

THE STUDY OF SEX DIFFERENCES IN ANTERIOR AND POSTERODORSAL SUBREGIONS OF THE MEDIAL NUCLEUS (NM) OF THE AMYGDALOID COMPLEX (AC) IN THE RAT BRAIN. STEREOLOGICAL ANALYSIS

LOZANČE OLIVERA*, DREKIĆ D*, MALOBABIĆ S** and CVETKOVIĆ DIJANA*

*Department of Anatomy, Faculty of Veterinary Medicine, Beograd **Department of Anatomy, Faculty of Medicine, Beograd

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To determine the precise localization of sex differences in the subregions of the nucleus medialis (NM) of the amygdaloid complex (AC), morphometric-stereological parameters (the volume density $[V_v]$ of cell nuclei, of the cytoplasm and neuropil and the numerical density $[N_v]$ of the anterior and posterior parts of NM) in adult male and female rats were used. There were no significant differences in the investigated volume densities and numerical density of the anterior part of NM AC between male and female adult rats. Although, there were no significant differences in $[V_v]$ of cell nuclei and of cytoplasm, our investigation demonstrated that the posterodorsal subregion of NM of the rat showed sex differences in the volume density of the neuropil and in the numerical density of cells. These values were significantly greater ($p < 0.05$ and $p < 0.001$) in male than that in female posterodorsal subregions of NM AC of adult rats.

Key words: amygdaloid complex, nucleus medialis, sex difference, rats.

INTRODUCTION

It was not until the last few decades that structural sex differences were demonstrated in the brain of mammals and birds. Especially the paper of Raisman and Field (1973), but also those of Pfaff (1966) and Nottebohm and Arnold (1976), formed the impetus for the study of structural correlates of sex differences in the brain, using morphometric methods (Uylings *et al.*, 1984). The presence of sexual dimorphism has been demonstrated in certain brain regions which are known to control various reproductive functions, and have been found to contain a great number of target neurons for sex steroid hormones (Pfaff and Keiner, 1973., Sar and Strumph, 1975), such as the hypothalamus (Gorki *et al.*, 1980., Arnold, 1985., Matsumoto and Arai, 1986., Matsumoto, 1991) and the nuclei of the amygdaloid complex (AC) (Mizukami *et al.*, 1983., Kalimulina, 1988., Drekić *et al.*, 1988., Hines *et al.*, 1992).

The medial nucleus (NM) is one of the sexually dimorphic regions of the AC of the rat brain. The presence of sexual dimorphism in the NM has been demonstrated for various morphological parameters: in nuclear volume (Mizukami *et al.*, 1983., Hines *et al.*, 1992), in synaptic organization (Nishizuka and Arai, 1981), in the volume of cell nuclei (Drekić *et al.*, 1988); and in the neurotransmitter system (Micevych *et al.*, 1988a; 1988b., Malsbury and McKay, 1989).

The purpose of this study was to use morphometric analysis and stereological parameters (volume and numerical density) in order to confirm or to exclude the presence of structural sex differences in the subregions (anterior and posterodorsal) of the NM of the adult rat amygdaloid complex.

MATERIALS AND METHODS

For our investigations we use adult male and female Wistar rats (10 males and 10 females). The animals were sacrificed on the 62nd day of life, under ether narcosis; the brains were removed and the amygdaloid complex with surrounding parts of the brain was isolated and fixed in Bouin solution. After standard paraffin embedding, serial sections (5 μ m thick) were stained with hematoxylin-eosin and Herlant methods, and investigated using light microscopy (objective -50x) and stereological analysis.

For stereological analysis, two morphological parameters, the volume density V_v (mm^0) of anterior and posterior parts of NM cell nuclei, cytoplasm, neuropil and the numerical density N_v (mm^{-3}) of cell were followed, using the Weibel multipurpose test system (P:42). Sampling was performed so that the rostral (the first) and posterior (the last) sections of NM parts were used. For each, anterior and posterodorsal subregions of the NM AC, 25 test fields were chosen by intermittent sampling (Kališnik, 1985). Statistical significance was tested with Student's T-test.

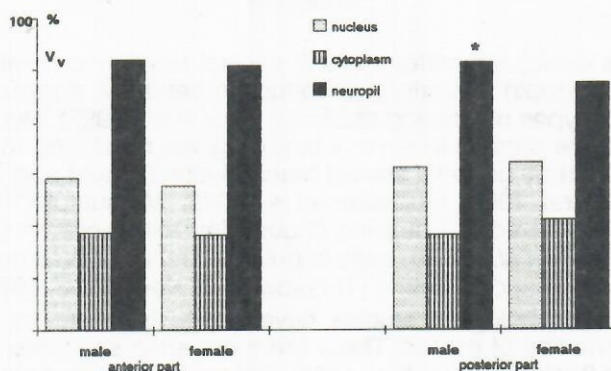
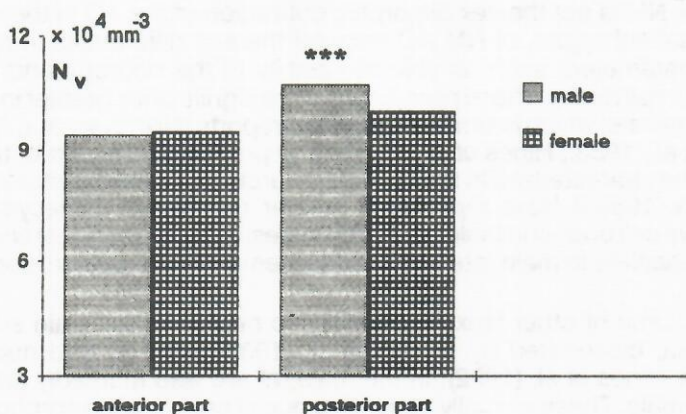
RESULTS

Anterior subregion of NM

The data demonstrated that there were no significant differences in the volume densities of the cytoplasm, the neuropil or the cell nuclei between adult male and female rats in the anterior subregion of NM AC. The numerical density of cells in the same anterior part ($N_{v_{ant}}$) also, did not show differences between values in males compared to female rats: $N_{v_{ant}}$ -in females was $9.51 \times 10^4 \text{ mm}^{-3}$ and in males $9.41 \times 10^4 \text{ mm}^{-3}$.

The results of stereological measurement of the anterior subregion of NM are summarized in *Figure 1*.

Posterodorsal subregion of NM

Figure 1. Volume density V_v of NM AC of male and female adult rats, sacrificed on 62nd day of lifeFigure 2. Numerical density N_v of NM AC of male and female adult rats, sacrificed on 62nd day of life

The volume density of the cytoplasm and the volume density of neuronal nuclei in the posterior of NM showed no significant difference between the adult male and female rats investigated. The volume density of the neuropil in the males was statistically significantly greater than that in females ($p < 0.05$). The relative increase of the volume density of neuropil in males was about 1.5% greater than that in females. The numerical density of cells in the posterodorsal subregion of NM also was significantly greater in males compared to female rats ($p < 0.001$); representing a percentage of about 7.14%.

The results of stereological measurements of the posterodorsal subregion of NM are summarized in Figure 2.

DISCUSSION

Mammals exhibit sex differences in several neurally controlled functions, including gonadotropin secretion, reproductive behavior, aggression, food intake and certain types of learning abilities (Hines *et al.*, 1992). Neuroanatomical sex differences are present in several brain regions regulating these functions and are influenced by gonadal steroid manipulation (Arnold and Gorski, 1984., Matsumoto and Arai, 1986., Guillaumon *et al.*, 1988., Matsumoto, 1991). The NM AC in general is involved in regulation of gonadotropin secretion (Sawer, 1972), aggression (Shaikh *et al.*, 1986), male copulatory behavior (Valcourt and Sachs, 1979., Kondo, 1992) and ovulation (Terasawa and Kawaikami, 1974).

Previous morphological studies have suggested different types of sex differences in the NM of the rat. There are more shaft synapses in the medial molecular layer (Nishizuka and Arai, 1981) and more dendritic spine synapses in the ventral molecular layer (Nishizuka and Arai, 1983) in male than in female rats. Also, the area of the NM AC as a whole has been reported to be about 19% greater in males than in females (Mizukami *et al.*, 1983). The findings of Hines *et al.* (1992) demonstrated that a specific posterodorsal subregion NM is almost 100% greater in male compared to female rats. Our findings indicated that the anterior subregion of the NM is not the sex dimorphic subregion of the AC in the rat but the posterodorsal subregion of NM AC showed the sex differences in some morphometric parameters such as volume density of the neuropil and numerical density of the cell nuclei. These parameters were significantly greater in male than in adult female rats, which is similar to previous reports (Nishizuka and Arai, 1981., Mizukami *et al.*, 1983., Hines *et al.*, 1992). The posterodorsal part of the NM AC of rats may be characterized in terms of the neurotransmitter system. Micevych *et al.*, (1988a, 1988b) have reported a greater number of cholecystokinin-immunoreactive cell bodies in males than in females; there is also greater substance P-immunoreactivity in male rats compared to female in the posterodorsal region of the NM.

The volume of other "sexually dimorphic nuclei" of the brain such as the preoptic area, designated by Gorski *et al.* (1980) and the bed nucleus striae terminalis by Hines *et al.* (1992), in the male rat are also markedly greater than that in the female. These sexually dimorphic nuclei are not only morphometrically and neurochemically dimorphic but are also anatomically interconnected (Simerly and Swanson, 1986., 1988). These nuclei are components of a neural circuit which relays olfactory information to regions of the hypothalamus known to regulate reproduction (Simerly *et al.*, 1989) and are located in areas of the brain that are known to have a great number of steroid hormone receptors.

Although, the real causes of sex differences in certain regions of the brain are still unknown, more and more authors think that gonadal steroid hormones (especially the presence or absence of androgen or androgen metabolites) have crucial organizational effects during the "critical periods" of perinatal development: removal of the rat testes during the first few neonatal days of life produced "feminine males" which are capable of secreting gonadotropin in a cyclic pattern and which can display female levels of feminine sexual behavior. Conversely, androgen administered to neonatal female rats causes masculinization of a number of neuroendocrine and behavioural parameters (Arai, 1973., Nishizuka and Arai, 1981). According to Weisz and Ward (1980) plasma androgen titers are

much higher in males than in females during the perinatal period. Matsumoto (1991) suggested that the brain sexual dimorphism is not determined genetically at birth, but rather is dependent on the perinatal sex steroid environment.

Our stereological study clearly indicated that the posterodorsal subregion is the main sex dimorphic area of NM AC of rats.

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**POLNI DIMORFIZAM ANTERIORNOG I POSTERODORZALNOG SUBREGIONA NUKLEUSA
MEDIJALISA (NM) AMIGDALOIDNOG KOMPLEKSA (AK) U PACOVA. STEREOLOŠKA
ANALIZA**

LOZANČE OLIVERA, DREKIĆ D., MALOBABIĆ S. I CVETKOVIĆ DIJANA

SADRŽAJ

Ispitivana je pojava polnog dimorfizma u okviru anteriornih i posteriornih subregiona nukleusa medijalisa (NM) amigdaloidnog kompleksa (AK) pomoću dva morfometrijska odn. stereološka parametra: volumenske gustoće /Vv/ (citoplazme, jedra neurona i međucelijskog prostora) i numeričke gustoće /Nv/ u odraslih mužjaka i ženki pacova. Rezultati su pokazali da nije bilo razlike u ispitivanim volumenskim parametrima i numeričkoj gustoći u anteriornom subregionu NM između mužjaka i polno zrelih ženki pacova. Iako nije bilo sig-nifikanantne razlike u /Vv/ jedra neurona i citoplazme, ispitivanje je pokazalo da posterodorzalni subregion NM AC poseduje izraženi polni dimorfizam u volumesnoj gustoći međucelijskog prostora i numeričkoj gustoći neurona. Vred-nosti ova dva parametra bile su signifikantno veće ($p < 0.05$ i $p < 0.001$) u odraslih mužjaka nego u ispitivanim odraslih ženki pacova.