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STUDY OF DENTATE GYRUS GRANULE CELLS OF MALE RATS NEONATALLY TREATED WITH ESTROGEN

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The proliferation of hippocampal dentate gyrus granule cells was investigated using 3H - thymidine incorporation in control and estrogen treated rats. Newborn 3 - day-old male Wistar rats were treated with a single dose of 1 mg of estradiol, and 30 μ Ci 3H-thymidine and sacrificed when 10 days old.

The total number of neurons and the number of labeled granule cells in the granular layer and its subdivisions of both suprapyramidal and infrapyramidal limb was analyzed using a stereological method. In both limbs, the total number of neurons as well as the total number of labeled granule cells in the granular layer was singificantly increased in treated rats compared to corresponding controls.

Moreover, in the infrapyramidal limb the increased number of labeled cells in treated animals was significant in all particular zones of the granular layer. In the suprapyramidal limb granule layer a significant increase of labeled cells was observed in SGZ and EGZ only.

Our results suggest a differential effect of estradiol on dentate gyrus granule cell proliferation through the early life of rats.

Key words: estrogen, dentate gyrus, neurons, rat

INTRODUCTION

Groups of sex steroid concentrating brain neurons are likely to be involved in the steroid feed - back mechanisms which regulate sexual behavior and in the sex dimorphism in the central nervous system. Each volumetrically sexually dimorphic cell group in mammals examined for gonadal hormone-binding neurons by autoradiography has been shown to exhibit labeling (Amold and Gorski, 1984). In fact, steroid regulation of cell survival may be a common means of generating sex differences in neuronal organization,for example, within the

rodent spinal cord (Nordeen et al., 1985) or within areas of the hypothalamus (Arnold and Gorski, 1984). We have also shown complex short and long term effects of estrogen in different regions of the rat brain (Pantić and Drekić, 1982; Drekić et al., 1990; 1995a, b).

Morphogenesis of the entire hippocampal region, including the dentate gyrus, in normal rats from embryonic (E) day E 10 to E 22 and on postnatal (P) days P 1, P 7, and P 21 was extensively described and correlated with autoradiographic data (Bayer, 1980; 1982; Bayer et al., 1982). During the embryonic period, postnatally and in adulthood, granule cells proliferate, migrate and degenerate (Cameron and Gould, 1996).

Neurogenesis of rat dentate granule cells persists prenatally and postnatally up to 11 months of age and even in adults (Seki and Arai, 1995; Kuhn et al., 1996).

The proliferating, developing and adult dentate granule cells have an affinity for and depend on steroid hormones i. e. adrenal corticosteroids (Teyler et al., 1980; Jaarsma et al., 1992; Watanabe et al., 1995; Rua et al., 1995; Cameron et al., 1995; Cameron and Gould, 1996). Also, sex steroids bind to, affect and maintain both morphological and functional properties of the hippocampal region (Wooley and Mc Ewen, 1993; O'Keffe et al., 1995). Estradiol treatment in ovariectomized rats significantly increases the number of NMDA receptor binding sites within the CA1 pyramidal cells and to a lesser extent, within the granule cell somata of the dentate gyrus (Gazzaley et al., 1996).

In this work we investigated postnatal development of the gyrus dentatus granular layer and its zones in neonatally estrogen treated male rats. We consider that the change in the number of dentate gyrus granule cells labeled by ^{3}H-thymidine autoradiography in treated male rats indicates their reactivity to estrogen.

MATERIALS AND METHODS

Experimental animals. Neonatal male Wistar rats (20 animals) were treated with a single dose of 1 mg of estradiol dipropionate, (ICN-Yugoslavia, Beograd), on the third day of postnatal life (P3). Simultaneously both treated (five male) and control (five male) rats were injected i/p with 30 μCi of methyl-3H-thyminidine (Amersham- TRK 120, sp. act. 21 Ci /mmol). The animals were killd under ether anesthesia at P 10.

Autoradiographic procedure. The brains were isolated, fixed in Bouin solution and processed for autoradiography using paraffin embedding. The hippocampal region was cut in 5μm thick, serial transverse sections which were covered with ILFORD L4 emulsion and exposed for 5 months at 4^{0}C. After development with KODAK 19, sections were counterstained with hematoxylin.

Stereological and statistical analysis. The total number of neurons and the number of labeled granule cells were determined using stereological analysis in both zones of the granular layer of the suprapyramidal and infrapyramidal limbs i. e. the subgranular (SGZ) and granular zones (GZ). Moreover, we found it necessary to describe and investigate an additional zone of the granular layer,

which we named the extragranular zone (EGZ). It is the periferal zone of the granular layer facing the molecular layer. Stereological analysis of the dentate granule cells was performed using Weibel's grid test system (P: 42). On each 10th section of the dentate gyrus, all cells in the granular layer, including labeled ones, were counted in all fields and the number of labeled granule cells was estimated. The total number of labeled granule cells was estimated according to Agduhr's formula: $N_V = N_A / D + t$ (mm^{-3}). Micrographs were taken on an NU_2 Carl Zeiss microscope Jena. Statistical significance was tested with Student's t-test.

RESULTS

In the newborn male control rats treated with 30 μCi of ^{3}H-thymidine on the third postnatal day and sacrificed seven days later (P10), labeled cells were found within both zones of the granular layer of the dentate gyrus. However, the majority of labeled cells was found in GZ.

A smaller number of larger (10-12 μm) ^{3}H-thymidine labeled cells was located deep in the granule cell layer facing the molecular layer. They had the main characteristics of dentate granule cells and both the nucleus and cytoplasm stained paler (in control sections). However, we considered it necessary to investigate these cells as a separate group and described this zone as an extragranular zone (EGZ).

In the newborn male rats treated with 30 μCi of ^{3}H-thymidine and 1 mg of estradiolon the third postnatal day (P3) and sacrificed seven days later (P10), there was an evident and significant increase in the number of labeled granule cells in the CGZ. These cells were characterized by their small size (6μm) and were darkly stained with heematoxylin in control sections. An increased number of labeled cells was found in EGZ in both the suprapyramidal and infrapyramidal limb.

Number of cells

I. Controls. The total number of granule cells in the suprapyramidal limb of the dentate gyrus was 14.663×10^4 (mm^{-3}) and 8.605×10^4 (mm^{-3}) were labeled. The labeled neurons were distributed in particular zones of the suprapyramidal limb as follows: the subgranular zone (SGZ) 2.378×10^4 (mm^{-3}), the granule zone (GZ) 2.417×10^4 (mm^{-3}), and the. extragranular zone (EGZ) 1.263×10^4 (mm^{-3}).

The total number of granule cells in the infrapyramidal limb of the dentate gyrus was 22.250×10^4(mm^{-3}) and 11.125×10^4 (mm^{-3}) were labeled. The labeled neurons were distributed in particular zones of the infrapyramidal limb as follows: in SGZ 6.433×10^4 (mm^{-3}), in GZ 2.299×10^4 (mm^{-3}) and in EGZ 2.393×10^4 (mm^{-3}).

II Treated rats. Under the influence of estrogen the total population of granule cells in the suprapyramidal limb of the dentate gyrus was increased to 16.012×10^4 (mm^{-3}) and the subpopulation of labelec cells increased to 10.083×10^4 (mm^{-3}). The increase of labeled neurons in the suprapyramidal limb was not distributed evenly in different zones. In the SGZ the increase was more than twice- to 5.929×10^4 (mm^{-3}), in the GZ it remained unchanged (very slightly

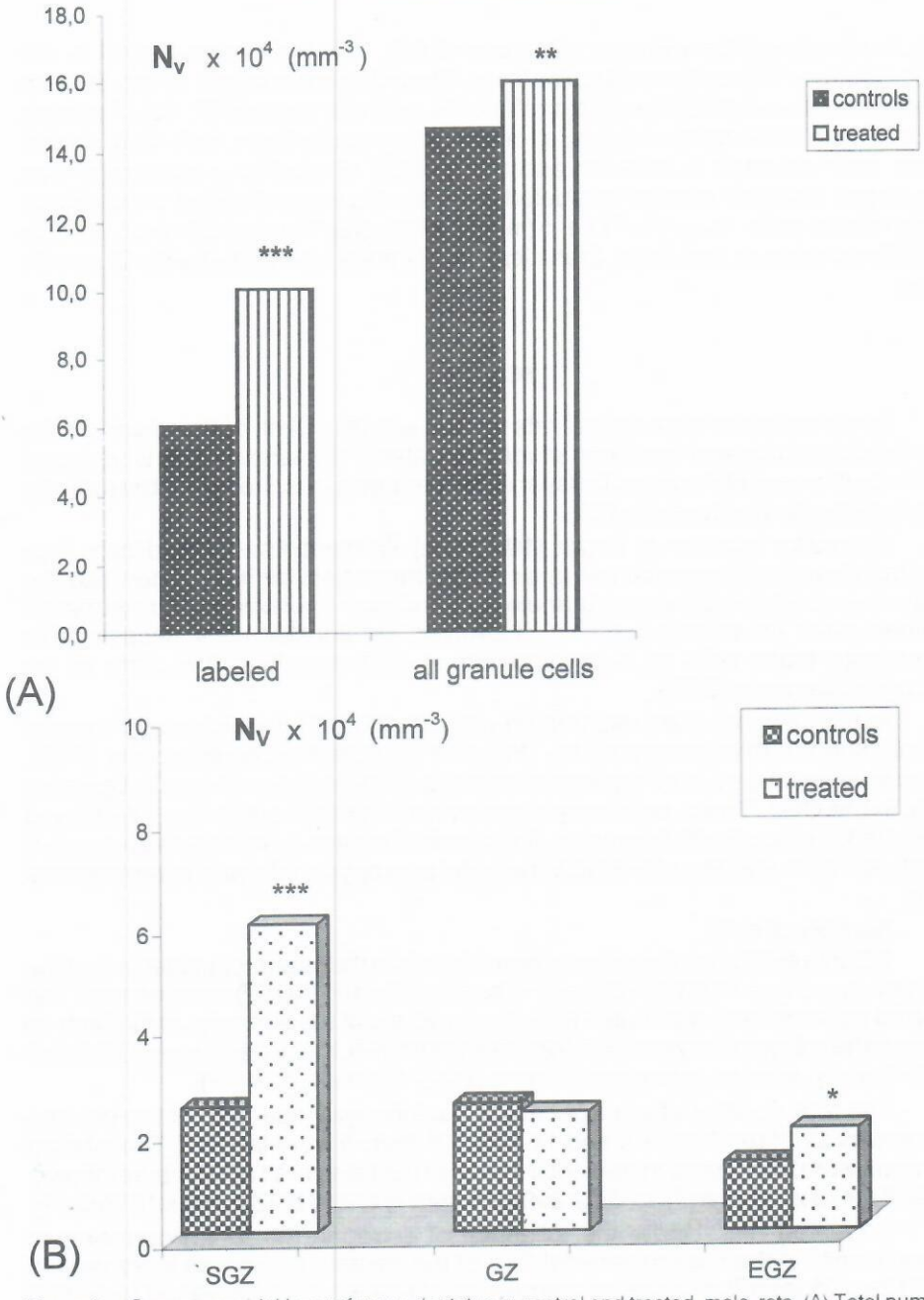
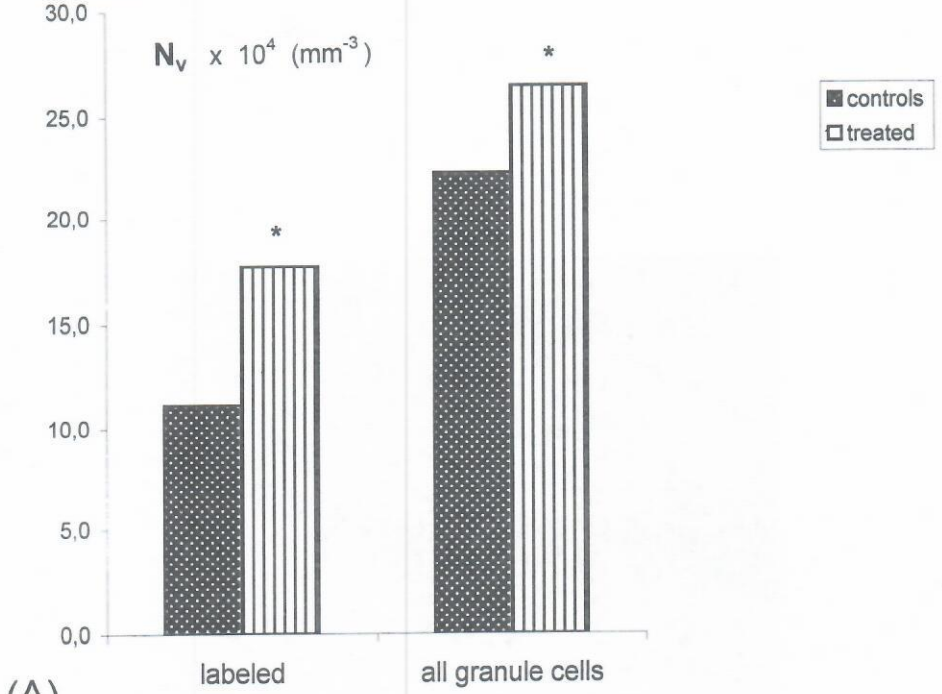


Figure 1. Suprapyramidal layer of gyrus dentatus in control and treated male rats. (A) Total number of granule cells and number of labelod granule cells. (B) Number of labeled granule cells in different zones: SGZ (subgranular), GZ(granular), EGZ (extragranular), *-p<0.05,** -p<0.01, ***-p<0.001.



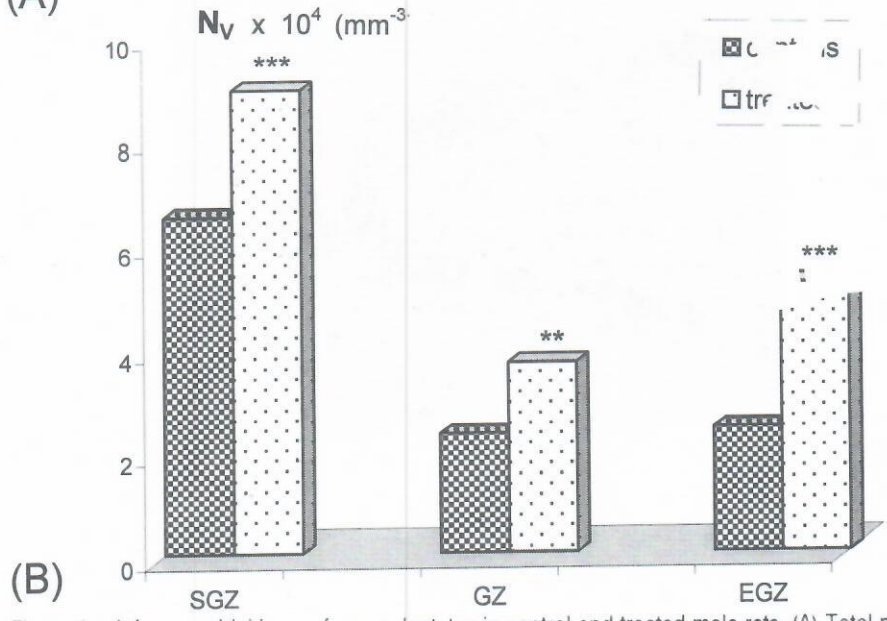


Figure 2 Infrapyramidal layer of gyrus dentatus in control and treated male rats. (A) Total number of all granule cells and number of labeled granule cells. (B) Number of labeled granule cells in different zones: SGZ (subgranular), GZ (granular), EGZ (extragranular), * -p<0.05, **-p<0.01, *** -p<0.001.

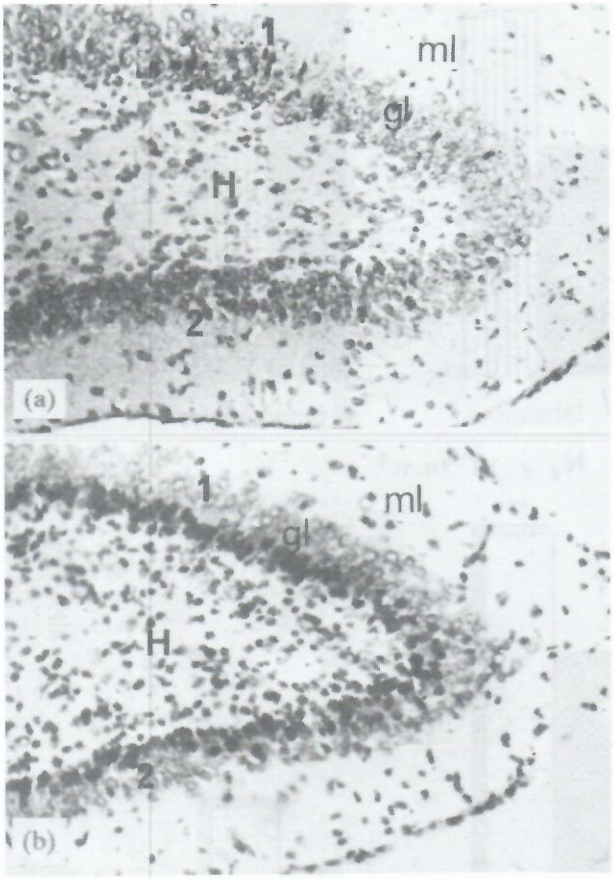


Figure 3a, b. Dentate gyrus of 10-day-old male rat. a) control b) treated with estrogen. (Autoradiography, x 150). 1. suprapyramidal limb. 2. infrapyramidal limb. ML-molecular layer. GL-granule layer. H-dentate hilus.

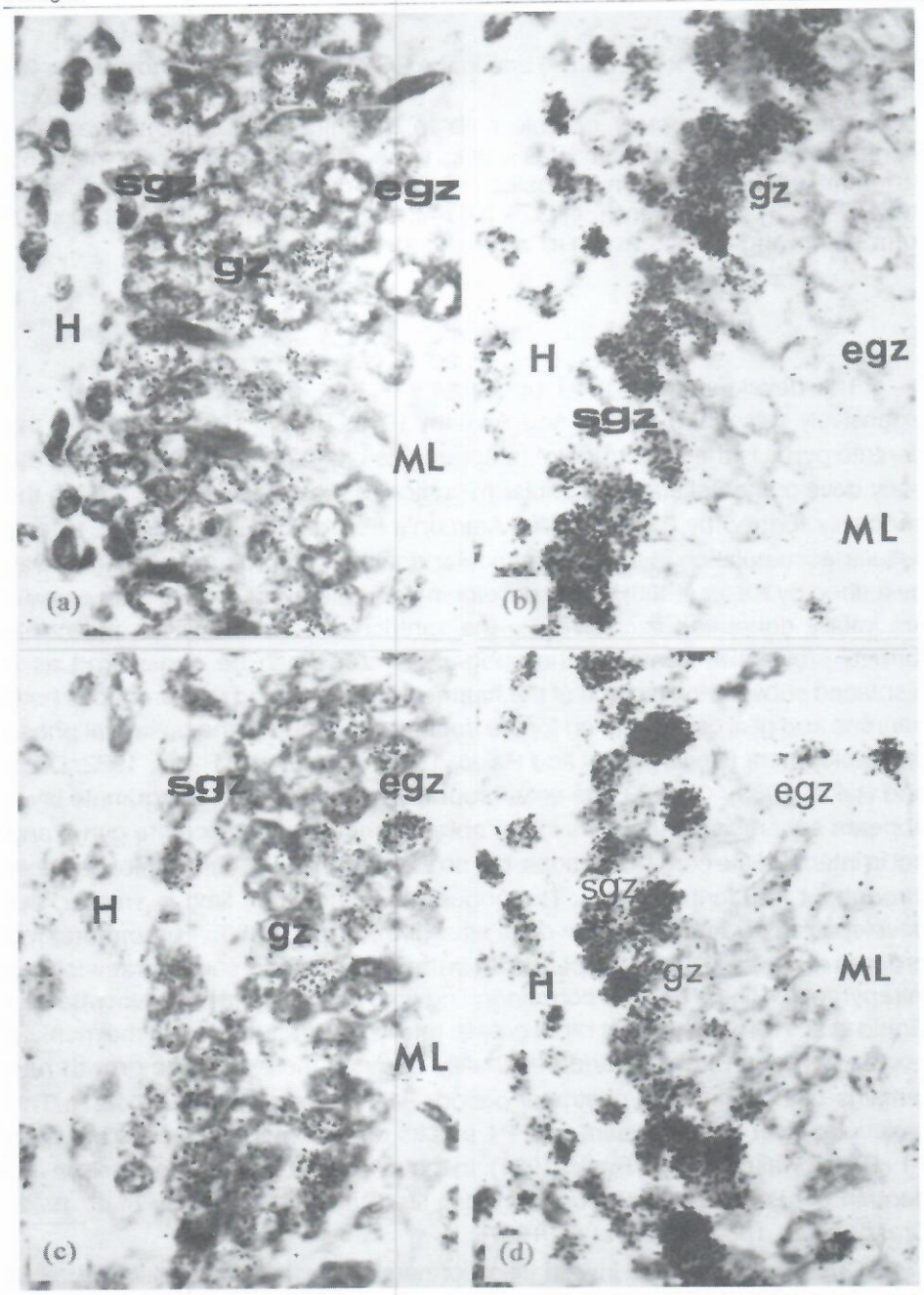


Figure 4a, b, c, d. Dentate gyrus of 10 day-old male rat. a) control - suprapyramidal limb c) control - infrapyramidal limb. b) treated - suprapyramidal limb. d) infrapyramidal limb. (Autoradiography, x 10240). SGZ- subgranular zone GZ-granular zone, EGZ - extraganular zone, ML - molecular layer, H-dentate hilus.

decreased) as 2.247 x 10^4 (mm^{-3}) and in the EGZ it was increased to 1.907 x 10^4 (mm^{-3}) (Figure 1).

The total number of granule cells in the infrapyramidal limb was also increased - 26.465 x 10^4 (mm^{-3}) as well as the number of labeled cells -17.675 x 10^4 (mm^{-3}). The distribution of labeled cells in the different zones was as follows: in SGZ - 8.881 x 10^4 (mm^{-3}), in GZ - 3.654 x 10^4 (mm^{-3}) and EGZ - 5.140 x 10^4 (mm^{-3}) showing an increase in all zones (Figure 2, 3ab, 4abed).

DISCUSSION

The development of the hippocampal dentate gyrusof the rat has been extensively described (Bayer and Altman, 1974; Bayer, 1980). Although the dentate gyrus is the last structure to appear, its origin can be followed from the early developmental stages. Cellular migration to the subventricular area in the concavity formed by the primordial Ammon's horn and subiculum at E16 and cellular accumulation in the subventricular dentate primordium at E18-E19 was described by Bayer (1980). In the developing dentate gyrus, young granule cells are intially generated exclusively in the ventricular zone. However, the newly formed prolifentive center in the subgranular zone can be considered as a displaced subventricular zone of the hippocampal formation that produces both neurons and glial cells destined for the dentate gyrus during the postnatal phase of development (Nowakowski and Rakić, 1981; Sidman and Rakić, 1982; Duffy and Rakić,.1983). On E20, the ectal (suprapyramidal) limb of the granule layer appears superficial to the hilus in the anterior and posterior dentate gyrus and not in intermediate coronal sections, but on E21 and E22 it becomes more distinet throughout the dentate gyrus. The ended (infrapyramidal) limb is smaller, but develops rapidly in the first few days after birth (Bayer, 1980). Furhermore, the process of naturally occurring cell death in the dentate gyrus suprapyramidal and infrapyramidal limbs has the corresponding time sequence to that described by Gould et al. (1991). The most rapid growth rate of the granule layer in the monkey occurs between E20 -E21 and continually declines thereafter (the growth rate remains high during the postnatal period, P1-P7, and between P7-P21). The molecular layer is not present until P1 and its development increases between P1 - P7 (Nowakowski and Rakić, 1981). In the rat and rabbit, neurogenesis in the dentate gyrus continues throughout adult life (Bayer, 1980; Bayer et al. 1982; Crespo et al., 1986; Parent et al., 1997).

During our previous investigation of neurogenesis in amygdala nuclei of neonatal rats we also monitored the neurogenesis of the cells of the dentate gyrus due to the anatomical vicinity of amygdala and hipocampal structures (Lozanče, 1995; Cvetković, 1995). Although unpublished, these observations are com-

parable with the results reported by Bayer (1980). The present examination of the dentate gyrus also represents a continuation of previous research on the influence of hormones, especially sex steroids, on the amygdala and other structures of the imbic system in the brain, including the hypocampus. The results presented in this paper clearly indicate that estrogen caused a significant increase in the total number of neurons in both the suprapyramidal and infrapyramidal limbs of the dentate gyrus of treated young rats. This increase was more pronounced in the suprapyramidal limb and in the labeled subpopulation of neurons than in the infrapyramidal limb.

It is well known that several cell types of the rat hippocampus are targets for steroid (glucocorticoid) hormones, including the pyramidal cells of Ammon's horm and granule cells of the dentate gyrus (Gerlach and McEwen, 1972; Cameron and Gould, 1996). Hippocampal estrogen receptors (ER) mRNA levels increased significantly between birth and P4, when peak concentrations were found, and then declined by P10. This suggests that the ontogeny of ER in the hippocampus is regulated by alterations in ER gene expression in specific neonatal populations. The postnatal rat hippocampus may be sensitive to estrogenic trophic and organizational influence during a "critical period" of sexual differentiation (O'Keefe et al., 1995). Estradiol increases spine density on rat hippocampal CA1 pyramidal cells (Wooley and McEwen, 1993). There are no related data for the dentate gyrus. The presence of labeled cells indicates the existence of DNA replication in investigated regions in the neonatal period (Drekić et al., 1990; 1995 a, b).

Although the greatest increase of labeled cells occurred in the SGZ of both limbs, the other layers of the dentate gyrus reacted differently. Thus the number of labeled neurons in the suprapyramidal limb GZ was unchaged, and in the EGZ was increased, while in the infrapyramidal limb GZ it was significantly increased. The number of labeled neurons in the EGZ was increased in both suprapyramidal and infrapyramidal limbs. This difference between the suprapyramidal ("less sensitive" in that period of development) and infrapyramidal limbs is well related to described neurogenetic gradients in development (Bayer, 1980) and cell death proceses in the dentate (Gould et al., 1991). Also, in X-ray irradiated rats at P1 the dorsal blade of thee dentate gyrus was reduced but present and the ventral blade was entirely missing or rudimentary (Czurko et al., 1997). In our experiment the number of labeled cells was not significantly changed in the GZ of the suprapyramidal limb only. Considering this fact, as well as the different quantitative relationships in reaction to estrogen in different layers of the supra- and infrapyramidal limbs which reacted by an increase in the number of labeled cells, we can conclude that these two regions of the dentate gyrus and their zones

react differently to administered estradiol in neonatal male rats. Generally we can conclude that estradiol increased ^{3}H-thymidine incorporation in deoxyribonucleic acid (DNA) of hippocampus dentate gyrus granule cells in the neonatal male rats. Furthermore, our findings suggest very fine specific local differences in proliferation and differentiation of nerve cells inside the hippocampal dentate gyrus in early postnatal days caused by the influence of estrogene.

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IZUČAVANJE PROLIFERACIJE ZRNASTIH ĆELIJA GIRUS DENTATUSA NEONATALNIH MUŽJAKA PACOVA TRETIRANIH ESTROGENOM

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SADRŽAJ

Izučavana je proliferacija zrnastih ćelija girus dentatusa korišćenjem ugrađivanja ^{3}H - thymidin-a u kontrolnih i estrogenom tretiranih pacova. Novorođeni, 3- dana stari mužjaci pacova Wistar soja tretirani su jednom dozom od 1 mg estradiola, i 30μ Ci ^{3}H-thymidin-a i žrtvovani su 10. dana.

Ukupan broj neurona i broj obeleženih zrnastih ćelija u granularnom sloju i u njegovim delovima (suprapiramidalni i infrapiramidalni deo) analizirani su korišćenjem stereološke metode.

U oba dela u poređenju sa kontrolama, ukupan broj neurona, kao i ukupan broj obeleženih zrnastih ćelija u granularnom sloju bio je značajno povećan u tretiranih pacova.U infrapiramidalnom delu povećan broj obeleženih ćelija u tretiranih životinja bio je signifikantan u svim zonama granularnog sloja. U suprapiramidalnom delu granularnog sloja signifikantni porast broja obeleženih ćelija je nađen samo u supragranularnoj (SGZ) i ekstragranularnoj zoni (EGZ).

Naši rezultati ukazuju na specifičan efekat estradiola na proliferaciju zrnastih ćelija girus dentatusa tokom ranog razvoja kod pacova.