

Original Article	Effects of Castration and Testosterone Substitution on the Hippocampus in Adult Male Albino Rats <i>Manal E. El-Sawaf</i> <i>Anatomy Department, Faculty of Medicine, Tanta University</i>
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ABSTRACT

Background: It was previously documented that hypogonadal men suffer from learning and memory impairment. Recent researches proved the role of the hippocampus in controlling learning and memory functions mediated by the action of gonadal hormones. However, the mechanism of this action is debatable.

Aim of the Work: To verify the effects of castration and testosterone substitution on the neuronal structure of the hippocampus in adult male albino rats.

Material and Methods: Twenty adult male albino rats were divided into 4 groups : control (group I), castrated nontreated rats (group II), castrated rats provided with immediate daily injections of dihydrotestosterone (group III) and castrated rats injected with daily dihydrotestosterone 3 weeks post surgery (group IV). Six weeks post surgery, all rats were sacrificed and their hippocampi were processed to be examined with the light microscope and transmission electron microscope.

Results: Significant structural changes of the hippocampi obtained from Groups II and IV were noticed. Meanwhile, rats of group III showed restoration of the normal hippocampal structure compared to that of the control.

Conclusion: Castration affected the structure of the hippocampus which may explain learning and memory impairment in cases of hypogonadism. Early testosterone substitution would prevent these effects. However, late substitution showed no protective role which may indicate that hippocampal damage, when occurs, is irreversible.

Key Words: Testosterone, castration, learning, memory, hippocampus, rat.

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INTRODUCTION

The hippocampus is a neural structure in the medial temporal lobe that is usually referred to cornu ammonis and dentate gyrus. The cornu ammonis is differentiated into fields CA1, CA2, and CA3 in which the principal cells are the pyramidal cells. The dentate gyrus contains the fascia dentata and the hilus. Its principal neurons are tiny granule cells (Standring et al., 2004).

The hippocampus is considered the area of the brain involved in learning and memory functions. Many authors agreed that the hippocampus has an important role in the formation of new memories about experienced events and it is a part of a larger memory system responsible for general declarative memory. Accordingly, damage to

the hippocampus usually results in profound difficulties in forming new memories, affecting access to memories prior to damage and impairing learning ability (Squire, 1992; O'Kane et al., 2004).

Many researches explored the effect of gonadal hormones on learning and memory. Whereas these researches have primarily focused on estrogen in females, recent evidence suggested that testosterone can also modulate learning and memory in males through its stimulation of the hippocampal neurons (Daniel et al., 2003; Hajszan, 2007 ; Leonard et al., 2007). Animal studies suggested that sex hormones play a role in the organization of the nervous system and

memory. Possible mechanisms to explain the relationship between testosterone and learning ability were based on preclinical observations of the neurotrophic and neuroprotective effects of testosterone (Hammond *et al.*, 2001). In the hippocampus, testosterone was found to bind exclusively to granule cells of the dentate gyrus and pyramidal cells of cornu ammonis. In these cells, testosterone is metabolized to dihydrotestosterone and bind to androgen receptors. Also, testosterone has been shown to increase concentrations of nerve growth factor, and its neuroprotective effects against oxidative stress and apoptosis could also help to protect the brain (Pouliot *et al.*, 1996; Beauchet, 2006).

Leranth *et al.* (2003) induced hypogonadism in adult male albino rats through their castration. They then declared that testosterone has been found to increase dendritic spine synapses in the CA1 region of the male rat hippocampus. Learning and memory impairment has been observed in men undergoing chemical castration, further indicating the role of testosterone (Chen & Petrylak, 2005). Moreover, many researchers documented that late-onset hypogonadism can be accompanied by changes in mood with concomitant reductions in intellectual activity and orientation ability, as well as fatigue and irritability which may be improved by testosterone substitution (Moffat *et al.*, 2002; Sherwin, 2003; Nieschlag *et al.*, 2005).

Because learning and memory processes are not directly observable, they cannot be measured directly and thus must be inferred from observed changes in behavior over time. Many assessment tests were designed in humans (Wolf & Kirschbaum, 2002). However, in animal studies, many different types of mazes and tasks had been used (Paul *et al.*, 1997). Recent interest in testosterone therapy has been fueled not only by increased medical awareness of the effects of hypogonadism, but also by media attention regarding hormone-replacement therapy in both men and women (Rhoden & Morgentaler, 2004). Although some studies have reported no benefit with testosterone substitution, others have found significant improvement in orientation and memory. Accordingly, many authors postulated that further investigation of the effects of testosterone substitution on memory is warranted (Beauchet, 2006; Gooren, 2007). To the best of my knowledge, researches describing the effect of testosterone on the histology and ultrastructure of the cells in the

hippocampus were hardly found. Thus, the present work was conducted to study whether castration affects the structure of hippocampal neurons and whether these changes can be restored with early or late testosterone substitution.

MATERIALS AND METHODS

The Animal housing:

Twenty adult male albino rats (weighing 250-300 gms) were used for this study. The animals were group housed, provided with unlimited access to food and water and maintained on 12-h light/12-h dark cycle.

Experimental design:

The 20 animals were divided randomly into 4 groups (five rats each). Rats of group (I) were sham operated, injected sc with 200 μ L sesame oil daily and served as controls. The remaining 15 rats of the other 3 groups were castrated under ether anesthesia. Then, beginning immediately after surgery; rats of group (II) were injected daily with 200 μ L sesame oil sc, rats of group (III) were injected daily with 500 μ g 5-dihydrotestosterone dissolved in 200 μ L sesame oil while rats of group (IV) were injected daily with 200 μ L sesame oil for 3 weeks then with 500 μ g 5-dihydrotestosterone dissolved in 200 μ L sesame oil till death. Treatments were adjusted on the basis of previously published studies in which this dose of 5-dihydrotestosterone is sufficient to reproduce the density of hippocampal spine synapses observed in intact male rats (Leranth *et al.*, 2003). All rats were sacrificed after six weeks from the operation day.

Twenty-four hours after the last injection, rats were anaesthetized with Diethyl ether. Thereafter, the chest was opened, the descending aorta clamped and the right atrium opened. After rinsing the vascular system via transcatheter perfusion with 0.9% saline, animals were further perfused for 10 min with fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed overnight at 4^o C in the same fixative used for perfusion. The brains of 3 animals from each group were processed for light microscopic study (routine histological and immunohistochemistry) while the other brains were directed to electron microscopic study.

Castration surgery:

All animals were anesthetized with inhalation of Diethyl ether. A horizontal incision was performed in the scrotum and both testes were tied off and removed with a cut distal to the ligature, then the incision was sutured.

Tissue processing for light microscopic study:

The anterior and posterior parts of the brain were removed by coronal cuts at the rostrum and splenium of the corpus callosum respectively. Tissues were dehydrated in ascending concentrations of alcohol, cleared in xylene and embedded in paraffin. Sections of 5 µm thick were cut coronally at the dorsal hippocampus and either stained with Hx.&E for routine examination or prepared for immunostaining for glial fibrillary acidic protein (GFAP).

GFAP immunohistochemistry:

The sections were deparaffinized, hydrated and incubated with blocking solution TBT (Tris Base Saline (TBS) 0.5M, pH7.4, containing 3 % (w/v) bovine serum albumin (BSA) and 0.05% (v/v) Triton X-100) for 3 min at room temperature to reduce non specific binding. The tissue sections were subjected to a preliminary heat induced antigen retrieval step. Sections were then incubated overnight at 4^o C in a humidified chamber with the anti-glial fibrillary acidic protein (GFAP) mouse antibody at a 1:50 dilution. The slides were washed for 5min in TBS. Immunodetection was performed using biotinylated anti-mouse immunoglobulins followed by incubation with 3,3'-diaminobenzidine (DAB) chromogen in hydrogen peroxide for 5-10 min for brown staining. The sections were lightly counterstained with Mayer's hematoxylin, dehydrated and mounted (Rollin *et al.*, 2004).

Light microscopic analysis and quantitative study:

Twenty-five serial sections of the hippocampus of each animal were cut, from which five sections were chosen randomly. The sections were mounted and stained with Hx.&E. Brain sections were visualized by the use of an Olympus microscope Bx50 provided with an Olympus digital camera and the hippocampal anatomy was defined using an x40 objective. In each section, fixed parts of

the CA1 subfield stratum pyramidale and exposed blade of dentate gyrus were chosen to ensure uniform sampling. The peripheral areas of the CA1 (at its junction with CA2 zone at one end and its junction with the subiculum at the other end) were excluded to avoid the overlap of these areas (Standring *et al.*, 2004). The sheet of neurons was centered in the high power field x400 (for pyramidal cells) and x1000 (for granule cells) and all neurons were counted. Data were obtained as means and standard deviations (Roy *et al.*, 2002). Statistical analysis was done by using SPSS program to calculate student's- t test and p values. The histological structure of pyramidal cells and granule cells in different sections were examined and micrographs were taken. Immunoreactive glia cells and processes in the stratum radiatum of the CA1 hippocampal subfield were examined and photographed.

Electron microscopic study:

The hippocampi were cut in small cubes 1mm³ and kept in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, at pH 7.4 overnight. The specimens were postfixed in 1% osmium tetroxide for 30 min, dehydrated in ascending grades of alcohols then cleared in propylene oxide and embedded in epon. Semithin sections of 2 µm thick were prepared and stained with toluidine blue then examined by light microscope to localize the CA1 subfield of the hippocampus. Ultrathin sections (40-50nm) were cut with glass knife from the chosen regions. Grids were then stained with uranyl acetate and lead citrate and examined by electron microscope JEOL 100SX E/M equipped with a camera. Electron micrographs were taken at magnifications of X3,000 and X8,000.

RESULTS**Light microscopic results:**

Light microscopic examination of Hx& E-stained sections revealed consistent structure of the hippocampus for all brains in the different groups with easily identifiable cornu ammonis and dentate gyrus. Cornu ammonis was formed of stratum pyramidale sandwiched between stratum oriens externally and stratum radiatum internally. The CA1, CA2 and CA3 subfields of stratum pyramidale were demonstrated. The dentate gyrus showed an outer molecular layer, an intermediate

Table 1: Means and standard deviations of pyramidal cell numbers in controls and castrated rats under different treatment regimens (per power field x400).

Animal groups	Group I (control)	Group II	t ₁	Group III	t ₂	Group IV	t ₃
Mean	91.4	33.47	19.4	98.07	-2.04	36.73	22.65
SD ±	8.77	5.71	**	10.28	*	4.59	**

SD: standard deviation; t₁: group II versus control; t₂: group III versus control; t₃: group IV versus control; **: significant (p≤0.05); *: non significant.

Table 2: Means and standard deviations of granule cell numbers in controls and castrated rats under different treatment regimens (per power field x 1000).

Animal groups	Group I (control)	Group II	t ₁	Group III	t ₂	Group IV	t ₃
Mean	79.3	55.3	20.35	98.4	-14.46	61.8	19.73
SD ±	2.99	2.79	**	4.81	**	1.78	**

SD: standard deviation; t₁: group II versus control; t₂: group III versus control; t₃: group IV versus control; **: significant (p≤0.05).

granular layer and an inner polymorphic layer arranged in exposed and buried blades. The hilus of the dentate gyrus was demonstrated between the two blades (Fig. 1). Quantitative analysis of the CA1 pyramidal cells in different groups revealed significant reduction of the mean number of these cells in both castrated untreated rats (group II) and castrated rats treated with late testosterone substitution (group IV) (Fig. 2-b,d). On the other hand, the pyramidal cell number was restored by early testosterone substitution in rats of group III compared to the controls (Fig. 2-a,c). Statistical data were analyzed in Table (1).

Histological study of the pyramidal cells in the CA1 subfield of the hippocampus in controls showed that the pyramidal cells possessed huge rounded nuclei with relatively pale- stained, dispersed nuclear chromatin and prominent nucleoli. The cytoplasm was basophilic and cell processes could not be identified from the surrounding neuropil. Neuroglial cells showed small dark nuclei and undistinguished cytoplasm. Histological appearance of pyramidal cells in group III resembled that of the controls (Fig. 3- a, b). Many histological changes were manifested in group II. Apparent neuronal loss with some damaged cells among a number of normal cells were seen. The nuclei of damaged cells showed either condensed nuclear chromatin, pyknosis, irregular contours or complete absence.

Multiple basophilic masses of degenerated cells and unstained areas were seen scattered in the neuropil. Some cells appeared shrunken (Fig. 4 -a, b, c). Similar results were seen in pyramidal cells in group IV (Fig. 5- a,b).

Meanwhile, quantitative study of the granule cells of the dentate gyrus showed significant reduction of the mean number in groups II and IV and significant increase in group III compared to the controls. Data were represented in Table (2).

Comparison between groups II and IV showed t- value equal -7.11 which was significant.

Histological study of the granule cells of dentate gyrus in the controls showed closely packed small cells with dark- stained nuclei. The same characters appeared in group II with only apparent reduction in number. However, granule cells appeared less packed with vacuolated cytoplasm in groups III and IV. In both groups, two different types of cells appeared: one ordinary type with dark- stained nucleus and the other type with pale nucleus. In group III, apparent increase of granule cells appeared (Fig. 6-a,b,c,d).

Immunohistochemistry results suggested a difference in the density of astroglial processes between different groups. In the CA1 stratum radiatum of group II and group IV, increase of

GFAP immunoreactive glia profile was expressed compared to control group. On the other hand, GFAP expression in group III was as that of the controls (Fig. 7-a,b,c,d).

Electron microscopic results:

In electron microscopic study, neurons in the control group showed the ordinary ultrastructure of these cells. The nucleus was huge, round or oval with dispersed chromatin. It was surrounded by extensive free ribosomes. The cytoplasm was rich in rough endoplasmic reticulum (rER), free ribosomes and mitochondria (Fig. 8). Hippocampal formation in groups II and IV showed focal alterations compared to the control group. In group II, some cells showed change of the nuclear contour and reduction of the intracellular organelles including mitochondria, rER and free ribosomes. However, increased lysosomes and granulovacuolar bodies were seen (Fig. 9). Other cells in the previous group showed irregular nuclear outline with some mitochondria showing loss of their normal cisternae (Fig. 10). Cells in group III showed restoration of the cytoplasmic organelles as that in intact animals (Fig. 11). Cells in group IV showed marked reduction of cytoplasmic organelles (Fig. 12). No obvious qualitative differences could be observed by comparing the ultrastructure of the CA1 stratum radiatum of control rats and other animals of different groups of treatments.

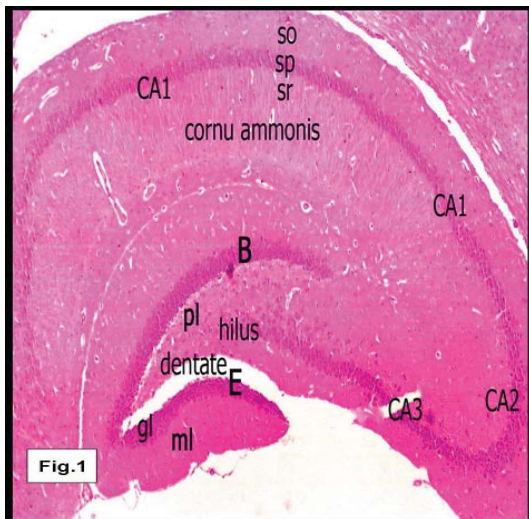


Fig. 1: The hippocampus of a control rat showing its normal structure (cornu ammonis & dentate gyrus). Cornu ammonis is formed of stratum pyramidale (sp) merged between stratum oriens (so) and radiatum (sr). CA1-CA3 are subfields of cornu ammonis. Dentate gyrus is formed of molecular (ml), granular (gl) and polymorphic (pl) layers arranged in exposed (E) and buried (B) blades. Note the hilus of dentate gyrus. Hx. &E.; X40

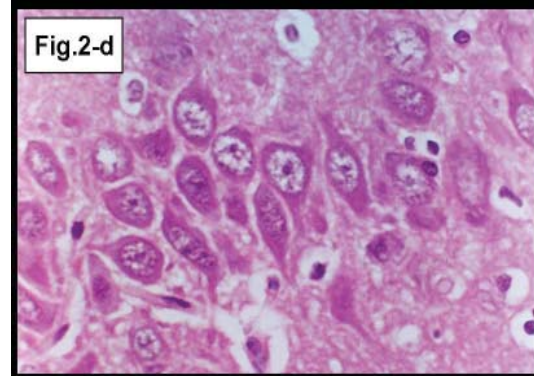
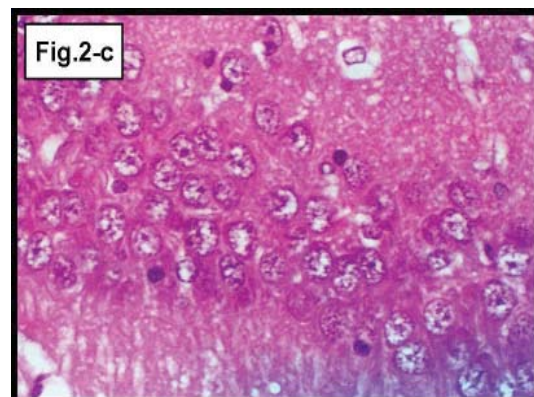
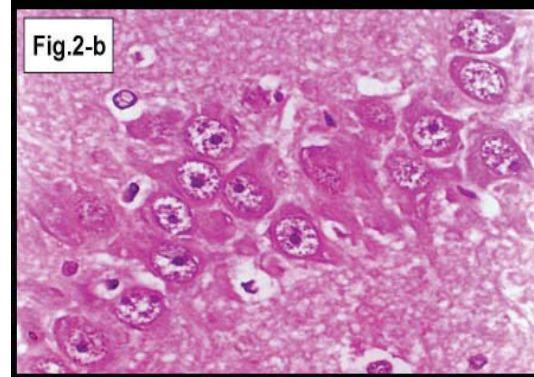
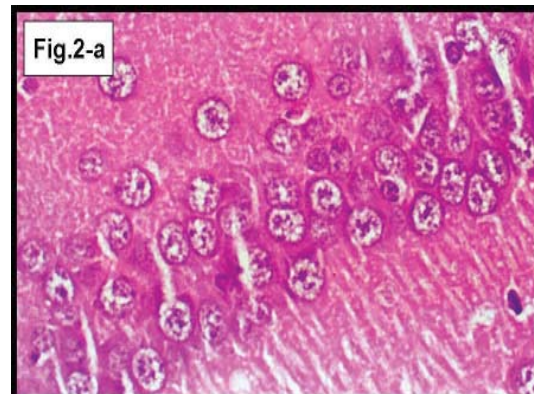


Fig. 2: Apparent reduction of pyramidal cell number denoting neuronal loss are seen in the hippocampus of group II rats (b) and that of group IV rats (d) compared to the control group (a). Group III (c) shows apparent restoration of normal number. Hx. &E.; X400

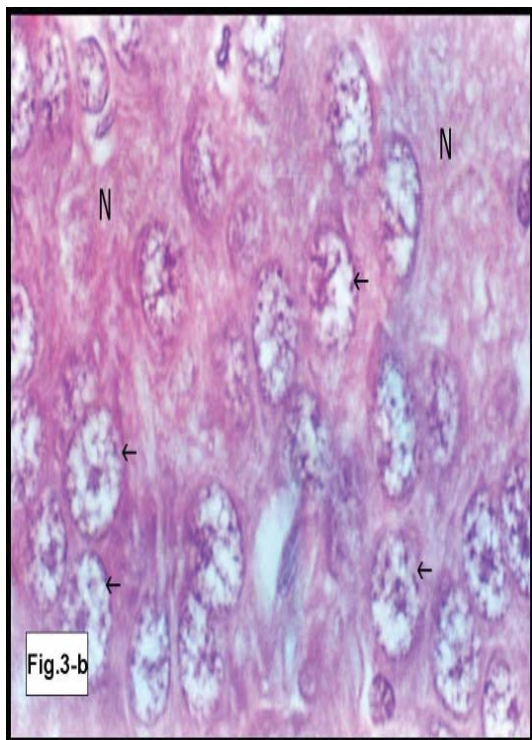
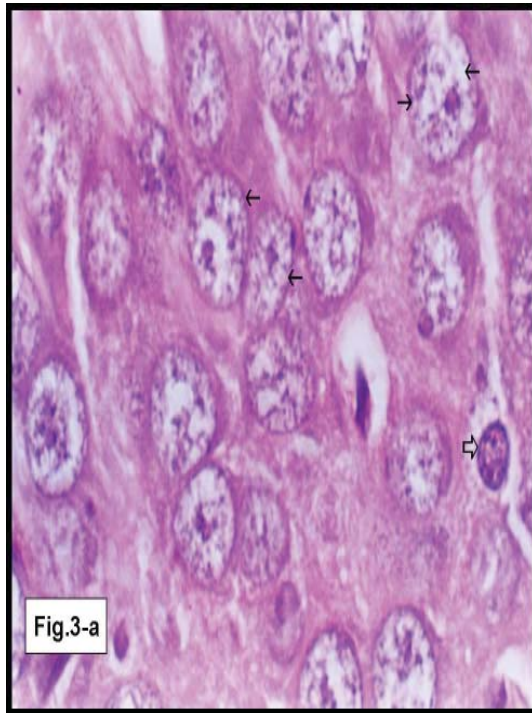


Fig. 3: CA1 pyramidal cells (arrow) in a hippocampus of a rat from the control group (a) and a rat from group III (b) showing huge rounded nuclei with pale stained dispersed chromatin and prominent nucleoli. The cytoplasm is basophilic and the cell processes are undistinguishable from the surrounding neuropil (N). The neuroglial cells (open arrow) show small dark nuclei. Hx. &E.; X1000

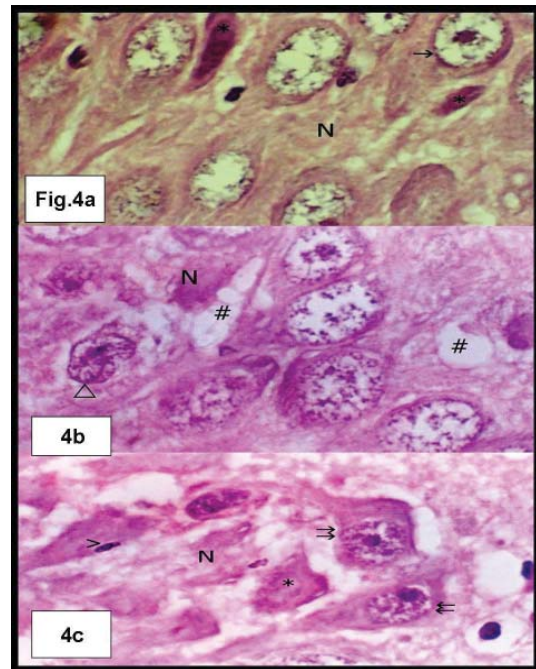


Fig. 4: CA1 field of a hippocampus in a rat from group II showing: (a) Dark basophilic masses of destructed cells (*) among normal pyramidal cells (arrow) in the neuropil (N). (b) One of the pyramidal cells shows irregular contour of its nucleus (open arrow head) and unstained areas (#) of the neuropil. (c) Some pyramidal cells are shrunken and show condensed nuclear chromatin (double arrows). One cell shows pyknotic eccentric nucleus (arrow head) and the nucleus is absent in another cell (*). Hx. &E.; X1000

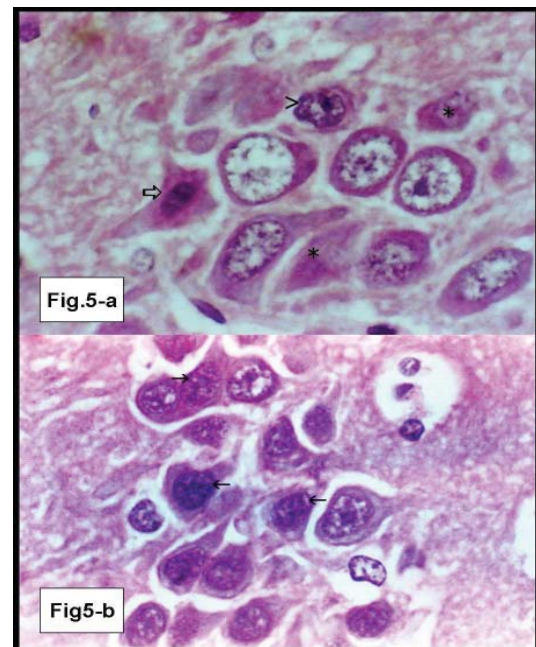


Fig. 5: CA1 field of a hippocampus from a rat of group IV showing: (a) a pyramidal cell with a pyknotic nucleus (open arrow) and another one with shrunken irregular nucleus (arrow head). Basophilic masses are seen (*). (b) A group of pyramidal cells (arrows) appear shrunken with unstained surroundings and dark nuclei. Hx. &E.; X1000

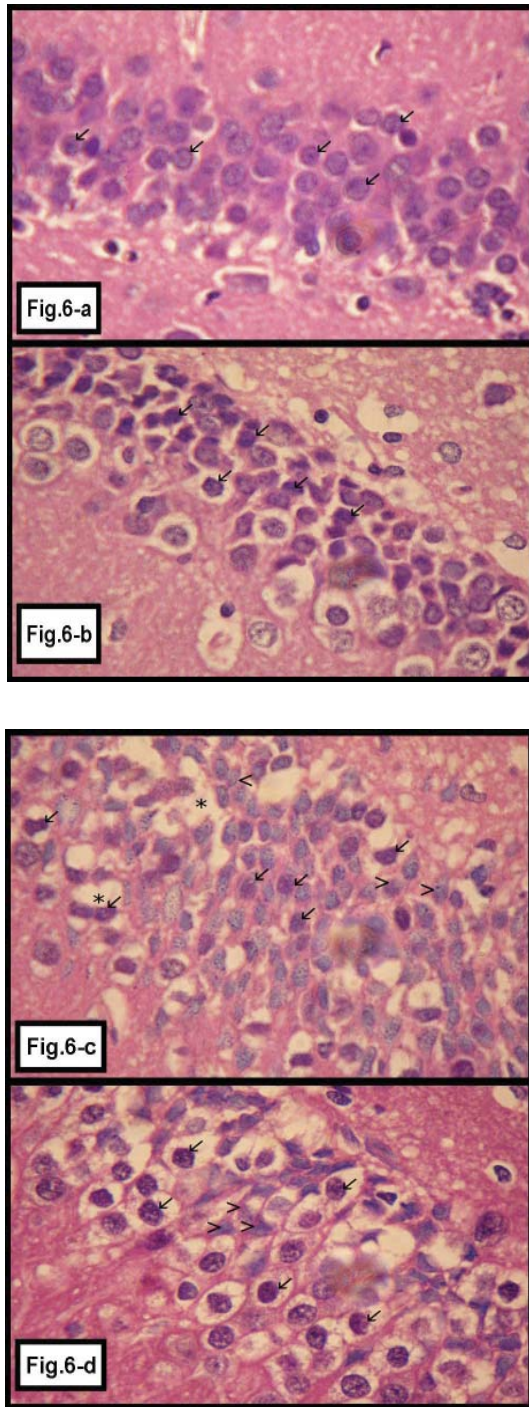


Fig. 6: Photomicrographs for the granular layer of the dentate gyrus in different groups showing (a) The granule cells (arrow) in the control group are closely packed small cells with dark stained nuclei. (b) The granule cells (arrow) in group II appear closely packed with apparent reduction in number compared to that of the control group. (c) Group III rats show apparent increase of the granule cells. Some cells have dark nuclei (arrow). Others appear with pale nuclei (arrow heads). Vacuolated cytoplasm can be seen (*). (d) The granule cells in group IV are less packed with vacuolated cytoplasm. The cells either have dark nuclei (arrow) or pale nuclei (arrow heads). Hx. &E.;X1000

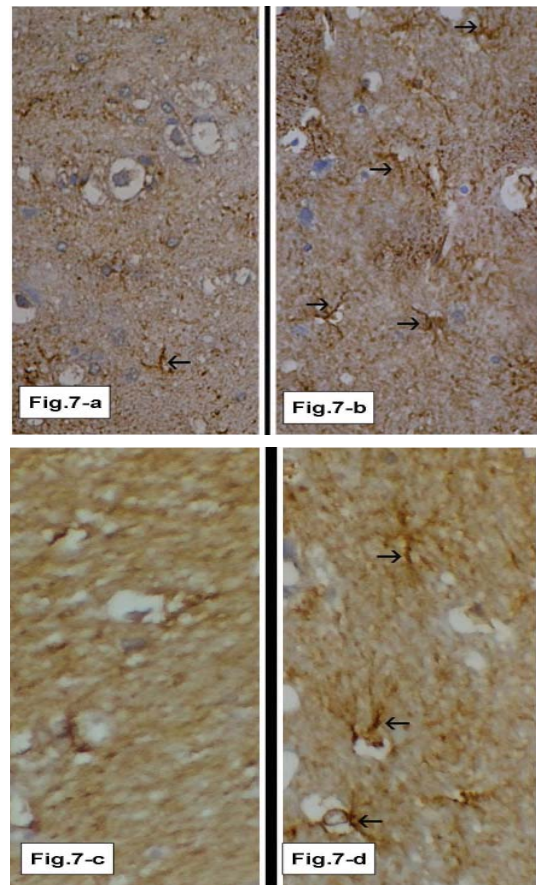


Fig. 7: Photomicrographs of the CA1 stratum radiatum of the hippocampal region in different groups show increase of GFAP immunoreactive glia profile (arrow) in group II(b) and group IV (d) compared to that of control group (a). On the other hand, GFAP expression in group III (c) resembles that of the control group. GFAP; X400

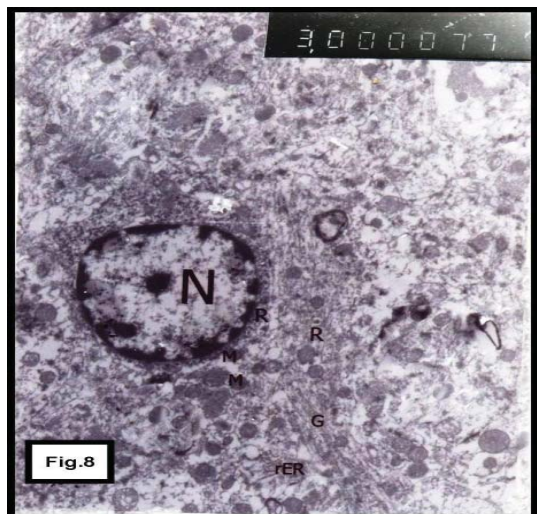


Fig. 8: A transmission electron micrograph of a pyramidal neuron in the control group shows a huge rounded nucleus (N) with dispersed chromatin and prominent nucleolus. It is surrounded by extensive free ribosomes (R). The cytoplasm is rich in rough endoplasmic reticulum (rER), free ribosomes (R) and mitochondria (M). Note: (G) golgi apparatus. X 3,000

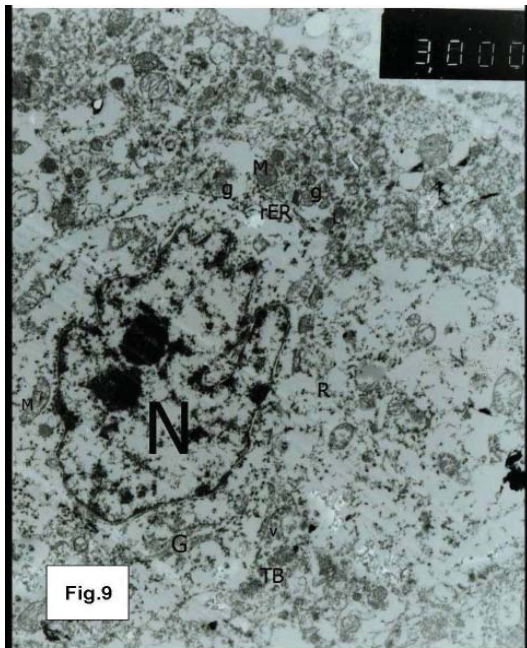


Fig. 9: A transmission electron micrograph of a pyramidal cell in group II with irregular nuclear contour of its nucleus (N). It shows reduction of the intracellular organelles including mitochondria (M), rER and free ribosomes (R). However, increased lysosomes (L) and granulovacuolar bodies (g) are seen. Note: TB (terminal button) contains (v) vesicles. (G) golgi apparatus. X 3,000

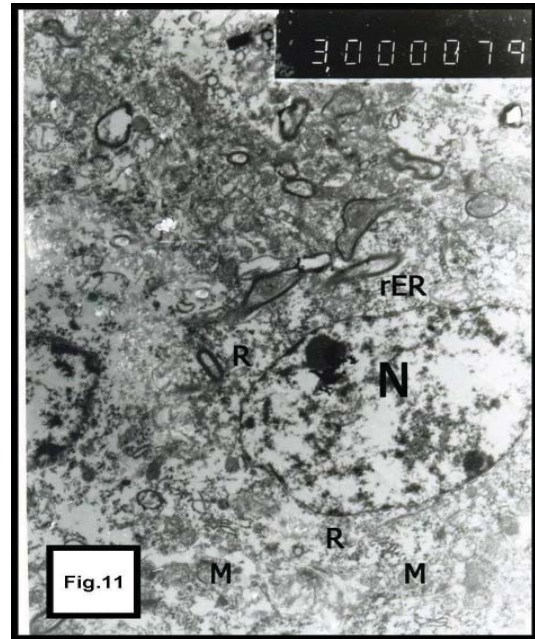


Fig. 11: A transmission electron micrograph of a pyramidal cell in group III shows a huge oval nucleus (N) with prominent nucleolus and dispersed chromatin. It is surrounded by extensive free ribosomes (R). The cytoplasm is rich in rough endoplasmic reticulum (rER), free ribosomes and mitochondria (M). X 3,000

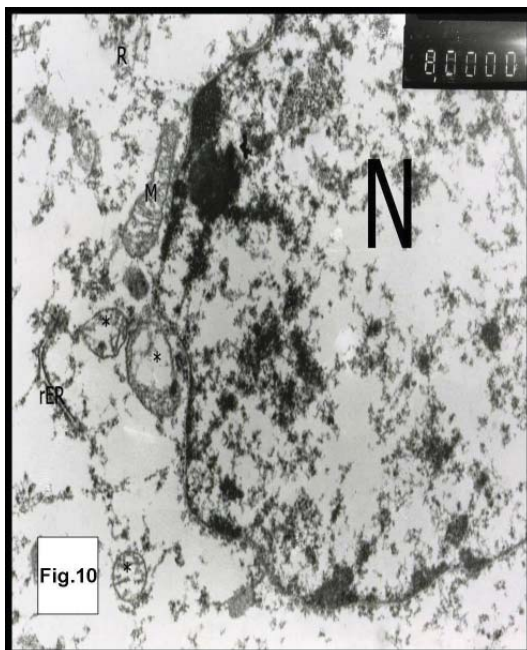


Fig. 10: A transmission electron micrograph of a pyramidal cell in group II shows irregular nuclear outline of its nucleus (N) and reduction of the free ribosomes (R) and rER. Some mitochondria appear normal (M) while others show loss of their normal cisternae (*). X 8,000



Fig. 12: A transmission electron micrograph of a pyramidal cell in group IV shows a huge rounded nucleus (N) with dispersed chromatin. Reduction of free ribosomes (R) and mitochondria (M) are seen. The arrows point to the cell membrane. X 3,000

DISCUSSION

As previously documented, learning and memory are affected by gonadal hormones. Meanwhile, these functions are known to be mastered by the hippocampus. The present study was designed to correlate between testosterone deprivation in castrated adult male albino rats and structural changes in hippocampal neurons. Also, the effects of either early or late testosterone substitution on restoration of normal hippocampal structure were studied. A positive correlation was found between early testosterone administration and the number of pyramidal cells in the hippocampus. In fact, rats of group III showed the highest number of pyramidal cells in the CA1 subfield of stratum pyramidale. On the other hand, rats of group II (castrated nontreated) and rats of group IV (castrated with late testosterone injection) showed significant reduction of the pyramidal cell number compared to controls. This came contradictory with Leranthe et al.(2003), who suggested that gonadectomy had no significant effect on the number of CA1 pyramidal cells, but reduced CA1 spine synapse density, 2 days following castration. The contradiction may be explained on the basis of the long period of the experiment designed in the current study. However, the present results are in agreement with *Beauchet (2006)* who mentioned that memory impairment due to testosterone deprivation has been associated with significant neuronal loss in the hippocampus. Meanwhile, *Cherrier et al. (2003)* and *Frye et al. (2004)* suggested that dihydrotestosterone has been shown to improve hippocampal-dependent functions in male rodents and humans. Moreover, *Yildirim et al. (2008)* suggested that gonadal hormones had profound effects on learning and memory mediated by hippocampus as well as hippocampal anatomy and hippocampal cell function.

The results found in this study proved that castration of adult male rats caused focal lesions of the hippocampus in the examined CA1 subfield. However, some pyramidal cells appeared normal while others showed different grades of degeneration and damage. These changes were manifested also in castrated rats receiving late onset testosterone (3 weeks post surgery) in spite of long term treatment. On the other hand, castrated rats receiving early onset testosterone replacement showed restoration of pyramidal cell structure compared to the control. *Leranth et al.(2003)* declared that removal of circulating gonadal steroids by gona-

ductomy resulted in a decrease in CA1 pyramidal cell dendritic spine density that was prevented with hormone replacement treatment. They also suggested that this effect might be specific to CA1 neurons within the hippocampus which possessed receptors for gonadal hormones. They added that the entire CA1 pyramidal cells were not uniformly responsive to these hormone manipulations. Interestingly, they explained this finding on the basis that a variety of afferents to the hippocampus form synapses on sharply defined regions of CA1 pyramidal cells.

On the same context, *Romeo et al.(2005)* mentioned that testosterone, acting through its androgenic metabolite 5 α -dihydrotestosterone (DHT), could restore dendritic spine density in the CA1 region of the male rat hippocampus. In this study, however, castrated rats receiving late onset testosterone substitution showed no restoration of the number of pyramidal cells suggesting irreversible damage. Moreover, degenerative process was going on in spite of testosterone therapy. According to *Gooren (2007)*, the duration of hypogonadism affects the efficacy of testosterone substitution. *Eriksson et al. (1998)* stated that loss of neurons is thought to be irreversible in the adult human brain, which is the cause of neurological disease and impairment. The present results suggested that early testosterone administration is essential for maintaining normal structure of the pyramidal cells in the hippocampus. This could be explained after *Clancy et al.(1992)* and *Kerr et al. (1995)* who stated that in the CA1 area of the male rat hippocampus, androgen receptors appear to be primarily located in the pyramidal neurons. Our results are in agreement with *Sakata et al. (2000)* and *Shors et al.(2001)* who demonstrated that androgens have powerful neuroprotective and homeostatic effects on the hippocampus.

In this study, significant reduction in the mean numbers of the granule cells in the dentate gyrus in group II and IV in comparison to controls was found. However, the difference in mean number between both groups II and IV was significant. In contrast, group III showed significant increase in the mean number of cells in comparison to controls. Unexplained vacuolated cytoplasm was manifested in castrated rats receiving testosterone therapy either of early or late onset. Both groups III and IV showed two different granule cell populations; cells with dark- stained nuclei and others with pale- stained nuclei. These results

indicated that testosterone stimulated neurogenesis of granule cells in the dentate gyrus. *Eriksson et al. (1998)* declared that the granule neurons are generated throughout life from a population of continuously dividing progenitor cells residing in the subgranular zone of the dentate gyrus in the rodent brain. Further, they suggested that testosterone stimulate neuronal regeneration and neurotrophin growth factor. According to *Sun & Bartke (2007)*, neurogenesis that occurs in the dentate gyrus of mammalian hippocampus may play an important role in restoration of some aspects of memory functions.

Conejo et al. (2005) stated that expression of glial fibrillary acidic protein (GFAP) as an astrocyte-specific marker can be regulated by the levels of circulating gonadal steroids during post-natal development. In addition, astrocytes play an important role in the physiology of the hippocampus. In the current study, immunohistochemistry for GFAP proved the much higher density of immunoreactive glia profiles in the hippocampus of the castrated nontreated rats and rats receiving late onset testosterone compared to the control group. These results simulate what was found by *Leranth et al. (2004)* who found that in the CA1 of castrated animals, the fiber density was increased compared to the control. However, early testosterone substitution preserves the GFAP expression as that of the control. It has been shown that GFAP is a sensitive and specific biomarker of neuronal damage. Diverse damaging insults result in proliferation and hypertrophy of astrocytes (reactive gliosis). The hallmark of this response is enhanced expression of the major intermediate filament protein GFAP of astrocytes (*O'Callaghan & Sriram, 2005*).

In electron microscopic results, no obvious qualitative differences could be observed by comparing the ultrastructure of the CA1 stratum radiatum of control rats and castrated animals belonging to different experimental groups. These results resemble what was found by *Leranth et al. (2003)*. However, ultrastructure of neurons in CA1 stratum pyramidale confirmed the light microscopic results. The irregular nuclear outline and abnormal contour seen in group II castrated rats were indicator for early degenerative processes of pyramidal cells. Depletion of free ribosomes and reduction of rough endoplasmic reticulum might indicate impaired protein synthesis. Damaged mitochondria indicate the

impairment of vital processes of the neuronal cells. Increased lysosomes and granulovacuolar bodies point to an ongoing degenerative process (*Garcia et al., 1997*). These results confirm previous suggestions that testosterone is essential for the normal structure and integrity of the neuronal cells (*Leranth et al., 2003*).

In conclusion, the data demonstrated in the current study indicate that testosterone is required for maintenance of normal neuronal structure of the male rat hippocampus. Androgen-induced changes in hippocampal structure may contribute to the effects of testosterone on hippocampally-mediated learning and memory function. Accordingly, early testosterone substitution may be recommended for hypogonadal men, taking in consideration that they do not suffer from any risk factors.

REFERENCES

- Beauchet, O. 2006.* Testosterone and cognitive function: Current clinical evidence of a relationship. *European Journal of Endocrinology / European Federation of Endocrine Societies* 155(6):773-781.
- Chen, A. C., and Petrylak, D. P. 2005.* Complications of androgen-deprivation therapy in men with prostate cancer. *Current Urology Reports* 6(3):210-216.
- Cherrier, M. M., Craft, S., and Matsumoto, A. H. 2003.* Cognitive changes associated with supplementation of testosterone or dihydrotestosterone in mildly hypogonadal men: a preliminary report. *Journal of Andrology* 24(4):568-576.
- Clancy, A. N., Bonsall, R. W., and Michael, R. P. 1992.* Immunohistochemical labeling of androgen receptors in the brain of rat and monkey. *Life Sciences* 50(6):409-417.
- Conejo, N. M., Gonzalez Pardo, H., Cimadevilla, J. M., et al. 2005.* Influence of gonadal steroids on the glial fibrillary acidic protein-immunoreactive astrocyte population in young rat hippocampus. *Journal of Neuroscience Research* 79(4):488-494.
- Daniel, J. M., Winsauer, P. J., and Moerschbaeher, J. M. 2003.* Castration in rats impairs performance during acquisition of a working memory task and exacerbates deficits in working memory produced by scopolamine and mecamlamine. *Psychopharmacology* 170(3):294-300.

- Eriksson, P. S., Perflieva, E., Bjork Eriksson, T., et al. 1998.** Neurogenesis in the adult human hippocampus. *Nature Medicine* 4(11):1313-1317.
- Frye, C. A., Edinger, K. L., Seliga, A. M., and Wawrzycki, J. M. 2004.** 5 alpha-Reduced Androgens may have actions in the hippocampus to enhance cognitive performance of male rats. *Psychoneuroendocrinology* 29(8):1019-1027.
- Garcia, J. H., et al. 1997.** Dementia and other neurodegenerative diseases: in *Neuropathology, the diagnostic approach*. Edited by J. H. Garcia, et al., Philadelphia, Mosby-Yearbook, 637-668.
- Gooren, L. 2007.** Testosterone and the brain. *Journal of Men's Health and Gender* 4(3):344-351.
- Hajszan, T., MacLusky, N. J., Johansen, J. A., et al. 2007.** Effects of androgens and estradiol on spine synapse formation in the prefrontal cortex of normal and testicular feminization mutant male rats. *Endocrinology* 148(5):1963-1967.
- Hammond, J., Le, Q., Goodyer, C., et al. 2001.** Testosterone-mediated neuroprotection through the androgen receptor in human primary neurons. *Journal of Neurochemistry* 77(5):1319-1326.
- Kerr, J. E., Allore, R. J., Beck, S. G., and Handa, R. J. 1995.** Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. *Endocrinology* 136(8):3213-3221.
- Leonard, S. T., Moerschbaecher, J. M., and Winsauer, P. J. 2007.** Testosterone potentiates scopolamine-induced disruptions of nonspatial learning in gonadectomized male rats. *Experimental and Clinical Psychopharmacology* 15(1):48-57.
- Leranth, C., Petnehazy, O., and MacLusky, N. J. 2003.** Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *The Journal of Neuroscience* 23(5):1588-1592.
- Leranth, C., Prange Kiel, J., Frick, K. M., and Horvath, T. L. 2004.** Low CA1 spine synapse density is further reduced by castration in male non-human primates. *Cerebral Cortex* 14(5):503-510.
- Moffat, S. D., Zonderman, A. B., Metter, E. J., et al. 2002.** Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. *The Journal of Clinical Endocrinology and Metabolism* 87(11):5001-5007.
- Nieschlag, E., Swerdloff, R., Behre, H. M., et al. 2005.** Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM and EAU recommendations. *European Urology* 48(1):1-4.
- O'Callaghan, J. P., and Sriram, K. 2005.** Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. *Expert Opinion Drug Safety* 4(3):433-442.
- O'Kane, G., Kensinger, E. A., and Corkin, S. 2004.** Evidence for semantic learning in profound amnesia: an investigation with patient H.M. *Hippocampus* 14(4):417-425.
- Paul, C. A., Beltz, B., and Berger-Sweeney, J. 1997.** Testing spatial and nonspatial learning using a morris water maze in neurons coordinating behavior: in *discovering neurons: the experimental basis of neuroscience*. Edited by C. A. Paul, B. Beltz and J. Berger-Sweeney, Cold Spring Harbor Laboratory Press, 324-337.
- Pouliot, W. A., Handa, R. J., and Beck, S. G. 1996.** Androgen modulates N-methyl-D-aspartate-mediated depolarization in CA1 hippocampal pyramidal cells. *Synapse* 23(1):10-19.
- Rhoden, E. L., and Morgentaler, A. 2004.** Risks of testosterone-replacement therapy and recommendations for monitoring. *The New England Journal of Medicine* 350(5):482-492.
- Rollin, R., Mediero, A., Roldan Pallares, M., et al. 2004.** Natriuretic peptide system in the human retina. *Molecular Vision [Computer File]* 10:15-22.
- Romeo, R. D., Staub, D., Jasnaw, A. M., et al. 2005.** Dihydrotestosterone increases hippocampal N-methyl-D-aspartate binding but does not affect choline acetyltransferase cell number in the forebrain or choline transporter levels in the CA1 region of adult male rats. *Endocrinology* 146(4):2091-2097.
- Roy, T. S., Seidler, F. J., and Slotkin, T. A. 2002.** Prenatal nicotine exposure evokes alterations of cell structure in hippocampus and somatosensory cortex. *The Journal of Pharmacology and Experimental Therapeutics* 300(1):124-133.

Sakata, K., Tokue, A., and Kawai, N. 2000. Altered synaptic transmission in the hippocampus of the castrated male mouse is reversed by testosterone replacement. *The Journal of Urology* 163(4):1333-1338.

Sherwin, B. B. 2003. Steroid hormones and cognitive functioning in aging men: a mini-review. *Journal of Molecular Neuroscience* 20(3):385-393.

Shors, T. J., Chua, C., and Falduto, J. 2001. Sex differences and opposite effects of stress on dendritic spine density in the male versus female hippocampus. *The Journal of Neuroscience* 21(16):6292-6297.

Squire, L. R. 1992. Memory and the hippocampus: A synthesis from findings with rats, monkeys and humans. *Psychological Review* 99(2):195-231.

Standring, S. 2004. Gray's anatomy: The anatomical basis of clinical practice. 39th ed., Churchill Livingstone.

Sun, L. Y., and Bartke, A. 2007. Adult neurogenesis in the hippocampus of long-lived mice during aging. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 62(2):117-125.

Wolf, O. T., and Kirschbaum, C. 2002. Endogenous estradiol and testosterone levels are associated with cognitive performance in older women and men. *Hormones and Behavior* 41(3):259-266.

Yildirim, M., Mapp, O. M., Janssen, W. G. M., et al. 2008. Postpubertal decrease in hippocampal dendritic spines of female rats. *Experimental Neurology* 210(2):339-348.

تأثيرات نزع الخصيتين واحلال التيستوستيرون على قرن آمون فى ذكور الجرذان البيضاء البالغة

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ملخص البحث

لقد برهنت الأبحاث السابقة على أن مرضى ضعف المناسل الوظيفي من الرجال يعانون من اضطراب فى وظائف التعلم و الذاكرة و لقد اثبتت الأبحاث الحديثة دور قرن آمون فى التحكم فى هذه الوظائف بواسطة عمل الهرمونات التناسلية، إلا أن آلية هذا العمل لازالت موضع للمناقشة. و لقد كان الهدف من اجراء هذه الدراسة هو اثبات تأثير نزع الخصيتين واحلال التيستوستيرون على بنية الخلايا العصبية لقرن آمون فى ذكور الجرذان البيضاء البالغة. من أجل هذا الغرض، أجرى هذا البحث على عشرين جرذا من الجرذان الذكور البيضاء البالغة تم تقسيمها الى اربع مجموعات؛ مجموعة ضابطة (المجموعة الأولى)، مجموعة تم اخصائها (المجموعة الثانية)، مجموعة تم اخصائها ثم تم حقنها مباشرة بالداى هيدروتستوستيرون يوميا (المجموعة الثالثة)، و مجموعة تم حقنها يوميا بالداى هيدروتستوستيرون بعد اخصائها بثلاثة اسابيع (المجموعة الرابعة). بعد الجراحة بستة أسابيع تم التضحية بكل الجرذان و تم معاملة قرون آمون ليتم فحصها بالميكروسكوب الضوئى و الميكروسكوب الإلكتروني النافذ. و لقد أثبتت النتائج تغيرات ذات دلالة احصائية فى بنية قرون آمون التى تم الحصول عليها من المجموعتين الثانية و الرابعة بينما أظهرت جرذان المجموعة الثالثة المحافظة على البنية الطبيعية لقرن آمون عند مقارنتها بالمجموعة الضابطة. نستخلص من هذا البحث ان الإخفاء أثر على بنية قرن آمون و الذى قد يفسر اضطراب التعلم و الذاكرة فى مرضى اضطراب المناسل الوظيفي. قد يمنع الإمداد المبكر للداى هيدروتستوستيرون هذه التأثيرات. بينما الإمداد المتأخر له ليس له تأثير واق والذى من شأنه أن يدل على أن تلف قرن آمون غير قابل للشفاء.