STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE AMYGDALA DURING THE ESTROUS CYCLE

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The modulating influence of the amygdala (AM) on activity of the hypothalamic centers controlling gonadotropin secretion and the role of the olfactory stimuli in this phenomenon have been proved. However, existing information on amygdalo-hypothalamic relations formed during regulation of the estrous cycle is general in character and requires more detailed analysis.

The aim of this investigation was to identify zones of AM responding to changes in circulating hormone levels by the method of karyometry in the course of the estrous cycle, and also to discover differences between them with respect to levels of estrogen binding.

EXPERIMENTAL METHOD

Experiments were carried out on mature Wistar rats weighing 220-300 g, kept under identical animal house conditions, with 14 h of daylight daily. In the first stage of the investigation, regularity of the estrous cycle was studied in 30 females, and on that basis 10 rats were selected with a precise cycle for karyometry of the neurons. These animals were killed by decapitation at a particular time of day (from 11 a.m. to 1 p.m.) in the stages of estrus and metestrus (five rats in each case), which were differentiated on the basis of the vaginal cytology. The brain was fixed in 10% neutral formalin. Paraffin sections 8-10 μ thick were stained with hematoxylin and eosin and with cresyl violet. Karyometry was carried out in 22 zones of the corticomedial and 25 zones of the basolateral group of structures of AM, a detailed list of which was described previously [1, 3]. Projections of the nuclei of the neurons were drawn for on average 150 in each zone from each animal. The volume of the nuclei was determined assuming them to be ellipsoids of rotation.

The rostro-caudal gradient, discovered in the structural and functional organization of AM on the basis of the karyometric data predetermined the program of investigation of binding of ^{3}H-estradiol in the cytoplasmic cell fraction in this formation (stage 2 of the investigation). This stage was conducted on AM isolated from the brain, and the accuracy of its excision was confirmed morphologically (Fig. 1). The isolated AM was divided in rostral and caudal parts. To ensure that the weighed samples were of the necessary size (100-120 mg) the corresponding parts of AM were taken from four rats and the number of receptors was determined [5] in three parallel tests. In group 1 (12 males and eight females) the mean parameters characteristic of the above-mentioned parts of AM in male and female rats were determined; groups of the latter were formed from animals at all stages of the cycle. Statistical analysis of the results was carried out by parametric and nonparametric methods.

EXPERIMENTAL RESULTS

In the course of the estrous cycle a neuronal response was found in several zones of the corticomedial and basolateral groups of structures of AM, in which a statistically significant increase in the mean size of the cell nuclei was found during estrus compared with metestrus (Fig. 2). On the basis of differences in their structural and functional organization, they can be combined into three groups. The zones of group 1 (the anterior cortical nucleus, the basomedial nucleus) lies at the rostral pole of AM and is characterized by di-

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Fig. 1. Morphological verification of excision of AM. Cresyl violet, 35×.

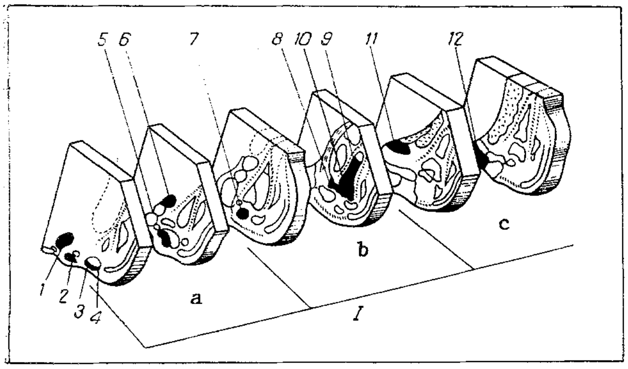


Fig. 2. Zones of AM responding during the estrous cycle (shaded black). 1) Anterior amygdaloid region, 2) lateral olfactory tract, 3) anterior cortical nucleus (medial part of superficial cellular zone at the rostral level), 4) anterior cortical nucleus (lateral part of superficial cellular zone at the rostral level), 5) anterior cortical nucleus (medial part of superficial cellular zone at the caudal level), 6) central nucleus, 7) basomedial nucleus, 8) intercalary masses, 9) basolateral nucleus (main part), 10) basolateral nucleus (gigantocellular part), 11) dorsomedial nucleus, 12) posterior cortical nucleus (medial part); I) zones of AM: a) anterior, b) central, c) posterior.

rect connections with the main olfactory bulb [10]. Group 2 (the dorsomedial nucleus and medial part of the posterior cortical nucleus) lies in the posterior part of AM and has direct connections with the accessory olfactory bulb [10]. Group 3 (the central nucleus and intercalary masses) have direct connections with zones of the above-mentioned groups and with nuclei of the brain stem [8, 11]. A response of neurons of the basolateral nucleus at the caudal level of the central division was discovered in the basolateral group of struc-

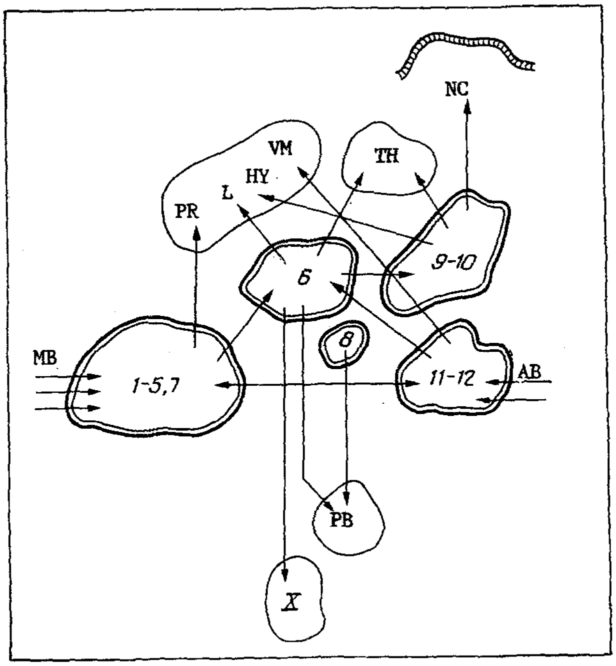


Fig. 3. Principal connections of nuclei of AM responding during the estrous cycle. Legend: 1-12) the same as in Fig. 2. PR) Preoptic region, HY) hypothalamus, L) lateral hypothalamus, VM) ventromedial hypothalamic nucleus, TH) thalamus, NC) neocortex, X) nucleus of vagus nerve, PB) parabrachial nucleus of pons, MB) main olfactory bulb, AB) accessory olfactory bulb.

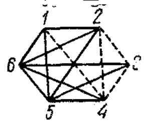
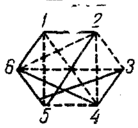
tures of AM. On the basis of analysis of the literature on the characteristics of the anatomical connections of the nuclei of AM responding in the course of the estrous cycle, the existence of the following relations between the brain formations mentioned above can be postulated (Fig. 3).

Comparison of these results with those of previous investigations [1, 3, 4] shows that the rostro-caudal gradient in the structural and functional organization of AM of rats, discovered in this brain formation during a study of zones of sexual dimorphism, is manifested in studies of neuronal reactivity differently, depending on the character of the hormonal shifts arising in the animal. Whereas the latter are characterized by their stability (1 month after ovariectomy the rise of the gonadotropin level flattens out on a plateau), one region responding to gonadectomy was identified, and was located in the posterior parts of AM [1]. If fluctuations of hormone levels follow a rapid course (estrous cycle), the response of the neurons is determined not only in the posterior, but also in the anterior part of AM (Fig. 2: 1-5, 7), connected with the medial preoptic region, whereas zones of the posterior part are connected with the ventromedial nucleus [7, 9].

The results of determination of estrogen binding levels in the cytoplasmic fraction of the cells of AM are given in Table 1. They show that the level of estrogen receptors is significantly higher in the cell cytosol of AM (both for the structure as a whole and for its regions) in female rats than in males. Six differences in the content of estrogen receptors in the cell cytosol of the piriform lobe, most of which is a component of AM, was first stated by Takeshita [14] in 1976. The results of the present investigation on the isolated AM not only confirmed his data, but also revealed differences in the concentration of estrogen receptors between the rostral and caudal parts of this brain formation, which exist in both female and male rats. The level of estradiol binding in the cytoplasmic fraction of the caudal parts of AM in this case (in both male and female rats) is about twice as high as in the rostral parts, i.e., there is the same ratio between cytoplasmic estrogen receptors as in the hypothalamus between the preoptic and mediobasal regions [2].

The existence of dependence of ^{3}H-estradiol-binding activity of the cells of the abovementioned parts of AM on hormonal changes taking place in the body during the estrous cycle

TABLE 1. Binding of Estrogen in AM of Male Rats (M ± m for the group)

Group of animals	Regions of amygdala	
	rostral	caudal
	femtomoles/mg DNA	
1. Males	332±55,6	518±11,5*
2. Females, total for all stages of cycle	564,5±75	823±68*
3. In diestrus II	460±46	754,5±35,5*
4. In proestrus	453±46,5	485±34
5. In estrus	55±12	514±81*
6. In metestrus	1861±557	1007±154
Significance of differences between groups region by region $p<0,05$		

Legend. Asterisk indicates significance of differences between regions within groups, $p < 0.01$.

was demonstrated for the first time. As Table 1 shows, the number of cytoreceptors for estradiol changed in both anterior and posterior parts of AM. The greatest differences in the content of cytosol receptors were found in the anterior part of AM between the stages of estrus (55 ± 12 femtomoles/mg DNA) and metestrus (1861 ± 557).

It is worth noting that the greatest fluctuation in size of the cell nuclei of neurons of AM during the estrous cycle also occurred between the stages of estrus and metestrus [13]. Under these circumstances an increase in size of the nuclei in the neurons of AM during estrus was accompanied by enhancement of their functional activity [6, 12] and is in good agreement with the hypothetical mechanism of translocation of the cytoplasmic receptor complex into the nucleus. The number of receptors in the cytoplasm rises sharply in metestrus, and this may be explained by the recycling of the spent receptors from the nuclei into the cytoplasm and also by synthesis of cytosol receptors de novo.

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