 0.68	2.3 ± 1.22	1.2 ± 0.24	93.57 ± 10.30
14 ≥ 16	14.34 ± 3.46	3.46 ± 0.23	1.14 ± 0.24	134 ± 9.62
16 ≥ 18	16.8 ± 0.76	4.15 ± 0.24	2.2 ± 1.9	215 ± 10.8
18 ≥ 20	18.55 ± 0.42	4.85 ± 0.19	2.75 ± 0.24	235 ± 10.80
20 ≥ 22	18.4 ± 0.36	5.06 ± 0.11	3.26 ± 0.25	275 ± 18.02
22 ≥ 24	19.75 ± 0.35	5.6 ± 0.14	4.25 ± 0.35	333.25 ± 35.35
24 ≥ 26	20.5 ± 0.77	5.85 ± 0.21	4.75 ± 0.35	455 ± 63.63
26 ≥ 28	20.5 ± 0.77	5.85 ± 0.21	4.75 ± 35	455 ± 0.63
28 ≥ 30	24 ± 1.41	6.25 ± 0.35	5.25 ± 0.35	555 ± 63.63
37	32 ± 0	8.6 ± 0	6.5 ± 0	3400 ± 0

Please cite this article in press as: S. Mishra, et al., Morphological and morphometrical maturation of ventral cochlear nucleus in human foetus, J. Chem. Neuroanat. (2017), http://dx.doi.org/10.1016/j.jchemneu.2017.03.002