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THE AMYGDALA IN OLD MALE RATS REPEATEDLY TREATED WITH TESTOSTERONE

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The amygdaloid complex in control and testosterone treated old male rats was studied using light microscopy and morphometric analysis. The volume of the cell nuclei (μm^3) and number of neurons per unit area (mm^2) were examined in nuclei (NBL and NLP) of the basolateral part, as well as in nuclei (NM, NCO and NCE) of the corticomedial part. It was evident that testosterone induced an increase in cell nuclear volume and a decrease in the number of cells per unit area. These changes were more pronounced in the corticomedial part, especially in NM and NCO. This suggests that the amygdala of old, male rats respond to repeated doses of exogenous androgen with morphological changes similar to those in younger, neonatally estrogenized animals.

Key words: amygdala, testosterone, aging, male rats

INTRODUCTION

Gonadal steroids play a significant role in modulating neuronal development during the perinatal period (Goy and McEwen, 1980, MacLusky and Naftolin, 1981; Arnold and Gorski, 1984). Moreover, it has been well documented that hormones of testicular origin affect the development of higher brain structures (such as the cortex and hippocampus), even if they are not directly involved in reproductive processes (Milner and Loy, 1982; Kolb, 1988; Stewart and Kolb, 1988). In contrast, effects of sex steroids on adult brain tissues are considered to be impermanent and reversible, although evidence suggests that even impermanent effects of sex steroids can involve major structural alterations in neural structures. Androgen has been found to regulate nuclear volume (Commins and Yar, 1984), neuronal size (Breedlove and Arnold, 1981), and neuronal number (Simerly and Swanson, 1987) in several areas of the adult rodent brain.

Amygdaloid nuclear cells as a part of the limbic system are well known to interact in reproductive processes (Beltramino and Taleisnik, 1978; Carrer et al, 1978; Mizukami et al.,1983; Akesson et al., 1988). Neonatally injected estrogen affects neuronal development in pubertal male and female rats as assessed by stereological analysis (Pantić and Drekić, 1982; Drekić et al., 1990).

Drekić et al. (1988) followed the long term effect of neonatal estrogenization of male and female rats in 6 and 12 month old animals and described significant changes in the volume of cell nuclei in almost all amygdaloid nuclei.

The purpose of this study was to use light microscopy and morphometric analysis in order to establish whether repeated doses of testosterone affect the morphology of neurons and volume of cell nuclei as well as the number of neurons per unit area in particular amygdaloid nuclei of old male rats.

MATERIAL AND METHODS

Male Wistar rats, 24 months old, average body mass 429 g, were housed in transparent plastic cages, 4 animals per cage, and exposed to a normal rhythm of natural light and dark. Commercial laboratory food (Veterinarski Zavod Zemun, Yugoslavia) and tap water were available ad libitum. Five animals were treated subcutaneously with 3 mg of testosterone propionate (ICN Galenika, Yugoslavia) every second day during one month, while five animals treated in the same time intervals with solvent (sterilized *ol. olivae*) served as controls.

The animals were sacrificed under ether narcosis 24 hours after the last injection. The brain was removed immediately from the scull, the amygdaloid complex was isolated and fixed in Bouin solution. After standard paraffin embedding serial sections (5-6 μm thick) were stained with hematoxylin and eosin (HE) and investigated using light microscopy and morphometric analysis. Two morphometric parameters were followed, the volume of neuronal nuclei (μm^3) and number of neurons per unit area (mm^2) in the following amygdaloid nuclei: *n. basolateralis* (NBL) and *n. lateralis posterior* (NLP) of the basolateral part and *n. medialis* (NM), *n. corticalis* (NCO) and *n. centralis* (NCE) of the corticomedial part.

The volume of cell nuclei was determined using the formula for a rotatory ellipsoid conus and number of cells per unit area (mm^2) using Weibel's multipurpose test system (P:42). Sampling was performed so that the first, middle and last sections of each particular amygdala was used. In each nucleus 50 test fields were chosen by intermittent sampling (Kališnik, 1982). Classification of neurons was performed according to de Olmos (1990). The statistical significance was determined using Student's t-test.

RESULTS

Histology

Control animals. The nunclei of basolateral (NBL, NLP) and corticomedial (NM, NCO, NCE) amygdala in 25 month old intact male rats revealed characteristic cytoarchitecture with a predominant form of pyramidal neurons. Most of the neurons, especially the pyramidal, in both regions of the amygdala contained a heterochromatic nucleus, while some of them had a typical euchromatic nucleus with a prominent and centrally positioned nucleolus (especially in NBL,NCO and NCE - Figures 1a, 1c, 1e). The perikaryon was well delineated and in the cells of the basolateral part had a basophilic cytoplasm

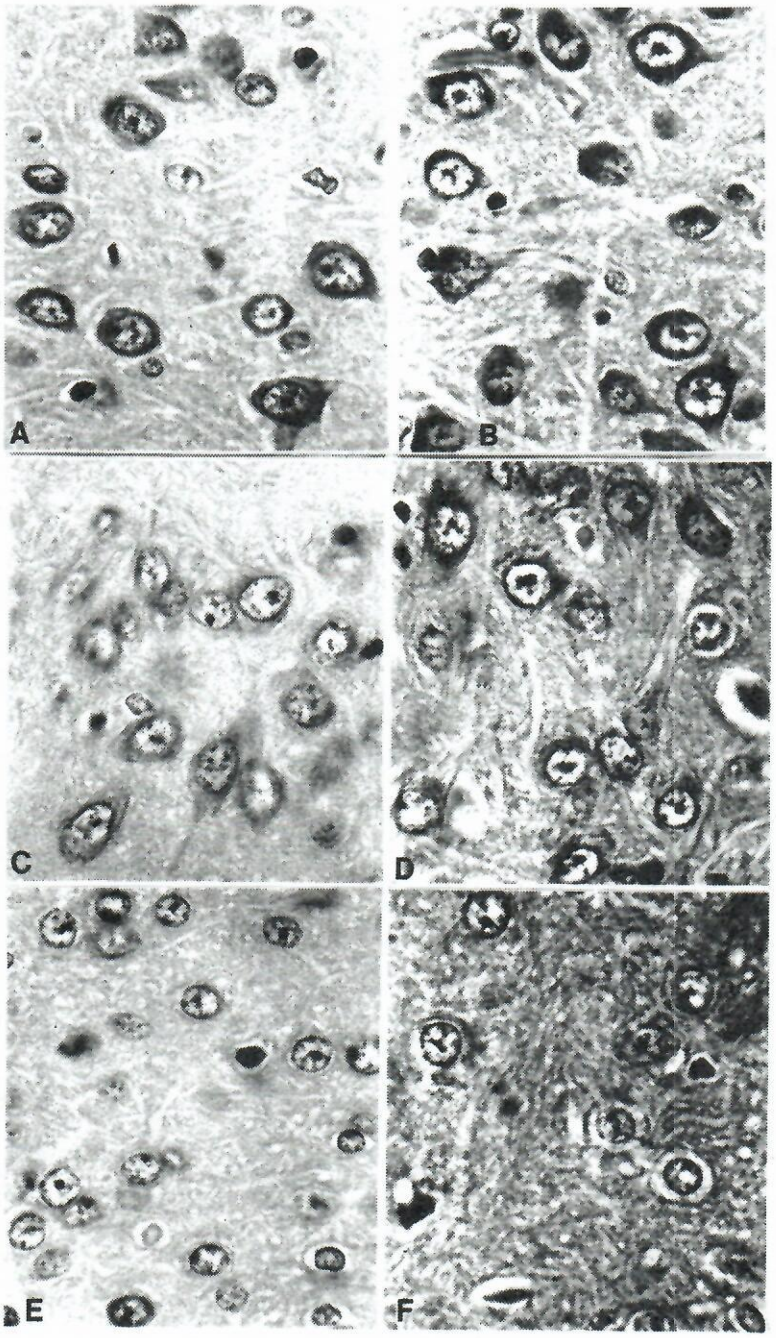

Figure. 1. Amygdaloid nuclei of aged male rats: NBL in control (A) and treated (B) rats; NCO in control (C) and treated (D) rats; NCE in control (E) and treated (F) rats. HE, 1024x.

(Figure 1a). This was less pronounced in the corticomedial part (Figure 1e). Apical dendrites with a smooth surface were the salient feature in pyramidal neurons of the basolateral part (Figure 1a) or just distinguishable in the corticomedial part (Figure 1c). The neuropil was homogenous or finely granular.

Treated animals. In the basolateral part many pyramidal neurons revealed a large "pale" nucleus with an enlarged and centrally positioned nucleolus (Figure 1b). The other group were neurons of all types with a "dark" nucleus and one or two eccentric nucleoli. "Pale" neurons had apical dendrites thinner than those in controls while the "dark" ones had long and thick apical dendrites (Figure 1b). The perikaryon was well delineated and basophilic especially in NBL (Figure 1b). The neuropil was granular.

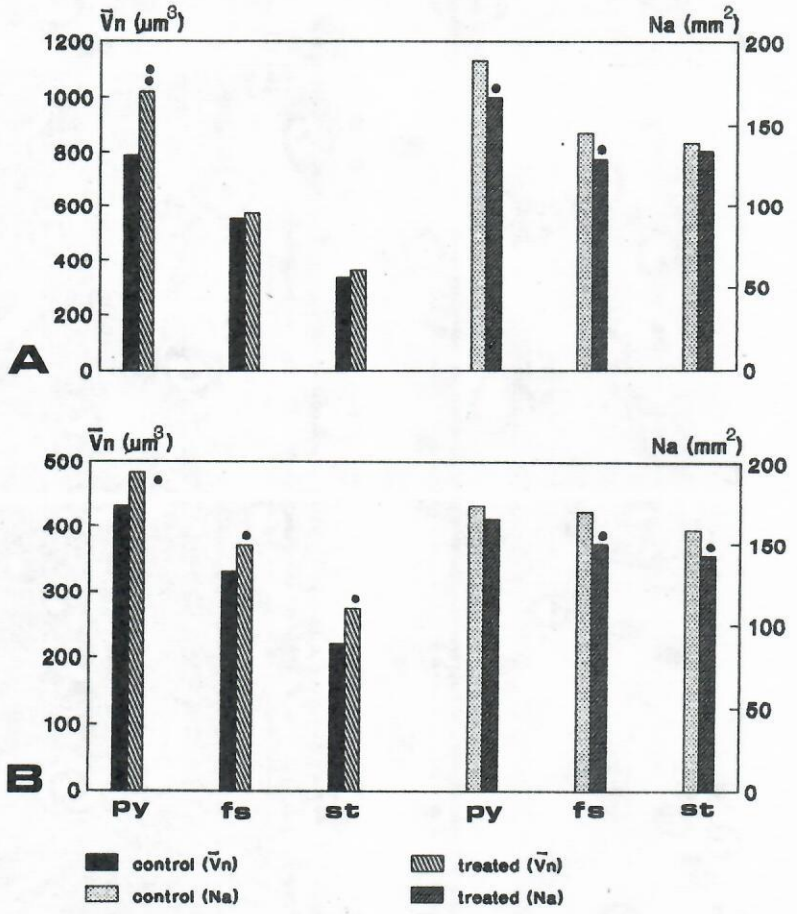

Figure. 2. Volume of cell nuclei and number of particular types of neurons in control and treated aged male rats. (A) - NBL; (B) - NLP. py-pyramidal; fs-fusiform; st-stellate * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$).

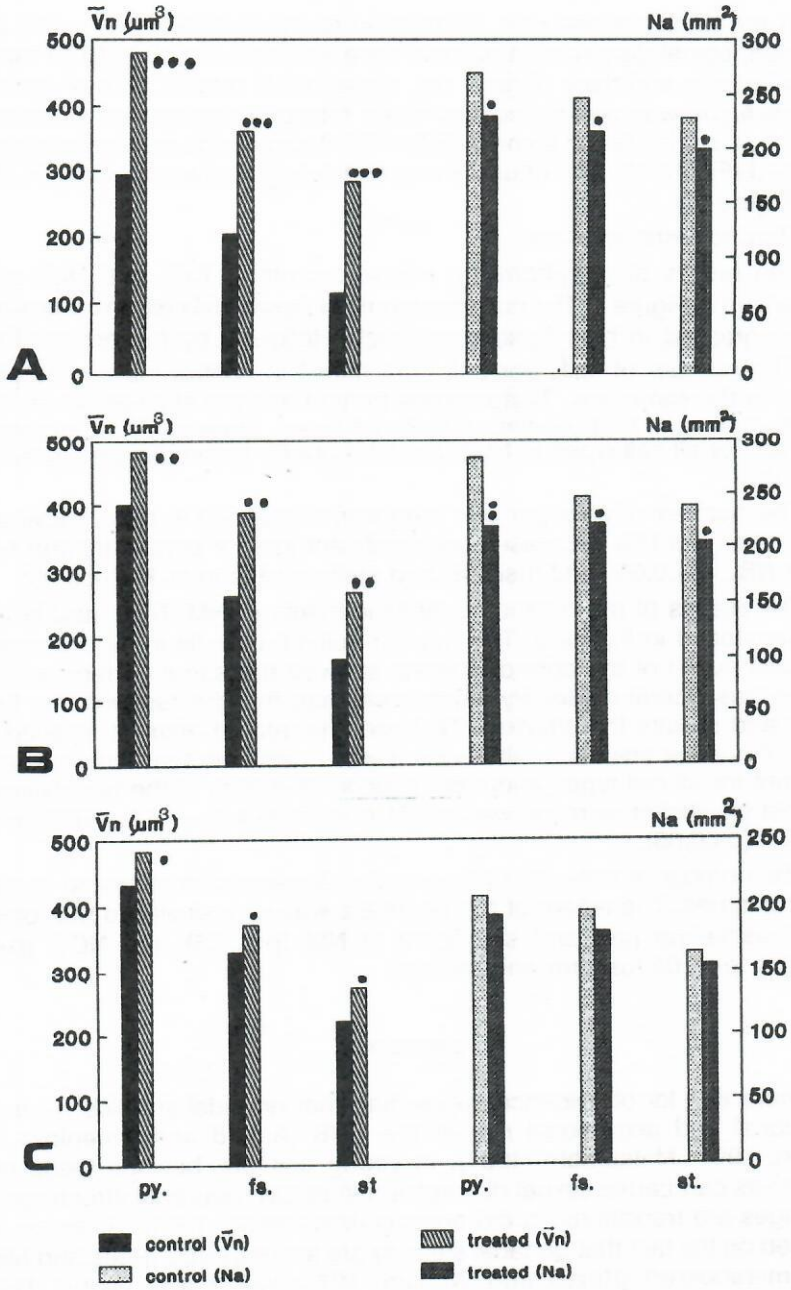

Figure. 3. Volume of cell nuclei and number of particular types of neurons in control and treated aged male rats. (A) - NM; (B) -NCO; (C) -NCE. py-pyramidal; fs-fusiform; st-stellate. * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$).

In the corticomedial part, in addition to the two forms of nuclei already described, apical dendrites in all cells were well developed. In NCO they were especially long and thick (Figure 1d), while in NM and NCE they were short. The perikaryon was well delineated with a basophilic cytoplasm in most of the cells and all nuclei, but in some cells of NCE it seemed to be almost completely unstained (Figure 1f). The neuropil was coarsely granular especially in NM and NCE (Figure 1f).

Morphometric analysis

The results of morphometric measurements of NBL and NLP cells are summarized in Figure 2. The nuclear volume of pyramidal cells in control animals was the greatest in both basolateral nuclei, followed by fusiform and stellate cells. The number of cells per unit area (mm^2) in control males was also distributed in the same way. Testosterone treated animals showed an increase of nuclear volume in both nuclei and all cell types. However, this increase was significant for all cell types in NLP ($p<0.05$) but for pyramidal cells only in NBL ($p<0.01$).

The number of cells per unit area was decreased in both nuclei and for all cell types but this decrease was significant just for pyramidal and fusiform cells in NBL ($p<0.05$) and fusiform and stellate cells in NLP ($p<0.05$).

The results of morphometric measurements of NM, NCO and NCE cells are summarized in Figure 3. The nuclear volume of cells in all examined corticomedial nuclei of the control animals showed the same pattern as the one found in basolateral nuclei. Pyramidal cells had the greatest volume, fusiform smaller and stellate the smallest. Testosterone treated animals showed an increase of nuclear volume in all nuclei. It was noticeable that this increase was significant for all cell types and more pronounced than in the basolateral part. The most significant increase was in NM ($p<0.001$) followed by NCO ($p<0.01$) and NCE ($p<0.05$).

The number of cells per unit area was decreased in all tested nuclei and for all cell types. The extent of the decrease was very similar to that observed in the basolateral part and significant in NM ($p<0.05$) and NCO ($p<0.01$-pyramidal; $p<0.05$-fusiform and stellate).

DISCUSSION

There is a lot of evidence suggesting that gonadal steroids have an organisational and activational role in the CNS (Arnold and Breedlove, 1985; McEwen, 1991; Matsumoto, 1991). In young animals, besides these effects, sex steroids can cause sexual dimorphism in certain neuronal structures, while all changes are transitional in old animals (Matsumoto, 1991). All these effects are based on the fact that gonadal steroids are accumulated (Rees and Michael, 1982), metabolized (Rezek and Whalen, 1978; Weisz and Gibbs, 1974) or aromatized (Weisz and Gibbs; 1974; Rhoda et al, 1984) in many brain regions well known to interact in reproductive processes. One of these regions is the amygdaloid complex.

It has been previously described that neonatal estrogenization induces a significant increase in the relative volume of cell nuclei in all amygdaloid nuclei of juvenile and prepubertal male rats (Pantić and Drekić, 1982). The authors suggested that this increase was a result of the suppressive offect of estradiol and retarded maturation. In adult animals neonatally treated with estrogen Drekić et al. (1988) found that the volume of cell nuclei is significantly increased in 6 month old and without changes in 12 month old rats. These results are comparable with data of other authors who reported that estradiol induces synaptic remodelling in adult rat brain by the reorganization of neuronal membranes (Olmos et al., 1987), i.e. the effect is impermanent and reversible. In our experiment the volume of cell nuclei in 25 month old control animals was not significantly different from the 12 month old intact rats described by Drekić et al. (1988). It was increased significantly in treated animals compared to corresponding controls but the extent of increase was different in certain amygdaloid nuclei. The most significant increase was in NM (Figure 3 A).Bearing in mind the data about aromatization of testosterone to estradiol it could be assumed that some of these effects are a result of estradiol effects on amygdaloid neurons and not testosterone alone. In accordance witt this, some data indicate a more discrete localization of aromatizing enzymes in the limbic system than of 5 α-reductases (Naftolin et al., 1972; Weisz and Gibbs, 1974), which suggests that estrogen produced in situ may have a more specific function in the regulation of limbic system function than testosterone.

Some authors reported that the decreased sexual activity of old rats may be partly attributed to the loss of hypothalamic neurons during aging (Hsu and Peng, 1978; Sabel and Stein, 1981). However, Witkin (1987) reported that aging causes no decrease in the total number or size of LHRH neurons or their distribution in the brain, but it causes significant changes in synaptic organization, i. e. increased input to LHRH perikarya. We have also found that the number of cells in our control animals was comparable to that in 12 month old intact rats observed by Drekić et al. (1988). The decreased number of cells per unit area in our treated animals, in our opinion, might be a result of neuronal loss and/or increased volume of cell nuclei (Figure 2,3).

The other important fact is that in certain brain regions which are known to control various reproductive functions sexual dimorphism has been demonstrated concerning some morphologic parameters such as nuclear volume (Gorski et al., 1978; Nottebohm, and Arnold, 1976), and synaptic organization (Nishizuka and Arai, 1981). Mizukami et al. (1983) indicate that the nuclear volume is sexually different in the medial nucleus of the amygdala and its differentiation occurs during the early postnatal period under the influence of the organizational action of sex steroids. They showed that n. medialis is a sexually dimorphic region and that the volume of cell nuclei in n. lateralis of female rats chronically treated with estrogen was not changed compared with the control group. Drekić et al. (1988) also pointed out the sexual dimorphism of *n. medialis* in control and treated animals and not only in adults but also in the earlier periods of life. Finally, Kondo (1992) reported that rats with medial amygdala lesions showed a severe deficit of copulatory behaviour, whereas

rats with basolateral amygdala lesions showed no change in the performance of copulation. In rats with cortical amygdala lesions, copulatory behaviour was impaired, too, but to a much lesser extent. In our experiment it is obvious that the most pronounced changes in cell nuclear volume were observed in n. medialis, too. Having in mind that the pituitary of aged male rats is still capable of responding to injections of LHRH, LH or testosterone (Bruni et al., 1977; Riegle and Meites, 1976; Pantić et al., 1982) and that the brain of impotent aged male rats is capable of responding to fetal hypothalamic transplants by restoration of sexual function and fertility (Huang et al., 1987), our results suggest that particular amygdaloid structures of aged male rats are still capable of responding to exogenous androgen wiith morphological reactions and/or changes characteristic for neonatally estrogenized younger animals.

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D. Gledić et al.: The amygdala in old male rats repeatedly treated with testosterone

AMIGDALA U STARIH MUŽJAKA PACOVA VIŠEKRATNO TRETIRANIH TESTOSTERONOM

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SADRŽAJ

Izučavan je efekat ponovljenih doza testosterona na ćelije pojedinih jedara amigdaloidnog kompleksa starih mužjaka pacova koristeći svetlosnu mikroskopiju i neke stereološke parametre. Volumen jedara neurona i broj neurona na jedinicu površine određivani su u NBL i NLP bazolateralnog dela i NM, NCO i NCE kortikomedijalnog dela amigdale. U svim izučavanim jedrima višekratno tretiranje testosteronom izazvalo je povećanje volumena jedara neurona i smanjenje broja ćeliija na jedinicu površine. Ove promene su bile jače izražene u kortikomedijalnom delu amigdale, posebno u NM i NCO. Naši rezultati ukazuju da amigdala starih mužjaka pacova reaguje morfološki na egzogene steroide slično kao amigdala mlađih životinja neonatalno tretiranih estrogenom.