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Sexual dimorphism in rat oxytocinergic hypothalamic regions

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SUMMARY

Oxytocin (OT), a nonapeptide of the hypothalamo-neurohypophyseal system is known to be important for milk ejection and uterus contraction in females and for erection and seminal emissions in males. These sex-specific functional properties imply differential distribution of OT in the hypothalamus. In the present study, complete series of vibratome sections of male and female rat brains were stained for OT with either immunoperoxidase or immunofluorescence in order to evaluate such sex differences. While no significant differences were found in the classical magnocellular nuclei, females had more OT neurons in the zona incerta and the retrochiasmatic portion of the supraoptic nucleus. In males, the lateral subcommissural nucleus and the lateral preoptic area contained significantly more OT perikarya. These sexually dimorphic locations of OT-expressing neurons may be the neuroanatomical correlates of known gender-specific functions of OT.

Key Words: Male and female rat – Sex differences – Oxytocin – Hypothalamus

Abbreviations: al ansa lenticularis; CA anterior comissure; CAI capsula interna; F fornix; FO perifornical neurons; lh laterale intersupraoptic-paraventricular neurons; lo laterale olfactory tract; LPO lateral preoptic area; LSN lateral subcomissural nucleus; mh medial intersupraoptic-paraventricular neurons; MPO medial preoptic area; NPE periventricular nucleus; NPV paraventricular nucleus; SON supraoptic nucleus; NST nucleus septi triangularis; ox optic chiasm; OT Oxytocin; pome medial preoptic nucleus; rSON retrochiasmatic supraoptic nucleus; SM rostral supraoptic nucleus; TS stria terminalis; ZI zona incerta.

INTRODUCTION

The distribution of hypothalamic neurons, expressing the milk-ejecting and labor-inducing posterior pituitary peptide oxytocin (OT), has been studied extensively (Broadwell and Bleier, 1976; Sofroniew, 1985). OT is synthesized in the classical magnocellular nuclei SON (supraoptic nucleus), NPV (paraventricular nucleus) and in other groups of neurons, often referred to as "accessory neurons". OT has been shown to act as a peripheral hormone, as a neurotransmitter and as a neuromodulator in part of the limbic system (for review see Ganten and Pfaff, 1985).

The function of oxytocin in lactation and parturitation is well known (Swaab, 1997; Evans, 1997). OT is also involved in sexual (Caldwell et al., 1989), maternal (Pedersen et al., 1982) and social behavior (Panksepp, 1992; Carter, 1992; Insel et al., 1993; Fahrbach et al., 1985). An inhibitory action on learning and memory has also been shown for OT (Fehm-Wohlsdorf et al., 1984; Arletti et al., 1995).

OT-immunoreactive brain topography is malleable to experimentally and physiologically altered steroid conditions (Rhodes et al., 1981; Jirikowski et al., 1988; Caldwell et al., 1988; Greer et al., 1986). The firing rate of oxytocinergic neurons is increased by estradiol treatment (Akaishi and Sakuma, 1985). In females, OT

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immunoreactive brain topography is affected by sexual stimulation (Caldwell et al., 1988; 1986). The OT-dependent onset of maternal behavior is estrogen dependent (Pedersen et al., 1982; Fahrbach et al., 1984; Kovács, 1986; Caldwell et al., 1986).

While the role of OT in females is quite evident, this nonapeptide also seems be important in the male part of reproduction, controlling erection, ejaculation, smooth muscle contraction and sperm motility, in addition to the above-mentioned behavioral changes (Argiolas et al., 1989; Insel et al., 1993). The dependence of OT function on steroid levels implies the presence of sex dimorphism in the oxytocinergic systems and, indeed, some qualitative and quantitative differences in the distribution of hypothalamic OT have been observed in mice (Häußler et al., 1990). No differences, however, have been found in the size or number of OT neurons in the SON and NPV in the human hypothalamus (Ishunina and Swaab, 1999).

In the present study, we examined the distribution of OT in male and female rats by immunocytochemistry of complete series of vibratome sections.

Materials and Methods

Ten young adult unmated male rats (bw. 300g) and ten intact cycling female rats (bw. 300g) were housed under standard conditions.

Rats were killed by prolonged ether anesthesia, prior to cardiac perfusion with 4% paraformaldehyde in phosphate-buffered saline (PBS). After fixation, brains were removed and postfixed over night in the same fixative at 4°C. Brains were then cut on a vibratome (Cambridge instruments) into series of 100 µm thick frontal sections.

Complete series of sections were incubated with anti-oxytocin antibody (rabbit antiserum, Chemicon, diluted in PBS 1:1000) ovemight at 4° C. Immunocomplexes were visualizted by incubation with anti-rabbit IgG (diluted in PBS 1:100, 30min at RT) followed by peroxidase-antiperoxidase-complex (PAP, diluted 1:100 in PBS, 30 min at RT). Staining was performed with 3',3'–diamino benzidine (DAB) and H_2O_2. All reagents were obtained from Sigma. After staining, sections were mounted on microscopic slides, dehydrated through ascending series of ethanol and mounted in Entellan (Merck).

Another set of sections was stained for immunofluorescence: After incubation with the primary antibody as mentioned above, sections were incubated with goat anti-rabbit IgG-Fab Fragment, conjugated with Cy™3 (Jackson Labs, diluted in PBS 1:1000) for 2 hours. Stained sections were rinsed in 4mM Na_2CO_3, pH9, and mounted in glycerol containing 0.5% propyl gallate.

Immunocytochemical controls were performed on some sections with normal rabbit serum instead of the OT antibody.

Sections were examined with an Olympus BX 50 photo-microscope. Numbers of immunostained perikarya were counted in various hypothalamic regions. Anatomical locations were determined according to the stereotaxic atlas by Paxinos and Watson (1985). Statistical significance was determined using the Mann Whitney's U-test.

Results

OT-immunoreactive perikarya and processes could be seen in both genders in several hypothalamic regions. The PAP method and immunofluorescence revealed identical locations of immunostaining. OT immunoreactivity was in all cases confined to the perinuclear cytoplasm and to processes. Long processes with numerous varicosities were considered axonal pathways. Immunocytochemical control incubations were devoid of staining in all cases. Mean perikaryal diameter was about 15 µm throughout the hypothalamus and no parvocellular OT neurons were observed. OT-positive fibers could be seen especially in the fluorescence preparations. Dense bundles of fibers occurred in the lateral hypothalamus, extending from the NPV towards the median eminence and parallel to the optic tract. Fibers were also seen parallel to the third ventricle, and in the preoptic region, in the bed nucleus of the stria terminalis, and the lateral septum. The distribution of OT-immunoreactive axons appeared to be similar in males and females. The staining intensity and distribution of OT-stained perikarya in the classical magnocellular nuclei NPV and SON as well as in the NPE were comparable in males and females. The same observation was made for perivascular groups of neurons in the lateral hypothalamus. Projections of these neurons stretched along the blood vessels and OT neurons in the PEN seemed to be close apposed to the ventricular lumen as observed with immunofluorescence. Single magnocellular OT neurons could also be seen within the OT-negative parvocellular neurons in the suprachiasmatic nucleus. OT immunoreactivity was found in groups of neurons in the LPO and in the LSN. The numbers of these neurons were much greater in males than in females (Fig. 3 A,B, and C,D). The retrochiasmatic portion of the SON contained large groups of OT-positive cells in females. The numbers of OT cells were much smaller in the male rSON (Fig. 3 E,F). Groups of scattered neurons in the ZI were also more numerous in females than in males, extending intensely stained bundles of fibers laterally to the internal capsule (Fig. 3 G,H). The topographical location of male and female OT-positive perikarya is given in Fig. 2. The numbers of immunostained neurons

were counted in the various hypothalamic regions. The numerical distribution of these observations is given in Fig. 1. While OT-positive fibers were seen in several brain regions stretching to the preoptic region, the CAI, the brain stem and the medulla oblongata, immunostained neuronal perikarya could not be seen outside the hypothalamus.

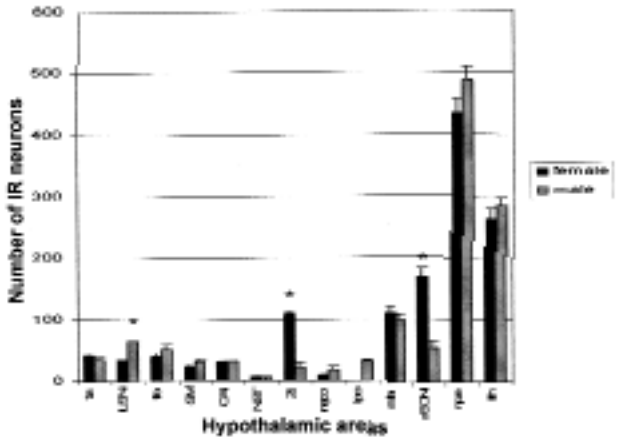

Fig. 1.- Distribution of immunostained neuronal perikarya in the rat hypothalamus. Numbers of neurons in the different nuclei are given as mean ± SEM. Asterisk indicate significance p<= 0,01.

DISCUSSION

Neuroanatomical and neurochemical sex differences have been shown to be the morphological correlates of known neuroendocrine and behavioral differences between males and females. In a previous study, we observed a gender specific distribution of OT-immunoreactive neuronal systems in the mouse hypothalamus (Häußler et al., 1990). While OT probably involved in the control of both the male and female part of reproduction, in females OT has been shown to control not only milk ejection during lactation and labor but also smooth muscle contraction in the pelvic organs during copulation. In addition, OT has a facilitating effect on sexual arousal and maternal behavior. In males, OT is involved in the control of erection and ejaculation by stimulating contractions in the pelvic organs. Furthermore, behavioral changes in the male, associated with mating and parental behavior, are in part dependent on OT. Oxytocinergic neurons in the hypothalamus contribute only in part to the neurohypophysial system. These perikarya are located in the magnocellular portions of the NSO and the NPV. Neuroendocrine projections of these cells are known to project to the posterior lobe to release OT into the systemic circulation. In the present study, we did not find significant gender differences in these nuclei. This is in contrast to our observations in mice, which showed a clear quantitative sex dimorphism in the magnocellular nuclei. Here we used adult and sexually mature but naive rats, which probably had comparatively high levels of OT expression. Due to the enhancing effect of the PAP staining method, quantitative differences of OT immunoreactivity may have escaped detection. We did not perform radioimmunoassays of microdissected NPV and SON to obtain absolute quantitative data. Due to the high numbers of OT Neurons in the NPV and SON, it was not possible to obtain reliable cell counts.

Clear topographical differences were observed in the ZI, the LSN and the lateral MPO. All these regions contain scattered immunoreactive OT neurons with predominantly central projections and no direct connection to the neurohypophysial system. Neurons in the preoptic region and the LSN were more abundant in males than in females. OT neurons in this area have been shown to have projections to the septum and limbic system (Sofroniew, 1985). These neurons seem to be involved in the control of sexual (Caldwell et al., 1988; 1989) and maternal (Pedersen et al. 1982) behavior. While in males gonadotopin release and hence sexual activity is tonic, they may show higher levels of OT in this area than females, which display a cyclic pattern of gonadotropin secretion. Females showed more OT-immunoreactive perikarya in the retrochias-

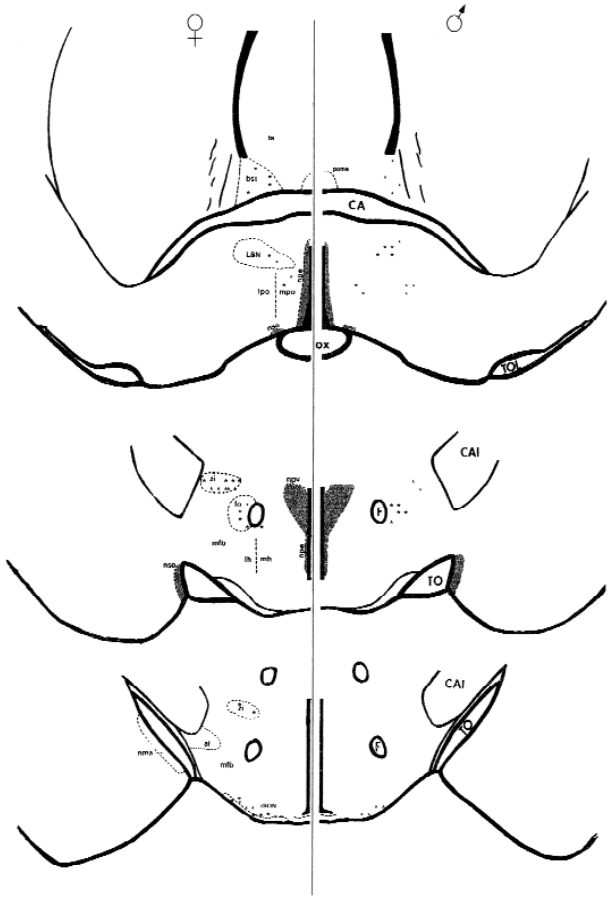

Fig. 2.- Three consecutive planes of the female (left half) and the male (right half) rat hypothalamus. Approximate locations of OT-immunoreactive perikarya are indicated with an asterisk.

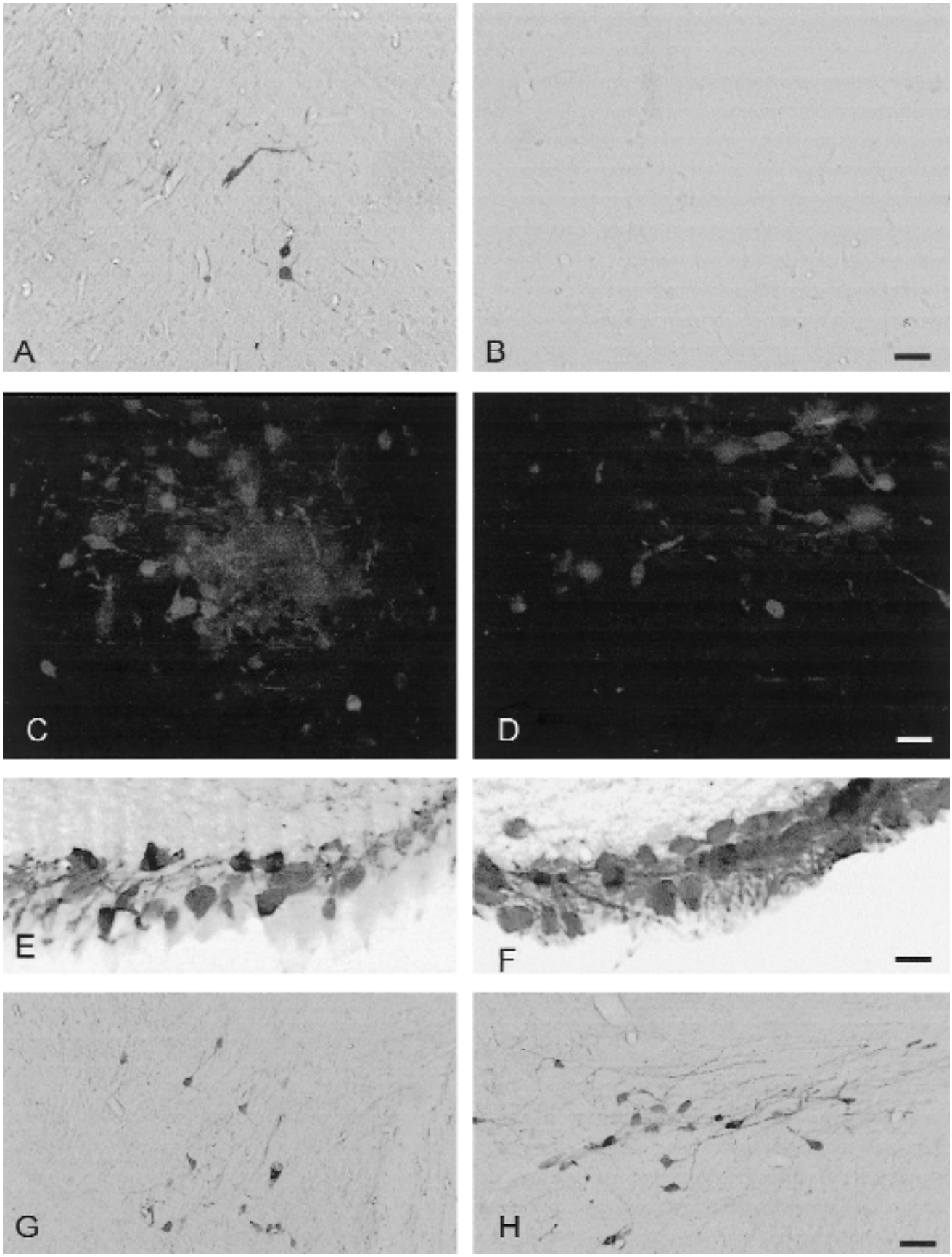

Fig. 3.- OT-immunoreactive neurons in the MPOA (**A**) and the LSN (**C**) of males are more numerous than in females (**B**,**D**). In the rNSO of male rats (**E**) and in the ZI (**G**) the numbers of OT neurons are smaller than in females in the respective areas (**F**,**H**). Scale bars: A, B = 50 µm; C, D = 30 µm; E, F = 15 µm, G, H = 50 µm.

matic portion of the NSO. Neurons in this region project to the internal zone of the median eminence, to gain access to the hypophysial portal circulation. OT is likely to act as prolactin-releasing factor (Johnston and Negro-Vilar, 1988). OT also potentiates the stimulating effects of corticotropin-releasing factor on adenohypophysial corticotrophic cells (Lang et al., 1983) and it enhances LH release (Johnston et al., 1990). These neuroendocrine functions are probably more pronounced in females, accounting for the greater number of OT cells in the rNSO. The ZI also contained many more OT neurons in females than in males. ZI neurons project towards the bed nucleus of the stria terminalis and the internal capsule. To date, the functional importance of the ZI is unclear and so far no correlation with reproductive functions has been observed.

Our present findings indicate clear gender differences in OT distribution in the rat, which may in part parallel differences in neuroendocrine and behavioral function. It is likely that similar differences exist throughout mammalian species, including humans, thus pointing to further sexual dimorphism in the brain.

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