Effect of experimental orchiectomy on hippocampus of adult albino rat and the role of testosterone supplementation: a histological and immunohistochemical study
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Introduction
The hippocampus is an important and sensitive region of the brain. It is divided into four areas (known as cornu Ammonis), CA1, CA2, CA3 and CA4, on the basis of their cellular variances (density, size and branching of axons and dendrites) [1,2]. Each of these regions consists of three layers: stratum molecular, stratum pyramidale and stratum multiforme. The stratum pyramidale contains bodies of pyramidal cells. It was reported that the dominant neurons in the hippocampus are the pyramidal cells [2].

The hippocampus plays a crucial role in certain aspects of learning and memory [3]. Short-term memory, which is recognized as the location for the storage of new information, is closely related to the hippocampus [4,5]. Furthermore, the functions of the right hippocampus are related to visual memory, whereas those of the left hippocampus are related to verbal memory. Hence, any lesions that occur at this region of the brain lead to loss of memory in varying degrees [4,6]. It was documented that the hippocampus is sexually differentiated as a

Materials and methods
Thirty-five adult male albino rats were divided into control group (group I) and two experimental groups (groups II and III). Rats in groups II and III were subjected to orchiectomy. The orchiectomized rats in group III were treated daily with testosterone propionate (0.5 mg/kg/day) and both groups were left alone for 30 days. At the end of the experiment, all rats were anaesthetized and their brains were removed and processed. Sections were stained by H&E and immunohistochemically for Bax, Bcl2 and glial fibrillary acidic proteins (GFAP). Further, the serum level of testosterone was measured. The results were statistically analysed.

Results
Examination of the hippocampus of orchiectomized rats showed decreased thickness of the pyramidal layer, which contained many apoptotic cells. Minute haemorrhage, cellular infiltration and dilated capillaries were also seen. Immunohistochemically, intense Bax and GFAP with minimal Bcl2 reactions were detected. The hippocampi of orchiectomized rats treated with testosterone were less affected. The pyramidal cell layer thickness was relatively normal. Few nerve cells with dark cytoplasm appeared among the normal ones. Further, minimal Bax and GFAP with moderate Bcl2 reaction were detected. Statistically, there was a significant decrease in the level of testosterone in group II compared with group I.

Conclusion
The results demonstrated that decrease in the level of testosterone had deleterious histological effects on rat hippocampi. Testosterone replacement ameliorated these histological changes after orchiectomy.

Keywords:
hippocampus, orchiectomy, testosterone replacement, ultrastructure
result of developmental androgen exposure in male individuals [7].

Testosterone is an androgenic hormone derived from cholesterol. It is formed by glial cells in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system [8]. Therefore, testosterone is considered a neurosteroid that plays an important role in the function of the CNS and in behavior control [9,10]. In addition, testosterone is capable of providing analgesic and anxiolytic activities, leading to variation in personal mood and awareness [11,12]. Moreover, it has been reported that testosterone affects the differentiation of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes in astroglia during brain development [13]. However, in the adult brain, testosterone regulates the expression of GFAP in the hippocampus and reduces its production as a result of aging in the cerebellum [12].

Physiologically, androgens are involved in neuronal differentiation, neuroprotection, neuronal survival and development [14]. It was reported that disorders interrupting the secretion of testosterone may trigger apoptosis in neural tissues [13,14] and lead to the development of neurodegenerative diseases in humans [15–17].

Apoptosis is the genetically regulated form of cell death (programmed cell death) that permits the safe disposal of cells when they are damaged or have fulfilled their intended biological function [18]. Death signals cause release of some proteins that are related to the Bcl2 family. Some proteins in this family are proapoptotic (Bax), whereas some are antiapoptotic (Bcl2). To sum up, immunohistochemical Bax staining in the cytoplasm of cells indicates that apoptosis has occurred [19].

On a more global scale, functions of testosterone are mediated through intracellular androgen receptors (ARs). Hence, the gradual loss of testosterone leads to functional changes in androgen responsive tissue. These tissues include those areas of the body in which ARs are abundant [20,21], such as the prostate, heart, skin and musculoskeletal tissue [22]. Several studies [23] have reported that the brain is highly responsive to androgens such as testosterone, with the hippocampus being one of the most strongly affected regions. The level of AR expression in the hippocampus has been shown to be of the same magnitude as expression in the prostate. In addition, animal studies have demonstrated a significant positive association between testosterone and hippocampal volume, as well as effects of the hormone on hippocampal neural plasticity, synaptic density and neurogenesis [24,25].

Interestingly, there was a strong correlation between memory impairment and atrophy of the hippocampus that supported the new, recently proposed criteria for diagnosis of Alzheimer’s disease (AD) [26,27]. In addition, it was believed that a low testosterone level might lead to the development of AD through hippocampal atrophy and gliosis [14,28]. Considerable attention has been given to studying hippocampal volume as a sensitive and specific indicator of Alzheimer’s neuropathology in cases of testosterone deficiency. Thus, this study was conducted to throw more light on the histological changes in the hippocampus (memory and learning area) that are associated with testosterone deficiency and evaluate the possible role of testosterone replacement.

Materials and methods

Thirty-five adult male albino rats of 3 months of age, weighing 180–200g each, were used in this study. They were obtained from Animal House, Faculty of Medicine, Zagazig University. The animals were housed in individual cages and maintained at room temperature. They were allowed standard diet and tap water ad libitum. The light cycle was fixed at 12 h. The rats were divided into three groups: group I, group II and group III.

Group I (C): This group served as the control group and was subdivided into three subgroups of five rats each:

- Ca: Rats not subjected to surgical procedure or treatment.
- Cb: Rats that were subjected to sham surgical procedure. Under anaesthesia, the testes were pulled out through a scrotal incision and then replaced without removal (sham-operated untreated).
- Cc: Rats subjected to sham surgical procedure and that received subcutaneous injections of sesame oil daily for 30 days (sham-operated treated).

Group II: Rats subjected to orchietomy (OrchX) and left alone for 30 days (n=10 rats).

Group III: Orchietomized rats that received daily subcutaneous injections of testosterone propionate (Teikoku Hormone Mfg. Co. Ltd, Tokyo, Japan) 1 week after orchietomy (0.5 mg/kg in 0.1 ml of sesame oil) for 30 days (n=10 rats).

After 1 week of acclimatization, orchietomy and sham operation were performed in the experimental groups. The animals were anaesthetized with intraperitoneal injection of pentobarbital sodium (15 mg/kg body weight). In the orchietomized group, a small midline scrotal raphe incision was made and the testicles were exposed through it. The ductus deferens was isolated and ligated. The testicles were then removed bilaterally. The incision was closed and sutured with 4-0 nylon sutures [29]. The sham operation involved the exposure of the testicle without isolation.

At the end of the experiment, all the rats were sacrificed. Blood samples were collected and brains were removed and subjected to the following:

Histological study

The brain of each rat was divided into two hemispheres, fixed in 10% formal saline, and processed to prepare 5-µm-thick paraffin sections for H&E staining [30] and for immunohistochemical staining for Bax, Bcl2 proteins [31] and GFAP [32]. For these stains, the paraffin sections were placed in xylene, hydrated and treated with 3% hydrogen peroxide.
Sections were incubated with a monoclonal antibody against Bax and Bcl2 (Dako, Carpinteria California, USA) and GFAP proteins (Sigma, St. Louis, Missouri, USA). Detection of the antibody was performed using a biotin–streptavidin detection system with 3-aminobenzazide as chromogen (Dako) for Bcl2 and Bax and with 0.05% diaminobenzidine as chromogen (Amersham, Little Chalfont, UK) for GFAP. Negative control sections were treated according to the protocol, omitting exposure to primary antibodies.

**Serum testosterone level analysis**

The collected blood samples were allowed to clot and then centrifuged at 3000g for 10 min. Serum concentrations of testosterone were quantified using Euleess (Roche, Mannheim, Germany) commercial immunoassays. To avoid the difference in testosterone level, all blood samples are taken at a fixed time in the mornings [33].

**Morphometric study**

The thickness of the pyramidal layer was measured in serial sections stained with H&E. In addition, area percentages for Bcl2, Bax and GFAP immunoreactions were measured. Five nonoverlapping fields from five different sections were randomly chosen from each animal. All data were obtained using a Leica Qwin 500 image analyzer computer system (UK) at the Histology and Cell Biology Department, Faculty of Medicine, Cairo University.

**Statistical analysis**

Data were expressed as mean ± SD (X overline{X} ± SD). The results were computed statistically using SPSS program (Chicago, USA), version 15. One-way analysis of variance was performed to compare among all studied groups. P values less than 0.05, less than 0.001 and greater than 0.05 were considered significant, highly significant, and nonsignificant, respectively.

**Results**

**Morphologically**

**Group I (control group)**

Examination of the H&E sections of the hippocampus of the rats of all control subgroups (Ca, Cb and Cc) revealed that the hippocampus was C-shaped and formed of three areas: CA1, CA2 and CA3. The dentate gyrus and parahippocampal region were also seen (Fig. 1). Each area of the hippocampus was composed of three layers: molecular, pyramidal and polymorphic (Fig. 2). The molecular and polymorphic layers contained few cells, whereas the pyramidal layer was formed of numerous rounded nerve cells containing large vesicular nuclei with prominent nucleoli and pale basophilic cytoplasm (Fig. 3). Immunohistochemically, no Bax-stained cells were seen (Fig. 4a). In contrast, strong reaction for Bcl2 protein could be detected in this group (Fig. 4b). Few GFAP-reactive cells were seen in this group (Fig. 5).

Examination of the H&E-stained sections of the hippocampus of experimentally induced orchiectomy rats (OrchX) showed decreased thickness of the pyramidal layer (Fig. 6). Some nerve cells contained small condensed nuclei and dark cytoplasm. Minute haemorrhage and dilated capillaries were also seen (Fig. 7). Most nerve cells had dark condensed nuclei, whereas the other cells had vesicular nuclei. Glial cells appeared with small nuclei (Fig. 8). Intense Bax-stained cells and weak reaction of Bcl2 protein were also seen (Fig. 9a and b). Many GFAP-reactive cells could be seen among the different layers (Fig. 10).

Examination of the H&E-stained sections of the hippocampus of orchitectomized rats that were treated with testosterone (the treated group) revealed relatively normal thickness of the pyramidal layer in comparison with the OrchX group (Fig. 11) (Table 1). Irregular faintly stained nerve cells were seen among normal cells with vesicular nuclei and prominent nucleoli (Fig. 12). Some nerve cells had dark basophilic cytoplasm. Glial cells were also seen (Fig. 13). Minimal Bax-stained cells and moderate Bcl2 reaction were observed in this group (Fig. 14a and b). A statistically relative number of GFAP-reactive cells were observed in this group (Fig. 15).

**Statistical and morphometrical results**

Statistical analysis of the results showed highly significant difference in the thickness of the pyramidal layer and in the area percentage of Bcl2 and Bax immunoreactions between the control and experimental groups (Tables 2 and 3). Further, the area percentage of GFAP immunoreactions revealed significant increase in the operated group and nonsignificant increase in the treated group when compared with the control group (Table 4). Finally, the level of testosterone showed a highly significant decrease and a significant decrease in the operated and treated groups, respectively, in comparison with the control group.

**Serum testosterone level**

Compared with the control group, a significant (P ≤ 0.05) decrease in the mean value of serum testosterone was detected in group II. Further, there was a nonsignificant difference in the mean value of this hormone level in group III compared with group I (Table 5).
Figure 2. A section of the hippocampus of the control group (C) showing the three layers: molecular (M), pyramidal (P) and polymorphic (PP).
H&E, × 200.

Figure 3. A section of the hippocampus of the control group (C) showing few cells in molecular and polymorphic layers (arrow head).
The pyramidal layer is formed of numerous nerve cells with large vesicular nuclei with prominent nucleoli and pale basophilic cytoplasm (arrow).
H&E, × 400.

Figure 4. A section of the hippocampus of the control group (C) showing (a) no Bax-stained cells and (b) strong Bcl2 reaction (arrowhead) in nerve cells.
Bax&Bcl2 immunostaining, × 400.

Figure 5. A section of the hippocampus of the control group (C) showing few glial fibrillary acidic proteins-reactive cells (arrow).
Avidin–biotin peroxidase system, × 400.

Figure 6. A hippocampal section of the orchiectomized group (OrchX) showing decreased thickness of the pyramidal layer. PP (clarify different layer of hippocampus).
H&E, × 200.

Figure 7. Hippocampal section of the orchiectomized group (OrchX) showing some nerve cells with small condensed nuclei and dark cytoplasm (arrow). Note minute haemorrhage (h) and dilated capillaries (arrow head).
H&E, × 400.
Figure 8. The hippocampal section of the orchiectomized group (OrchX) showing nerve cells with dark condensed nucleus and pale cytoplasm (arrow). Few nerve cells have vesicular nuclei (arrow head). Note glial cells (GC) with small nuclei. H&E, × 400.

Figure 9. A hippocampal section of the orchiectomized group (OrchX) showing (a) intense Bax-stained cells (arrows) and (b) weak Bcl2 reaction (arrow head). Bax&Bcl2 immunostaining, × 400.

Figure 10. A hippocampal section of the orchiectomized group (OrchX) showing many glial fibrillary acidic proteins-reactive cells among the different layers of the hippocampus (arrow). Avidin–biotin peroxidase system, × 400.

Figure 11. Hippocampus of the orchiectomized group treated with testosterone (the treated group) showing relatively increased thickness of the pyramidal layer. PP (clarify different layer of hippocampus). H&E, × 200.

Figure 12. Hippocampus of the orchiectomized group treated with testosterone (the treated group) showing irregular faintly stained cells (arrow). Other nerve cells have vesicular nuclei with prominent nucleoli (arrow head). H&E, × 400.
Figure 13. Hippocampus of the orchiectomized group treated with testosterone (the treated group) showing relatively normal nerve cells (NV) with vesicular nuclei and dark basophilic cytoplasm. Glial cells are also seen (GC).

H&E, × 400.

Figure 14. Hippocampus of the orchiectomized group treated with testosterone (the treated group) showing (a) minimal Bax-stained cells (arrow) and (b) moderate Bcl2 reaction (arrow head).

Bax&Bcl2 immunostaining, × 400.

Figure 15. Hippocampus of the orchiectomized group treated with testosterone (the treated group) showing relative number of glial fibrillary acidic proteins-reactive cells among the different layers (arrow).

Avidin–biotin peroxidase system, × 400.

Table 1. The mean values of the thickness of the pyramidal layer

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (range)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>64.75 ± 14.01</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Group II (operated)</td>
<td>35.64 ± 8.89</td>
<td>5.530</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group III (treated)</td>
<td>45.19 ± 8.62</td>
<td>3.674</td>
<td>&lt;0.005</td>
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P < 0.001, F=18.872.
Testosterone is the main endogenous anabolic/androgenic steroid hormone, and it plays fundamental roles in development, differentiation and cellular growth [12]. In neurons, testosterone acts as a neurosteroid and can induce changes at the cellular level, which in turn lead to changes in behavior, mood and memory [30].

Neurosteroids have been implicated as components essential for the normal function of the CNS. The gonadal steroid also affects areas of the brain that are not primarily involved in reproduction, such as the hippocampus preoptic area, amygdala and medial hypothalamic area [31].

The male rat hippocampus is rich in AR-expressing cells [32], indicating that it is a target for testosterone action. In the CA1 area, the ARs appear to be primarily located in pyramidal neurons [31,33].

As early as the fourth decade of life, testosterone level in men begin to decline at a steady rate [34,35]. Although
there has been minimal investigation of the relationship between testosterone and the hippocampus in humans, numerous studies have found a positive correlation between testosterone and hippocampally mediated cognitive processes (e.g. episodic memory and visual–spatial ability) in middle-aged and older adults [36,37].

In the present study, coronal section of the brain showed that the hippocampus was a C-shaped region composed of three areas: CA1, CA2 and CA3. Each of them was formed of three layers: molecular, pyramidal and polymorphic. The molecular and polymorphic layers contained few cells. The pyramidal layer contained numerous nerve cells with vesicular nuclei and basophilic cytoplasm. It was reported that the dominant neurons in the hippocampus are the pyramidal cells. The size and density of these neurons appeared variable throughout the hippocampus proprius. This fact was used to differentiate the hippocampus. Hence, the hippocampus was divided into four regions according to density, size and branching of axons and dendrites of the pyramidal cells [2].

In orchietomized rats, examination of the hippocampus showed nerve cells with small condensed nuclei and dark cytoplasm. Minute haemorrhage and dilated capillaries were also noticed. Glial cells had small nuclei. It was reported that gonadectomy caused a decrease in the antioxidant enzyme levels and, consequently, initiated an apoptotic process inside the cells, leading to cellular death and increase in lipid peroxidase levels [38]. The antioxidant defence system was shown to have collapsed after gonadectomy and the synaptic intensity of neurons was seen to have decreased and cell death to have occurred because of apoptosis [39,40].

It is well recognized that steroids act through nuclear receptors that are ligand-regulated transcription factors and that participate in many cellular processes such as proliferation, differentiation and cell death [41]. Also, the steroids had a protective role against cell damage through their antioxidant properties by means of a membrane mechanism of action [42,43].

In the current work, a significant decrease in the mean value of serum testosterone was detected in group II ($P \leq 0.05$) in comparison with the control one. Moreover, a nonsignificant difference was detected in the mean value of this hormone level in group III compared with group I. It was mentioned that testosterone has neuroprotective effects in the hippocampus in vitro and in vivo because it can attenuate amyloid toxicity and oxidative stress-induced cell death in its neurons. Furthermore, gonadectomized male rats supplemented with testosterone had significantly fewer pyknotic cells in their hippocampus compared with the vehicle-administered ones [14,44].

Examination of the hippocampus of orchietomized rats that were treated with testosterone (the treated group) revealed fewer pyknotic cells with few darkly stained nerve cells. Nerve cell nuclei retained their vesicular appearance and basophilic cytoplasm. Several studies have demonstrated that testosterone administration prevents tissue damage and improves antioxidant enzyme levels [45,46]. Furthermore, testosterone could reduce the survival of reactive glial cells or decrease the activated phenotype of astrogliosis and microgliosis. Hence, this hormone could control the gliosis in brain tissue, including the hippocampal region [12,39]. Other experimental studies [45,47] have proved that testosterone is capable of increasing the glutathione peroxidase activity in the hippocampus and also in preventing the apoptosis that occurs because of streptozotocin in pancreatic cells.

Morphometrically, the width of the granular and pyramidal cell layers in orchietomized animals (OrchX) showed a strong decrease compared with control and sham-operated animals. However, there was no significant difference in the width of the examined areas between the control and testosterone-treated groups. The difference in width of these layers was attributed to the decreased number of nerve cells, as the size of the cells remained constant [48]. Other researchers have suggested that hippocampal volumes may be valuable not only in distinguishing individuals with AD from the nondemented elderly but also in identifying individuals with AD neuropathology who have not demonstrated dementia or even memory impairment [49].

In this study, the hippocampus sections were immunohistochemically stained with Bax and Bcl2. The results revealed cellular apoptosis that was determined by assessing the intensity of these reactions. No Bax-stained cells were observed in the control and sham-operated groups. However, a large number of Bax-stained cells were observed in the hippocampus of orchietomized rats. A few of these cells were detected with testosterone treatment. It was mentioned that the Bax staining occurred to a severe extent in the cellular cytoplasm of the hippocampus after orchietomy [50]. The hippocampus is vulnerable to injury in several neurodegenerative conditions. Neuronal loss in these conditions was explained by apoptosis and related to low testosterone level [50]. In contrast, a high level of this hormone could induce programmed cell death with decreased cell viability [51].

Moreover, examination of Bcl2-stained sections revealed strong reaction for Bcl2 (antiapoptotic) protein in the control and sham-operated groups in comparison with the OrchX group. However, the orchietomized group revealed weak reaction for this protein as compared with the control one. In the treated group, the reaction appeared to be relatively similar to the control group. It was reported that physiological levels of testosterone protect against serum deprivation-mediated neuronal apoptosis through interaction with ARs. Moreover, they confirmed the presence of ARs in human neuron cultures. Testosterone was used in the treatment of conditions associated with neuronal death because of its ability to improve and maintain neuronal cell viability [1,14].

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With regard to GFAP immunoreactions, this study revealed many GFAP-reactive cells in the OrchX group in comparison with the control group. In the testosterone-treated group, these GFAP-reactive cells were relatively decreased. Previous studies have shown that systemic administration of testosterone to orchietomized male rats on days 0, 1 and 2 after a stab wound in the cortex and hippocampus significantly decreases the number of GFAP immunoreactive astrocytes in the border of the wound [52]. Some researchers [13] reported that control of glialis may be one of the mechanisms involved in the neuroprotective effects of testosterone. Moreover, testosterone may exert its effects on reactive glialis by acting on ARs or after local conversion to oestradiol. The identification of factors that regulate reactive glialis is of practical interest for the development of therapeutic strategies to reduce neural damage and promote regeneration after CNS injuries and to decrease neuronal death in neurodegenerative disorders. The activation of glial cells is modulated by local factors and by substances transported by the systemic circulation, such as the hormones secreted by the gonads and adrenals [53].

Importantly, accumulating data raise the possibility that androgens could help in the treatment of AD in a manner similar to oestrogen replacement therapy in women. Hence, the current data focus on the hippocampus as an established target of the androgen hormone that is involved in several aspects of memory and other functions. It is not only critical for learning and memory but also vulnerable to injury in several neurodegenerative conditions, including AD.

Conclusion
From the results of this study we have concluded that orchietomy causes deleterious effects on the histological structure of the rat hippocampus. Testosterone administration could protect the structure of the hippocampus of orchietomized rats. It was documented that age and different aspects of an individual’s environment affect brain structure. Our results refer to another endogenous factor: decreased testosterone level that affects brain structure including the hippocampus. The hippocampus has an important role in memory and learning and is seriously affected in neurodegenerative conditions such as AD. Therefore, testosterone is considered as a therapeutic hope for those suffering from AD and its normal level must be maintained in elderly men.

Acknowledgements
Conflicts of interest
No conflict of interest to declare.

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الملخص العربي

تأثير الاخصاء التجريبي على الهيبوكامبس في ذكور الجرذان البيضاء البالغة ودور التعويض لهرمون التيستوستيرون (دراسة هستولوجية و هستوكيميائية مناعية)

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المقدمة: يعد المخ من أهم الأعضاء المستهدفه للهرمون التناسلي التيستوستيرون. و يعتبر الهيبوكامبس (قرن آمون) الذي يؤدي دوراً هاماً في الذاكرة و التعليم منطقة حساسة لتأثير هذا الهرمون، ولذلك يتأثر بإخفاقه التجريبي المرتبط بالعمر في الرجال.

الهدف من البحث: يهدف هذا العمل لدراسة التغيرات النسيجية المحتملة التي يحدثها الاخصاء التجريبي في الجرذان البيضاء في الهيبوكامبس كما يقيم الاستفادة المحتملة من الامداد بهرمون التيستوستيرون.

المETHODS: أجريت هذه الدراسة على خمسة و ثلاثين من ذكور الجرذان البيضاء البالغة، مقسمة إلى ثلاث مجموعات: المجموعة الأولى اتخذت كمجموعة ضابطة (I) والمجموعتين التجريبتين (II، III) كل منهما عشرة جرذان، وتم إخصاء الجرذان في المجموعة الثانية، ثم حُقدت الجرذان المخصودة في المجموعة الثالثة ببروبيوتات التيستوستيرون (0.5 مجم/كم/يوم) و تركت المجموعتين لمدة 30 يوماً. وفي نهاية التجربة تم تخدير الجرذان, وأخذت عينات من الدم لقياس مستوى هرمون التيستوستيرون به كما أخذت عينات من الهيبوكامبس تجسيماً وصبغت عينات للدراسة الهستوكيميائية المناعية لتحديد الباكس و حسب رؤية الفرديةIRT و حسب رؤية الفردية.

النتائج: أظهر فحص عينات الهيبوكامبس في الفئران المخصودة انخفاض سمك طبقة الخلايا الهرمية مع ظهور العديد من الخلايا ذات الأنوية الداكنة و شوه نزيف بسيط و خلايا متخيلة و شعارات دموية متسعة, و صبغت الخلايا العصبية داكناً بالباكس و بالبروتين الحمضى الخيطى الدقيق و ظهرت باهتة مع الباكس التري-ال. كما أظهر الإمداد بهرمون التيستوستيرون حماية ضد التغيرات الهستولوجية السابقة، و ظهرت الخلايا الهرمية بجودة و ساقية متسقة بين الخلايا الطبيعية. وقد أظهرت النتائج انخفاض ذو دلالة إحصائية لمستوى هرمون التيستوستيرون في المجموعة 2 مقارنة بالمجوم.

الاستنتاج: تشبي نتائج هذه الدراسة إلى أن نقص هرمون التيستوستيرون له تأثير ضار على التركيب النسيجي للهيبوكامبس (قرن آمون) و أن التعويض بهذا الهرمون يحمي من هذه التغيرات الهستولوجية في ذكور الجرذان بعد الإخصاء.