DISTRIBUTION OF OXYTOCIN IN THE SPINAL CORD AND ITS PRESENCE IN THE PERIPHERAL NERVOUS SYSTEM

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Ph. D. Thesis

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Publications related to the thesis

Referred Papers

I. M. Vecsernyés, I. Jójárt, **J. Jójárt**, F. Laczi, F. A. László: Presence of chromatographically identified oxytocin in human sensory ganglia. Brain Res. 414: 153-154 (1987).

II. I. Jójárt, M. Vecsernyés, **J. Jójárt,** F. Laczi, G. Szabó, G. L. Kovács, T. Janáky, F. A. László, G. Telegdy: Acute effects of peripheral histamine administration on arginine-8-vasopressin and oxytocin levels in rat spinal Cord. Endocrinologia Expermentalis 23: 229-233 (1989).

III. M. Vecsernyés, I. Jójárt, **J. Jójárt,** F. Laczi: Chromatographically identified oxytocin in the human peripheral nervous system. Prog In Brain Res. 91: 85-87 (1992).

IV. **J. Jójárt**, I. Jójárt, K. Boda, M. Gálfi, A. Mihály, Zs. B.-Baldauf, M. Vecsernyés: Distribution of oxytocin-immunoreactive neuronal elements in the rat spinal cord. Acta Biol Hung. 60 (4): 333-346 (2009).

Abstract

I. **J. Jójárt,** I. Jójárt, M. Vecsernyés, F. Laczi, G. K. Tóth: Distribution of immunoreactive oxytocin in the rat spinal cord: immunohistochemical localization and quantification by radioimmunoassay. Versammlung der Anatomischen Gesellschaft 86. Anat. Anz. Erg. 172: 132. (1991).

Abbreviations

Astro	astrocyte
b. wt.	body weight
cc	central canal
DGC	dorsal gray commissure
DH	dorsal horn
DR	dorsal root
Ep	ependyma
HPLC	high-performance liquid chromatography
IML	intermediolateral column
LF, Lf	lateral funiculus
LH	lateral horn
LT	Lissauer's tract
mRNA	messenger ribonucleic acid
PAP	peroxidase-antiperoxidase method
PF, Pf	posterior funiculus
pf	pars funicularis of the nucleus intermediolateralis
PLF	posterolateral funiculus
PM	pia mater
pp	pars principalis of the nucleus intermediolateralis
RIA	radioimmunoassay
RT	room temperature
TFA	trifluoroacetic acid
Vmf	ventromedian fissure

Contents

1. Introduction

Oxytocin is a neuropeptide synthesized mainly in the paraventricular and supraoptic nuclei of the hypothalamus. As one of the classical neurohormones, oxytocin is transported to the neurohypophysis where it is stored and released to the blood circulation. It plays an important role in neuroendocrine regulation of parturition and lactation (Silverman and Zimmerman, 1983; Sofroniew, 1985). Oxytocin is present not only in the hypothalamo-neurohypophyseal system, but is also located in various areas of the central and peripheral nervous system (Buijs, 1978, 1980; Swanson and McKellar, 1979; Jenkins et al., 1984; Sequeira and Chaiken, 1984; Kai-Kai et al., 1985; Sofroniew, 1985). Furthermore, numerous studies have shown that oxytocin mRNA is present in peripheral tissues such as decidua, chorion, amnion, ovary, testis and heart (Chibbar et al., 1993; Furuya et al., 1995; Einspanier and Ivell, 1997; Inaba et al., 1999; Gutkowska et al., 2000). Locally synthetized oxytocin can stimulate uterine contractions in the time of parturition in a paracrine fashion without increasing circulating maternal oxytocin concentrations (Chibbar et al., 1993). Actions of local oxytocin in the ovary are linked to luteinization, luteolysis and steroidogenesis (Einspanier et al., 1997). Oxytocin may be involved in the local control of androgen biosynthesis and spermatogenesis in the testis (Assinder et al., 2000). The heart and large vessels like aorta and vena cava are also sites of oxytocin synthesis and the locally produced oxytocin may have important regulatory functions within the heart and the vascular system (Gutkowska et al., 2000).

1.1 Oxytocin in the spinal cord

A growing body of knowledge has accumulated on the location, origin, putative roles and regulation of oxytocin in the spinal cord. The majority of oxytocin-containing fibers in the spinal cord were shown to arise from the hypothalamic paraventricular nucleus (Sawchenko and Swanson, 1982; Lang et al., 1983; Hawthorn et al., 1985; White et al., 1986; Halbeck et al., 2001). Hosoya et al. (1995) reported that oxytocinergic fibers descending from the paraventricular nucleus make contacts with preganglionic neurons in the rat spinal cord, using a combination of retrograde cell body labelling and immunocytochemistry. Rousselot et al. (1990) described oxytocinergic

terminals in the dorsal horn, central gray and intermediolateral column by means of electron microscopic immunocytochemistry. The distribution of the oxytocinergic hypothalamo-spinal neuronal projections shows a good overlap with that of the spinal oxytocin receptors (Loup et al., 1989; Reiter et al., 1994; Tribolet et al., 1997), supporting the view that oxytocin acts as a neurotransmitter in the central nervous system (Buijs, 1983; Raggenbass, 2001). Several lines of evidence suggest that central oxytocin is an endogenous modulator of various spinal functions. It has been shown to induce spinal antinociception through activation of oxytocin receptors (Arletti et al., 1993; Robinson et al., 2002; Condes-Lara et al., 2003; Yu et al., 2003; Yang et al., 2007). In addition, several studies indicate the involvement of oxytocin in the regulation of spinal autonomic functions: it can inhibit sympathetic preganglionic neurons of the spinal cord (Gilbey et al., 1982), plays an important role in the control of penile erection and male sexual behaviour (Argiolas et al., 2004), mediates the pupillary dilatation response to vaginocervical stimulation (Sansone and Komisaruk, 2001), modulates the micturition reflex (Pandita et al., 1998) as well as uterine motility (Benoussaidh et al., 2004), and it can increase the heart rate and renal sympathetic activity (Yang et al., 2002). Experiments have also been directed at defining physiological and pharmacological stimuli that can alter the spinal levels of oxytocin. Spinal oxytocin displays cyclic variations during the stages of the rat estrous cycle, suggesting that it may be regulated by ovarian hormones (Miaskowski et al., 1987). Evidence has been provided for a direct estrogen-dependent release of oxytocin within the spinal cord in response to vaginocervical stimulation (Sansone et al., 2002). Various other stimuli, such as immobilization stress (Miaskowski et al., 1988), isotonic or hypertonic saline (Lukic and Haldar, 1993) also affect the oxytocin content in the spinal cord. In addition, oxytocin release from spinal cord synaptosomes seems to be under inhibitory control of opioid peptides (Daddona and Haldar, 1994)

1.2 Oxytocin in the peripheral nervous system

Data available on the occurrence, origin and role of oxytocin in the peripheral system is yet scanty. Oxytocin immunoreactivity was localized by immunohistochemistry in neurons of rat sensory ganglia and characterized by high-performance liquid chromatography (Kai-Kai et al. 1985, 1986). Immunoreactive oxytocin detected in extracts of human lumbar sympathetic paravertebral ganglia was

identified chromatographically as being authentic oxytocin (Vecsernyés et al., 1990). Occurence of oxytocin has been also revealed in peripheral nerves (Sequeira and Chaiken, 1984). Recently, transcripts for oxytocin and its receptor was found in ganglion cells of myenteric and submucosal plexuses. In addition, oxytocin and oxytocin receptor proteins have been demonstrated by immunohistochemistry in the enteric nervous system of the rat (Welch et al., 2009).

1.3 Rationale and objectives

I. Although several studies have reported the localization of oxytocin- and/or neurophysin-immunoreactive fibers in the rat spinal cord (Buijs, 1978; Sofroniew and Weindl, 1978; Swanson and McKellar, 1979; Nilaver et al., 1980; Sofroniew, 1980; Gibson et al., 1981; Sofroniew et al., 1981; Krukoff et al., 1985), most of them lack a detailed analysis of their segmental distribution. A truly detailed and comprehensive study was reported by Swanson and McKellar (1979), although they demonstrated only neurophysin I (the carrier protein of oxytocin) staining, presumably due to more intense staining obtained for neurophysin as compared to oxytocin. We have therefore sought to examine the distribution of oxytocin-immunoreactive neuronal elements in the rat spinal cord, using a specific oxytocin antibody for light and electron microscopic immunocytochemical localization and quantitation by RIA.

II. Central histaminergic neurons have been shown to be involved in the control of oxytocin secretion under various physiological conditions including parturition and lactation, and both central and peripheral administration of histamine increases plasma oxytocin level (Knigge and Warberg, 1991; Bealer and Crowley, 1999; Knigge et al., 1999). However, the literature falls short of providing sufficient data about the effect of peripheral histamine administration on the oxytocinergic neuronal systems of the central nervous system (Mens, 1982; Mens et al., 1983). Our objective was to examine whether oxytocin levels change in the spinal cord after peripheral administration of histamine.

III. There is scanty information available on the occurrence of oxytocin in the peripheral nervous system (Sequeira et al., 1984; Kai-Kai et al., 1985, 1986; Vecsernyés et al., 1990). In order to obtain data in the human, sensory and sympathetic ganglia as well as peripheral nerves have been examined for the presence of oxytocin using high-performance liquid chromatography and radioimmunoassay.

2. Subjects and methods

2.1 Identification of immunoreactive oxytocin in rat spinal cord

2.1.1 Light microscopic immunohistochemistry

The experiments were carried out on 12 male CFY rats weighing 200 - 310 g. The animals were anaesthetized with sodium pentobarbital and perfused through the heart with isotonic saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The spinal cords were then removed and kept in the same fixative for 4-5 hours. Transverse and horizontal sections 50-70 µm thick were cut on a Vibratome (Oxford Instruments). Oxytocin was localized by means of the peroxidase-antiperoxidase (PAP) method (Sternberger et al., 1970), using a rabbit antiserum to oxytocin (kindly provided by Prof. Tj. B. Van Wimersma Greidanus, Utrecht, The Netherlands). Briefly, the following incubations were performed: (1) 10% normal goat serum containing 1.5% Triton X-100 for 30 minutes at room temperature (RT), (2) rabbit antiserum to oxytocin (diluted 1/2000) containing 1% normal goat serum and 1.5 % Triton X-100 for 40-48 hours at 4 oC, (3) goat antiserum to rabbit IgG (diluted 1/20; Serono) for 2 hours at RT, (4) rabbit PAP (diluted 1:50; Human Oltóanyagtermelő Vállalat, Budapest) containing 1% normal goat serum for 2 hours at RT, and (5) 0.05% 3,3'-diaminobenzidine tetrahydrochloride containing 0.005% H_20_2 for 5-15 minutes at RT. All reagents were diluted in 0.1 M Tris-saline, pH 7.6. Between the successive incubations, sections were washed with 0.1 M Tris-saline, pH 7.6 at RT. After visualization of the immunoreactivity in step (5), sections were washed, mounted, Nissl-counterstained in some cases, dehydrated, cleared in xylene and coverslipped. When the specificity of the staining was tested, no staining could be found after replacement of the primary antiserum by normal rabbit serum at the same dilution. In immunohistochemical blocking experiments, consecutive sections were incubated with either anti-oxytocin serum or anti-oxytocin serum preincubated with arginine vasopressin or oxytocin for 24 hours at 4 oC. Preincubation of the diluted antiserum with as little as 20 µM oxytocin produced a complete blockade of staining, whereas as much as 200 µM arginine vasopressin did not influence the immunoreactivity.

2.1.2 Electron microscopic immunocytochemistry

Four male CFY rats weighing 200-310 g were anaesthetized with sodium pentobarbital and perfused through the heart with isotonic salt solution followed by a fixative containing 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Subsequently, the spinal cords were removed and kept in the same fixative for 4-5 hours. For preembedding staining, 40-50 µm thick cross-sections were cut with Vibratome. The sections were infiltrated with 30% sucrose, rapidly frozen in liquid nitrogen and thawed in 0.1 M phosphate buffer. Oxytocin immunoreactivity was localized by means of the PAP method (see 2.1.1). The Vibratome sections were osmicated, dehydrated and embedded in Durcupan (Fluka). Finally, ultrathin sections were made and examined under the electron microscope.

2.1.3 Radioimmunoassay (RIA)

Twenty male CFY rats of 220-250 g body weight were decapitated and their spinal cords were quickly dissected into cervical, thoracic, lumbar, sacral and coccygeal regions. Tissue samples were sonicated in ice-cold 1.0 M HCl. Subsequently, phosphate buffer was added and the pH was adjusted to 4.0. An aliquot of the homogenate was kept for determination of the protein content (Lowry et al., 1951). The remainder was centrifugated, and oxytocin was extracted from the supernatant using thermally activated Vycor glass powder. The evaporated residues of the extraction were redissolved in RIA buffer. Oxytocin was determined in duplicate by RIA. Details of the assay procedure has been described elsewhere (Dogterom et al., 1977). The same antiserum to oxytocin was used as that for immunocytochemistry. The antiserum was highly specific, its cross-reactivity with arginine vasopressin being less than 0.1%, and that with arginine vasotocin less than 0.01%. The limit of detection was 2 pg oxytocin per assay tube. Oxytocin concentrations were given in pg/mg protein. Data are expressed as mean ± SE. Results were calculated statistically by two-way ANOVA without interaction, followed by Scheffe’s test for multiple comparison.

2.1.4 Measurement of oxytocin after histamine treatment

The experiments were performed in male Wistar rats weighing 180-220 g. The animals were maintained on a 14 h light/10 h dark cycle (lights on between 06.00 and 20.00 h) and allowed to consume standard food and tap water ad libitum. They were injected intraperitoneally (i.p.) with an aqueous solution of histamine dihydrochloride (Peremin, CHINOIN, Budapest, Hungary) in a dose of 20 mg/kg b. wt. in a volume of 0.04 ml/10 g b. wt. The controls received physiological saline in the same volume. Fifteen min after the treatment the animals were killed by decapitation. Subsequently, the spinal cords were removed and their oxytocin contents were determined by RIA as described under 2.1.3. The limit of detection was 1.99 fmol oxytocin per assay tube. Oxytocin concentrations were given in fmol/mg protein. The statistical evaluation was performed with the Student's t-test. Data are expressed as mean ± SE.

2.2. Chromatographical characterization of oxytocin in human ganglia and peripheral nerves

Four pieces of Gasserian ganglia, two pieces of L_5 ganglia, two pieces of celiac ganglia and four pieces of vagal nerves (cumulative specimens from cervical and thoracic portions) were obtained at routine post-mortem dissection from males who died without a history of any neuropsychiatric and endocrine illness. The age of the subjects ranged from 50 to 71 years, and the time elapsed from death to removal of the ganglia was 10-42 h. After removal, the samples were stored on dry ice until homogenization.

2.2.1 HPLC/RIA method applied to samples of Gasserian and L5 ganglia

The ganglia were cut into small pieces and sonicated in 1 M HCl (1 ml HCl/100 mg wet weight tissue). The acid extracts were centrifugated and the supernatants were applied to SEP-PAK C_{18} cartridges (Waters Assoc., U.S.A.) which were then washed with 0.1% trifluoroacetic acid (TFA). The peptides were eluted from the cartridges with 3 ml aqueous methanol of gradually increasing methanol concentrations (25-50-75% v/v) containing 0.1% TFA. A 1 ml aliquot of each eluted fraction was evaporated to dryness under a nitrogen stream and subjected to RIA analysis for oxytocin (Dogterom et al., 1977; Kovács et al., 1985). The immunoreactive material eluted with 0.1% TFA-

methanol (50%, v/v) was identified with a high-performance liquid chromatography (HPLC) system. The eluate was evaporated to dryness under a nitrogen stream and the residue was dissolved in 100 μl distilled water. A Knauer 64 high-pressure liquid chromatograph was used to characterize oxytocin-like immunoreactivity. The samples were loaded onto a Lichrosorb 10 RP-18 column (260 mm x 4.6 mm; Merck), and the immunoreactive material was eluted with an isocratic eluent containing methanol (42%, v/v) in 0.1% TFA. The flow rate was 1.0 ml/min and fractions for RIA were collected at 1 min intervals to 50 min. To avoid contamination, the samples were run in the sequence: blank (distilled water), ganglion extracts and standard.

2.2.2 HPLC/RIA method applied to samples of celiac ganglia and peripheral nerves

Essentially, the same procedure was used as that described under 2.2.1, but with some modifications. Tissues were cut into small pieces and homogenized in 1 M HCl (1 ml HCl/100 mg wet weight tissue). The acid extracts were centrifugated and the supernatants were applied to SEP-PAK C_{18} cartridges (Waters Assoc., U.S.A.) which were then washed with 0.1% TFA. The immunreactive material was eluted from the cartridges with 3 ml aqueous methanol (80% v/v) containing 0.1% TFA. 1 ml aliquot of each eluted fraction was evaporated to dryness under a nitrogen stream and subjected to RIA analysis for oxytocin (Dogterom et al., 1977; Kovács et al., 1985). The remaining 2 ml eluates were pooled, partially evapurated and liophylized. The residue was dissolved in 1000 μl aqueous methanol (30% v/v) containing 0.1 % TFA. A Knauer 64 HPLC equipped with an AX-II computer system controlling the gradient elution was used to characterize oxytocin-like immunoreactivity. An aliquot of the dissolved samples was loaded onto a Si-100-S 5 RP-18 column (260x 4.6 mm; BST, Budapest, Hungary) and eluted in a gradient system with 30-60 % aqueous methanol containing 0.1% TFA. To avoid contaminations (Fischman et al., 1984), specimens were run in the sequence: blank (30% aqueous methanol containing 0.1% TFA), pooled nervus vagus extract, pooled ganglion extract and oxytocin standard (approx. 0.5 ng). The flow rate was 1 ml/min. The fractions were collected at 1-min intervals for up to 50 min and subjected to RIA.

Results

3.1 Immunoreactive oxytocin in the rat spinal cord

3.1.1 Light microscopic distribution of oxytocin-immunoreactive fibers

Each segment of the spinal cord contained oxytocin-immunoreactive fibers. However, no cell bodies were labelled. The schematic distribution of oxytocin-immunoreactive fibers is presented in Fig. 1. In the gray matter, the most intense immunoreactivity was observed at C_8-T_4 and T_{13}-L_4 levels. The superficial dorsal horn (Rexed laminae I and II) displayed moderate to intense immunoreactivity throughout the entire spinal cord. Immunoreactive fibers were found mainly in the marginal zone (Rexed lamina I) with extensions into the subjacent laminae II and III (Fig. 2). A longitudinally oriented network of labelled fibers was detected around the central canal (Fig. 3A, B). The strongest labelling was observed in the upper and lower thoracic, and lumbar segments. Some fibers were seen to closely appose the ependymal layer. A preferential accumulation of labelled fibers was observed in the dorsal gray commissure (Figs. 3B, 4A) in the thoracic, lumbar and upper sacral spinal cord. At the same levels, strong patchy accummulations of fibers were found in the intermediolateral column (Fig. 4B, C, D, E). These immunoreactive patches were interconnected by a network of horizontally running fibers (Fig. 4B, D). The perikarya of the preganglionic autonomic areas seemed to be surrounded by immunoreactive fibers (Fig. 3B, 4E). In the lamina VII of segments C_8-T_4 and T_{13}-L_4, strong immunoreactive bundles at irregular intervals coursed between the dorsal gray commissure and the intermediolateral column (Fig. 4A, B). This location corresponded to the area of nucleus intercalatus spinalis; for designation of specific autonomic nuclei, see Petras and Cummings (1972). Fibers around motoneurons of the anterior horn were labelled mostly in the C_5-C_7 and L_4-L_6 segments (Fig. 5). The rest of the gray matter contained very few labelled fibers.

In the white matter, the majority of labelled fibers were detected in the dorsal part of the lateral funiculus in the entire spinal cord (Fig. 6C, D). We frequently observed stained fibers in the Lissauer's tract in all spinal segments (Fig. 6C). Some of these fibers were found to enter the marginal zone of the dorsal horn. Fibers were often extending between the dorsal part of the lateral funiculus and superficial dorsal horn,

Lissauer's tract, intermediolateral column and dorsal gray commissure. In the lateral funiculus, reactive fibers were seen to leave the intermediolateral column and appear around the preganglionic neurons of the pars funicularis of nucleus intermediolateralis (Fig. 6A).

Numerous fibers formed a longitudinally oriented network observed in horizontal sections. This network was the densest below the pia mater, and some fibers did enter it (Fig. 7A, B). Oxytocin-immunoreactive fibers were also seen close to the subarachnoid space at the Lissauer's tract and dorsal part of the lateral funiculus (Fig. 6C, D). Occasional fibers were labelled in the anterior funiculus (Fig. 6B), mostly in the lumbar segments. These fibers entered the anterior funiculus from the network around the central canal, and a few of them run to the surface of the ventral median fissure (Fig. 6B). The posterior funiculus contained only a few scattered reactive fibers.

In horizontal sections of the spinal cord longitudinally oriented oxytocin-immunoreactive networks were detected around the central canal (Fig. 3A), in the dorsal gray commissure (Fig. 4A), superficial dorsal horn, Lissauer's tract, dorsal part of the lateral funiculus and lateral funiculus (Fig. 7B).

3.1.2 Electron micropscopic identification of oxytocin-immunoreactive terminals

Oxytocin-immunoreactivity was seen in unmyelinated fibers of small diameter and axon terminals in the laminae I, II, VII and X of the spinal gray matter. No immunoreactive cell bodies were labelled. Oxytocin-containing axon terminals formed mainly axo-dendritic synapses (Fig. 8, 9). We also found a few axo-somatic synaptic contacts with presumptive neuronal cell bodies located in the ependymal lining of the central canal (Fig. 10). In most cases, the electron dense peroxidase immunoprecipitate completely filled the cytoplasm of the axon terminals and masked the subcellular organelles and synaptic densities. When the reaction product was lightly distributed, it was possible to see that the immunoreactive terminals contained numerous small clear vesicles and a few large dense-cored vesicles (Fig. 9).

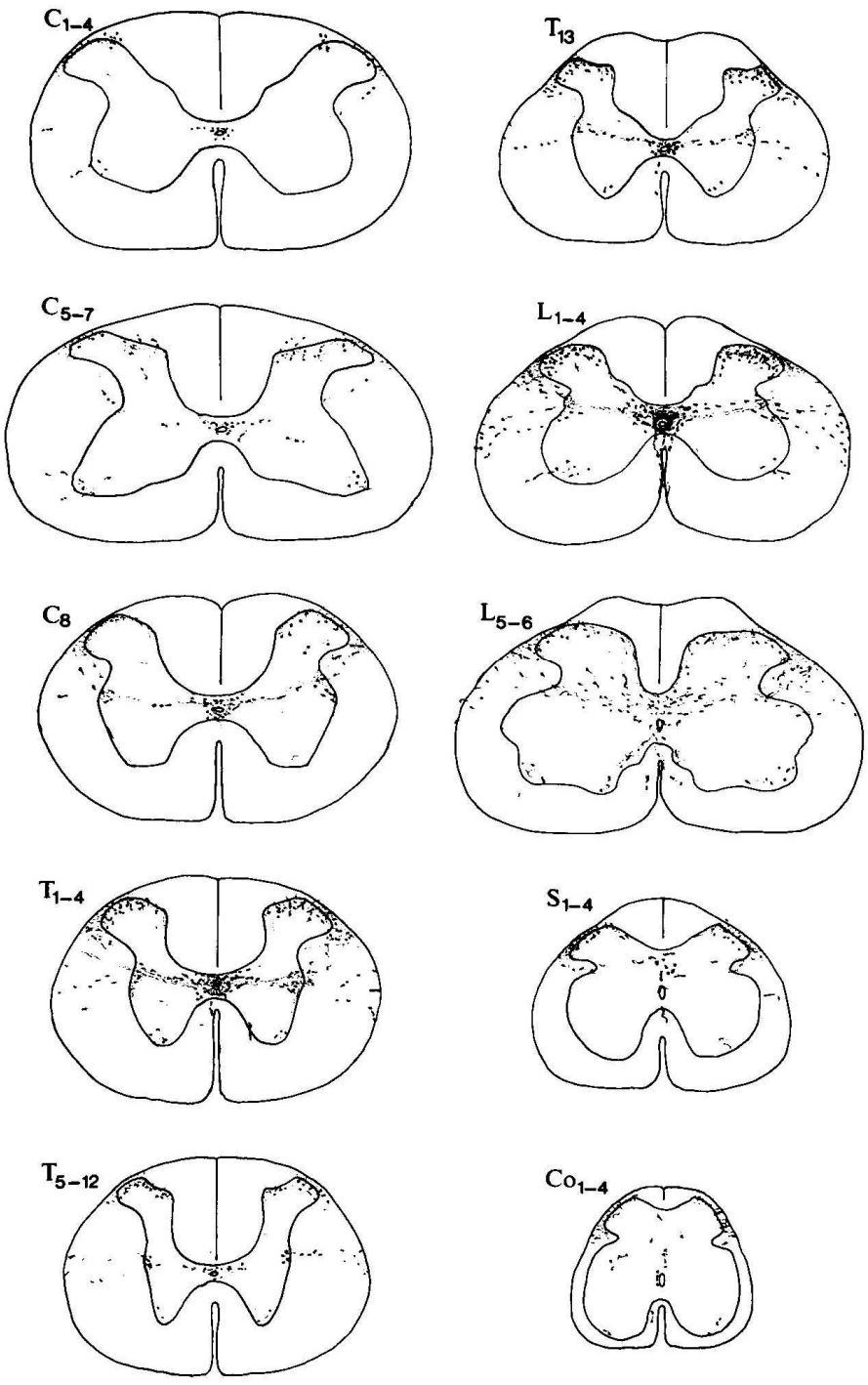


Fig. 1. Schematic distribution of oxytocin-immunoreactive fibers in the rat spinal cord.

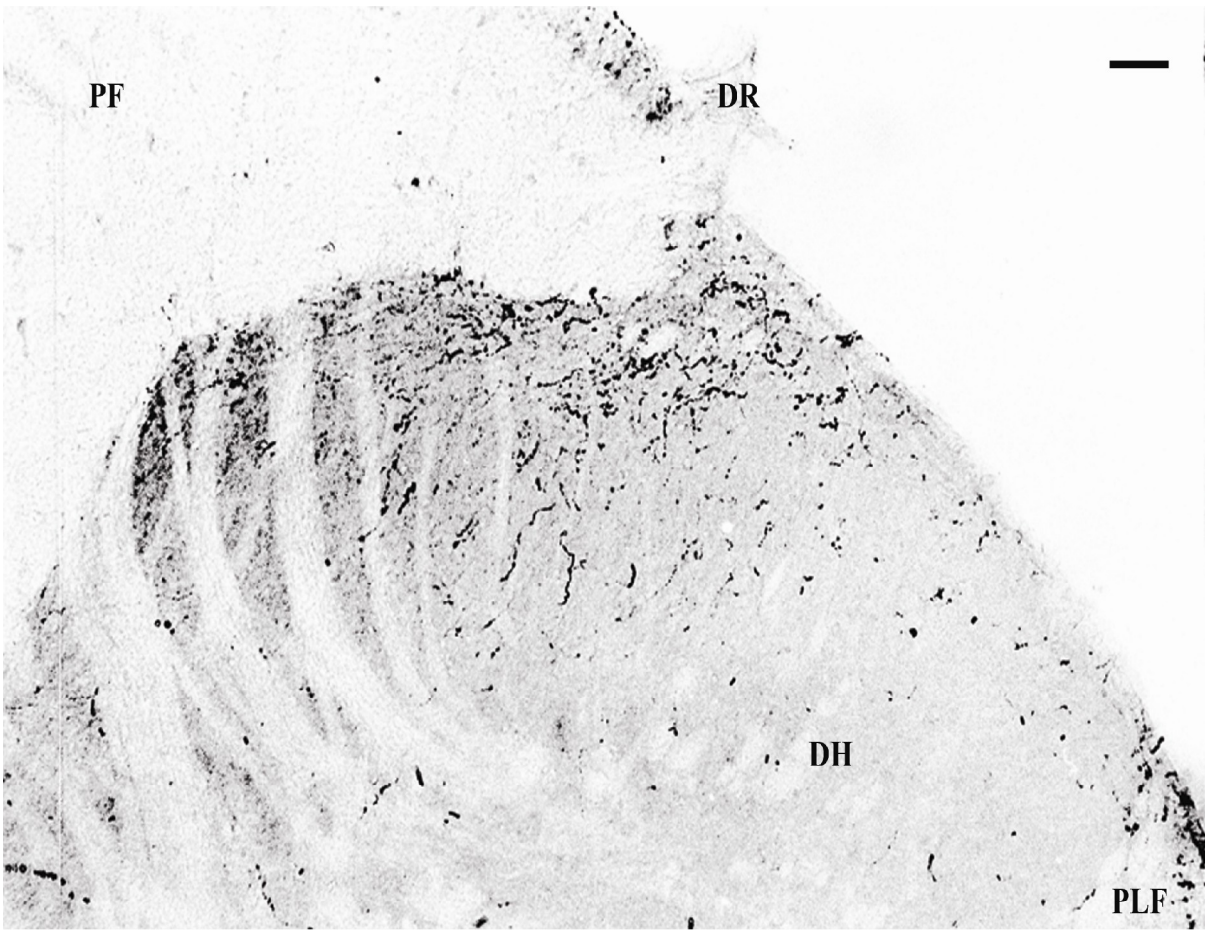


Fig. 2. Photomicrograph showing oxytocin labelled fibers in the upper dorsal horn. Abbraviations: DH, dorsal horn; DR, dorsal root; PF, posterior funiculus; PLF, posterolateral funiculus. Scale bar: 100 µm.

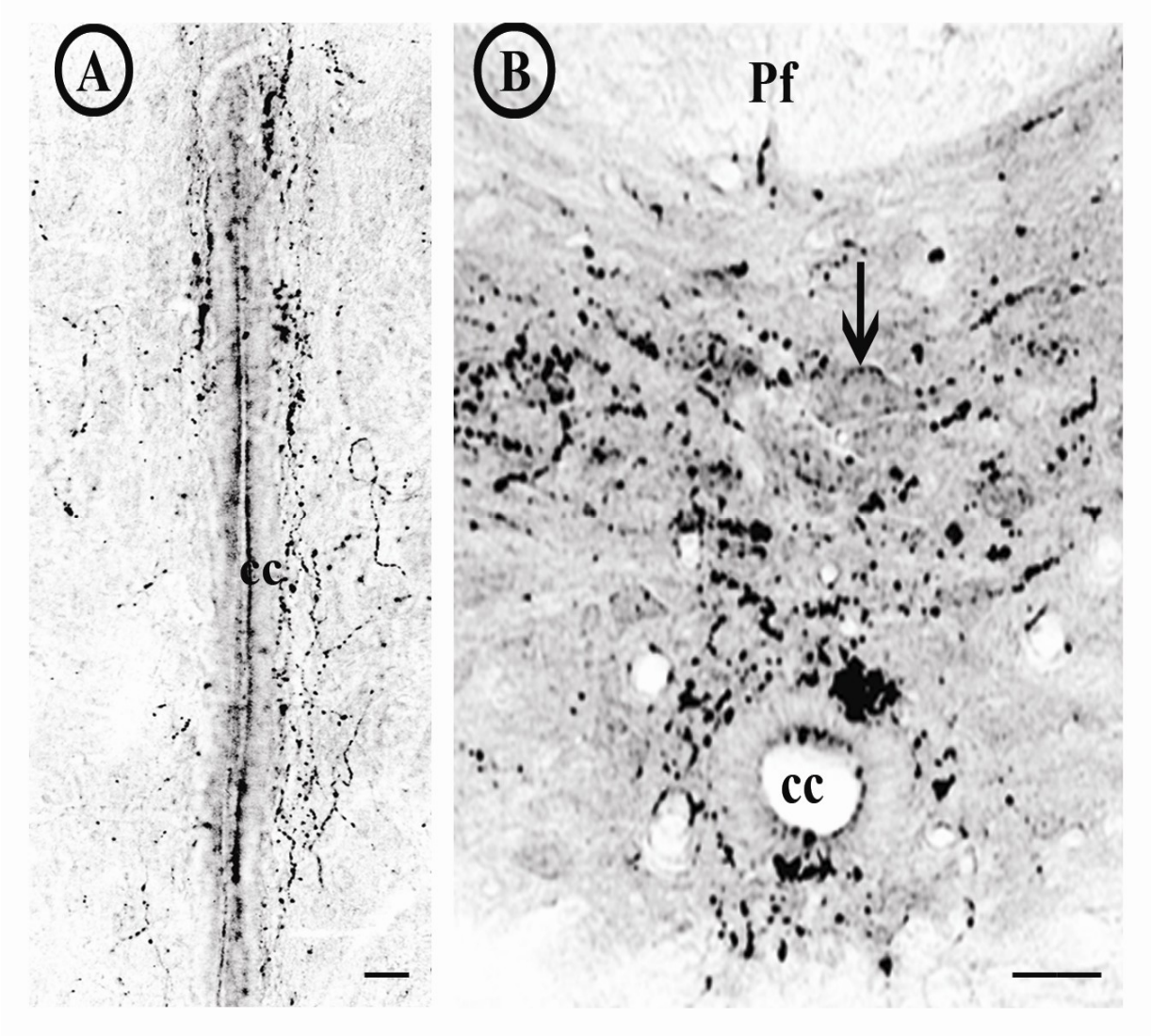


Fig. 3. Horizontal (A) and transverse sections (B) demonstrating a dense network of fibers around the central canal. Many fibers appear to contact the ependymal cells. Numerous labelled fibers can be observed in the dorsal gray commissure (B). A neuronal soma is encircled with oxytocin-immunoreactive varicosities (arrow). Abbreviations: cc, central canal; Pf, posterior funiculus. Scale bar: 100 µm.

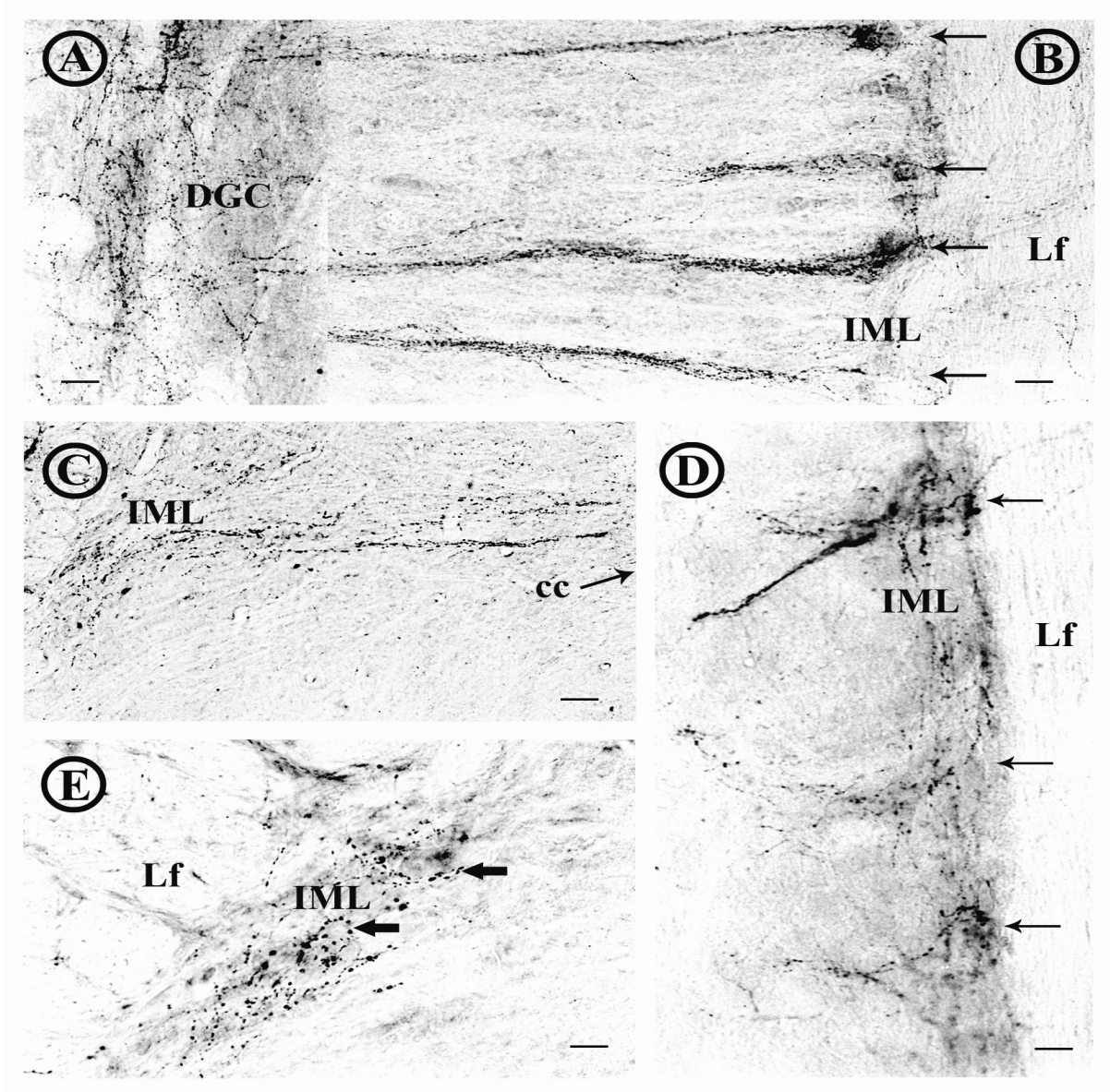


Fig. 4. Distribution of oxytocin in autonomic areas of the spinal cord shown in transverse (C, E) and horizontal sections (A, B, D). Patchy accummulation of labelled fibers can be seen in the intermediolateral column (B, C, D, E). The immunoreactive patches (arrows) are interconnected by a network of horizontally running labelled fibers (B, D). The perikarya of the preganglionic autonomic areas seem to be surrounded by immunoreactive fibers (arrows) (E). Strong immunoreactive bundles course between the dorsal gray commissure and intermediolateral column (A, B). Abbreviations: DGC, dorsal gray commissure; IML, intermediolateral column; cc, central canal; Lf, lateral funiculus. Scale bar: 100 μm (A, B, C, D), 50 μm (E).

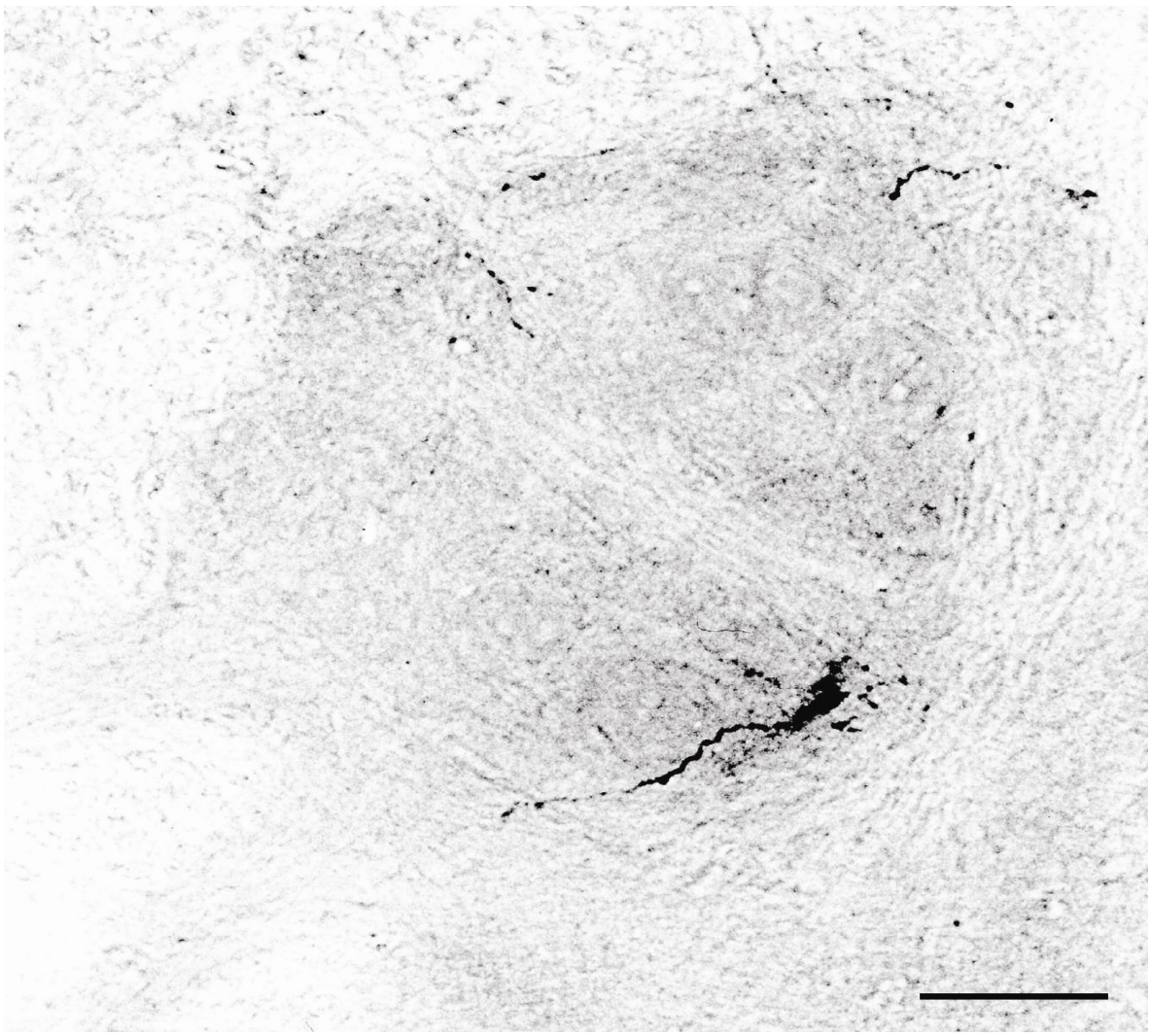


Fig. 5. Immunoreactive fibers scattered around motoneurons of the anterior horn in the lumbar spinal cord. Scale bar: 50μm.

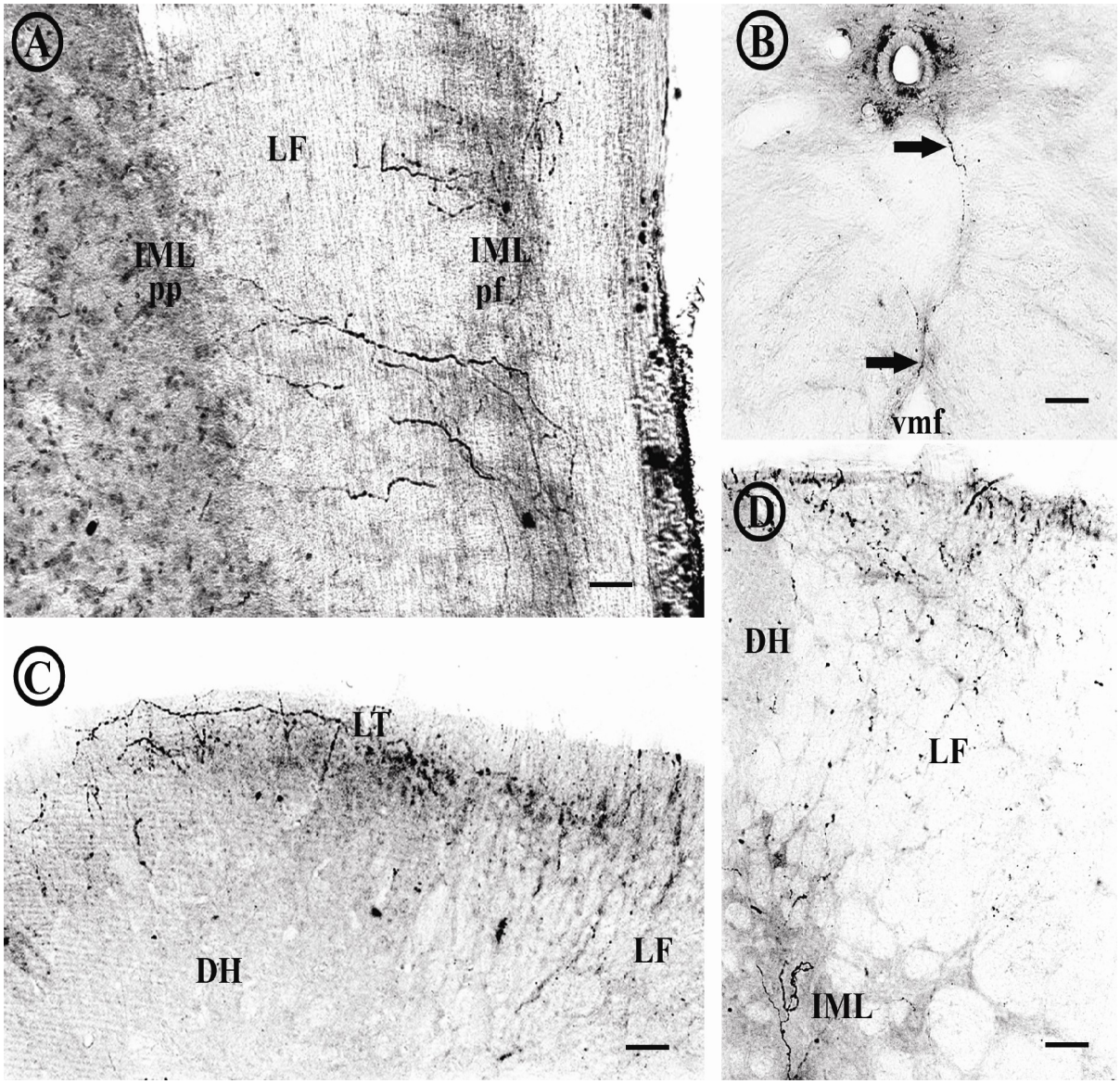


Fig. 6. Distribution of oxytocin-immunoreactive fibers in the white matter of the spinal cord shown in a counterstained (Nissl) horizontal section (A) and in transverse sections (B, C, D). The photomicrographs display oxytocin-immunoreactive fibers in the pars funicularis of the nucleus intermediolateralis (A), the Lissauer's tract (C), anterior funiculus (B), and in the dorsal part of the lateral funiculus (C, D). In the lateral funiculus, reactive fibers extend between the pars principalis (pp) and the pars funicularis (pf) of the nucleus intermediolateralis (A). In the anterior funiculus, the arrows indicate labelled fibers running toward the ventral median fissure (B). Abbreviations: IML, intermediolateral nucleus; LF, lateral funiculus; vmf, ventral median fissure; LT, Lissauer's tract; DH, dorsal horn. Scale bar: 100 μm.

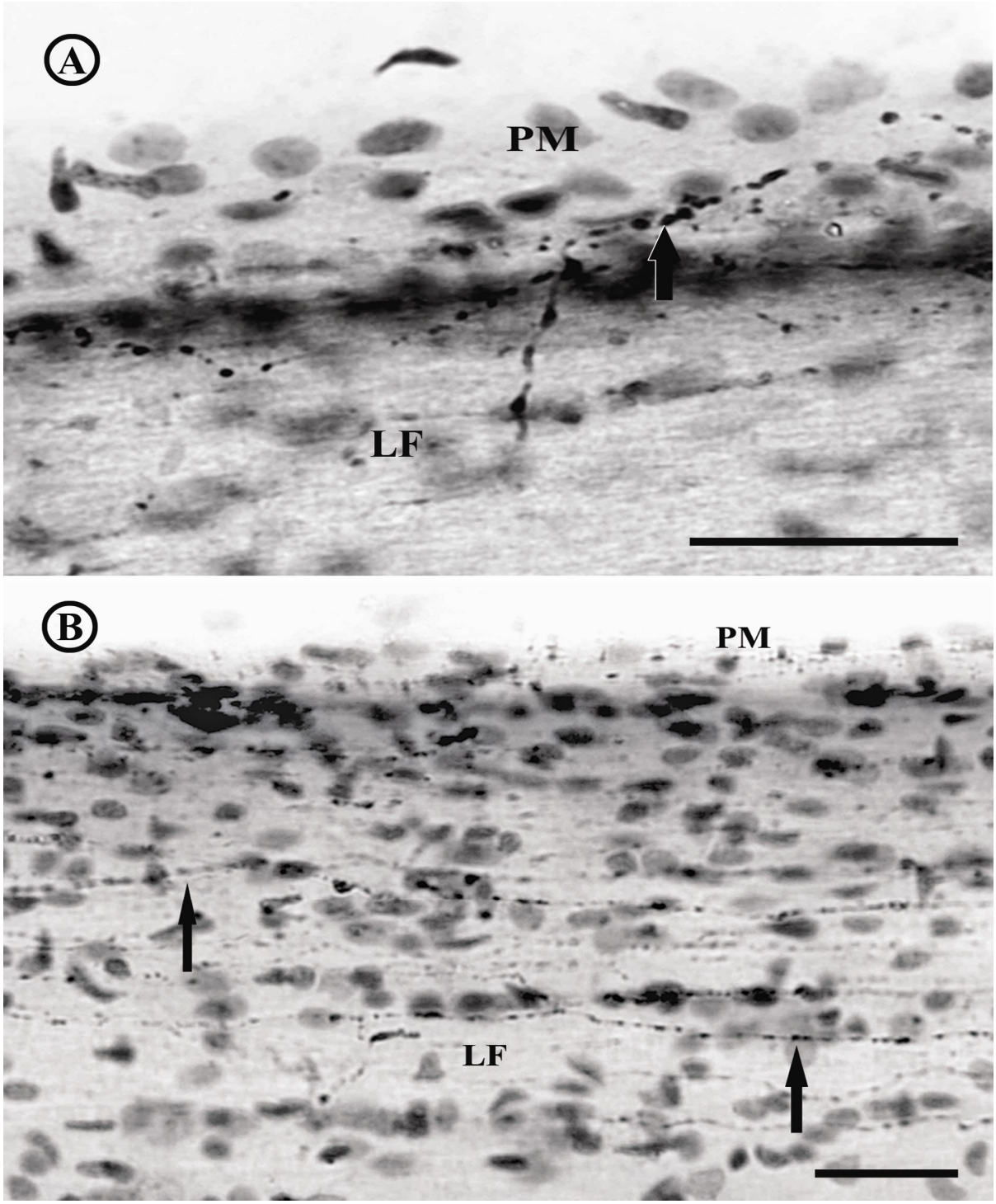


Fig. 7. Oxytocin-immunoreactive fibers (arrow) in the pia mater of the spinal cord (A), and a longitudinally oriented immunoreactive network (arrows) below the pia mater in the lateral funiculus (B) shown in Nissl-counterstained horizontal sections. LF, lateral funiculus; PM, pia mater. Scale bar: 50 µm.

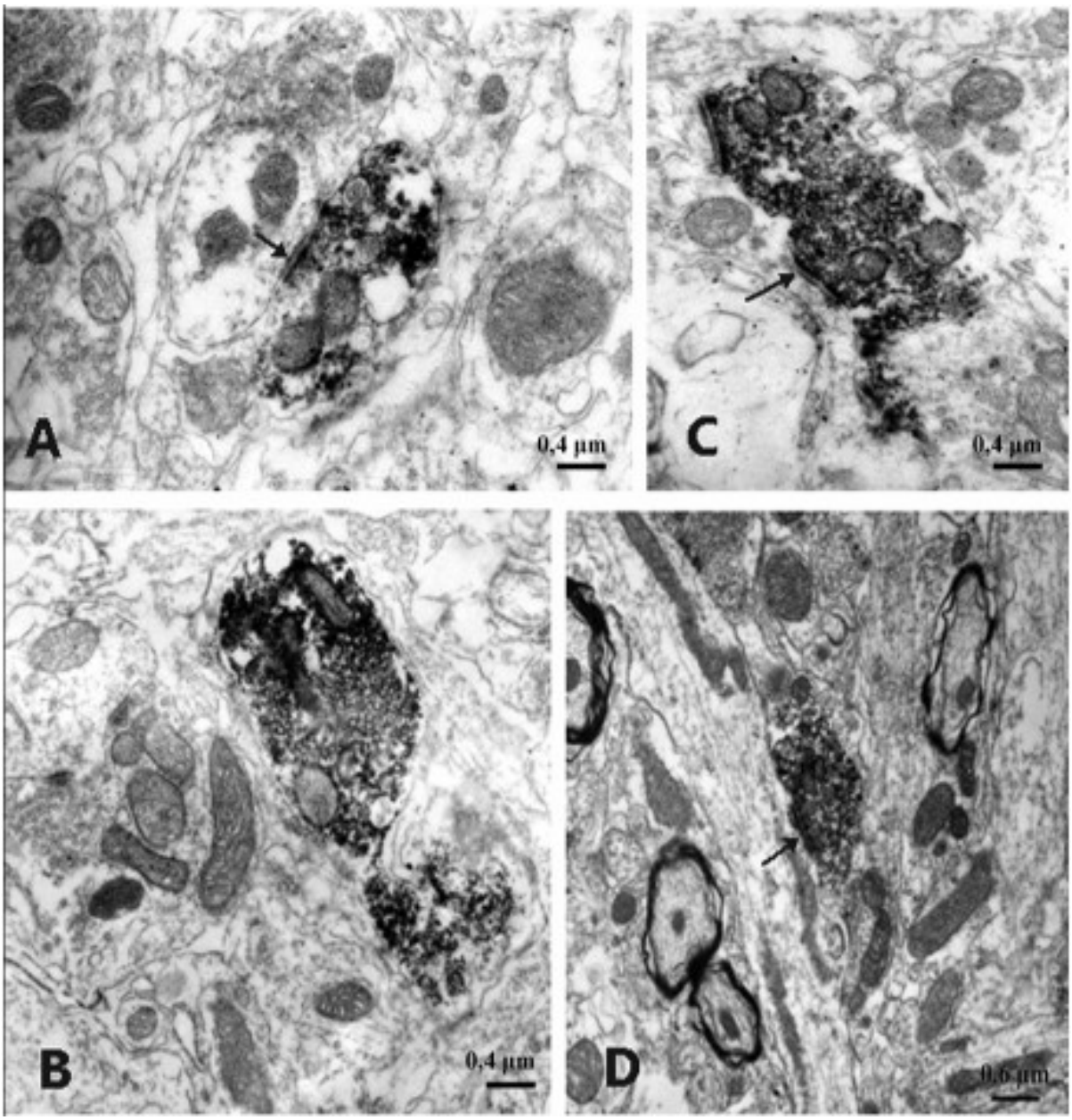


Fig. 8. Oxytocin-immunoreactive axon terminals in laminae VII and X of rat spinal cord. The electron dense peroxidase reaction product has diffused throughout the cytoplasm encircling the synaptic vesicles. The thickened postsynaptic membranes of dendritic profiles (arrows) in Fig. 8A, C, D are clearly visible.

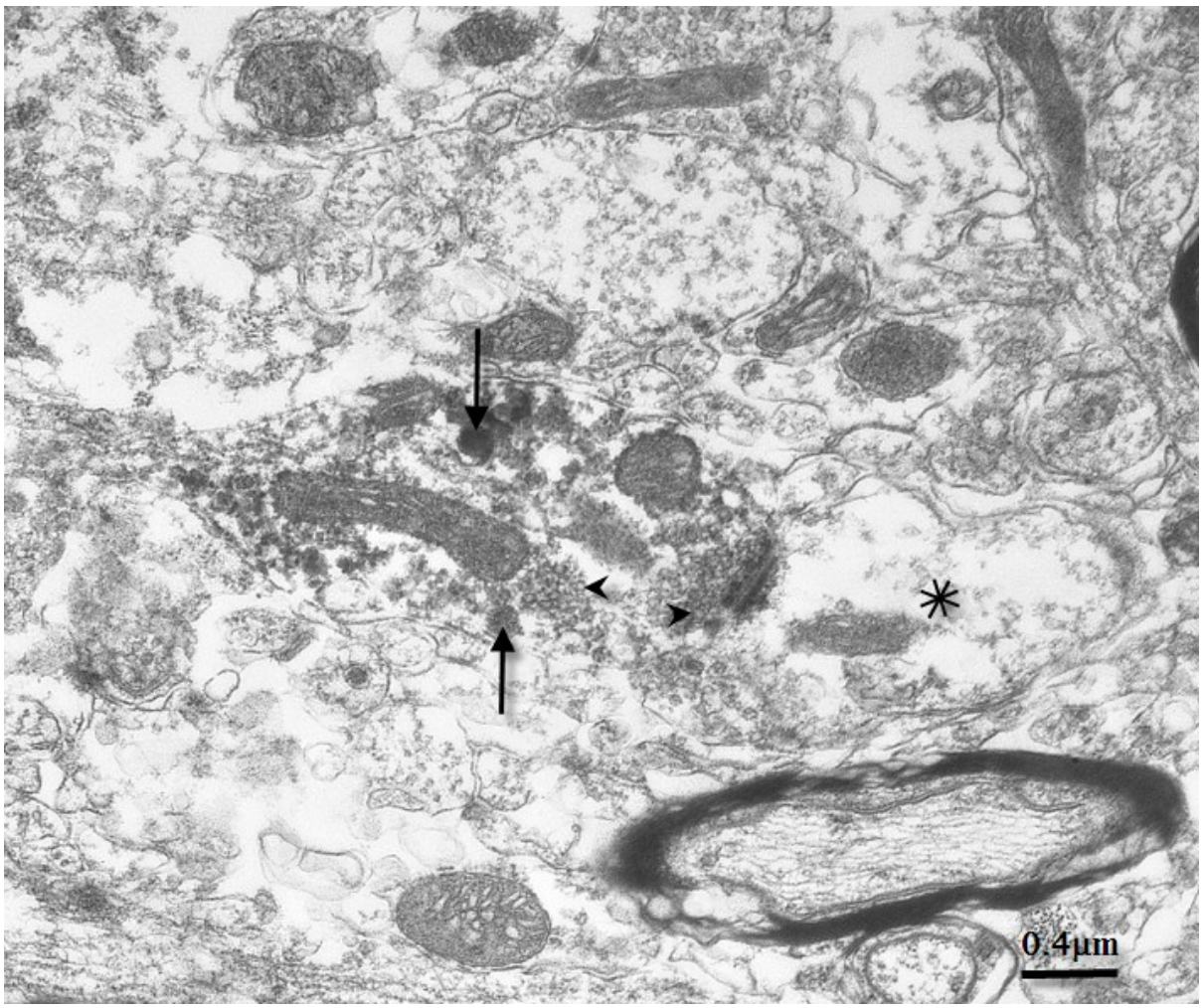


Fig. 9. Electron micrographs displaying an oxytocin-immunoreactive terminal that makes synaptic contact with a dendritic profile (asterisk). The axon terminal contains both small clear (arrowheads) and large dense-cored (arrows) synaptic vesicles.

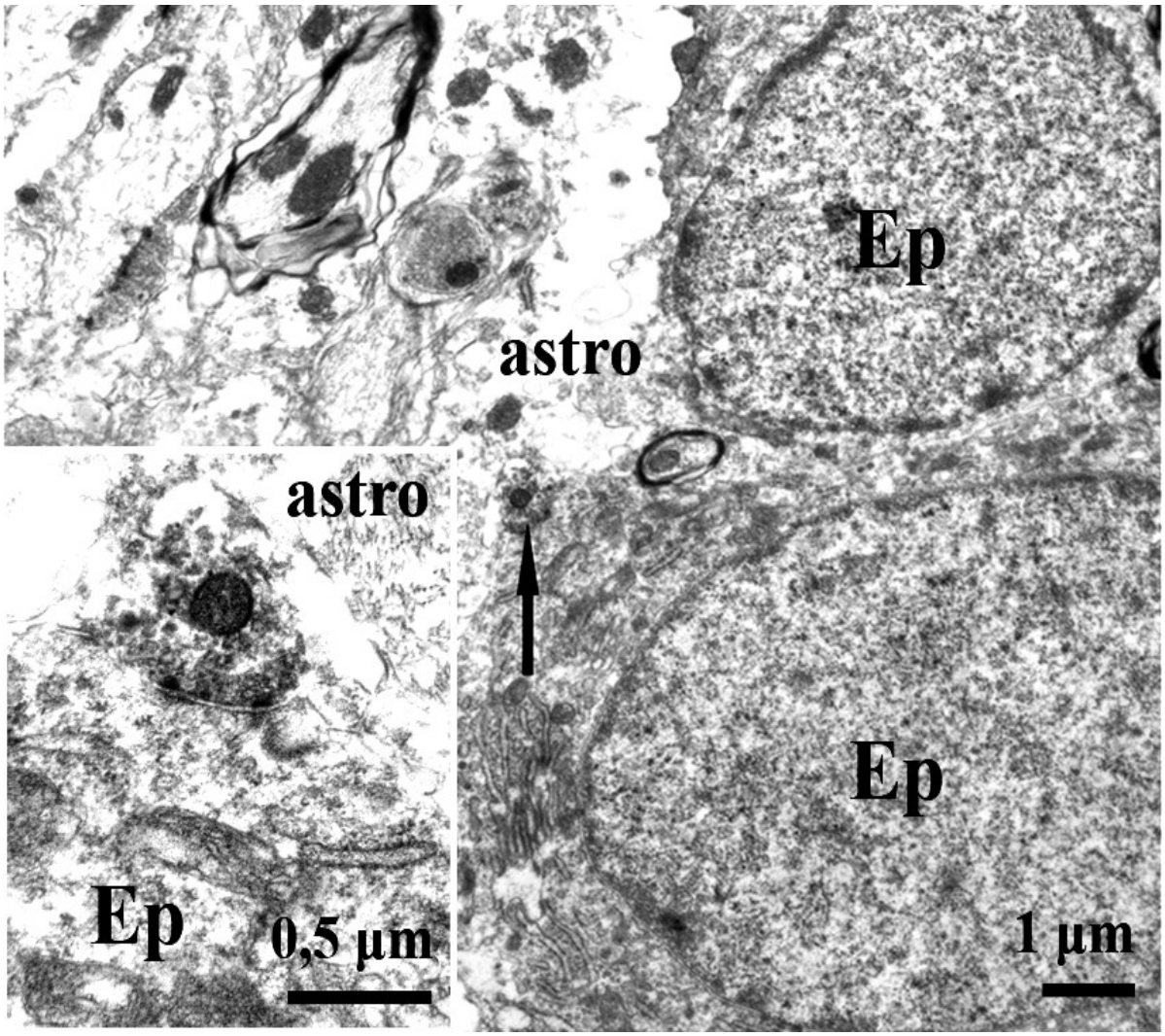


Fig. 10. An immunoreactive terminal (arrow) forms a synapse with a cell soma in the ependymal layer of the central canal (shown at higher magnification in the inset). Abbreviations: astro, astrocyte; Ep, ependyma.

3.1.3 Quantitative distribution of oxytocin

Our RIA measurements revealed that oxytocin was concentrated in the caudal regions of the rat spinal cord. The oxytocin content in the cervical spinal cord was significantly lower than those found in the thoracic, lumbar, sacral and coccygeal regions. The highest quantity was measured in the lumbar spinal cord (*Table 1*).

Table 1

Oxytocin content of rat spinal cord regions (n = 8)

Spinal cord regions	*Oxytocin (pg/mg protein)*
Cervical	64.41 ± 5.33^{a}
Thoracic	130.6 ± 12.46^{b}
Lumbar	235.08 ± 33.15^{c}
Sacral	116.04 ± 14.91^{d}
Coccygeal	162.24 ± 22.27^{e}

Oxytocin concentrations are given in pg/mg protein. The data are expressed as means ± SE. The statistical analysis was performed by two-way ANOVA followed by Scheffe's test for multiple comparison.

[a]Significantly different from thoracic ($p < 0.001$), lumbar ($p < 0.001$), sacral ($p < 0.05$) and coccygeal ($p < 0.001$) levels.

[b]Significantly different from cervical ($p < 0.001$) and lumbar ($p < 0.05$) levels.

[c]Significantly different from cervical ($p < 0.001$), thoracic ($p < 0.05$) and sacral ($p < 0.001$) levels.

[d]Significantly different from cervical ($p < 0.05$) and lumbar ($p < 0.001$) levels.

[e]Significantly different from cervical ($p < 0.001$) level.

3.1.4 Acute effect of peripheral histamine administration on spinal oxytocin content

Spinal cord contents of oxytocin in control and histamine-treated animals are shown in *Table 2.* The i.p. injection of histamine induced a 36% decrease in oxytocin content in the rat spinal cord.

Table 2

Effect of histamine (20 mg/kg b. wt., i.p.) on oxytocin content of the spinal cord

Animal groups	*Oxytocin (fmol/mg)*
Control	*129.4 ± 17.0 (10)*
Histamine	*83.4 ± 3.0* (9)*

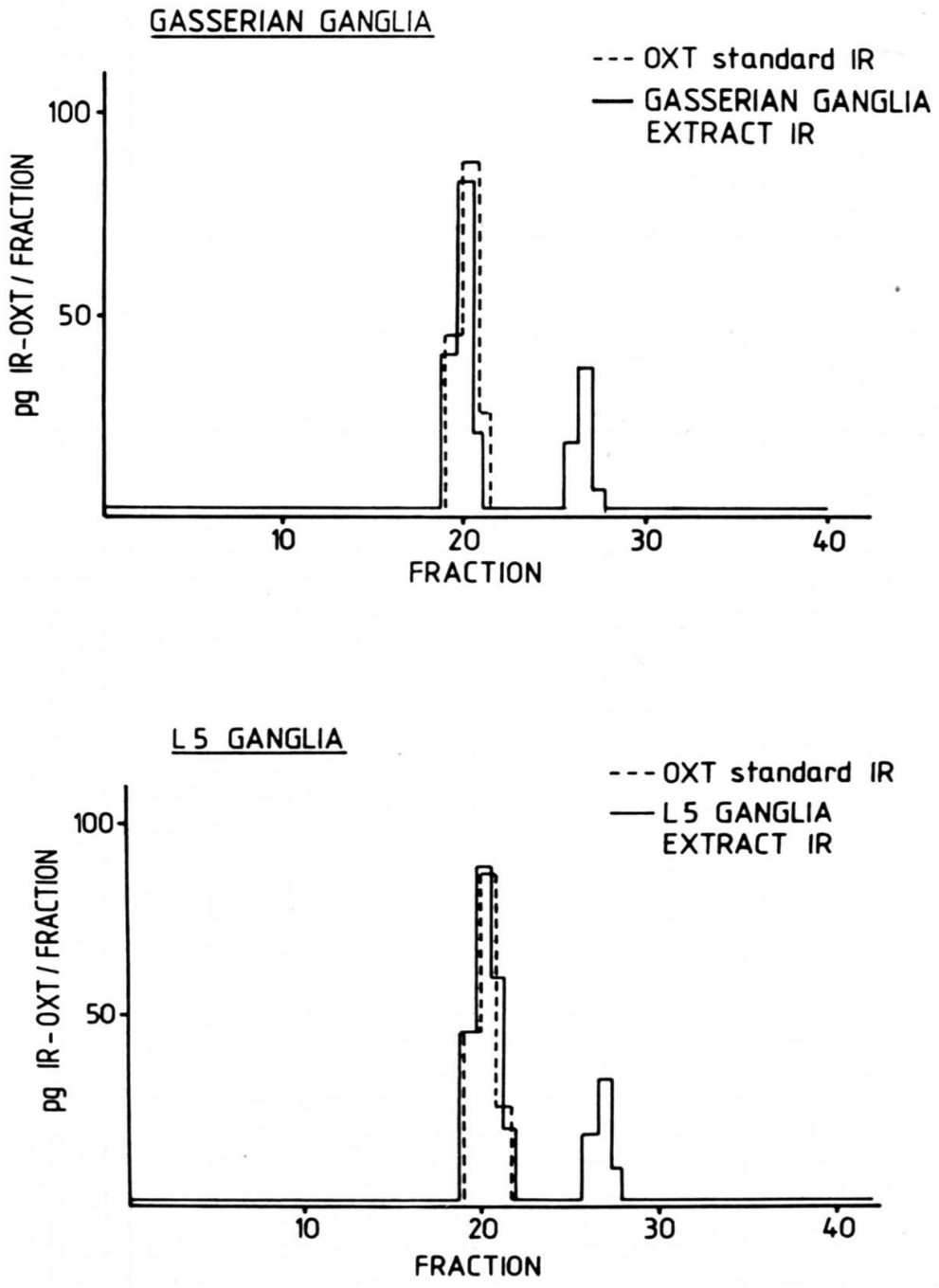
*The results are expressed as ± S. E. The numbers of animals are shown in parenthesis. * Significantly different from control level (P <0.05, Student's t-test).*

3.2 Chromatographically identified oxytocin in the human peripheral nervous system

Oxytocin-like immunoreactivity was detected in extracts of human sensory (spinal L5, Gasserian) ganglia, sympathetic (celiac) ganglia and peripheral nerves (nervus vagus) by radioimmunoassay (RIA) specific to oxytocin and was characterized by high-performance liquid chromatography (HPLC). HPLC-RIA measurements demonstrated that the Gasserian, L5 ganglia and the celiac ganglia of humans contained significant levels of immunoreactive oxytocin (*Table 3, 4*). However, our results showed that the immunoreactive oxytocin migrated as two individual peaks. The first immunoreactive peak was eluted at the same position as the authentic oxytocin

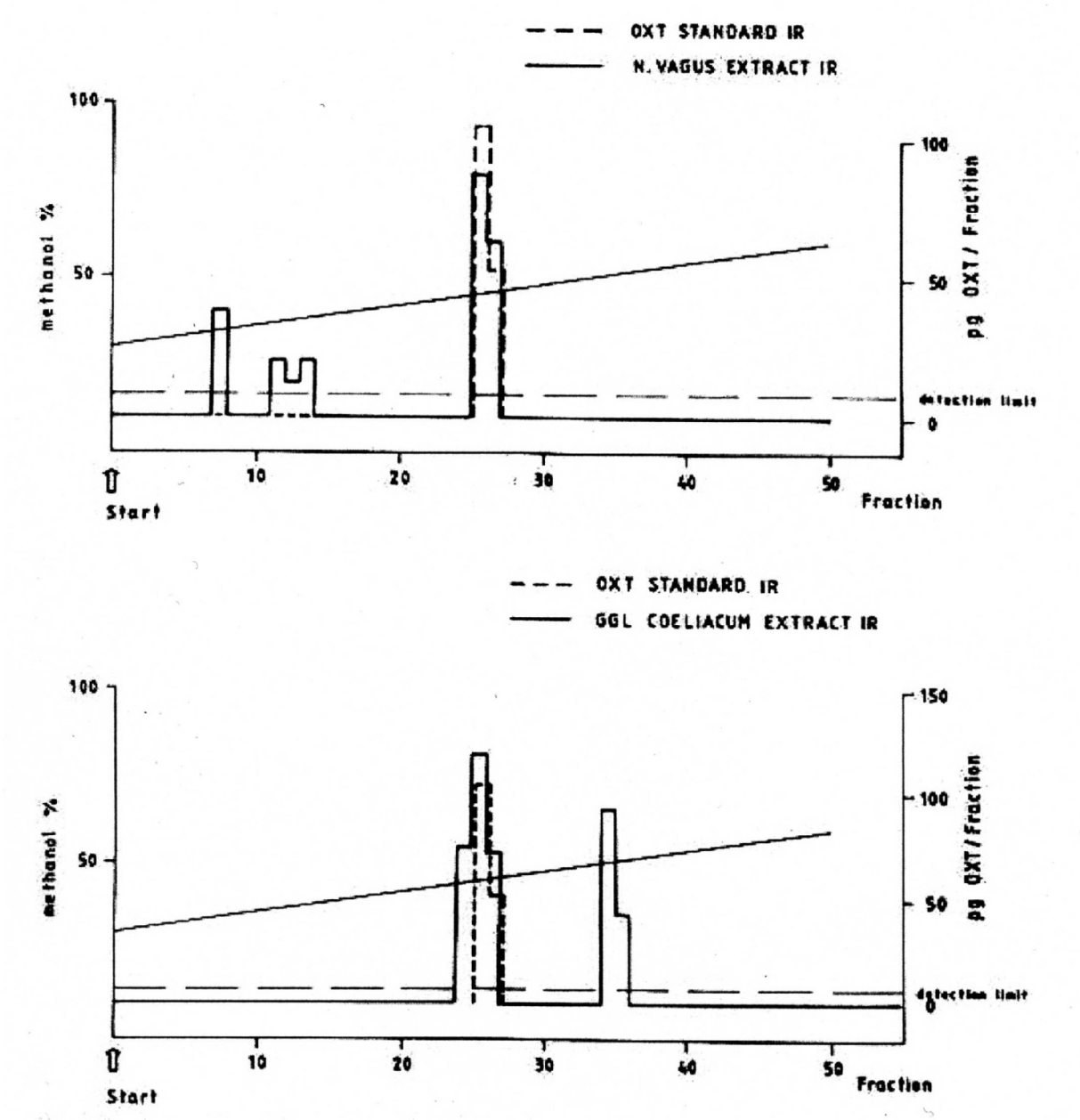
standard. The identity of the second immunoreactive peak is not known, but it might be a degradation product of oxytocin.

Table 3



Elution profile of Gasserian and L5 sensory ganglia extracts. A Lichrosorb C_{18} column was used. The elution was performed with an isocratic methanol (42%, v/v)-0. 1% TFA eluent, and the fractions were radioimmunoassayed for immunoreactive oxytocin.

Table 4



Elution profiles of the extracts of celiac ganglia and vagal nerves on a BST C_{18} column. The elution was performed with a methanol-0.1% TFA gradient system, and fractions were radioimmunoassayed for oxytocin. The great majority of the immunoreactive material was eluted at the same position as the reference oxytocin in extract of the vagal nerves, whereas in the extract of celiac ganglia a second immunoreactive peak was also observed.

4. Discussion

This study was undertaken to examine the distribution of oxytocin in the spinal cord and reveal its occurence in various parts of the peripheral nervous system. Our results show that oxytocin is localized exclusively in axons, and the distribution of oxytocin-immunoreactive fibers in the spinal cord correlates with structures related to nociceptive, autonomic and motor functions. The oxytocin-immunoreactive fibers clearly display segmentally uneven distribution. The significant rise in oxytocin content in the thoracic, lumbar and sacral segments is associated with the dense innervation in the spinal autonomic centers. In accordance with previous investigations of Swanson and McKellar (1979), we conclude that the preferential sites of accumulation of oxytocin-containing fibers in the spinal cord bear close resemblance to the locations of sympathetic and parasympathetic preganglionic neurons, suggesting oxytocinergic modulation of autonomic functions. An important finding of this study is that oxytocin-containing fibers innervate the nucleus intermediolateralis pars funicularis in the lateral funiculus, which has not been noticed by previous investigators. Our results demonstrate that oxytocin-immunoreactive fibers get close to the internal and external liquor spaces of the spinal cord. Although at the light microscopic level, many fibers seemed to appose the ependyma of the central canal, the electron microscopic immunocytochemistry revealed only occasional synaptic contacts with presumptive neuronal perikarya in the ependymal layer. Our experiments also showed that some fibers from the lateral funiculus entered the pia mater. In the study of Swanson and McKellar (1979), neurophysin I-stained fibers were found adjacent to the ependyma of the central canal in the rat. Furthermore, the authors found immunoreactive fibers in the pia-arachnoid surrounding the filum terminale of the monkey. To our knowledge, however, no oxytocin-containing fibers have been reported in the pia mater of the rat spinal cord. The close proximity of oxytocin-containing fibers to the spinal liquor spaces suggests that their terminals may release oxytocin into the cerebrospinal fluid. This possibility is supported by physiological experiments in which electrical stimulation of the hypothalamic paraventricular nucleus yielded increased concentration of oxytocin in perfusates obtained from the subarachnoid space of the spinal cord (Pittman et al., 1984). In accordance with our light microscopic results, in ultrathin electron microscopic sections we found numerous oxytocin-immunoreactive axon

profiles in the gray matter of the spinal cord, but could not detect labeled cell bodies. Our electron microscopic findings confirm those of Rousselot et al. (1990) and Hosoya et al. (1995) that many labelled axon terminals made synaptic contacts with dendrites in the posterior horn, around the central canal and the autonomic areas of the spinal gray matter. Moreover, we also found a few axo-somatic synaptic contacts in the area of the central canal. Since the pre-embedding immunoperoxidase technique that we applied is not suitable for a more precise analysis of the subcellular structure of oxytocin-labeled synapses, we could not confirm the results of Rousselot et al. (1990) showing that the immunolabelled presynaptic vesicles belong to the electron dense type.

Several studies indicate the role of the central histaminergic system in the control of oxytocin and vasopressin secretion (Dogterom et al., 1976; Bealer and Crowley, 1999; Knigge et al., 1999;). However, the effect of peripheral histamine on the oxytocin and vasopressin content of the central nervous system has been less studied (Mens et al., 1983). Our results show that peripheral histamine administration can produce a significant decrease in the spinal content of oxytocin. The dose of histamine used in this study and the time-point for checking its effect are similar to those found by Dogterom et al., (1976) and Mens et al., (1983) to have strong effects on arginine-8-vasopressin and oxytocin release, explaining our choice regarding these parameters. The mechanism of the action of peripheral histamine administration on the spinal oxytocin system is not clear. We put forward three possible explanations how peripherally administered histamine may influence the central oxytocinergic system the perikarya of which are located mainly in the hypothalamic paraventricular nucleus:

- The presence of histamine receptors has been revealed in sensory ganglia and sensory nerve endings (Ninkovic et al., 1982; Ninkovic and Hunt, 1985; Kashiba et al., 1999). Histamine as an inflammatory mediator stimulates nerve fibers that conduct nociceptive impulses to dorsal horn of the spinal cord. Spinal neurons give rise to spinothalamic tract that gives off collateral fibers terminating in the reticular formation. Paraventricular nucleus is richly innervated by catecholaminergic pathways from reticular formation (Yamano et al., 1985; Liposits, 1993; Knigge et al., 1999)
- Since histamine does not affect the plasma osmolality (Laczi et al., 1986), the possibility of osmotic stimulation of oxytocinergic neurons may be excluded. Instead, histamine might exert its effect through baroreceptor and chemoreceptor activity alterations in the vascular system (Koerner et al.,

2004; Lazarov et al., 2006; Rio et al., 2009). This haemodynamical information is carried through the vagus and glossopharyngeal nerves to the solitary nucleus and ventral lateral medulla, and then to the paraventricular nucleus through the ascending catecholaminergic pathways (Yamano et al.,1985; Liposits, 1993).

- Histamine is not readily capable of crossing the blood-brain barrier (Schwartz et al., 1979). However, the circumventricular organs such as the subfornical organ, organum vasculosum of the lamina terminalis, area postrema, subcommissural organ and median eminence lack a blood brain barrier and at these regions of the brain neurons are exposed to the chemical environment of the systemic circulation (Johnson and Gross, 1993). Thus, histamine in blood circulation may act directly on brain functions through the circumventricular organs.

Our RIA measurements revealed significant levels of immunoreactive oxytocin in selected samples of the human peripheral nervous system, including sensory (spinal L5, Gasserian) and sympathetic (celiac) ganglia as well as peripheral (vagal) nerves. HPLC showed that the immunoreactive oxytocin was eluted at the same position as the authentic oxytocin standard. Our results provide the first evidence for the presence of oxytocin in the human peripheral nervous system. The presence of oxytocin was later confirmed in human sympathetic paravertebral ganglia, too (Vecsernyés et al., 1990). The occurrence of neurohypohysial peptides has been detected in several components of the peripheral nervous system of mammals. It was found by Hanley et al., (1984) that a bioactive and immunoreactive vasopressin-like peptide is present in the sympathetic nervous system of the rat. Authentic arginine vasopressin and oxytocin were identified by chromatography in sensory neurones (Kai-Kai, 1985) and peripheral nerves (Sequeira, 1984). Oxytocin was reported to be present in both the small dark and the large light sensory neurones of L5 dorsal root ganglia and to coexist with arginine vasopressin in the rat. Arginine-vasopressin-like immunoreactivity was axonally transported in the dorsal root and sciatic nerve. Furthermore, observations obtained in animals neonatally treated with capsaicin suggests that vasopressin is located in primary afferent terminals in upper dorsal horn of the spinal cord (Kai-Kai et al., 1986). Finally, oxytocin and oxytocin receptor transcripts and protein have been recently found in the enteric nervous system (Welch et al., 2009). The origin and functional significance of oxytocin in the peripheral nervous system are not clear yet. Although it is very likely

that oxytocin is synthesized locally in neurons of sensory and autonomic ganglia, further studies should be performed to confirm this assumption. The axonal transport of oxytocin from ganglion cells also remain to be studied. Although the great majority of oxytocin in the spinal cord has been shown to stem from the hypothalamic paraventricular nucleus, there is a minor amount the source of which is unclear (Hawthorn et al., 1985). The possibility that it originates from dorsal root ganglion cells should thus be tested. There are only scarce data available on the role of oxytocin in the peripheral nervous system. Oxytocin is thought to depress the ganglionic transmission in superior cervical ganglia (Király, 1985). In other experiments it has been shown to activate intrathoracic ganglionic neurons involved in efferent sympathetic cardiac regulation (Armour, 1990). Further studied are obviously needed to elucidate the precise role and functional significance of oxytocin in different parts of the peripheral nervous system.

5. Summary

We investigated the distribution of immunoreactive oxytocin in the rat spinal cord using immunocytochemistry and RIA. The presence of authentic oxytocin in the human peripheral nervous system was demonstrated by means of HPLC and RIA.

By light microscopic immunohistochemistry, each segment of the spinal cord from cervical to coccygeal ones contained oxytocin-immunoreactive fibers. The Rexed laminae I and II of the dorsal horn showed moderate to intense immunoreactivity. A dense network was found around the central canal where some fibers apposed the ependyma. The autonomic centers of the spinal cord at the thoracolumbar and sacral segments were heavily innervated. Few fibers were seen around the motoneurons. In the white matter, the immunoreactivity was localized mainly in the dorsal part of the lateral funiculus, in the pars funicularis of the nucleus intermediolateralis and in a longitudinal network of the lateral funiculus below the spinal cord surface. Some fibers from this network were found to enter the pia mater.

Our electron microscopic investigations showed that the oxytocin-immunoreactive fibers were unmyelinated and most of the immunoreactive axon terminals made synaptic contacts with dendrites. A few oxytocin-containing axon terminals were also observed synapsing with neronal somas located in the ependymal lining of the central canal.

RIA measurements revealed that the oxytocin content was preferentially accumulated in the caudal regions of the rat spinal cord. The cervical spinal cord had lower oxytocin content than that found in either the thoracic, lumbar, sacral or coccygeal region.

Experiments made with peripheral administration of histamine demonstrated that this agent can bring about a significant decrease in the oxytocin content of the rat spinal cord.

Oxytocin immunoreactivity was detected in extracts of human spinal L_5, Gasserian and celiac ganglia as well as vagus nerve by RIA and characterized by HPLC. HPLC/RIA examinations showed that a major part of the immunoreactive material co-eluted with a reference synthetic oxytocin, but in the extracts of spinal L5, Gasserian and celiac ganglia a second immunoreactive peak was also observed. The identity of the latter material is unknown, but it might well be a degradation product of oxytocin.

In conclusion, our results demonstrate that the distribution of oxytocin in the rat spinal cord correlates with anatomic locations related to nociceptive, autonomic and motor functions. Oxytocin-containing axon terminals shown to make synaptic contacts with dendrites and cell somas are thought to be the structures through which oxytocin modulates spinal functions. We assume that oxytocin-containing axon terminals situated in close proximity of spinal liquor spaces play a role in secreting oxytocin into the cerebrospinal fluid. Our experiments also showed that peripherally administered histamine alters oxytocin content in the spinal cord which may have relevance under physiological and/or pathological conditions. Finally, this study revealed the occurrence of oxytocin in various parts of the human peripheral nervous system. The morphological aspects and functional significance of this finding remain to be elucidated.

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7. References

1. Argiolas, A., Melis, M. R. (2004) The role of oxytocin and the paraventricular nucleus in the sexual behaviour of male mammals. Physiol. Behav. 83, 309-317.
2. Arletti, R., Benelli, A., Bertolini, A. (1993) Influence of oxytocin on nociception and morphine antinociception. Neurpeptides 24, 125-129.
3. Armour, J. A. and Klassen, G. A. (1990) Oxytocin modulation of intrathoracic sympathetic ganglionic neurons regulating the canine heart. Peptides, 11, 533-537
4. Assinder S. J., Carey M., Parkinson T., Nicholson H. D. (2000) Oxytocin and vasopressin expression in the ovine testis and epididymis: changes with the unset of spermatogenesis. Biol. Reprod. 63 (2), 448-456.
5. Bealer, S. L., Crowley, W.R.(1999) Stimulation of central and systemic oxytocin release by histamine in the paraventricular hypothalamic nucleus: evidence for an interaction with norepinephrine. Endocrinology, 140 (3) 1158-1164.
6. Benoussaidh, A., Maurin, Y., Rampin, O. (2004) Spinal effects of oxytocin on uterine motility in anesthetized rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287, R446-R453.
7. Buijs, R. M. (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord. Cell Tiss. Res. 192, 423-435.
8. Bujis, R. M. (1980) Vasopressin and Oxytocin Innervation of the Rat Brain. A Light- and Electronmicroscopical Study. PhD Thesis, Amsterdam.
9. Buijs, R. M. (1983) Vasopressin and oxytocin—their role in neurotransmission. Pharmac. Ther. 22, 127-141.
10. Chibbar, R., Miller, F.D., Mitchell, B.F. (1993) Synthesis of oxytocin in amnion, chorion and decidua may influence the timing of human parturition. J. Clin. Invest. 91, 185-192.
11. Condés-Lara, M., González, N. M., Martínez-Lorenzana, G., Delgado, O. L., Freund-Mercier, M. J. (2003) Actions of oxytocin and interactions with glutamate on spontaneous and evoked dorsal spinal cord neuronal activities. Brain Res. 976, 75-81.
12. Daddona, M. M., Haldar, J. (1994) Opioid modulation of oxytocin release from spinal cord synaptosomes. Neuroreport 5, 1833-1835.

13. Dogterom, J., Van Wimersma Greidanus Tj. B., De Wied, D. (1976) Histamine as an extremely potent release of vasopressin in the rat. Experientia 32, 659-660.

14. Dogterom, J., Van Wimersma Greidanus Tj. B., Swaab, D. F. (1977) Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: measurements in plasma and CSF of intact and hypophysectomized rats. Neuroendocrinology 24, 108-118.

15. Einspanier, A., Ivell, R. (1997) Oxytocin and oxytocin receptor expression in reproductive tissues of the male marmoset monkey. Biol Reprod. 56 (2), 416-422.

16. Einspanier, A., Jurdzinski, A., Hodges, J. K. (1997) A local oxytocin system is part of the luteinization process in the preovulatory follicle of the marmoset monkey (Callithrix jaccus). Biol. Reprod. 57 (1), 16-26.

17. Fischman, A. J., Kastin, A. J. and Graf, M. V. (1984) HPLC shadowing: artifacts in peptides characterization monitored by RIA. Peptides, 5, 1007-1010.

18. Furuya, K., Mizumoto, Y., Makimura, N., Mitsui, C.,Murakami, M., Tokuoka, S., Ishikawa, N., Imaizumi, E.,Katayama, E., Seki K.(1995) Gene expressions of oxytocin and oxytocin receptor in cumulus cells of human ovary. Horm. Res. 44 (suppl.2) 47-49.

19. Gibson, S. J., Polak, J. M., Bloom S. R., Wall, P. D. (1981) The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (lamina X). J. Comp. Neurol. 201, 65-79.

20. Gilbey, M. P., Coote, J. H., Fleetwood-Walker, S., Peterson, D. F. (1982) The influence of the paraventriculo-spinal pathway, and oxytocin and vasopressin on sympathetic preganglionic neurones. Brain Res. 251, 283-290.

21. Gutkowska, I., Jankowski, M., Mukaddam-Daher, S., McCann, S. M. (2000) Oxytocin is a cardiovascular hormone. Braz. J. Biol. Res. 33 (6), 625-633.

22. Hallbeck, M., Larhammar, D., Blomqvist, A. (2001) Neuropeptide expression in rat paraventricular hypothalamic neurons that project to the spinal cord. J Comp. Neurol. 433, 222-238.

23. Hanley, M. C., Benton, H.P. Lightman, S.L., Todd, K., Bone, E.A., Fretten, P., Palmer, S., Kirk, C.J., Michell, R.H. (1984). A vasopressin like peptide in the mammalian sympathetic nervous system. Nature 309. 258-261

24. Hawthorn, J., Ang, V. T. Y., Jenkins, J. S. (1985) Effect of lesions in the hypothalamic paraventricular, supraoptic and suprachiasmatic nuclei on vasopressin and oxytocin in rat brain and spinal cord. Brain Res. 346, 51-57.
25. Hosoya, Y., Matsukawa, M., Okado, N., Sugiura, Y., Kohno, K. (1995) Oxytocinergic innervation to the upper thoracic sympathetic preganglionic neurons in the rat. A light and electron microscopical study using a combined retrograde transport and immunocytochemical technique. Exp. Brain Res. 107, 9-16.
26. Inaba T., Nagayama Y., Tani H., Tamada H., Kawate N., Sawada T. (1999) Oxytocin gene expression and action in goat testis. Theriogenology 52 (3), 425-434.
27. Johnson, A. K., Gross, P. M. (1993) Sensory circumventricular organs and brain homeostatic pathways. FASEB J. 7, 678-686.
28. Jenkins, J. S., Ang, V. T., Hawthorn, J., Rossor, M. N., Iversen, L. L. (1984) Vasopressin, oxytocin and neurophysins in the human brain and spinal cord. Brain Res. 291, 111-117.
29. Kai-Kai, M. A., Anderton, B.H. and Keen, P. (1986) A quantitative analysis of the interrelationships between subpopulations of rat sensory neurons containing arginine-vasopressin or oxytocin and those containing substance-P, fluoride-resistent acid phosphatase or neurofilament protein. Neuroscience, 18, 475-486.
30. Kai-Kai, M. A., Swann, R.W. and Keen, P. (1985) Localization of chromatographically characterized oxytocin and arginine-vasopressin to sensory neurones in the rat. Neurosci. Lett. 55, 83-88.
31. Kashiba, H., Fukui, H., Morikawa, Y.,Senba, E. (1999) Gene expression of histamine H1 receptor in guinea pig primary sensory neurons: a relationship between H1 receptor mRNA-expressing neurons and peptidergic neurons. Mol Brain Res 66 (1) 24-34.
32. Király, M., Maillard, M., Dreifuss, J. J. and Dolivo, M. (1985) Neurohypophyseal peptides depress cholinergic transmission in a mammalian sympathetic ganglion. Neurosci. Lett. 62, 89-95.
33. Knigge, U., Warberg J. (1991) The role of histamine in the neuroendocrine regulation of pituitary hormone secretion. Acta Endocrinol (Coppenhaga) 124, 609-619.

34. Knigge, U., Willems E., Kjaer, A., Jorgensen, H., Warberg, J. (1999) Histaminergic and catecholaminergic interactions in the central regulation of vasopressin and oxytocin secretion. Endocrinology 140 (8), 3713-3719.
35. Koerner, P., Hesslinger, C.,Schaefermeyer, A., Prinz, C., Gratzl, M. (2004) Evidence for histamine as a transmitter in rat carotid body sensor cells. J Neurochem. 91 (2), 493-500.
36. Krukoff, T. L., Ciriello, J., Calaresu, F. R. (1985) Segmental distribution of peptide- and 5HT-like immunoreactivity in nerve terminals and fibers of the thoracolumbar sympathetic nuclei of the cat. J. Comp. Neurol. 240, 103-116.
37. Kovács, G. L., Vecsernyés, M., Laczi, F., Faludi, M., Telegdy, G., László, F. A. (1985) Acute morphine treatment and morphine tolerance/dependence alter immunoreactive oxytocin levels in the mouse hippocampus. Brain Res. 328, 158-160.
38. Laczi, F., Iványi, T., Julesz, J., Janáky, T., László, F. A (1986) Plasma arginine-8-vasopressin responses to osmotic or histamine stimulation contribute to the differential diagnosis of central diabetes insipidus. Acta Endocrinologia (Kbh.) 113, 168-174.
39. Lang, R. E., Heil, J., Ganten, D., Hermann, K., Rascher, W., Unger, T. (1983) Effect of lesions in the paraventricular nucleus of the hypothalamus on vasopressin and oxytocin contents in brainstem and spinal cord of rat. Brain Res. 260, 326-329.
40. Lazarov, N., Rozloznik, M., Reindl, S., Rey-Ares, V., Dutschmann, M., Gratzl, M. (2006) Expression of histamine receptors and effect of histamine in the rat carotid body chemoafferent pathway. Eur J Neurosci. 24 (12), 3431-3444.
41. Liposits Z. (1993) Ultrastructure of hypothalamic Paraventricular Nucleus. Critical Reviews in Neurobiology 7 (2), 89-162.
42. Loup, F., Tribollet, E., Dubois-Dauphin, M., Pizzolato, G., Dreifuss, J.J. (1989) Localization of oxytocin biding sites in the human brainstem and upper spinal cord: an autoradiographic study. Brain Res. 500, 223-230.
43. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.
44. Lukic, D., Haldar, J. (1993) Isotonic and hypertonic saline act as stressful stimuli for oxytocinergic system of the pituitary, hypothalamus and spinal cord. Life Sci. 53, 579-584.

45. Mens, W. B. J. (1982) Neurohypophyseal Hormones in Blood, Cerebrospinal Fluid and Brain of the Rat. PhD Thesis, Utrecht.
46. Mens, W. B. J., Laczi F., Tonnaer, J. A. D. M., De Kloet, E. R., Van Wimersma Greidanus, Tj. B. (1983) Vasopressin and oxytocin content in cerebrospinal fluid and various brain areas after administration of histamine and pentylenetetrazol. Pharmac. Biochem. Behav. 19, 587-591.
47. Miaskowski, C., Ong, G. L., Haldar, J. (1987) Cyclic variations in spinal cord levels of oxytocin and vasopressin during the stages of the rat estrous cycle. Endocrinology 120, 1685-1687.
48. Miaskowski, C., Ong, G. L., Lukic, D., Haldar, J. (1988) Immobilization stress affects oxytocin and vasopressin levels in hypothalamic and extrahypothalamic sites. Brain Res. 458, 137-141.
49. Nilaver, G., Zimmerman, E. A., Wilkins, J., Michaels, J., Hoffman, D., Silverman, A. J. (1980) Magnocellular hypothalamic projections to the lower brain stem and spinal cord of the rat. Immunocytochemical evidence for predominance of the oxytocin-neurophysin system compared to the vasopressin-neurophysin system. Neuroendocrinol. 30, 150-158.
50. Ninkovic, M., Hunt, S. P., Gleave, I. R. W., (1982) Localization of opiate and histamine H1- receptor sin the primate sensory ganglia and spinal cord. Brain Res. 241 (2) 197-206.
51. Ninkovic, M., Hunt, S. P. (1985) Opiate and histamine H1- receptors are present on some substance P-containing dorsal root ganglion cells. Neuroscience Letters 53 (1) 133-137.
52. Pandita, R. K., Nylén, A., Andersson, K.-E. (1998) Oxytocin-induced stimulation and inhibition of bladder activity in normal conscious rats—influence of nitric oxide synthase inhibition. Neuroscience 85, 1113-1119.
53. Petras, J. M., Cummings, J. F. (1972) Autonomic neurons in the spinal cord of the rhesus monkey: a correlation of the findings of cytoarchitectonics and sympathectomy with fiber degeneration following dorsal rhizotomy. J. Comp. Neurol. 146, 189-218.
54. Pittman, Q. J., Riphagen, C. L., Lederis, K. (1984) Release of immunoassayable neurohypophyseal peptides from rat spinal cord, in vivo. Brain Res. 300, 321-326.

55. Raggenbass, M. (2001) Vasopressin- and oxytocin-induced activity in the central nervous system: electrophysiological studies using in-vitro systems. Prog. Neurobiol. 64, 307-326.
56. Reiter, M. K., Kremarik, P., Freund-Mercier, M. J., Stoeckel, M. E., Desaulles, E., Feltz, P. (1994) Localization of oxytocin binding sites in the thoracic and upper lumbar spinal cord of the adult and postnatal rat: a histoautoradiographic study. Eur. J. Neurosci. 6, 98-104.
57. Rio, R.D., Moya, E. A., Alcayaga, J., Iturriaga, R. (2009) Evidence for histamine as a new modulator of carotid body chemoreception.Adv. Exp. Med. Biol. 648, 177-184.
58. Robinson, D. A., Wei, F., Wang, G. D., Li, P., Kim, S. J., Vogt, S. K., Muglia, L. J., Zhuo, M. (2002) Oxytocin mediates stress-induced analgesia in adult mice. J. Physiol. 540, 593-606.
59. Rousselot, P., Papadopoulos, G., Merighi, A., Poulain, D. A., Theodosis, D. T. (1990) Oxytocinergic innervation of the rat spinal cord. An electron microscopic study. Brain Res. 529, 178-184.
60. Sansone, G. R., Gerdes, C. A., Steinman, J. L., Winslow, J. T., Ottenweller, J. E., Komisaruk, B. R., Insel, T. R. (2002) Vaginocervical stimulation releases oxytocin within the spinal cord in rats. Neuroendocrinology 75, 306-315.
61. Sansone, G. R., Komisaruk, B. R. (2001) Evidence that oxytocin is an endogenous stimulator of autonomic sympathetic preganglionics: the pupillary dilatation response to vaginocervical stimulation in the rat. Brain Res. 898, 265-271.
62. Sawchenko, P. E., Swanson, L. W. (1982) Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. J. Comp. Neurol. 205, 260-272.
63. Schwartz, J. C. (1979) Minireview: histamine receptors in brain. Life Sci. 25, 895-912.
64. Sequeira, R.P. and Chaiken, I. M. (1984) Occurrence of oxytocin, vasopressin and neurophysins in peripheral nerves. In F. Labrie and L. Proulx (Eds.), Proceedings of the 7th International Congress on Endocrinology, Quebec, Elsevier, Ansterdam, p.1320.
65. Silverman, A. J., Zimmerman, E. A. (1983) Magnocellular neurosecretory system. Ann. Rev. Neurosci. 6, 357-380.

66. Sofroniew, M. V. (1980) Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. J. Histochem. Cytochem. 28, 475-478.
67. Sofroniew, M. V. (1985) Vasopressin, oxytocin and their related neurophysins. In: Björklund A., Hökfelt T. (eds) Handbook of Chemical Neuroanatomy. Vol. 4: GABA and Neuropeptides in the CNS, Part I, Elsevier, Amsterdam, pp 93-165.
68. Sofroniew, M. V., Weindl, A. (1978) Extrahypothalamic neurophysin-containing perikarya, fiber pathways and fiber clusters in the rat brain. Endocrinology 102, 334-337.
69. Sofroniew, M. V., Weindl, A., Schrell U., Wetzstein, R. (1981) Immunohistochemistry of vasopressin, oxytocin and neurophysin in the hypothalamus and extrahypothalamic regions of the human and primate brain. Acta Histochem. Suppl. 24, 79-95.
70. Sternberger, L. A., Hardy, P. H., Jr., Cuculis, J. J., Meyer, H. G. (1970) The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem. 18, 315-333.
71. Swanson, L. W., McKellar, S. (1979) The distribution of oxytocin- and neurophysin-stained fibers in the spinal cord of the rat and monkey. J. Comp. Neurol. 188, 87-106.
72. Tribollet, E., Barberis, C., Arsenijevic, Y. (1997) Distribution of vasopressin and oxytocin receptors in the rat spinal cord: sex-related differences and effect of castration in pudendal motor nuclei. Neuroscience 78, 499-509.
73. Vecsernyés, M., Jójárt I., Pepó, J., Laczi F. (1990) Characterization of oxytocin immunoreactivity in human sympathetic paravertebral ganglia. Brain Res. 522, 325-326.
74. Welch, M. G., Tamir, H., Gross, K.J., Chen, J., Anwar, M., Gershon, M. D. (2009) Expression and developmental regulation of oxytocin (OT) and oxytocin receptors (OTR) in the enteric nervous system (ENS) and intestinal epithelium. J. Comp. Neurol. 10, 512 (2), 256-270.
75. White, J. D., Krause, J. E, McKelvy, J. F. (1986) In vivo biosynthesis and transport of oxytocin, vasopressin and neurophysin from the hypothalamus to the spinal cord. Neuroscience 17, 133-140.

76. Yamano, M., Bai, F. L., Tohyama, M., Shiotani, Y. (1985) Ultrastructural evidence of direct synaptic contact of catecholamine terminals with oxytocin-containing neurons in the paraventricular nucleus. Brain Res. 336. 176.
77. Yang, J., Yang, Y., Chen J.-M., Liu, W.-Y., Wang, C.-H., Lin, B.-C. (2007) Central oxytocin enhances antinociception in the rat. Peptides 28, 1113-1119.
78. Yang, Z., Wheatley, M., Coote, J. H. (2002) Neuropeptides, amines and amino acids as mediators of the sympathetic effects of paraventricular nucleus activation in the rat. Exp. Physiol. 87, 663-674.
79. Yu, S.-Q., Lundeberg, T., Yu, L.-C. (2003) Involvement of oxytocin in spinal antinociception in rats with inflammation. Brain Res. 983, 13-22.

8. Appendix

Papers related to the thesis