Morphogenesis of the Dorsomedial Nucleus of the Amygdaloid Complex in Early Juvenile Period in Rats

A. V. Akhmadeev and L. B. Kalimullina

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We studied the dynamics and gender differences in the formation of the dorsomedial nucleus of the amygdaloid complex in early juvenile period (postnatal days 21, 24, 28 and 31) by determining its planimetric parameters, count of neural and glial cells, and glial and apoptotic indexes.

Key Words: *dorsomedial nucleus, cerebral amygdaloid complex, sex steroids, early juvenile period, sexual dimorphism*

Sexual dimorphism in the dorsomedial nucleus (DMN) of the amygdaloid complex (AC) was revealed in experiments where the response of DMN neurons to sex steroid deficit was studied [1]. Later it was confirmed by studies of sex steroid replacement therapy in gonadectomized rats and by autoradiography on adult animals with administration of radiolabeled sex steroids followed by recording of some morphometric parameters [1,6]. This zone of sexual dimorphism in AC attracts much interest because of its involvement into regulation of reproduction. However, the mechanisms of DMN formation during pubertal period are still not investigated.

The aim of this study was to characterize sex differences of structural changes observed in the DMN of AC in early juvenile period of rat ontogeny.

MATERIALS AND METHODS

Studies were performed on 40 Wistar rats; the brains were examined on postnatal days 21, 24, 28 and 31 (5 males and 5 females per point). For evaluation of cytological and planimetric parameters, serial brain frontal sections were prepared and stained with cresyl violet after Nissl. The areas of brain hemispheres, AC, and DMN were measured on sketches of their projections using a planimeter and expressed in arbitrary units. The number of neurons containing nucleoli and glial cells per filed of view (0.035 mm^2) was counted under an MBI-11 microscope (Lomo) on 10-μ sections at magnification ×600 (lens ×40, ocular ×15). Apoptotic cells were detected by TUNEL technique on 5-μ paraffin sections poststained with hematoxylin. The apoptotic index was calculated according to the formula [12] using summarized cell count in three consecutive sections. Statistical analysis was performed using Statistica 5.5 software. Significance of numerical data was evaluated using Student's *t* test.

RESULTS

Evaluation of the cytoarchitectonics and cytological parameters of neural and glial cells in males on postnatal day 21 showed that DMN was formed primarily by medium-size neurons located on the medial surface of the hemisphere dorsally to its mediobasal angle. Stria terminalis lied laterally to the nucleus. The lower boundary of the nucleus was formed by a fibrous layer, which separated DMN from the complex of posterioir medial nucleus and posterior cortical nucleus located beneath it. Separate large neurons and groups of small neu-

Department of Human and Animal Morphology and Physiology, Bashkir State University, Ufa, Russia. ***Address for correspondence:*** mpha@ufanet.ru. A. V. Akhmadeev

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rons were seen within DMN. The density of neurons was higher in central zones of the nucleus and decreased at the periphery, this creates a picture of their gradual dissemination. Perikaryons were round or oval, the greater part of the protoplasm was occupied by light cell nucleus with intensively stained nucleolus in its center. Neuronal cytoplasm contained fine-grained basophilic substance, which penetrated into initial segments of dendrites. The glia was presented by olygodendrocytes and astrocytes distinguished by peculiar nuclei. In contrast to males, DMN neurons in females were arranged more compactly and the aggregation they formed contained less fibrous layers.

On postnatal day 24, relative position of the lower horn of the lateral ventricle and DMN changed in both males and females. Due to enlargement of the hemisphere and ventricle, this nucleus was displaced under the ventricle and was located subventricularly. This led to changes in the configuration of both DMN (it stretches along the dorsomedial axis) and medial angle of the hemisphere (it assumes an angulate shape) took place. In contrast to female DMN, in males it contained pronounced fibrous layers and was characterized by dispersed arrangement of neurons. In females, the nucleus was formed by a compact group of small- and medium-size neurons and occupied a smaller area than in males. Neuron cytoplasm was more intensively stained with cresyl violet than at the previous observation term. Among the dust-like granulation of the basophilic substance there occurred groups of small tigroid bodies.

On postnatal day 28, DMN in males was located under the lower horn of the lateral ventricle being separated by fibrous layers. Fibrous tracts were seen both inside and around the nucleus as if encapsulating it. Neural and glial cells were arranged dispersely with well-detectable intercellular space between the neurons. Cell nucleus contained euchromatin and clear-cut nucleolus located in the center of the nucleus. Basophilic substance was finely grained, chromophilic bodies were regularly spread in different zones of the cytoplasm. The area of the nucleus was smaller in females compared to males and the neurons forming it were arranged more densely. This was clearly seen at high magnification power when bodies of adjacent neuron were visible and glial cells were located on their surface.

On postnatal day 31, neuron aggregation formed in males had cytoarchitectonical and cytological characteristics analogous to those observed on postnatal day 28. Unlike DMN in males, in females it consisted of very compact group of neurons with closely adjacent cells. Neuronal groups in the perivascular and pericapillar zones were well-distinguishable.

Analysis of planimetry data for hemisphere, DMN and AC areas (Table 1) showed that specific area of AC in relation to hemisphere area gradually increased from postnatal 21 to day 31 both in males and females, but significant difference between males and females was revealed only on day 31. Significant differences in values of specific area of DMN were recorded starting from day 24 (on day 24 p=0.054), in males it was higher on postnatal days 28 (p<0.01) and 31 (p<0.05).

Neural and glial cell count in DMN (Table 2) showed that neuronal density was significantly higher in females starting from day 24. Pronounced sexual dimorphism was revealed in the value of glial index on postnatal day 24 although at all observed terms glial index was higher in males.

Apoptotic cells were extremely rare at all terms of postnatal development. They were absent in females on days 21 and 31 and in males AI on day 21 was 3.42±0.95, on day 24 this parameter in females and males was 2.27±0.74 and 2.40±0.29, respectively, on day 28 AI in females and males was 1.38±0.44 and 2.47±0.51, respectively, and on day 31 AI in males was 2.66±0.39. The revealed difference in AI values for all observed terms were insignificant.

Current investigation demonstrated sex differences in DMN morphogenesis in the early juvenile period of rat development. They included increased

TABLE 1. Specific Area of AC and DMN at Different Terms of Early Juvenile Development in Rats

Term, days	AC		DMN	
	females	males	females	males
21	16.83±0.29	17.11±0.50	2.79±0.21	3.23±0.25
24	15.40±0.31	17.66±0.72	1.74±0.19	2.72±0.29
28	15.84±0.21	16.76±0.32	2.09±0.11	3.18±0.20**
31	16.75±0.34	18.18±0.47*	2.78±0.14	3.25±0.17*

Note. Here and in Table 2: *p<0.05, **p<0.01 compared to females of the same stage of postnatal development.

TABLE 2. Count of Neural and Glial Cells and Glial Index for DMN at Different Terms of Early Juvenile Development in Rats

Term, days	Neurons		Glia		Glial index	
	females	males	females	males	females	males
21	21.06±3.35	18.06±3.54	34.06±5.85	31.66±0.71	1.83±0.49	2.04±0.39
24	21.86±2.59	16.66±2.43**	32.24±4.75	31.46±4.62	1.45±0.05	1.89±0.04*
28	24.06±1.40	16.66±1.41*	26.66±4.18	31.46±4.55	1.09±0.12	1.91±0.28
31	28.06±4.03	16.46±0.91*	38.46±5.65	26.86±3.20	1.36±0.01	1.65±0.21

specific area of DMN in males by the end of this period and in the formation of differences in specific area of DMN: on days 28 and 31 DMN area was significantly larger in males; the decrease of DMN area in females was accompanied by an increase of neuron packing density. It seems likely that dense packing of DMN neurons in females and formed interneuronal and neuroglial contacts promote synchronization of the function of neuroendocrine neurons in this nucleus which becomes involved in the formation of the positive feedback and presumably in mechanisms of estrous cycle regulation [1,8,9]. Previous electron-microscopic study of this nucleus in sexually mature rats revealed the presence of extended inter-neuron contacts [2]. It is known that DMN neurons express cell adhesion molecules; the interaction of these molecules with calcium channels leading to an increase of intracellular calcium concentration is mediated by estrogens [4,11].

AI values indicate that programmed cell death in the early juvenile period of DMN development occurs in individual cells and is not critical for the formation of changes during this period of rat development. It seems likely that apoptosis during the recorded period of postnatal rat development is caused by specialization of synaptic targets and reflects the formation of neural connections between DMN and accessory olfactory bulb, DMN, and posterior medial and posterior cortical nuclei and also between DMN and hypothalamic reproductive centers [3,5,7,10].

Our findings add to previous research on sexual dimorphism of DMN in adult animals [1] and characterize plasticity in this area during pubertal period.

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