Original Article

Immunohistochemical Identification of Oxytocin Neurons in the Suprachiasmatic Nucleus of the Male and Female Albino Rats: A Morphometrical Study

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ABSTRACT

Background: The suprachiasmatic nucleus (SCN) is one of the nuclei in the anterior hypothalamus superior to the optic chiasma. It is well known that vasopressin and oxytocin neurons are located in the hypothalamic para-ventricular and the supraoptic nuclei. Extensive vasopressin neurons were observed in the rostral and middle regions of the suprachiasmatic nucleus.

Aim of the work: Although the presence of oxytocin immunoreactive neurons within the SCN is unusual, this study was designed to clarify immunohistochemical localization of oxytocin neurons in the suprachiasmatic nucleus in the male and pregnant female adult albino rat and to investigate morphometrical differences in those neurons in relation to the gender.

Material and Methods: Twenty adult rats (10male and 10 non pregnant female) were involved in this study. Rats were anaesthetized with ketamine, sacrificed and their brains were carefully dissected. The anterior portion of the hypothalmus containing the suprachiasmatic nucleus was taken. Coronal sections of the brain were prepared for histological stains (Hematoxylin and Eosin) and immunohistochemical stain (Oxytocin immunostaining). The diameters and number of oxytocin labelled neurons in the suprachiasmatic nucleus in each group were measured in a fixed field. The data will be used for morphometrical study.

Results: The SCN in rat was formed mainly of 2 regions according to the change in the shape, rostral SCN (rSCN) and caudal SCN (cSCN).The rSCN appeared as a collection of neurons basal to the third ventricle and superior to the optic chiasma. It showed mixture of bipolar and multipolar cells. The cSCN was formed of mixture of large rounded and small bipolar neurons. The rostral part of the nucleus showed positive OT immune-reactive neurons throughout its structure in both male and female rats, the caudal part of the nucleus exhibited OT immune-reactive neurons in the lateral part only on both sides of the third ventricle, while its medial potion showed negative reaction for OT in both groups.

Conclusion: The present findings proved immunehistochemical identification of OT neurons in the adult male and nonpregnant female rat SCN, and indicate clear sex differences in OT neurons morphometrical measurements which may be parallel to differences in the neuroendocrine and behavioral function in both sexes.

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Key Words: Morphometry, oxytocin, suprachiasmatic nucleus.

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INTRODUCTION

The suprachiasmatic nucleus (SCN) is one of the nuclei in the anterior hypothalamus superior to the optic chiasma[1]. In some animals, the SCN could be divided into two parts; the dorsomedial and ventrolateral[2]. The dorsomedial SCN is rich with arginine-vasopressin (AVP) neurons, while the ventrolateral part contains vasoactive intestinal polypeptide (VIP) neurons and gastrin releasing peptide neurons[3]. In Camel, SCN was described as a bilateral aggregation of neurons extending in rostro-caudal direction. Based on histological results, the SCN showed a distinct topography allowing to subdivide it into rostral (rSCN), main body (mSCN) and caudal (cSCN) divisions[4].

It was reported that the biological rhythms in mammals are controlled by a unique circadian clock in the hypothalamic suprachiasmatic nucleus[5,6]. The SCN has a powerful autonomous oscillator cycles with a period near to 24hours[7].

It is well known that vasopressin and oxytocin (OT) neurons are located in the hypothalamic para-ventricular and the supraoptic nuclei. In addition to these nuclei, those neurons are also located in the hypothalamic periventricular nucleus, perifornical area and the lateral hypothalamus. Extensive vasopressin neurons were observed in the rostral and middle regions of the suprachiasmatic nucleus[8].

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Although the presence of oxytocin immunoreactive neurons within the SCN is unusual, this study was designed to clarify immunohistochemical localization of oxytocin neurons in the suprachiasmatic nucleus in the male and pregnant female adult albino rat and to investigate morphometrical differences in those neurons in relation to the gender.

MATERIALS AND METHODS

Experimental animals

Twenty adult rats (male and non-pregnant female albino rats, 200-300gm weight) were bought from Animal house, Faculty of Pharmacy, Mansoura University, Egypt. The use of experimental animals was prospectively approved Institutional Research Board (IRB) at Faculty of Medicine, Mansoura University. The rats were provided with fresh food and water daily and inspected for any possible signs of infection during the period of acclimatization (2 weeks).

Experimental design

Animals were divided into two groups (10 male and 10 non pregnant female rats).

Histological analysis

After two weeks of acclimatization, rats of each group were anaesthetized with Ketamine (60mg/kg/ip), sacrificed and their brains were carefully dissected[9]. The anterior hypothalmus superior to the optic chiasma nucleus (at level of anterior commissure and continue in rostral direction) was taken and processed for paraffin block preparation. Coronal sections were cut using microtome at thickness 5 µm for histological stains (Hematoxylin and Eosin) and 10µm for immunohistochemical stain (Oxytocin immunostain)[10].

OT Immunohistochemistry

OT Immunohistochemistry was performed using the avidin-biotin method. After deparaffinization, rehydration, and inactivation of the endogenous hydrogen peroxidase, sections were washed for 5 min in phosphate buffer, then were incubated for 72 h at 4 c with primary rabbit polyclonal anti-oxytocin antibodies adilutied at 1:1000 (ABCAM CO.) followed by a biotinylated secondary antibody (goat anti-rabbit IgG, 1:400) for I h at room temperature. The OT immunoreactive neurons showed cytoplasmic brown colour[11].

Image analysis and statistical analysis

Sections were examined using Olympus R digital camera .The number of oxytocin labelled neurons in the suprachiasmatic nucleus in each group were measured in a fixed field in in serial sections. Using the interactive measuring menu, two diagonal diameters of oxytocin neurons were measured in each animal group using an objective lens of magnification x 400.

All measurements were carried out through ImageJ analysis software. Statistical analysis was carried out by SPSS program (statistical package for social science) version 10. The data obtained were subjected to statistical analysis using independent samples t-test. $P<0.05$ was accepted as a signifi¬cant difference.

RESULTS

SCN Morphology and Cytology

The SCN in rat was formed mainly of 2 regions according to the change in the shape, rostral SCN (rSCN) and caudal SCN (cSCN).The rSCN in the male and non-pregnant female rats could be clearly identified from other hypothalamic nuclei in H, E stained sections; It appeared as a collection of neurons basal to the third ventricle and superior to the optic chiasma. It showed mixture of bipolar and multipolar cells (Figure 1). Proceeding in caudal direction, two lateral collection of neurons in SCN, cSCN, could be observed on each side of the third ventricle superior to the optic chiasma. cSCN was formed of mixture of large rounded and small bipolar neurons (Figure 2).

OT immune-reactive neurons

The rostral part of the nucleus showed positive OT immune-reactive neurons (strong brown color extending sometimes to the processes) throughout its structure in both male and non-pregnant female rats (Figure 3). In the same time, the caudal part of the nucleus exhibited OT immune-reactive neurons in the lateral part only on both sides of the third ventricle (Figure 4), while its medial potion showed negative reaction for OT in both groups (Figure 5).

Morphometrical results

1. Number of OT neurons: The number of OT immunoreactive neurons is significantly increased in females in rostral and caudal parts of the nucleus (Table 1, Histogram 1).
2. Size of OT neurons: The size of OT immunoreactive neurons shows insignificant difference between adult male and adult non pregnant female rats in rostral and caudal parts of the nucleus. (Table 2, Histogram 2).

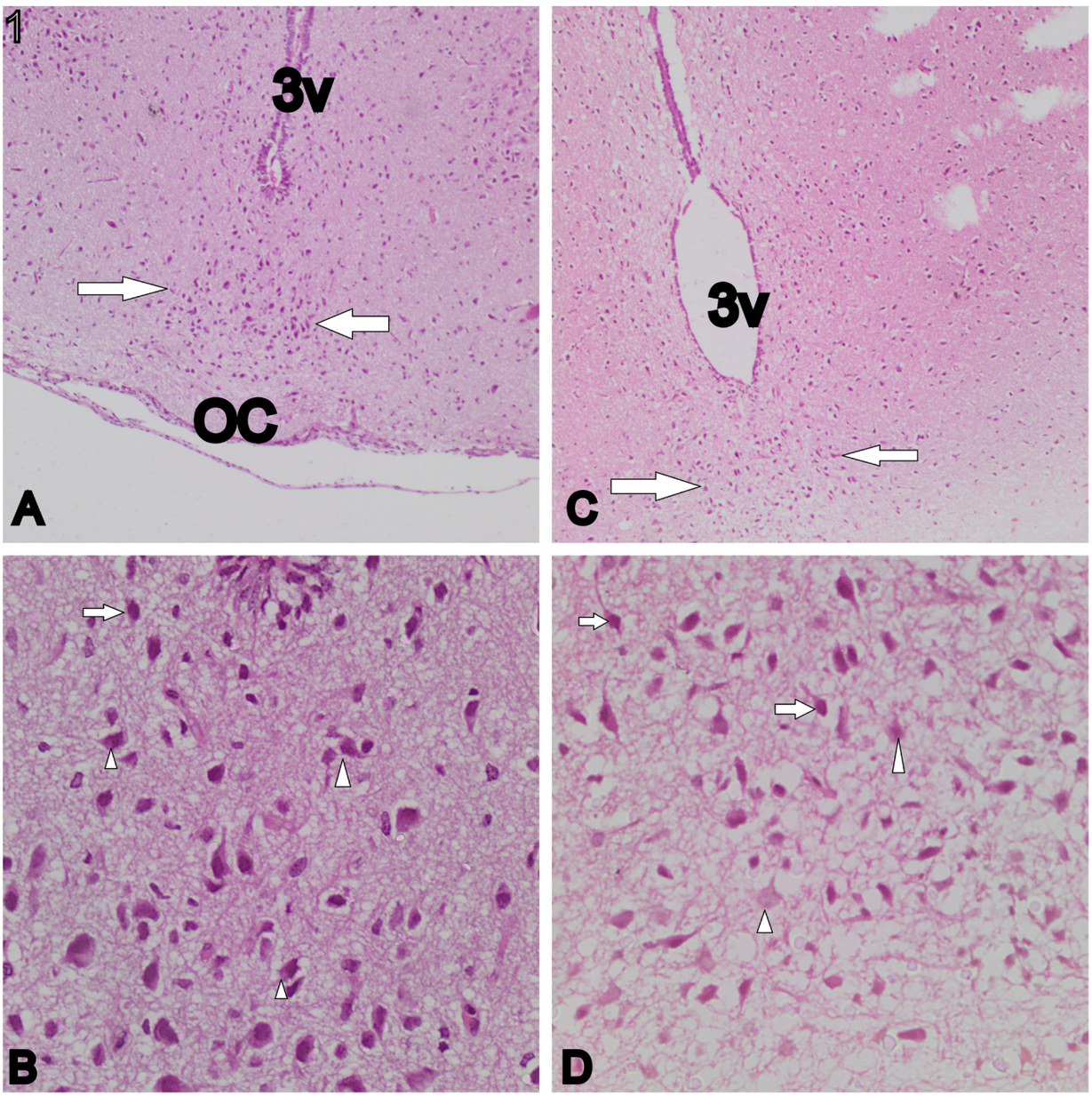


Fig. 1: A,C. photomicrograph of rostral SCN (rSCN) in the male and female rats receptively, appears as collection of neurons (arrows) basal to the third ventricle (3V) and superior to the optic chiasma (OC) (H,E X 100). B, D. higher magnification of Fig A,C, It shows mixture of bipolar (arrows) and multipolar cells (arrow heads) (H,E X 400).

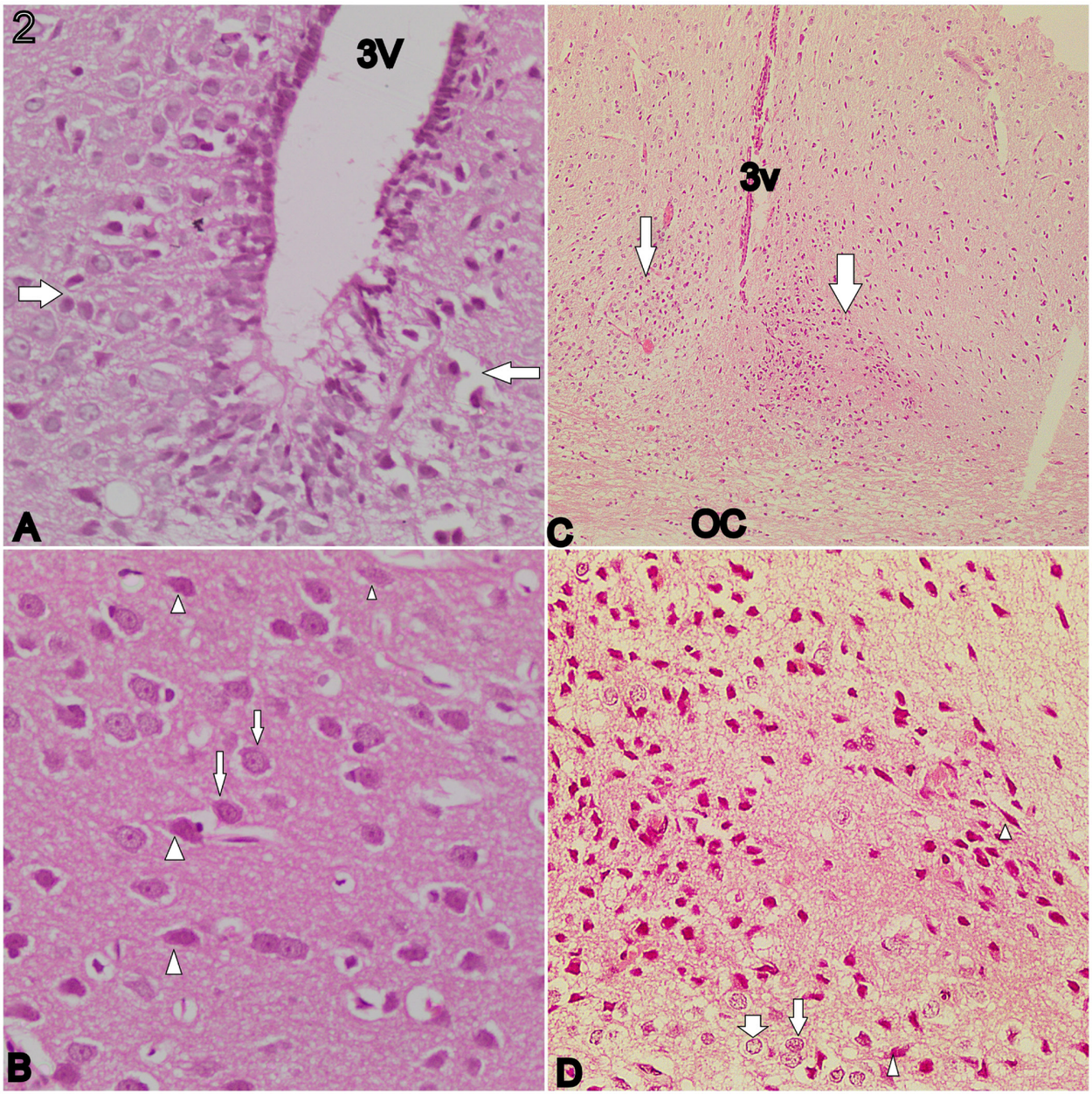


Fig. 2: A,C Photomicrograph of caudal SCN (cSCN) in the male and female rats receptively, appears as two lateral collection of neurons (arrows), could be observed on each side of the third ventricle(3V) superior to the optic chiasma (OC) (H,E X 100). B, D. higher magnification of Fig A, C, It shows mixture of large rounded (arrows) and small bipolar neurons (arrow heads) (H,E X 400).

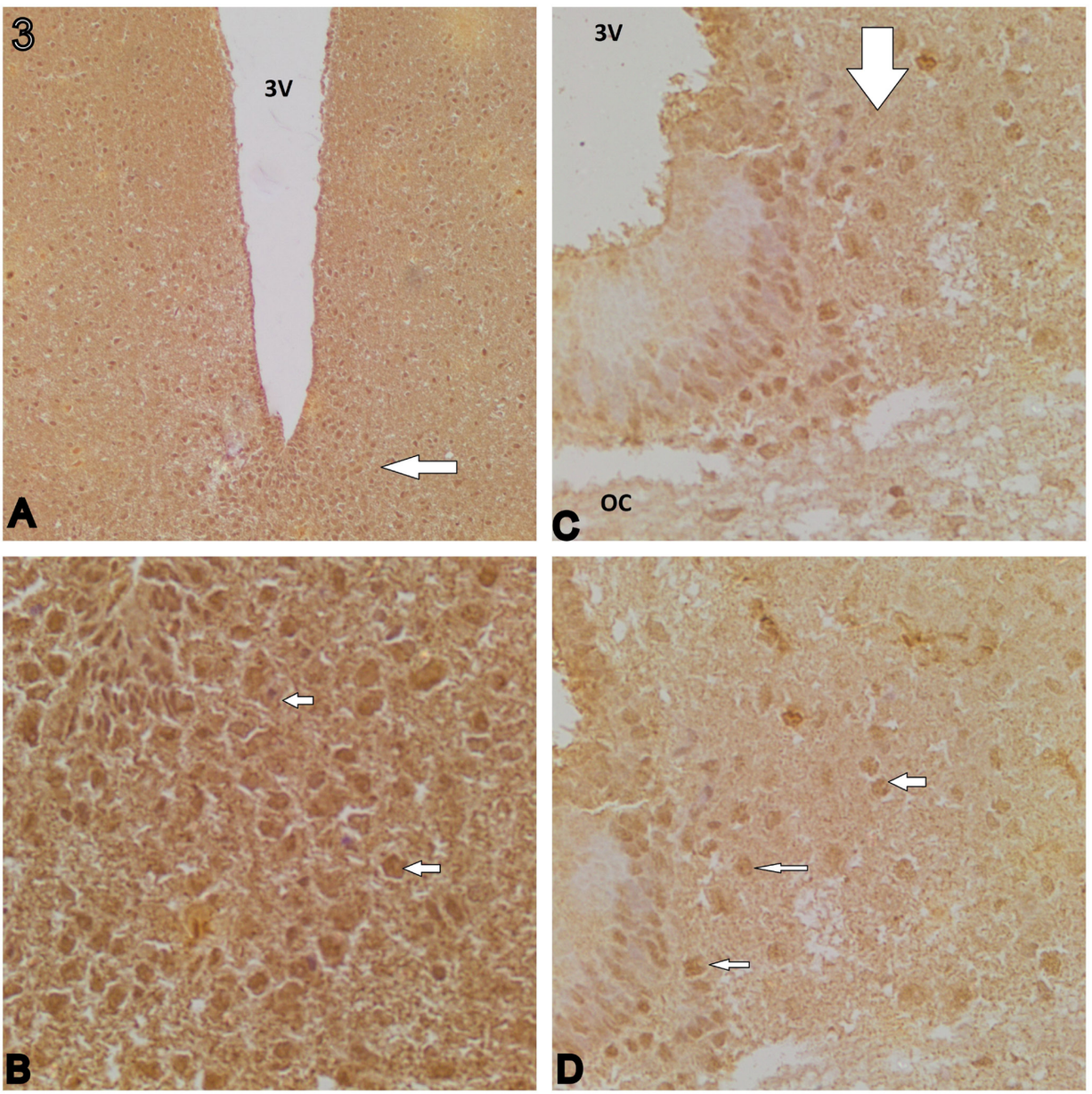


Fig. 3: A,C. Photomicrograph of rSCN in the male and female rats receptively showing positive OT immune-reactive neurons throughout the nucleus (arrows) (OT-immunereactivity X 100). B,C. higher magnification of Fig A,C, shows OT immne-reactive neurons (arrows) (OT-immunereactivity X 400).

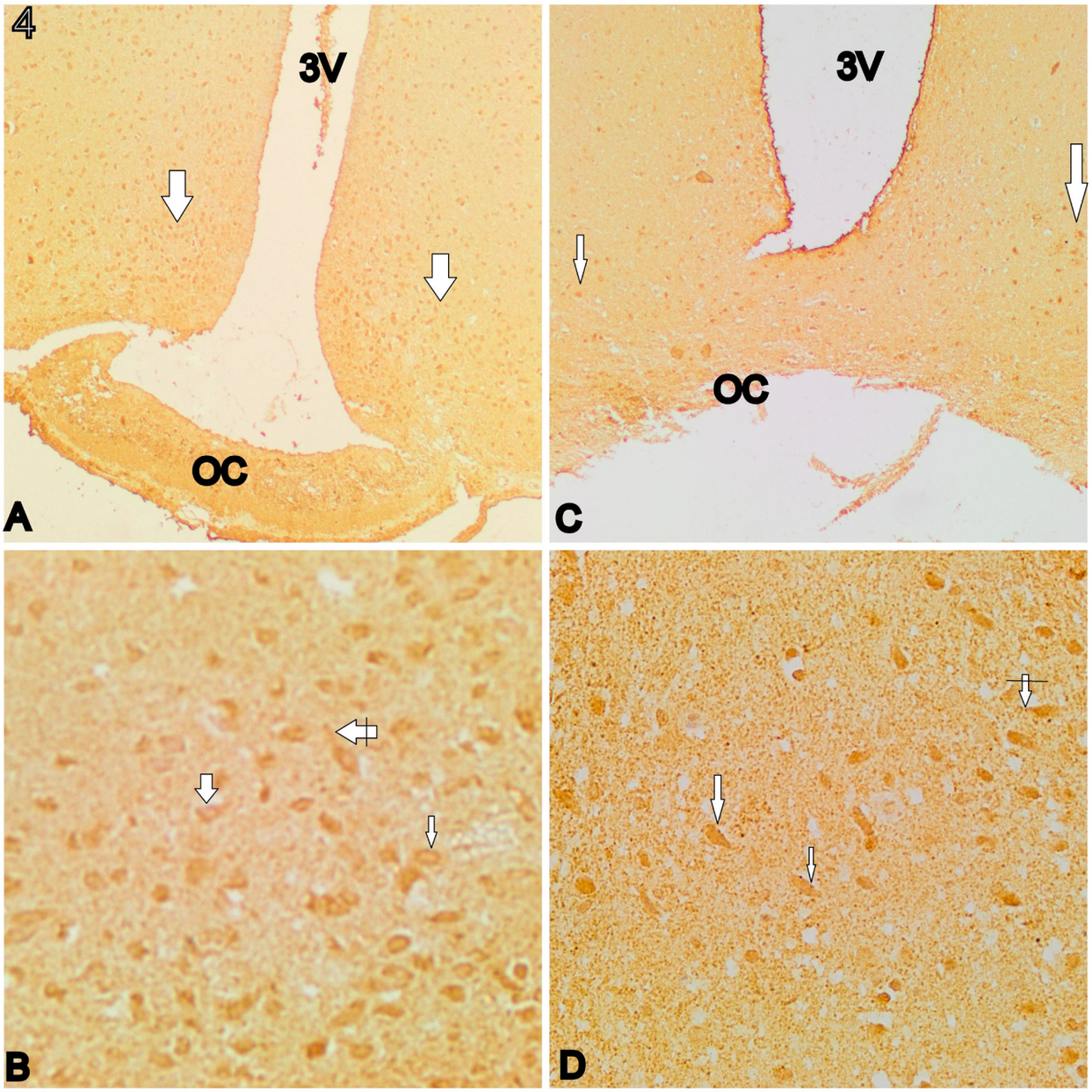


Fig. 4: A.C. Photomicrograph of cSCN in the male and female rats receptively showing positive OT immune-reactive neurons throughout the nucleus (arrows) (OT-immunereactivity X 100). B,C. higher magnification of Fig A,C, shows OT immne-reactive neurons (arrows) with strong brown colour extending sometimes to the processes (crossed arrows) mainly in the lateral part of the nucleus on each side of third ventricle (OT-immunereactivity X 400).

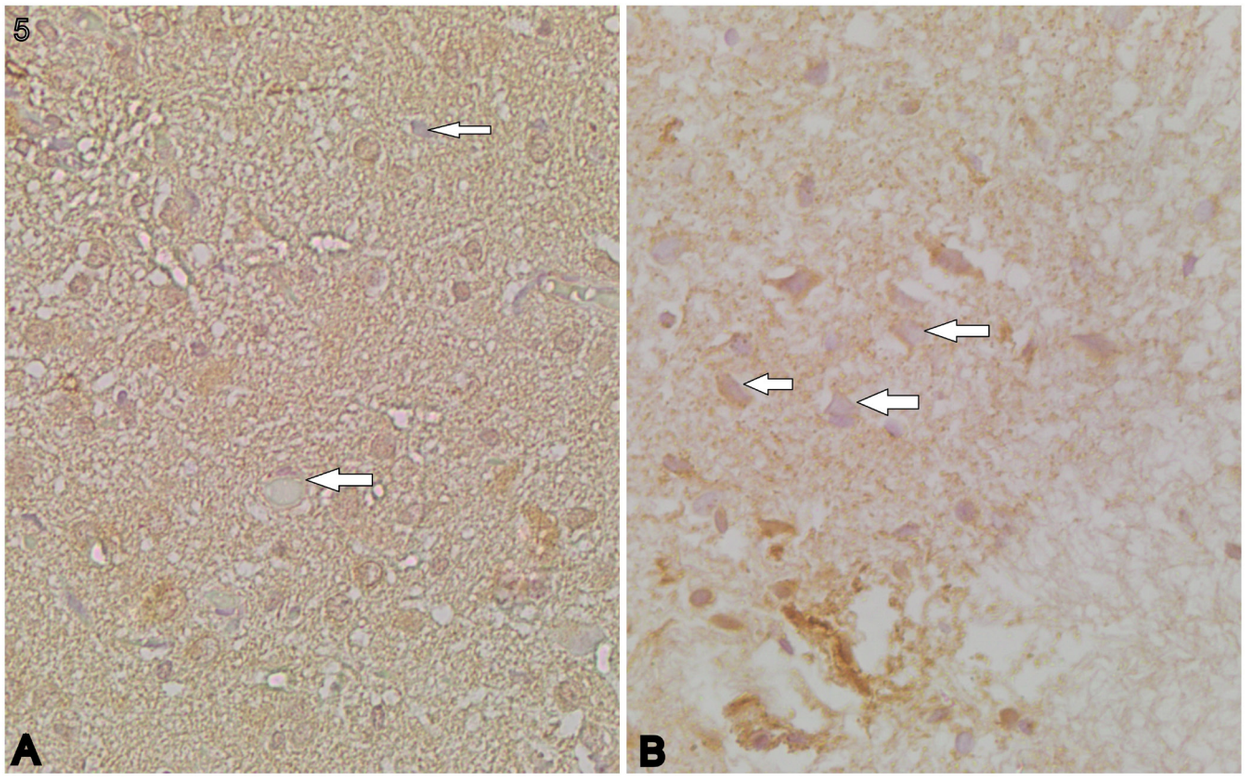


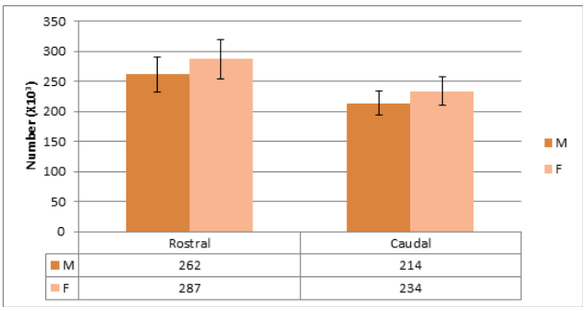
Fig. 5: A,B. Photomicrograph of cSCN in the male and female rats receptively shows its medial potion with negative reaction for OT (arrows) (OT-immunereactivity X 400).

Table 1. Morphometry of OT-immunoreactive neurons in rSCN and cSCN in male and non -pregnant female adult rats (number of neuronsX10³, *=significant ;*P*<.05)

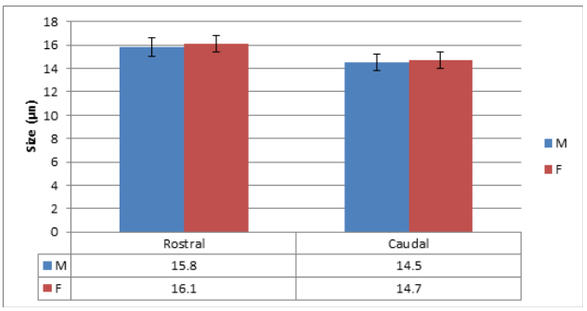
Group	Mean±SD (standard deviation)	SEM (standard error of the mean)	t- test for significance	
			T	P sig.(2-tailed)
rSCN				
Male	262±28.9	.139	59.789	<.000*
Female	287±32.4	.354		
cSCN				
Male	214±20.1	.135	21.123	<.000*
Female	234±23.6	.239		

Table 2: Morphometry of OT-immunoreactive neurons in rSCN and cSCN in male and non -pregnant female adult rats (size in µ, P >.05= insignificant)

Group	Mean±SD (standard deviation)	SEM (standard error of the mean)	t- test for significance	
			T	P sig.(2-tailed)
rSCN				
Male	15.8±3.55	.453	59.789	<.246
Female	16.1±4.71	.481		
cSCN				
Male	14.5±2.8	.202	2.123	<.238
Female	14.7±5.7	.187		



Histogram 1: The number of OT immunoreactive neurons is significantly increased in females in rostral and caudal parts of the nucleus



Histogram 2: The size of OT immunoreactive neurons shows insignificant difference between adult male and adult non-pregnant female rats in rostral and caudal parts of the nucleus.

DISCUSSION

Oxytocin is known to play an essential role in social care, and affiliation[12]. Although, The OT-dependent maternal behavior is estrogen dependent mainly[13], it seems to play also a major role in the male; control of erection, ejaculation and sperm motility[14]. The OT dependence on steroid hormones reflects the sexual dimorphism in the oxytocinergic systems[15]. Androgen receptors (ARs) have been identified in the SCN of the mouse, rat, and human[16]. In mouse, ARs are located mainly in the central region of the nucleus, that receives retinal input[17]. It was reported also that estrogen may act directly on the SCN due to expression of α and β estrogen receptors in the SCN in several mammalian species; humans and rats[18].

The OT immune-reactive neurons presence within the SCN is unusual. The neuropeptides in the SCN were studied in 13 species belonging to 6 mammals, (e.g: marsupials, rodents, lagomorphs, carnivores, artiodactyls, and primates), the SCN in all investigated animals didn't show oxytocin immune-reactive neurons[19,20].

In this study, the rostral part of SCN (rSCN) in the male and non pregnant female rats appeared as a collection of neurons basal to the third ventricle and superior to the optic chiasma. It showed mixture of bipolar and multipolar cells. Proceeding in caudal direction, two lateral collection of neurons in SCN (cSCN) could be observed on each side of the third ventricle superior to the optic chiasma. SCN was formed of mixture of large rounded and small bipolar neurons. Similar results were observed in the SCN of fruit eating bat, it was in the anterior hypothalamus dorsal to optic chiasma, the rostral part was triangular in shape and change caudally to be pear shaped[21]. Also, the SCN in camel was formed of mixture of small cells with triangular, elongated or rounded shape and large irregular cells[4 ,22].

The rostral part of the nucleus showed positive OT immune-reactive neurons throughout its structure in both males and females. In the same time, the cadual part of the nucleus exhibited OT immune-reactive neurons in the lateral part only on both sides of the third ventricle, while its medial potion showed negative reaction for OT in both male and female rats. The OT immune-reactive neurons showed strong brown colour extending sometimes to the processes. This comes in agreement with previous report in camel, OT-ir perikarya were located within the SCN mainly in the ventrolateral part, with round or multipolar soma[4].

In contrast to our finding, it was reported that the monkey SCN demonstrated only vasopressin neurons and fibers in the medial portion of the nucleus while oxytocin neurons and fibers were not identified at all in the nucleus[23].

In this work, numbers of OT neurons were significantly higher in female SCN when compared to male SCN in both rostral and caudal parts. In contrast to our result, no differences have been reported in the number or size of OT immune-reactive neurons in both SON and PVN in the hypothalamus of human[24]. While in animals' brain, the preoptic area showed that OT neurons were more in male than female rats.. However, females expressed more OT immuno-reactive neurons in the retrochiasmatic part of the SON. Those neurons project to the median eminence, to reach the hypophysial portal circulation. OT acts as prolactin - releasing factor[25], and it potentiates LH secretion[26]. These functions are more prevalent in females, this may give reason for the increased OT cells in the rSON of females when compared with males. In addition, estrogen can modulate the expression of oxytocin gene in the rat supraoptic and paraventricular nuclei[27].

In this study, the size of OT IR neurons showed insignificant difference between adult male and adult non pregnant female rats. This comes in agreement with previous reports in human[24] and in rat[28]. They reported that OT immune-reactive cells didn't differ significantly between adult male and female in the supraoptic nucleus.

There a tight connection between the SCN control of circadian rhythm and sexual behavior. Rats and hamsters with SCN lesion showed disturbed rhythm in the sexual behavior; LH surge before ovulation, steroid rhythms and the estrous cycle[29]. In humans, circadian rhythm was reported in LH surge; it occurs in day time for diurnal species[30], while in nocturnal rodents occurs in light off time[31]. The onset of labor also shows diurnal rhythmicity. The membranes rupture usually occur between midnight and 4 am[32]. It was reported that females which had labor induction in the morning with oxytocin had a shorter

duration from induction to labor, when compared to females who had labor induction in the evening[33].

The present findings proved immunehistochemical identification of OT neurons in the adult male and nonpregnant female rat SCN, and indicate clear sex differences in OT neurons morphometrical measurements which may be parallel to differences in the neuroendocrine and behavioral function in both sexes.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

التعرُّف المناعي الكيميائي للخلايا العصبية للأوكسيتوسين في النواة الفوق تصالبة لذكر وانثى الفأر الابيض: دراسة مورفومترية

هاجر عطاالله حشيش

التشريح الآدمي وعلم الأجنة، كلية الطب، جامعة المنصورة

الغرض من هذا البحث: يستهدف هذا البحث دراسة الخلايا العصبية المفرزه للأوكسيتوسين في النواة الفوق تصالبة لمنطقة الهيبوثالاموس لذكر وانثى الفأر الأبيض. وتوضيح الفروق المرفومترية بين الذكر والانثى فى هذه الخلايا.

المواد والطرق المستخدمة: تم استخدام ٢٠ من الفئران البيضاء البالغة، وقسمت الى مجموعتين : ١٠ فئران من الذكور و١٠ من الاناث. تمت التضحية بفئران كل مجموعة ليتم تجميع عينات من المخ وتم تجهيزها لعمل قطاعات من شمع البارافين لتصبغ بالهماتوكسيلين والآيوسين للدراسة الهستولوجية و أيضا بالصبغة المناعية المضادة للأوكسيتوسين وتم استخدام جهاز تحليل الصور لقياس حجم وعددالخلايا المفرزه للأوكسيتوسين، كما خضعت هذه القياسات للعمليات الاحصائية اللازمة.

النتائج: تكونت النواة الفوق تصالبة من جزئين اساسي: جزء امامى وجزء خلفى. احتوت النواة على الخلايا العصبية الصغيرة ثنائية ومتعددة الأقطاب. كونت الخلايا المفرزة للاوكسيتوسين جزءا من النواة الفوق تصالبة حيث احتوى الجزء الامامى للنواة على خلايا الاوكسيتوسين بشكل منتشر بينما الجزء الخلفي احتوى على هذه الخلايا فقط فى اجزاءه الخارجية واختفت خلايا الاوكسيتوسين فى الجزء الداخلي تماما.

يوضح هذا البحث وجود خلايا الاوكسيتوسين فى النواة الفوق تصالبة وكذلك اختلاف اعدادها بين ذكر وانثى الفار الابيض البالغ مما قد يوضح الاختلافات الهرمونية والوظيفية بين الجنسين.