Journal of Reproduction and Development, Vol. 49, No. 1, 2003

—Original—

Distribution of Glutamic Acid Decarboxylase, Neurotensin, Enkephalin, Neuropeptide Y, and Cholecystokinin Neurons in the Septo-Preoptic Region of Male Rats

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Abstract. Neurons in the lateral septum (LS) and preoptic area (POA) are known to play an inhibitory role in feminine sexual behavior regulation in male rats. In this study, the distribution of neurons containing glutamic acid decarboxylase (GAD) and of the peptidergic neurotransmitters neurotensin (NT), enkephalin (ENK), neuropeptide Y (NPY), and cholecystokinin (CCK), was examined immunohistochemically in the LS and POA of castrated male rats subcutaneously implanted with estrogen-containing Silastic tubes. Colchicine was injected into the lateral ventricle of the animals. The forebrain sections were immunostained for each substance. A large number of GAD-immunoreactive (ir) cells were found in the LS. Many NT-ir cells were seen in the intermediate and ventral parts of the LS at the rostral and middle levels. A considerable number of ENK-ir cells were scattered over the LS at the rostral and middle levels and were observed in the ventral part of the caudal LS. There were only a few NPY-ir cells in the LS. No CCK-ir cells were observed in the LS. In the POA, GAD-ir cells were observed in abundance. Many NT-ir cells were seen, especially in the medial preoptic nucleus. Some ENK-ir cells and a few NPY-ir cells were found in the medial POA. CCK-ir cells of the POA were restricted to the periventricular and paraventricular hypothalamic nuclei.
Key words: Lateral septum, Preoptic area, Glutamic acid decarboxylase, Peptidergic neurotransmitter, Male rat

(**J. Reprod. Dev. 49:** 67–77, 2003)

The lateral septum (LS) and preoptic area (POA) are known to regulate reproductive behavior in both male and female rats [1, 2]. The LS exerts an estrogen-dependent inhibitory influence on feminine sexual behavior in female rats [3] and a facilitative influence on masculine sexual behavior in males [4]. Like the LS, the POA plays similar roles in females [5] and in males [6–8].

Accepted for publication: October 5, 2002
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The septo-preoptic region of males is further known to play an important role in producing sex differences as regards the expression of feminine sexual behavior [9]. Although the expression of lordosis, a characteristic female sexual behavior, is very rare even in castrated male rats treated with estrogen, lesions of the LS or transections of the septal fibers make the males show lordosis [4, 10, 11]. Lesions of the POA also enhance lordosis behavior in male rats and guinea pigs [12, 13]. Recently, our previous study suggested that

neurons in the LS project axons directly to the midbrain central gray (MCG); this projection plays a critical role in inhibiting lordosis displayed by estrogen-treated castrated male rats [14]. In addition, the number of septal neurons in female rats is larger than that in male rats [15], suggesting the sexual dimorphism of the LS-MCG neural connection in rats.

Many types of neurotransmitter are found in the rat septo-preoptic region [16–18]. Gamma-aminobutyric acid (GABA) [19, 20], enkephalin (ENK) [21], neuropeptide Y (NPY) [22], and cholecystokinin (CCK) [23] have been reported to play inhibitory roles in regulating lordosis expression in female rats. On the basis of the current evidence, and as the first step to analyzing the lordosis-inhibiting influence in male rats, the present study investigated the precise locations of neurons containing glutamic acid decarboxylase (GAD), ENK, NPY, and CCK in the septo-preoptic region of castrated male rats administered estrogen. Furthermore, the distribution of neurotensin (NT) producing neurons was also examined, as the existence of NTergic neurons has been described in the LS and POA of intact male rats [18, 24].

Materials and Methods

Animals

Adult male Wistar rats weighing 250–340 g (Takasugi Animal Farm, Saitama, Japan) were used in accordance with the Guidelines for the Care and Use of Laboratory Animals in the Human Science Department of Waseda University. The animals were kept in a light (14 h light: 10 h dark, lights on at 0700 h) and temperature (23–25 C) controlled room with free access to water and food.

All animals were gonadectomized under ether anesthesia. Fourteen days after gonadectomy, the animals were anesthetized by ether and subcutaneously implanted with two Silastic tubes (inner diameter, 1.57 mm; outer diameter, 3.18 mm; length, 30 mm., Kaneka Medix Co., Osaka, Japan) containing 17β-estradiol (Sigma Chemical Co., St. Louis, MO, USA) in order to mimic the hormonal conditions reported in our previous studies investigating the lordosis-inhibiting system of male rats [14, 15].

Colchicine injection

Eleven days after estrogen treatment, colchicine (100 μg dissolved in 35 μl of saline per animal, Sigma) was infused into the lateral ventricle on the right side to enhance immunoreactivity. Under ether anesthesia, animals were placed in a stereotaxic instrument with the bregma and lambda at the same dorsoventral level. The tip (diameter, 30–40 μm) of a glass micropipette was lowered to a point 0.8 mm caudal and 3.6 mm below the bregma and 1.4 mm lateral to the midline. Colchicine was infused at a flow rate of 3.5 μl/min for 10 min by a microinfusion pump through a Hamilton microsyringe connected to a micropipette. The micropipette was kept in place for 5 min to complete the infusion.

Tissue preparation

Two days after the colchicine injection, animals deeply anesthetized by sodium pentobarbital (20–25 mg per animal) were perfused intracardially with 50 mM phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 50 mM phosphate buffer (pH 7.4). Brains were postfixed with the same fixative for 2 h and immersed in 30% sucrose in 50 mM phosphate buffer for 4–5 days at 4 C. Serial coronal sections (50 μm) of the forebrain were made with a cryostat and collected as five series of sections. One complete section series obtained from 4, 4, 6, 5, or 5 animals was used for immunostaining for GAD, NT, ENK, NPY, or CCK respectively.

Primary antibodies

The present study employed a rabbit anti-GAD 67 kDa antiserum (Chemicon International, Inc., Temecula, CA, USA), a rabbit anti-NT antiserum (Eugene Tech International Inc., Bridgefield, NJ, USA), a mouse anti-ENK antibody (Chemicon), a rabbit anti-NPY antiserum (Sigma) and a rabbit anti-CCK (26–33) antiserum (Research Biochemicals International, Natick, MA, USA). The specificities of anti-GAD [25, 26], anti-ENK [27], and anti-CCK [28] have already been described. The instructions for anti-NPY also mention the specificity confirmed by an absorption test with the antigen. To confirm the specificity of anti-NT, antiserum solution was preabsorbed with a synthetic NT (final concentration of 300 μg/ml, Sigma) at 4 C overnight before immunostaining. Immunoreactive signals were abolished by the

preabsorption.

Immunostaining

For the GAD-immunohistochemical analysis, free-floating sections were incubated with (1) 0.6% H_2O_2 in 50 mM PBS for 30 min at room temperature, (2) 5% normal goat serum (NGS) in 50 mM PBS for 1 h at room temperature, (3) anti-GAD (1:1000) in 50 mM PBS containing 5% NGS for 72 h at 4 C, (4) biotinylated goat anti-rabbit IgG (1:300, Vector Laboratory Inc., Burlingame, CA, USA) in 50 mM PBS at 4 C overnight, and (5) peroxidase-conjugated streptavidin (1:300, Dako, Carpinteria, CA, USA) in 50 mM PBS for 2 h at room temperature. Sections were rinsed in 50 mM PBS 2–3 times after each step, except between steps 2 and 3. After step 5, sections were rinsed in 100 mM Tris-HCl buffer (pH 7.2) and reacted with 0.05% 3, 3′-diaminobenzidine, 0.01% H_2O_2, and 0.08% ammonium nickel sulfate in the same buffer.

For NT-, NPY-, and CCK-immunohistochemistry, sections were processed according to the same protocol as that used for the GAD-immunohistochemistry, with the exception of steps 2, 3, and 4. Alternatively, sections were incubated with 5% NGS and 0.4% Triton X-100 in 50 mM PBS for 1 h at room temperature (step 2), with either anti-NT (1:1000), anti-NPY (1:5000), or anti-CCK (1:5000) in 50 mM PBS containing 5% NGS and 0.4% Triton-X-100 for 72 h at 4 C (step 3), and with biotinylated goat anti-rabbit IgG (1:300) in 50 mM PBS containing 0.4% Triton X-100 at 4 C overnight (step 4).

For the ENK-immunohistochemistry, our data obtained by using a dilution of anti-ENK (1:100, 1:300, and 1:600) indicated that dilution (1:100 and 1:300) detected ENK peptide in the sections, and that distribution of ENK-ir signals had no striking differences between the sections immunostained with anti-ENK diluted at 1:100 and 1:300. However, ENK-ir signals were weak when anti-ENK dilution (1:600) was used. Therefore, the present study presents the results of immunostaining with anti-ENK dilution (1:100 and 1:300, 3 animals for each). Free-floating sections were incubated with (1) 0.6% H_2O_2 in 50 mM PBS for 30 min at room temperature, (2) 5% NGS and 0.4% Triton X-100 in 50 mM PBS for 1 h at room temperature, (3) anti-ENK (1:100 or 1:300) in 50 mM PBS containing 5% NGS and 0.4% Triton X-100 for 72 h at 4 C, (4) biotinylated donkey anti-mouse IgG (1:2000, Chemicon) in 50 mM PBS containing 0.4% Triton X-100 at 4 C overnight, and (5) peroxidase-conjugated streptavidin (1:300) in 50 mM PBS for 2 h at room temperature. Sections were rinsed in 50 mM PBS 2-3 times after each step, except between steps 2 and 3. After step 5, sections were rinsed in 100 mM Tris-HCl buffer and reacted with 0.05% 3, 3′-diaminobenzidine, 0.01% H_2O_2, and 0.08% ammonium nickel sulfate in the same buffer.

Light microscopy

Immunostained sections were examined under a light microscope. Line drawings of the forebrain were rendered with the aid of a computer and a CCD camera connected to a light microscope, and immunoreactivities were mapped onto the drawings. Additionally, forebrain sections were prepared from males of the same age as those used for immunostaining, and the sections were stained with cresyl fast violet. The sections and the rat brain atlas [29] were used as references for the anatomical determination of the location of immunoreactivities.

Results

In the septo-preoptic region, GAD-, NT-, ENK-, NPY-, and CCK-immunoreactive (ir) cell bodies and fibers were seen with variations in amount and location. Figure 1 shows the distribution of GAD-, NT-, ENK-, NPY-, and CCK-ir cell bodies and fiber bundles in individual animals for each immunostaining. Line drawings indicate the rostral (a, e, i, m, q), middle (b, f, j, n, r), and caudal (c, g, k, o, s) parts of the LS in the anteroposterior axis. In addition, the LS is divided into dorsal, intermediate, and ventral parts (LSd, LSi, and LSv) in the dorsoventral axis. The POA is shown at two levels from rostral (c, g, k, o, s) to caudal (d, h, l, p, t) perspective.

LS

GAD-ir neurons: Numerous GAD-ir cell bodies were found in the LSd, LSi, and LSv in all sections with uniform spreading (Fig. 1a-c). The LS contained two types of GAD-ir cells (Fig. 2A). Immunoreactive signals in some GAD-ir cells were located only in the soma, whereas others had immunoreactivities in both the soma and the short fibers. However, dense GAD-ir fiber bundles were

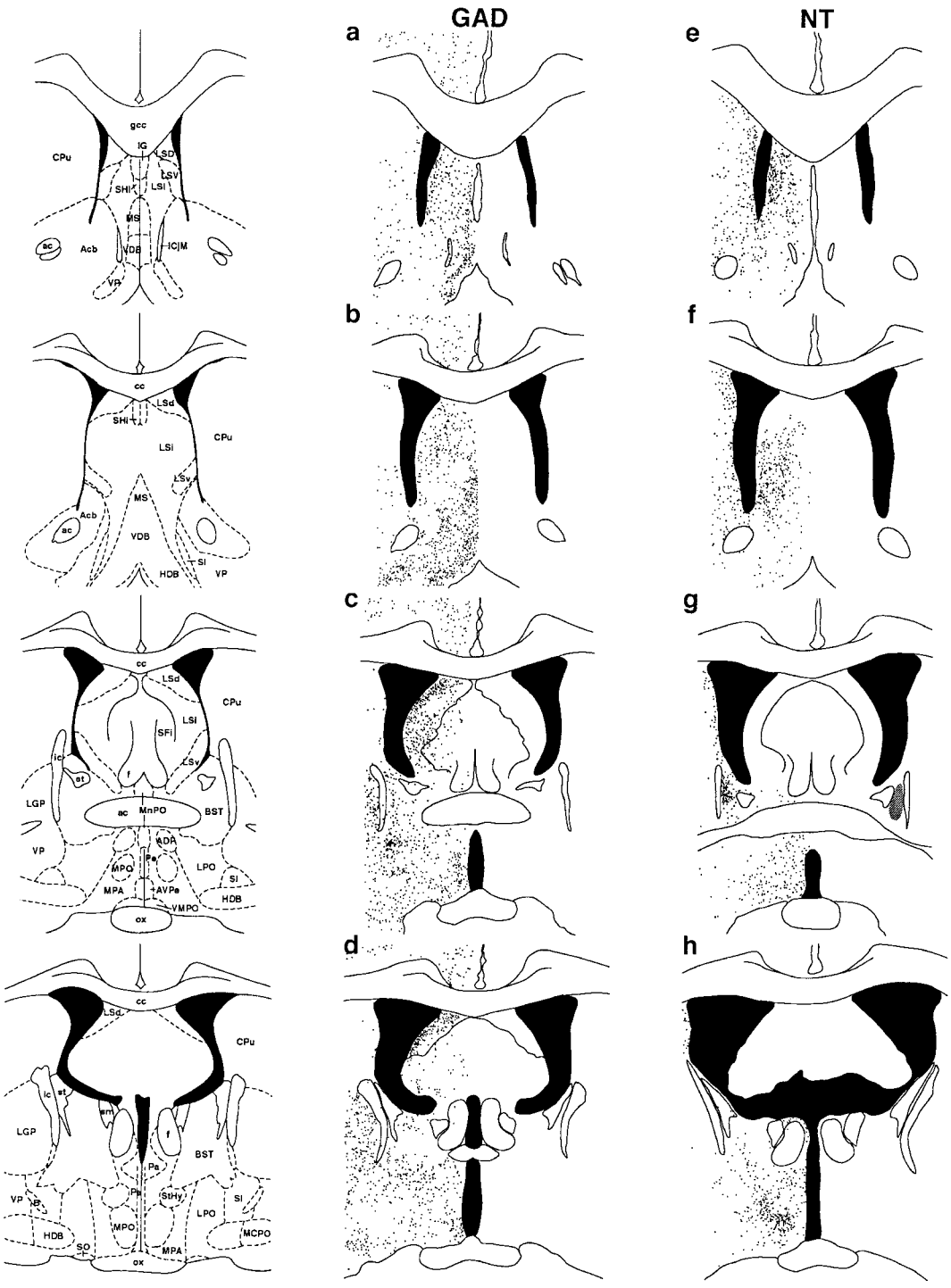
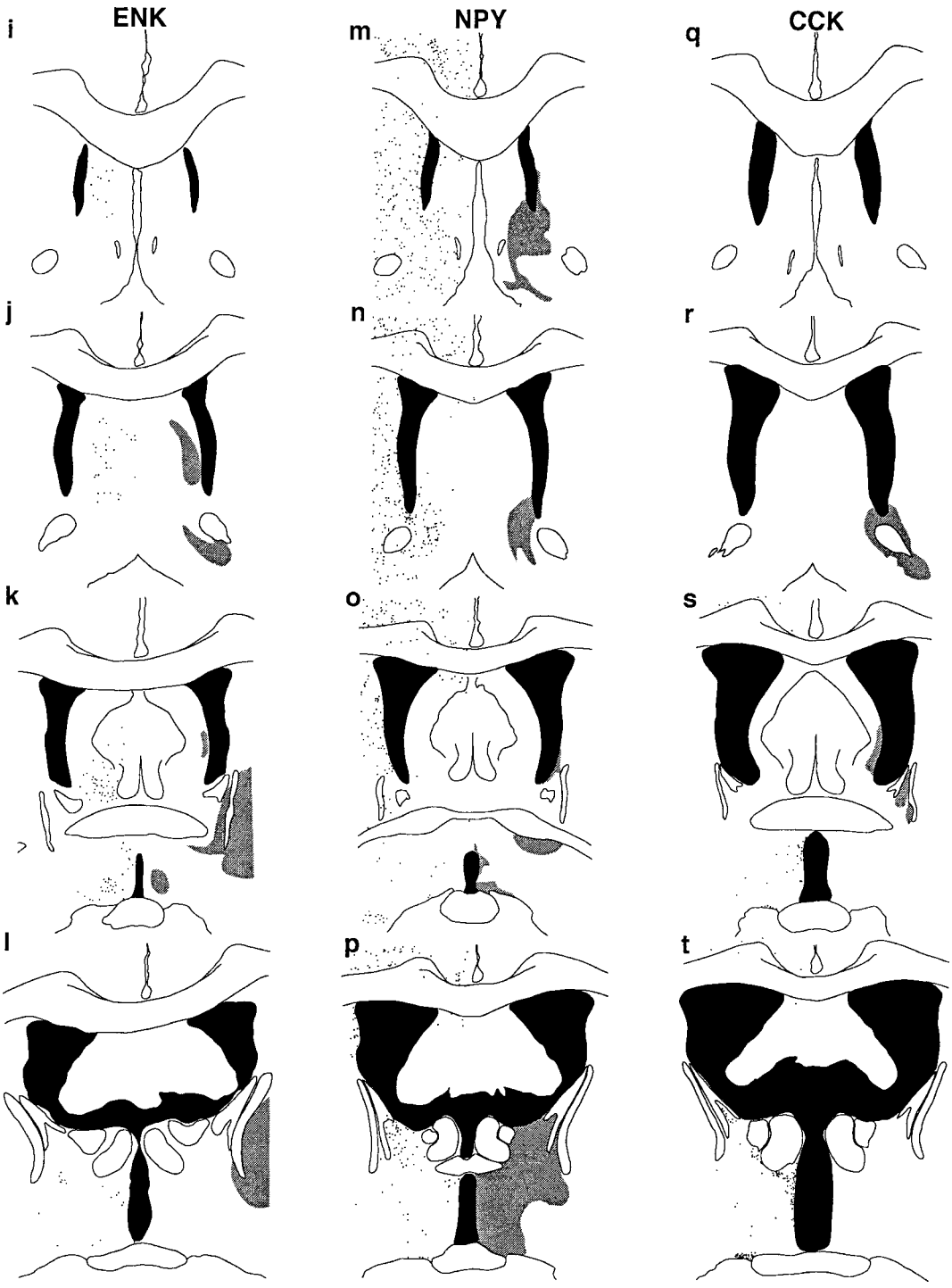


Fig. 1. Distribution of GAD-, NT-, ENK-, NPY-, and CCK-ir cell bodies and fiber bundles in the septo-preoptic region of estrogen-treated castrated male rats. Each dot indicates an immunoreactive cell body. Gray fields show the location of immunoreactive fiber bundles. The locations of immunoreactivities are noted in the left-hand column at equivalent levels. Abbreviations: ac, anterior commissure; Acb, accumbens nucleus; ADP, anterodorsal preoptic nucleus; AVPe, anteroventral periventricular nucleus; B, basal nucleus; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CPu, caudate putamen; f, fornix; gcc, genu of the corpus callosum; HDB, nucleus of the horizontal limb of the diagonal band; ic, internal capsule; ICjM, major island of Calleja; IG, indusium griseum; LGP, lateral globus pallidus; LPO, lateral preoptic area;



LSd, dorsal part of the lateral septum; LSi, intermediate part of the lateral septum; LSv, ventral part of the lateral septum; MCPO, magnocellular preoptic nucleus; MnPO, median preoptic nucleus; MPA, medial preoptic area; MPO, medial preoptic nucleus; MS, medial septum; ox, optic chiasm; Pa, paraventricular hypothalamic nucleus; Pe, periventricular hypothalamic nucleus; SFi, septofimbrial nucleus; SHi, septohippocampal nucleus; SI, substantia innominata; sm, stria medullaris of the thalamus; SO, supraoptic nucleus; st, stria terminalis; StHy, striohypothalamic nucleus; VDB, nucleus of the vertical limb of the diagonal band; VMPO, ventromedial preoptic nucleus; VP, ventral pallidum.

not observed.

NT-ir neurons: NT-ir cell bodies were abundant in the rostral and middle parts of the LSi and LSv (Fig. 1e, f). NT-ir cells were also seen in the rostral LSd. It was observed that most of the NT-ir cells had a large soma and multiple fibers (Fig. 2B). On the other hand, there were few NT-ir cells in the middle part of the LSd and in the caudal part of the LSd, LSi, and LSv (Fig. 1f, g). Dense NT-ir fiber bundles were not seen in the LS.

ENK-ir neurons: ENK-ir cell bodies were scattered over the rostral and middle parts of the LSd, LSi, and LSv (Fig. 1i, j). In the caudal part, a considerable number of ENK-ir cells were found only in the LSv (Fig. 1k). ENK-ir cell bodies were small and some of the ENK-ir cells had a few immunoreactive fibers (Fig. 2C). A large, dense ENK-ir fiber plexus was found in the LSi and LSv along the lateral ventricle (Fig. 1j, k and Fig. 2D).

NPY-ir neurons: A small number of NPY-ir cell bodies, which had multiple immunoreactive fibers, were seen in the LSd, but the LSi and LSv had few NPY-ir cells (Fig. 1m-o). No NPY-ir fiber bundles were observed in the LS.

CCK-ir neurons: CCK-ir cells were not found in any part of the LS (Fig. 1q-s). However, a small amount of CCK-ir fiber bundles were seen in the caudal LSv close to the lateral ventricle (Fig. 1s).

POA

GAD-ir neurons: Many GAD-ir cells were observed in the lateral and medial preoptic areas, medial and ventromedial preoptic nuclei, anteroventral periventricular nucleus, and periventricular hypothalamic nucleus (Fig. 1c, d). The POA, as well as the LS, had both GAD-ir cell bodies with and without a few short fibers, although prominent GAD-ir fiber bundles were not seen.

NT-ir neurons: A large number of NT-ir cells were seen in the medial preoptic area and medial preoptic nucleus (Fig. 1g, h). The periventricular hypothalamic nucleus, anteroventral periventricular nucleus, and ventromedial preoptic nucleus also contained NT-ir cells. In contrast, the lateral preoptic area contained a small number of NT-ir cells. Most of the NT-ir cells in the POA were found to have multiple fibers, but dense NT-ir fiber bundles were not observed.

ENK-ir neurons: Several ENK-ir cells were seen in the medial preoptic area (Fig. 1k, l). In this area, most of the ENK-immunoreactivities were observed in the small cell bodies, some of which had scant immunoreactive fibers. ENK-ir fiber bundles were observed in the medial preoptic nucleus and medial preoptic area (Fig. 1k).

NPY-ir neurons: There were few NPY-ir cells in the POA. In contrast, NPY-ir fiber bundles were found in the medial preoptic area, periventricular hypothalamic nucleus, ventromedial and medial preoptic nuclei, and striohypothalamic nucleus (Fig. 1o, p).

CCK-ir neurons: CCK-ir cell bodies were localized in the periventricular and paraventricular hypothalamic nuclei (Fig. 1s, t). These CCK-ir neurons were parvocellular neurons and most CCK-immunoreactivity was observed in the cell bodies. However, other preoptic regions had few CCK-ir cells. CCK-ir fiber bundles were not found in the POA.

Other regions

GAD-ir neurons: GAD-ir cells were abundant in the medial septum (MS) and nuclei of the vertical and horizontal limbs of the diagonal band (VDB and HDB) (Fig. 1a, b). The accumbens nucleus (Fig, 1a, b) and bed nucleus of the stria terminalis (BST) (Fig. 1c, d) had a considerable number of GAD-ir cells. GAD-ir cells were also seen in the cerebral cortex (Fig. 1a-d), lateral globus pallidus (Fig. 1c, d), ventral pallidum (Fig. 1b-d), septofimbrial nucleus (Fig. 1c), and substantia innominata (Fig. 1b-d). Prominent GAD-ir fiber bundles were not observed.

NT-ir neurons: Several NT-ir cells were observed in the MS, VDB, and HDB (Fig. 1e, f). Many NT-ir cells were found in regions surrounding the lateral ventricle, the caudate putamen, accumbens nucleus, and the BST (Fig. 1e-h). NT-ir fiber bundles were seen in the BST (Fig. 1g).

ENK-ir neurons: A few ENK-ir cells were found in the MS and septofimbrial nucleus (Fig. 1j, k). The VDB and HDB had few ENK-ir cells (Fig. 1i, j). ENK-ir fiber bundles were observed in the accumbens nucleus, lateral globus pallidus, and BST (Fig. 1j-l).

NPY-ir neurons: In the MS, VDB, and HDB, few NPY-ir cells were seen (Fig.1m, n). The accumbens nucleus (Fig. 1m, n) and BST (Fig. 1o, p) contained NPY-ir cells and fiber bundles. NPY-ir cells were widely observed in the cerebral cortex and caudate putamen (Fig. 1m-p).

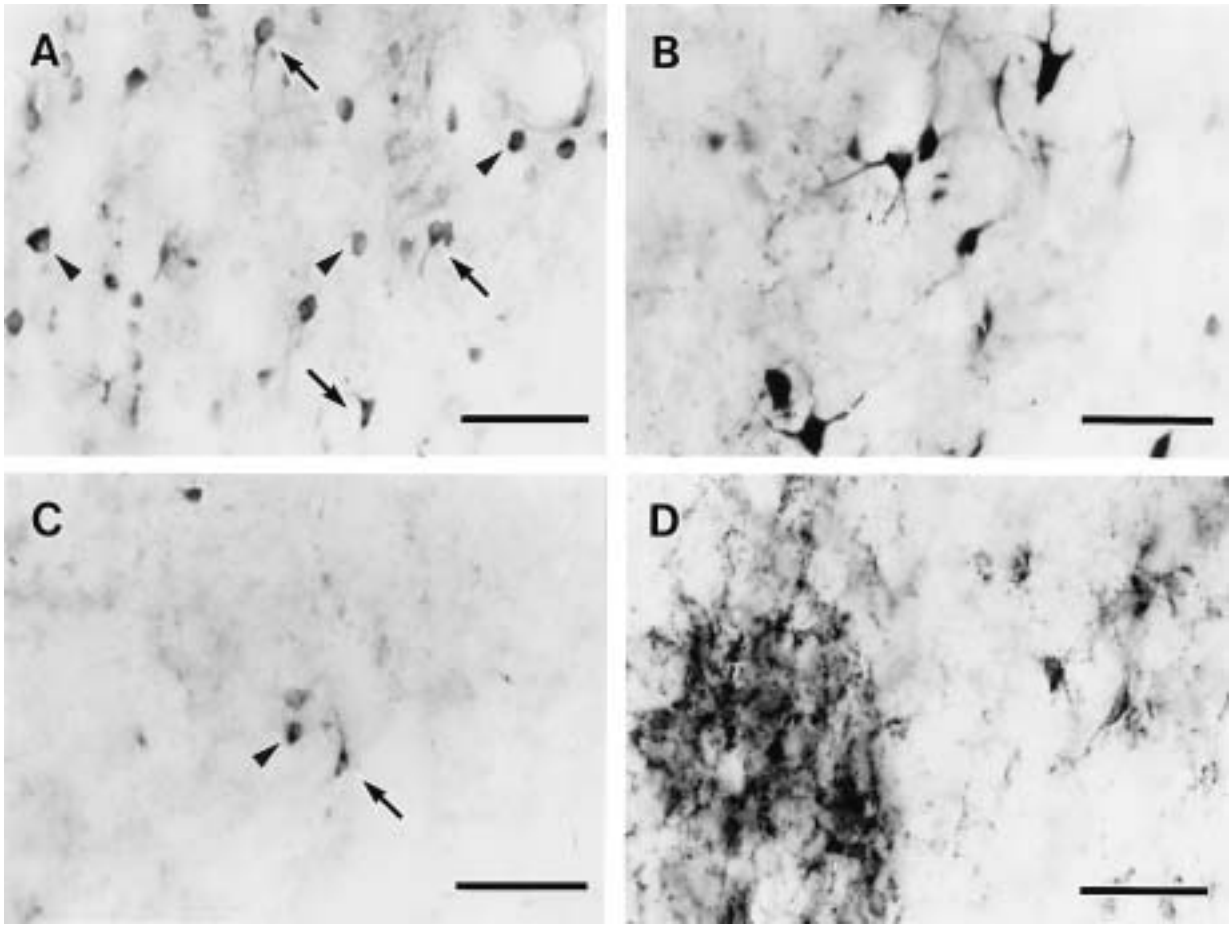


Fig. 2. Photomicrographs of the LSi immunostained for GAD (A), NT (B), or ENK (C and D) at the middle levels approximately corresponding to those in Fig. 1 b, f, and j, respectively. Scale bar: 50 μm. The LS contains GAD-ir perikarya with and without immunoreactive fibers (arrows and arrowheads in A, respectively), NT-ir perikarya with multiple fibers (B), ENK-ir perikarya with and without immunoreactive fibers (arrow and arrowhead in C, respectively), and ENK-ir fiber plexus (D).

CCK-ir neuron: Few CCK-ir cell bodies were found in the MS, VDB, and HDB (Fig. 1q-s). Many CCK-ir cell bodies were located in the supraoptic nucleus (Fig. 1t). The BST contained some CCK-ir cells. The accumbens nucleus and BST had CCK-ir fiber bundles (Fig. 1r, s).

Discussion

The widespread existence of GABAergic neurons in the rat brain is well-known [17]. Many GABAergic neurons are located in the LS and POA of intact male rats [18, 30]. The present study also observed a large number of GAD-ir perikarya in the LS and POA of estrogen-treated castrated rats. However, the number of GABAergic neurons may be decreased by castration, since it has been reported that GABAergic neural activity in the male POA decreases following castration [31, 32]. The POA is suggested to possess sex differences regarding GABA synthesis and activity, whereby GABA levels are generally larger in male rats than in female rats [33, 34]. However, there is one report suggesting that the GABAergic system in the POA exhibits no sex differences [30]. Although some GAD-ir neurons had short immunoreactive fibers in the LS and POA, prominent GAD-ir fiber bundles were not seen in the present study. GABAergic neurons are known to have short axons, with some exceptions [35–37]. There is no evidence for the long projection of GABAergic

neurons in the LS and the POA. GABAergic neurons may form a local circuit and contribute to various functions in these regions, although details of the GABAergic circuit are still unclear.

The present study showed that NT-ir cells were abundant in the LSi and LSv at the rostral and middle levels, but that they were scarce in the caudal LS. An *in situ* hybridization study revealed that many neurons expressing NT mRNA are found in the rostral and middle parts of the LSi and LSv of intact male rats [18]. This finding suggests that distribution of NTergic neurons in estrogen-treated castrated rats corresponds with that of normal male rats, although sex differences in NTergic neurons in the LS are nuclear. In contrast, the POA is known to contain the sexual dimorphic nucleus [38], and sexual dimorphic distribution of NTergic neurons was reported in the rat POA [24]. In the latter report, many NTergic neurons in the medial preoptic nucleus were found to be closer to the periventricular nucleus in females than in males. As shown in Fig. 1h of the present study, it seems that a larger number of NT-ir cells are found in the lateral portion of the medial preoptic nucleus rather than in the medial portion. The distribution of NTergic neurons in estrogen-treated castrated rats may be similar to that in intact males.

In this experiment, ENK-ir cells were seen in the rostral and middle parts of the LS and in the caudal part of the LSv in estrogen-treated castrated males. The number of ENK-ir cells was smaller than the number of GAD-ir or NT-ir cells. In intact male rats, a similar distribution of ENKergic neurons was reported, as well as the observation that the amount of ENKergic neurons was smaller than that of NTergic neurons [18]. However, in the experimental animals of the present study, the septofimbrial nucleus contained only a small number of ENKergic neurons, whereas intact males were shown to have many ENKergic neurons [18]. The discrepancy may have been due to the difference in the steroid hormonal regime, although the possibility that differences in histological techniques resulted in the discrepancy cannot be excluded. The existence of an ENKergic fiber plexus in the LS was demonstrated in intact male rats [18] and female guinea pigs [39], and corresponded to our results using estrogen-treated castrated male rats. Although the POA had ENK-ir cells and fiber bundles to a lesser extent in this study than in previous studies, it was reported that the medial preoptic nucleus of males and the anteroventral periventricular nucleus of females contain more ENKergic fibers compared with those of the other sex [40, 41], and that ENKergic neurons in both nuclei are larger in males than in females [40].

As described in intact male rats [42], NPYergic neurons are scarce in the LS and POA, whereas a large number of NPYergic neurons are scattered around the accumbens nucleus, cerebral cortex, and caudate putamen. This study also showed a similar distribution of NPYergic neurons in estrogen-treated castrated rats. In one study using intact male rats, NPY mRNA-expressing neurons in the septal region were restricted to the septohippocampal nucleus [18], although this distribution was not observed in this experiment or in another study [42]. In the POA, we observed the location of NPY-ir fibers as partly consistent with the location of NPYergic fibers reported in male and female rats [43–45], although persuasive histological studies are needed to determine sex differences in the NPYergic system in the septo-preoptic region. Since the NPY content in the POA is higher in female rats masculinized by neonatal testosterone treatment than in normal females [46], the NPYergic system in the POA may be sexually differentiated.

The distribution of CCKergic neurons has been reported in rats [17, 47]. Previous reports showing the existence of CCK-ir cells in the POA and CCK-ir fibers in the LS may support the present results. In this study, most of the CCK-ir cells were restricted to the periventricular hypothalamic nucleus of the POA. It has also been reported that the nucleus shows sex differences regarding the amount of CCKergic neurons, which were larger in females than in males [47].

In the present study, the locations of GAD-ir and NT-ir neurons partly overlapped with those of ENK-ir fibers in the LS and POA. In addition, the preoptic region possessing GAD-ir and NT-ir neurons was included in the region in which NPY-ir fibers were also observed. As regards the determination of the chemoarchitecture of the LS and POA, the present study indicated the possibility that the activities of GABAergic and NTergic neurons were modulated by ENKergic or NPYergic afferents to the LS and POA. Electron microscopic studies have demonstrated that GABAergic neurons in the LS receive synaptic

inputs from ENKergic axons [48, 49]. These studies provide at least partial support for the possibility that the ENKergic system modulates the GABAergic system in the LS. Further detailed studies employing confocal microscopy or electron microscopy will be needed.

For the purpose of investigating lordosis-inhibiting neurotransmitters in the male LS and POA, the present study prepared an experimental model of castrated male rats to remove the effects of androgen; these animals were then treated with estrogen. Besides roles in the inhibition of lordosis, the LS and POA are known to play important roles in the regulation of masculine sexual behavior in male rats [4, 6–8]. Androgen plays a key role in the regulation [2], and the male LS and POA contain a large number of androgen receptors [50]. Furthermore, many reports have indicated that the POA exerts an androgen-dependent facilitative influence on regulating masculine behavior [7, 8, 51]. On the other hand, we have shown that the lordosis-inhibiting influence remains in male rats castrated and treated with estrogen [14, 15]. Taken together, these results suggest that androgen-dependent masculine sexual behavior regulation follows the activity of the neurotransmitters affected by androgen, and that lordosis-inhibiting neurotransmitters in males are expressed, even under the influence of estrogen treatment after castration. We therefore conclude that the estrogen-treated castrated rat model enables valuable examination of the lordosis-inhibiting systems in male rats.

The LS is involved in regulation of autonomic functions and it projects axons to widespread regions, e.g., the hippocampus, lateral and anterior hypothalamic areas, and the MCG [16, 52]. In the LSi, neurons that directly project axons to the rostral MCG are involved in the lordosis-inhibiting system in male rats under the same hormonal treatment as that used in this study [14]. The present study showed NTergic and ENKergic neurons in the LSi. The MCG has NTergic [53, 54] and ENKergic [55, 56] nerve terminals and their receptors. Although it is premature to conclude which types of neurotransmitters are necessary for the lordosis-inhibiting pathway from the LS to the MCG, the neurotransmitters found in the LSi are possible candidates. In addition, since the GABAergic system plays an inhibitory role in regulating female sexual behavior, not only in female rats [19, 20] but also in male rats [57], GABAergic neurons in the LS may be involved in the inhibition of lordosis. It is possible that ENKergic and/or CCKergic afferents to the male LS play a role in the regulation of lordosis, the inhibitory influence of ENK [21] and CCK [23] on lordosis.

The medial part of the POA contains a large number of GAD-ir and NT-ir cells, a moderate number of ENK-ir cells, and a large amount of NPY-ir fibers. Several lines of evidence suggest the sexual dimorphism of these neurotransmitters in the POA [24, 33, 34, 40, 41, 46], and also their roles in the regulation of lordosis [19–22, 57]. As well as the LS, the medial preoptic area exerts an inhibitory influence on the regulation of lordosis and is involved in the determination of sex differences [9]. The POA neurons send axons to the lower brain stem, including the MCG, through the medial forebrain bundle [14, 58]. Further study is needed to understand the neurochemical determination of the neural tract regulating reproductive behaviors and sex differences.

Acknowledgements

This work was supported by Research Grants from Waseda University to S. T. (2001A-178) and K. Y. (2001A-611), Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to S. T. (12760189) and K. Y. (11640669), and the Promotion and Mutual Aid Corporation for Private Schools of Japan to K. Y.

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