

## Morphological Studies of Cardiac Ganglionic Neurons in Primary Cell Culture

Wann-Yee Her, Mei-Fang Yang, Tin-Hsin Hsiao and Keh-Min Liu<sup>1</sup>

Department of Anatomy, Kaohsiung Medical College, Kaohsiung, Taiwan 807, R.O.C.

(Accepted July 16, 1993)

**Wann-Yee Her, Mei-Fang Yang, Tin-Hsin Hsiao and Keh-Min Liu (1994)** Morphological studies of cardiac ganglionic neurons in primary cell culture. *Zoological Studies* 33(2): 108-113. In mammals, several groups of cardiac ganglia (CG) are sparsely distributed in the posterior wall of the atrium and the interatrial septum. The CG from early postnatal rats were cultured for optimum observation of cellular morphology and direct access to neurons for immunohistochemical analysis. During the two weeks observation period, CG neurons were immunocytochemically stained with neuron-specific enolase (NSE) or calcitonin gene-related peptide (CGRP). Results showed that one day after cultivation cells migrated out from the cultured cells mass. After 4 days of culture, isolated or aggregated neurons dispersed on the flattened fibroblasts layer. Most neurons are multipolar and contain an eccentric oval nucleus. Two neuron types were observed, type I neurons, characterized by a few, short cytoplasmic processes, were numerous; type II neurons, characterized by many long branching processes with beaded enlargements, were isolated and few in number. Some cultured neurons showed NSE immunoreactivity (IR), in which, both type I neurons and type II neurons expressed strong NSE-IR in soma and processes. Alternately, CGRP-IR was found in the perikarya of type I neurons only. This study showed that the tissue culture system could identify two types of growing CG neurons which may indicate that CG neurons do not play the same physiological role, and need more investigation. Further study is required to identify the relationship between the two types of neurons.

**Key words:** Cardiac ganglia, Tissue culture, NSE, CGRP.

Cardiac neurons are structurally and functionally related to the contractile myocardium. There have been numerous morphological studies of intramural myocardium ganglia neurons. King and Coakley (1958) reported that in rats, kittens and humans cardiac ganglia were widely distributed; but, most of them were aggregated in the sub-epicardium of the posterior wall of the right atrium, especially around the entrance of the vena cava and coronary sinus. Cardiac ganglia structure was also reported, from either light or electron microscopic observations of the ganglionic neurons, as small granular cells (SIF cells), myelinated and non-myelinated nerve bundles, and capillaries (Baluk and Gabella 1990).

Recently, cardiac ganglia were studied histochemically (Heathcote and Sargent 1987), immunohistochemically (Moravec et al. 1990), and electro-

physiologically (Fieber and Adams 1990). Immunohistochemical cardiac ganglia studies used the whole-mount or cryostat section method; however, results suffered from poor resolution due to excessive specimen thickness and numerous surrounding preganglionic nerve bundles. To overcome these obstacles, a new cell culture technique was developed by Hassall and Burnstock (1986); cardiac ganglionic cells from neonatal guinea-pigs were isolated in vitro facilitating observation and study. However, little is known about cardiac ganglionic cell morphologic structure after long-term in vitro cultivation. Therefore, in the present study, 6-8 day postnatal rat cardiac ganglia were cultivated over a long-term for the purpose of building a new model for further, more-detailed studies.

Whether or not cultivated cells exhibit the same immunoactivities as those of in vivo cardiac

1. To whom reprints request should be sent.

ganglionic neurons is not known. The nervous tissue-specific enzyme, neuron-specific enolase (NSE), has been found in neuroendocrine cells and neurons in the peripheral nervous system (Wood and Mayer 1978), as well as in some parts of the central nervous system (Schmechel et al. 1978). Similarly, calcitonin gene-related peptide (CGRP) exists extensively in both the central and peripheral nervous systems. In the rat, CGRP immunoreactive nerve fibers were found in the brain, the spinal cord, and the alimentary tracts (Gibson et al. 1984, Kawai et al. 1985, Ohtani et al. 1989). That certain neuronal populations of the mud-puppy cardiac ganglia contain CGRP has also been reported by Neel and Parsons (1986). These findings prompted us to investigate whether or not the cultivated rat cardiac ganglionic cells can be labelled with the anti-NSE and CGRP antibodies. We report here the selective labelling of cultivated ganglionic cells and their morphological characteristics.

## MATERIALS AND METHODS

### Preparation of cell cultures

Sprague-Dawley rats, 6-8 days postnatal, were used as a tissue source. Under aseptic conditions, the hearts were removed from the decapitated rats. The atrial portion was cut and washed with 4°C Hank's balanced salt solution (HBSS) and placed in a petri dish containing 4°C Dulbecco's phosphate buffered saline (DPBS). In the petri dish, the atrium was cut into small 1 mm<sup>3</sup> pieces and incubated in HBSS with 0.2% collagenase for 10 to 15 minutes at 37°C. Cell suspensions were washed in a M-199 medium (GIBCO) containing 10% fetal bovine serum, glucose (5 mg/ml), and penicillin (100 unit/ml). Freshly isolated cells were seeded on poly-L-lysine coated 35 mm petri dishes and cultured in a M-199 medium with 10% fetal bovine serum, at 37°C in 5% CO<sub>2</sub>. The medium was changed twice weekly. Observation was performed with a Nikon phase-contrast microscope; photographs used T-MAX 100 film.

### Characterization of cells

#### Immunohistochemistry

After a 7-14 day cultivation, the monolayers were washed briefly in 0.1 M PBS (pH 7.3) and fixed in 4% paraformaldehyde at 4°C for 1 hr. After being again washed with 0.1 M PBS (pH 7.3),

they were washed 6 times in 80% alcohol at room temperature each for 10 min. After being again washed with 0.1 M PBS (pH 7.3), the monolayers were then treated with 0.1% Triton X-100 for 10 min and washed with 0.1 M PBS (pH 7.3). They were then blocked with normal goat serum overlay for 20 min and then processed for immunoreactivity staining.

#### Staining for NSE

The monolayers were incubated with rabbit antiserum primary antibody against neuron-specific enolase (ready to used) at 4°C overnight and then washed with 0.1 M PBS (pH 7.3). They were then incubated at room temperature with biotin-conjugated goat anti-rabbit IgG secondary antibody for 10 min. This was followed by: a 0.1 M PBS (pH 7.3) washing, a streptavidin peroxidase (1 drop/ml PBS) incubation, and, a DAB (0.01 g/20ml Tris buffer) as a substrate for the peroxidase reaction staining.

#### Staining for CGRP

The monolayers were incubated with rabbit antiserum primary antibody against calcitonin gene-related peptide (diluted 1000X with 0.01 M PBS/0.1% Triton X-100/0.1% lysine/0.01% BSA/0.1% sodium azide), at 4°C overnight, followed by a biotinylated goat anti-rabbit IgG secondary antibody at room temperature for 10 min. Observations used streptavidin peroxidase and DAB peroxidase reactions.

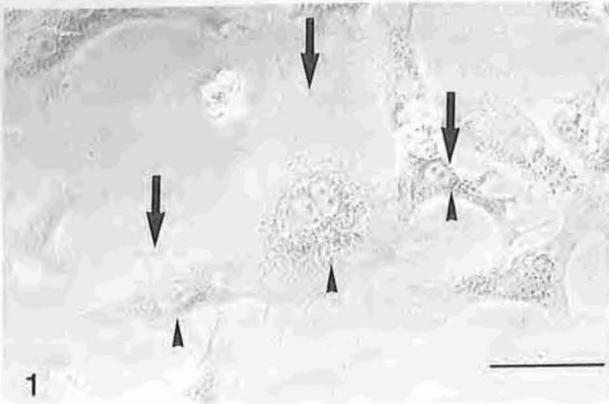
For the control staining, the monolayers were processed following the above procedures, with the exception that phosphate buffer solution (PBS) was used as the primary antibody.

## RESULTS

### Light microscopic observations

In phase-contrast microscopy, at the second day of culture, cells showed a typical fibroblast migration from tissue explants morphology. These irregular sized fibroblasts were characterized by flattened cell bodies, one to two nuclei, several nucleoli, and abundant perinuclear granular substance. Several sheets of membranous processes extended from the cell body (Fig. 1).

After four days of culture in vitro, the merging of proliferating fibroblasts formed several areas of monolayer cell carpet upon which isolated or aggregated neurons appeared. These small neurons,



**Fig. 1.** The phase-contrast microphotograph shows that 2 days after the culture, flattened fibroblasts possess one or more nuclei and several nucleoli (arrows). Numerous granular substances are distributed in the perikaryal region (arrowheads). Scale bar = 30  $\mu\text{m}$ .



**Fig. 2.** The phase-contrast microphotograph shows the type I neuron (arrow) is characterized by small oval shaped cell body and short processes with simple branches. Scale bar = 30  $\mu\text{m}$ .

classified as type I neurons (Fig. 2), possessed short processes with simple branches. From the 5th day of culture, a small number of large neurons with several long processes and complicated branches were observed and classified as type II neurons (Fig. 3). Most type I and type II neurons were multipolar and contained an eccentric oval nucleus. Beaded enlargements were found in the processes of type II neurons and some type I neurons (Figs. 3,4).

During early cultivation stages, fibroblasts, Schwann cells, endothelial cells and myocardial cells appeared in the petri dish; whereas, after two weeks in vitro, many active proliferating fibroblasts superseded other cell type.

### Immunohistochemistry

#### NSE-like immunoreactivity

Some of the cultured neurons showed NSE-like immunoreactivity (NSE-like IR) when both type I and type II neurons were immunostained with different intensity. Immunoreactivity was localized in the soma and processes of both isolated and aggregated neurons (Figs. 5A,5B,5C). Whereas, other cells did not immunostained.

#### CGRP-like immunoreactivity

In cultured cells, only some type I neurons showed CGRP-like immunoreactivity (CGRP-like IR) which was concentrated in the perikaryal region. These immunoreactive neurons were characterized by an oval-shaped cell body with short processes and an eccentric nucleus (Fig. 6).



**Fig. 3.** The phase-contrast microphotograph of higher magnification of the type II neuron (arrow) shows beaded varices in the processes (arrowheads). Scale bar = 30  $\mu\text{m}$ .



**Fig. 4.** The arrows indicate the beaded enlargements along the cytoplasmic processes of the type I neuron. Scale bar = 30  $\mu\text{m}$ .

5A

Fig. 5A. The immunoreactivity of NSE is localized in the soma and processes of the type I neuron. Scale bar = 30  $\mu$ m.

5B

Fig. 5B. The immunoreactivity of NSE is localized in the cell body and branched processes of the type II neuron. Scale bar = 30  $\mu$ m.

5C

Fig. 5C. The immunoreactivity of NSE in the aggregated neurons. Scale bar = 30  $\mu$ m.

6

Fig. 6. The strong immunoreactivity of CGRP can be found in the cell body of the type I neuron (arrow). Scale bar = 30  $\mu$ m.

## DISCUSSION

The culture of mammalian cardiac ganglionic cells as a study model has been done successfully in the guinea-pig (Hassall and Burnstock 1986, Kobayashi et al. 1986a,b) and in the human (Hassall et al. 1990). In our earlier experiments, the same methods were followed for the in vitro cultivation of rat cardiac ganglionic cells. However, we found that after having been incubated in 0.125% trypsin at 4°C overnight all the cells died. It is suggested that the cell death may either be due to the high sensitivity to trypsinization of rat cardiac ganglionic cells, or the physiological immaturity and vulnerability of those cells.

Alternately, Adams et al. (1987) reported that rat cardiac ganglionic cells had been in vitro cultured and survived as long as 72 hours. Therefore, Adams et al. (1987) culture method was modified and applied in our laboratory. Further avoiding the immaturity and vulnerability of neonatal rat cardiac ganglionic cells, 6-8 day postnatal rats were used instead of newborn rats.

In our culture system, fibroblasts, Schwann cells, endothelial cells, myocardial cells, and two types of neurons appeared. The earlier appearing type I neurons are small in size, and possess a few short processes with simple branches. The large type II neurons are characterized by several long processes with complicated branches. Throughout cultivation, no binucleated neurons were observed. The major difference between the culture of guinea-

pig cardiac ganglia (Hassall and Burnstock 1986) and the rat is that fewer neurons and well developed neurites were observed in rat cultures. It was thought that cardiac ganglia were removed completely during atria dissection, however, the paraffin or cryostat sections of the same tissue showed numerous cardiac ganglionic neurons. Suggested are ganglionic cells may be damaged by the tissue preparation or by poor culture conditions (such as insufficient nutrient components of the medium). Our recent experiments indicate that the addition of the nerve growth factor (NGF) to the medium do not promotes the neuron growth and the neurite complexity. However, the degree of environmental determination of neuron growth needs further investigation.

Two types of neurons, including large sized parasympathetic postganglionic neurons (principal neurons) and small sized catecholamine-containing fluorescent cells (intrinsic neurons or SIF cells) have been found in mudpuppy cardiac ganglia (McMahan et al. 1990, Neel and Parsons 1986). Whether or not the type I and type II neurons derived from cultured rat cardiac ganglia in the present experiment are exactly the same as those of the mudpuppy needs more morphological, functional, and electrophysiological study.

Different NSE-IR intensities were found in the cultured cardiac ganglionic neurons. It is well known that NSE is an enzyme for neuron glycolysis, and its concentration is dependent on the energy requirement of the neuron (Bishop et al. 1985). In addition, Whitehead et al. (1982) found that NSE-IR appeared only after the functional synapse of neurons was formed; Marangos et al. (1978) also reported that the NSE content is closely related to the neuron synaptic activity. Therefore it is suggested that NSE synthesis is initiated only in functioning neurons (Vinores et al. 1984). In the present study, the cultured cardiac ganglionic neurons are not immunostained by NSE which may indicate the necrosis or poor development of those neurons. Furthermore, during fixation, the different synaptic activities and metabolic requirements of neurons also showed different immunostaining intensities.

CGRP has been identified in different somatic and visceral afferent fiber systems in rats (Ju et al. 1987, Lee et al. 1985). Parsons and Neel (1987) reported that in organ cultures both preganglionic sympathetic nerve fibers and postganglionic sympathetic nerve fibers are lysed and disintegrated after in vitro cultivation for 8-10 days. Parsons and Neel (1987) also found that CGRP-IR in the

mudpuppy cardiac septum was located in the primary afferent fiber processes. Gerstheimer et al. (1986) reported that CGRP-IR nerve fibers formed the pericellular complex and surrounded the parasympathetic nerve cells. However, Gerstheimer et al. (1986) also found CGRP-IR in some guinea-pig atria intracardiac ganglionic cells. In the present study, the fact that no CGRP-IR nerve fibers were observed may be due to early degeneration of nerve fibers. CGRP-IR neuron and NSE-IR neuron structural similarity remains to be investigated. Further study is also required to identify the relationship between the two types of neurons.

**Acknowledgements:** This work was supported by a grant from the National Science Council of the Republic of China. NSC-81-0412-B037-508.

## REFERENCES

- Adams DJ, LA Fieber, B Konishi. 1987. Neurotransmitter action and modulation of a calcium conductance in rat cultured parasympathetic cardiac neurones. *J. Physiol.* **394**: 153.
- Baluk P, G Gabella. 1990. Some parasympathetic neurons in the guinea-pig heart express aspects of the catecholaminergic phenotype in vivo. *Cell Tissue Res.* **261**: 275-285.
- Bishop AE, F Carlei, V Lee, J Trojanowski, PJ Marangos, D Dahi, JM Polak. 1985. Combined immunostaining of neurofilaments, neuron specific enolase, GFAP and S-100. *Histochemistry* **82**: 93-97.
- Fieber LA, DJ Adams. 1990. Acetylcholine-evoked currents in cultured neurones dissociated from rat parasympathetic cardiac ganglia. *J. Physiol.* **434**: 215-237.
- Gerstheimer FP, J Metz. 1986. Distribution of calcitonin gene-related peptide-like immunoreactivity in the guinea-pig heart. *Anat. Embryol.* **175**: 255-260.
- Gibson SJ, JM Polak, SR Bloom, IM Sabate, PM Mulderry, MA Ghatel, GP McGregor, JFB Morrison, JS Kelly, RM Evans, MC Rosenfeld. 1984. Calcitonin gene-related peptide immunoreactivity in the spinal cord of man and eight other species. *J. Neurosci.* **4**: 3101-3111.
- Hassall CJS, G Burnstock. 1986. Intrinsic neurones and associated cells of the guinea-pig heart in culture. *Brain Res.* **364**: 102-113.
- Hassall CJS, R Penketh, C Rodeck, G Burnstock. 1990. The intracardiac neurones of the fetal human heart in culture. *Anat. Embryol.* **182**: 329-337.
- Heathcote RD, PB Sargent. 1987. Growth and morphogenesis of an autonomic ganglion. I. Matching neurons with target. *J. Neurosci.* **7**: 2493-2501.
- Ju G, T Hokfelt, E Brodin, J Fahrenkrug, JA Fischer, P Frey. 1987. Primary sensory neurons of the rat showing calcitonin gene-related peptide immunoreactivity and their relation to substance P-, somatostatin-, galanin-, vasoactive intestinal polypeptide- and cholecystokinin-immunoreactive ganglion cells. *Cell Tissue Res.* **247**: 417-431.
- Kawai Y, K Takami, S Shiosaka, PC Emson, CJ Hillyard, S Girgis, I MacIntyre, M Tohyama. 1985. Topographic localization of calcitonin gene-related peptide in the rat brain:

- an immunohistochemical analysis. *Neuroscience* **15**: 747-763.
- King TS, JB Coakley. 1958. The intrinsic nerve cells of the cardiac atria of mammals and man. *J. Anat.* **92**: 353-376.
- Kobayashi Y, CJS Hassall, G Burnstock. 1986a. Culture of intramural cardiac ganglia of the newborn guinea-pig. I. Neuronal elements. *Cell Tissue Res.* **244**: 595-604.
- Kobayashi Y, CJS Hassall, G Burnstock. 1986b. Culture of intramural cardiac ganglia of the newborn guinea-pig. II. Non-neuronal elements. *Cell Tissue Res.* **244**: 605-612.
- Lee Y, K Takmi, Y Kawai, S Girgis, CJ Hillyard, I MacIntyre, PC Emson, M Tohyama. 1985. Distribution of calcitonin gene-related peptide in rat peripheral nervous system with reference to its co-existence with substance P. *Neuroscience* **15**: 1227-1237.
- Marangos PS, AM Parma, FK Goodwin. 1978. Functional properties of neuronal and glial isoenzymes of brain enolase. *J. Neurochem.* **31**: 727-732.
- McKeon TW, RL Parsons. 1990. Galanin immunoreactivity in the mudpuppy cardiac ganglion. *J. Auto. Nerv. Syst.* **31**: 135-140.
- McMahan UJ, D Purves. 1976. Visual identification of two kinds of nerve cells and their synaptic contacts in a living autonomic ganglion of the mudpuppy (*Necturus maculosus*). *J. Physiol.* **245**: 405-425.
- Moravec M, J Moravec, S Forsgren. 1990. Catecholaminergic and peptidergic nerve components of intramural ganglia in the rat heart. *Cell Tissue Res.* **262**: 315-327.
- Neel DS, RL Parsons. 1986. Catecholamine, serotonin, and substance P-like peptide containing intrinsic neurons in the mudpuppy parasympathetic cardiac ganglion. *J. Neurosci.* **6**: 1970-1975.
- Ohtani R, T Kaneko, LW Kline, T Labedz, Y Tang, PKT Pang. 1989. Localization of calcitonin gene-related peptide in the small intestine of various vertebrate species. *Cell Tissue Res.* **258**: 35-42.
- Parsons RL, DS Neel, JW McKeon, RZ Carraway. 1987. Organization of a vertebrate cardiac ganglion: a correlated biochemical and histochemical study. *J. Neurosci.* **7**: 837-846.
- Schmechel D, PJ Marangos, AP Zis, M Brightman, FK Goodwin. 1978. The brain enolases as specific markers of neuronal and glial cells. *Science* **199**: 313-315.
- Vinore ST, MM Herman, LJ Rubinstein, PJ Marangos. 1984. Electron microscopic localization of neuron-specific enolase in rat and mouse brain. *J. Histochem. Cytochem.* **32**: 1295-1302.
- Whitehead MC, PJ Marangos, SM Connolly, DK Morest. 1982. Synapse formation is related to the onset of neuron-specific enolase immunoreactivity in the avian auditory and vestibular systems. *Dev. Neurosci.* **5**: 298.
- Wood JD, CJ Mayer. 1978. Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. *Nature* **276**: 834-837.

## 心臟神經節神經元的細胞培養及形態學研究

何宛怡 楊美芳 蕭廷鑫 劉克明

心臟神經節在哺乳類可分成幾群，主要分散在心房的後壁及心房間隔。爲了易於觀察細胞的形態及克服在免疫組織化學反應時，抗體不易進入的缺點，我們做出生6-8天大白鼠心臟神經節的體外培養，且在培養期間，用Neuron-specific enolase (NSE)及Calcitonin gene-related peptide (CGRP)抗體來做免疫細胞化學反應。在培養一天後，可見細胞由組織塊向外遷移；四天後，即可見單獨或成群的神經元在fibroblast layer上。神經元大部分爲多極性，且有一偏心的核，形態上可分爲兩種，Type I神經元數目較多，細胞突起較少且短；Type II神經元則數目較少，突起多而長，且有珠狀膨起。在免疫反應方面，Type I及Type II神經元皆有很強的NSE反應，集中在細胞本體及突起上，但只有Type I神經元在perikarya有CGRP的反應，其他細胞如fibroblast則無反應。故本實驗結果證明，組織培養可得兩種不同神經元，此兩種神經元在生理學上可能扮演不同的角色，因此需要進一步實驗找出彼此的關係。

關鍵詞：心臟神經節，組織培養，神經細胞專一烯醇酶(NSE)，降鈣素基因相關胜肽(CGRP)。

## The Distribution of Vasopressinergic and Oxytocinergic Neurons in the CNS of the Gerbil

Ching-Ming Wu and Ching-Liang Shen

Department of Anatomy, College of Medicine, National Cheng-Kung University, Tainan, Taiwan 701, R.O.C.

(Accepted September 9, 1993)

**Ching-Ming Wu and Ching-Liang Shen (1994)** The distribution of vasopressinergic and oxytocinergic neurons in the CNS of the gerbil. *Zoological Studies* 33(2): 114-125. This is the first immunohistochemical report on the distribution of the vasopressin (VP) and oxytocin (OT) producing neurons and their processes in the central nervous system of the gerbil. These neurons are primarily associated with the paraventricular, supraoptic and accessory neurosecretory nuclei. Two types of immunoreacted neurons, large multipolar (magnocellular) and small fusiform (parvocellular), coexist in some of these nuclei. The distribution of VP neurons is broader than that of OT neurons. Only VP neurons are observed in the suprachiasmatic nucleus, internal capsule, medial amygdala, dorsal hypothalamic area, dorsomedial nucleus, lateral hypothalamic area and dorsal capsule of the ventromedial nucleus within the hypothalamus. VP neurons in these nuclei where OT neurons do not coexist are all parvocellular type. By contrast, only OT neurons were detected in the medial preoptic area. VP and OT containing fibers were observed throughout the central nervous system of the gerbil. Most of the projections of these two neuropeptidergic fibers terminate in the neurohypophysis and median eminence. Extrahypophyseal projections have also been observed. The distribution of VP neurons and their fibers are more extensive in the gerbil than in other mammals. VP neurons distributed within the internal capsule, dorsal hypothalamic area and dorsal capsule of the ventromedial nucleus of the gerbil have not yet been described in other mammals. Moreover, the diffuse VP fiber distribution in the mammillary body of the gerbil is seldom observed in that of other mammals. Due to the antidiuretic role of VP, the well-developed VP system of the gerbil may be reflected by its excellent water reservation ability.

**Key words:** Immunohistochemistry, Vasopressin, Oxytocin.

The hypothalamic vasopressin (VP) and oxytocin (OT) neurons are well known for their respective neurohypophyseal hormone secretions, vasopressin and oxytocin. Many scholars have described the peripheral endocrine effects of VP and OT. VP affects antidiuresis regulation (Emmers 1973) and blood pressure (Sawyer 1971). OT effects uterine smooth muscle contraction during labor (Cross 1958, Munsick 1960) and mammary gland contraction during milk ejection (Peeters et al. 1960). In addition, increasing evidence confirms their neurotropic effects in liver metabolism (Martin and Baverek 1981), cardiovascular regulation (Mohring et al. 1981), thermoregulation (Cooper et al. 1979) and pain modulation (De Wied 1983). Other behavior, such as avoidance, reward, memory and maternal behavior are influenced by both neuropeptides (Van Wimersma Greidanus

1982).

The organization of the hypothalamo-hypophyseal system in many species has been studied with various methodologies (Scharrer and Scharrer 1954, Bargmann and Scharrer 1954, Dawson 1953, Peterson 1966). Technical limitations prevented earlier studies from revealing projecting fibers from this system clearly. Currently immunohistochemical techniques ease the identification of neuropeptide containing neurons as well as their neural pathways and targets. Immunohistochemical techniques have confirmed that VP and OT are produced by large perikarya of the supraoptic nucleus and several accessory neurosecretory nuclei as well as by large and small perikarya of the paraventricular nucleus. The hypothalamo-hypophyseal system has been described by using these techniques in the rat (Vandesande and

Dierickx 1975, Sokol et al. 1976), guinea pig (Sofroniew et al. 1979, Dubois-Dauphin et al. 1989a, b), cat (Reaves and Hayward 1979, Caverson et al. 1987), cow (Vandesande et al. 1975), horse (Melrose and Knigge 1989), pig (Van Eerdenburg et al. 1992), monkey (Zimmerman et al. 1977, Caffè et al. 1989), human (Dierickx and Vandesande 1977, Ulfig et al. 1990), and some nonmammalian vertebrates (Berk et al. 1982, Goossens et al. 1977). Among these, the system of the rat has been studied most pervasively.

In our study, we investigated the gerbil possession of a well-developed hypothalamo-hypophyseal system in order to explain its extraordinary water reservation ability. Besides, gerbil system data are unavailable; results of our study may be valuable in comparative studies of the hypothalamo-hypophyseal system in mammals.

## MATERIAL AND METHODS

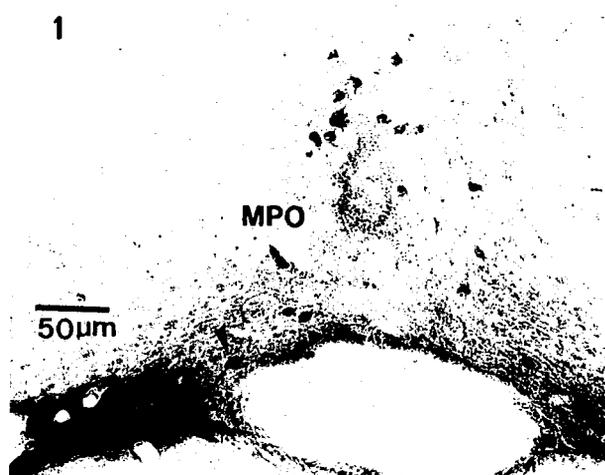
Six male Mongolian gerbils were used to study the central nervous system distribution of vasopressin and oxytocin immunoreactive cell bodies and fibers. We applied the indirect peroxidase-antiperoxidase (PAP) technique (Sternberger et al. 1970) in this study. Brief procedures are as follows:

The animals were anesthetized with Nembutal (35 mg/kg, I.P.) and fixed by intracardiac perfusion with a 4% peroxidate-lysine-paraformaldehyde (PLP) fixative. After removal and PLP postfixation (2 hrs) the brains and spinal cords were washed thoroughly with a 0.1M phosphate buffer (PB, pH 7.2) solution. Prior to sectioning, the brains and spinal cords were cryoprotected in 10% and 20% sucrose solutions each for a period of 2 hours, and then in a 30% sucrose solution in 0.1M PB overnight. Using a freezing microtome serial coronal sections of 40  $\mu\text{m}$  were collected and stored in six vials. Sections were then incubated at 4°C for 48 hours in primary antibody solutions: anti-rabbit vasopressin (vials 1, 3 and 5) or oxytocin (vials 2, 4, and 6) antisera (these antisera were pretreated by diluting antibody 1:1000 with 200  $\mu\text{g}$  of antisera and a 0.1M PB containing 0.3% Triton X-100 and 1.0% normal goat serum). After standing at room temperature for 30 minutes and a 0.1M PB rinse, sections were then incubated in goat-antirabbit-IgG secondary antibody solution (1:100) for 30 minutes, rinsed with 0.1M PB and exposed to rabbit peroxidase-antiperoxidase antibody (1:100) for 45 minutes. Sections were again

washed with a 0.1M PB. The incubated sections were then placed in a freshly prepared 0.05% diaminobenzidine (DAB) solution. A 0.3% hydrogen peroxide solution was added and the reaction was monitored under a low-power microscope for 20 minutes. Following the reaction stage, the sections were mounted on subbed slides, intensified with 0.1% osmic acid, dehydrated with ethanol, and cover-slipped with permount; they were then examined and under a light microscope a cell count was taken. All positive profiles with a soma were counted as a neuron. According to the specification sheet given supplied by Immuno Nuclear, all staining is blocked with absorption control for arginine and lysine indicative of VP and OT, respectively. Antisera specificities for VP (Lot No. V-5501, Sigma) and OT (Lot No. V-1627, Sigma) were further proven by preabsorption by some sections of a VP and OT antigen antisera (10  $\mu\text{l}$  concentration). No positive immunoreactivity was detected in these sections.

## RESULTS

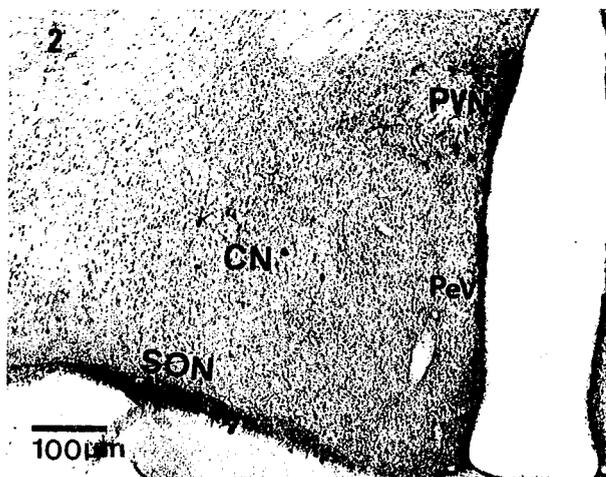
VP and OT antisera reacted neurons were brown or brownish and their processes were brown with beaded varicosities. Both neuropeptidergic neurons spreaded from the ventral of the forebrain (Fig. 1) to the diencephalon. Most VP neuron containing nuclei or areas also had OT neurons; they



**Fig. 1.** This is the most rostral level just before the anterior commissure (ac) where OT immunoreactivities begin to be found. Photomicrograph displays OT immunoreactive cell bodies in the medial preoptic nucleus (MPO) and on the ventral of the forebrain (arrow).

were concentrated mainly in the supraoptic nucleus, paraventricular nucleus and accessory neurosecretory nuclei of the hypothalamus (Fig. 2). Some neuron reaction in the bed nucleus of stria terminalis and periventricular zone was also noted. Two immunoreacted neuron types: large multipolar (magnocellular type, size 25-35  $\mu\text{m}$ ) and small fusiform (parvocellular type, size 12-20  $\mu\text{m}$ ), were observed. Some differences existed between the distribution patterns of immunoreactivities of both neuropeptides. The distribution of VP neurons was wider than that of OT neurons. VP neurons were observed in the suprachiasmatic nucleus, medial amygdala, dorsomedial nucleus, dorsal capsule of the ventromedial nucleus, dorsal and lateral hypothalamic area; while only OT neurons were detected in the medial preoptic area. The number and cell types of VP and OT neurons were enumerated in Table 1.

Both neuropeptidergic fibers and their respective terminals coexisted throughout the neuroaxes, but immunostaining density varied. Obviously, the majority of these fibers stemmed from the paraventricular nucleus and supraoptic nucleus towards the neurohypophysis and the median eminence portal capillary system (Figs. 3a, b). A few of the reacted fibers reach other neural and neurohemal targets. These VP and OT fiber targets were listed in Table 2. The disposition of both neuropeptidergic immunoreactivities were described below.



**Fig. 2.** A section just behind the anterior commissure. VP cell bodies appear on the wall of the periventricular zone of the 3rd ventricle. Note the sparsity of reacted neurons of the paraventricular nucleus (PVN).

## Immunoreacted perikarya

### Paraventricular nucleus

This nucleus contained many VP and OT neurons which were located in the medial, lateral and posterior areas. The reacted neurons in the medial and lateral parts of the paraventricular nucleus were closely packed together in a quasi conic formation. Although these two areas contained both large and small VP cell bodies, the medial part primarily consisted of small neurons, the lateral part contained a core of large ones. Posterior region VP neurons aligned themselves in a mediolateral direction to the fornical nucleus. VP cell bodies were more caudally and ventrally concentrated in the paraventricular nucleus, while OT neurons were more rostrally and dorsally located.

### Supraoptic nucleus

This nucleus included only large reacted neurons in its principal and retrochiasmatic parts. The principal part contained most of the reacted neurons. Like the distribution within the paraventricular nucleus, VP neuron arrangement was largely caudal and ventral, while OT neurons were mainly rostral and dorsal.

**Table 1.** The Distribution and Neuronal Number of Magnocellular (m) and Parvocellular (p) Containing Neurohypophyseal Peptidergic Neurons in the Brain of the Gerbil

	VP	OT
<b>Telencephalon</b>		
Bed nucleus of stria terminalis	42, m	16, m
Amygdala	12, p	—
Medial preoptic nucleus	—	78, m
Internal capsule	158, p	—
<b>Diencephalon</b>		
Suprachiasmatic nucleus	162, p	—
Paraventricular nucleus	2952, m, p	1540, m, p
Supraoptic nucleus	3288, m	2018, m
<b>Accessory neurosecretory nuclei</b>		
Fornical nucleus	414, m	230, m
Circular nucleus	152, m	74, m
Nucleus of medial forebrain bundle	198, m	84, m
<b>Dorsal hypothalamic area</b>		
Ventromedial nucleus	84, p	—
Dorsomedial nucleus	182, p	—
Lateral hypothalamic area	378, p	—
<b>Total</b>	<b>8164</b>	<b>4040</b>

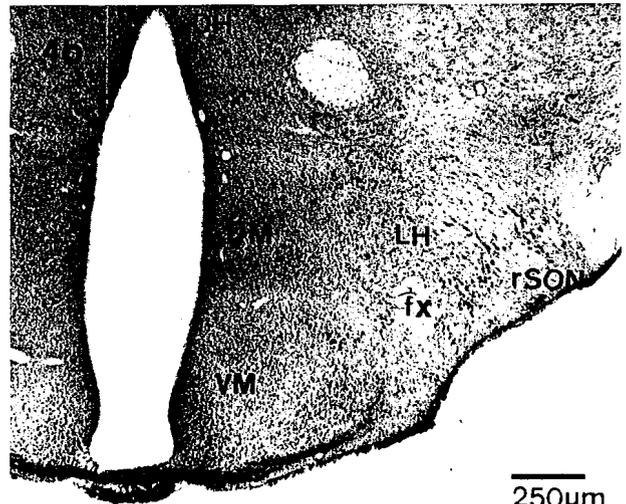
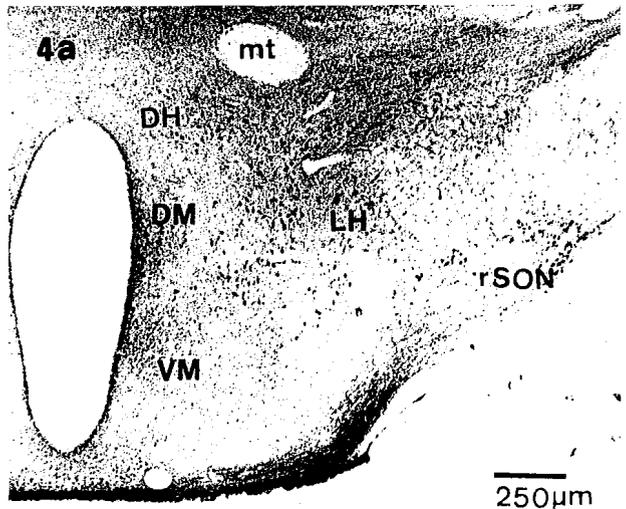
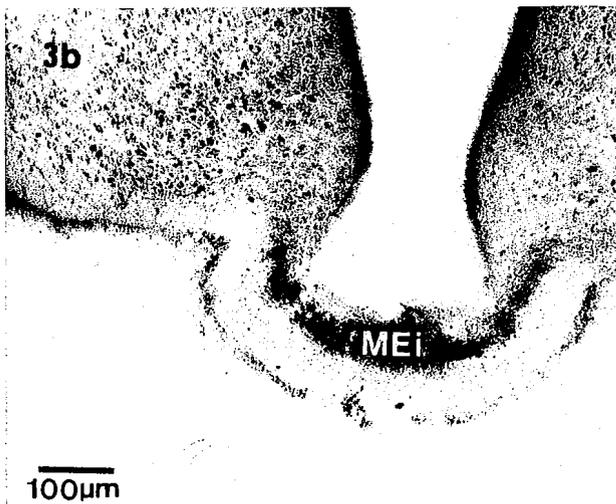
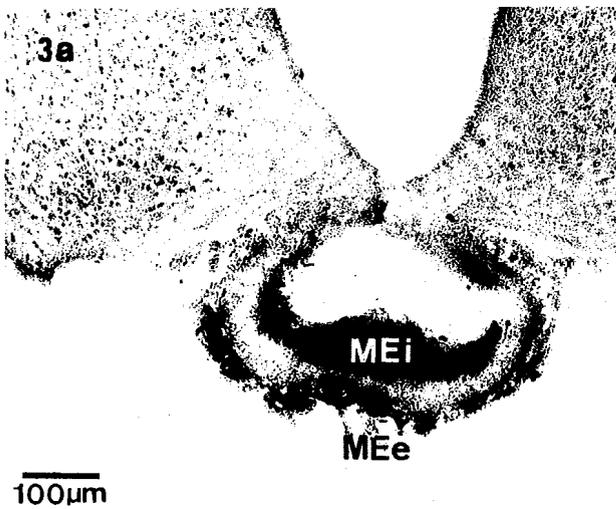
### Accessory neurosecretory nuclei

Only large VP and OT neurons were observed in the accessory neurosecretory nuclei. These were the fornical, circular (Fig. 2) and median forebrain bundle nuclei (Figs. 5, 6, 7). Among them, the fornical nucleus included the most numerous reacted neurons. A few VP neurons and fibers ran from the posterior part of the paraventricular nucleus to the fornical nucleus and intertwined with reacted neurons of the latter (Figs. 5a, b). The circular nucleus was a small nucleus

with some reacted neurons; blood vessels surrounded by these neurons within this nucleus were observed occasionally. The VP and OT neurons within the nucleus of median forebrain bundle were diffusely distributed in the internuclear zone and always paralleled the fibers of the paraventriculo-supraoptico-neurohypophyseal tract.

### Suprachiasmatic nucleus

A few small VP neurons appeared in the dorsomedial part of this nucleus (Figs. 6a, 7a). No



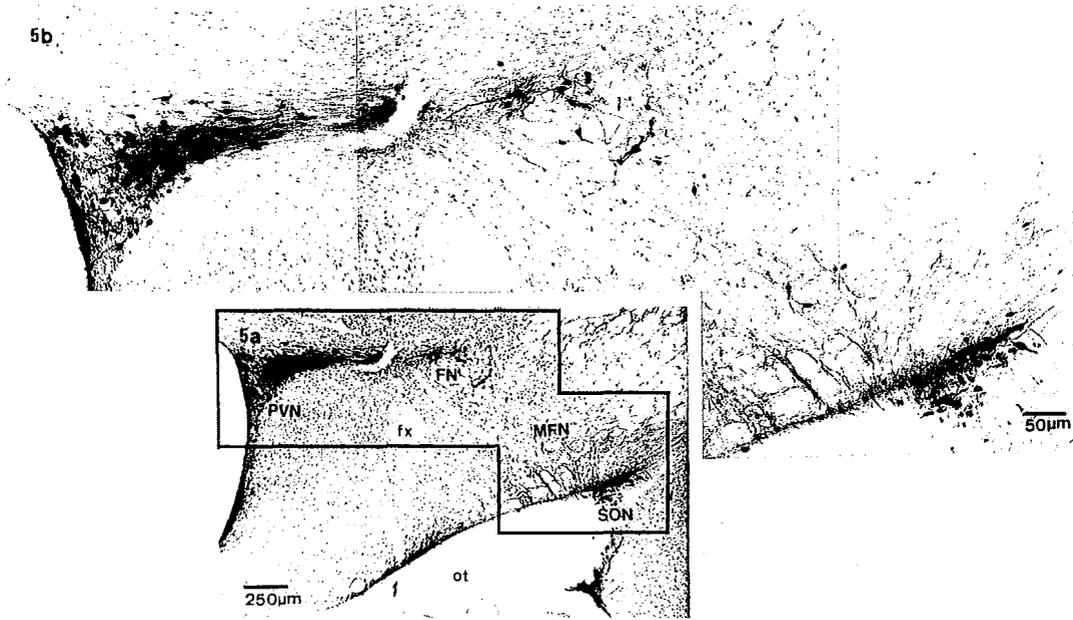
**Figs. 3a-b.** Photomicrographs reveal VP and OT containing fibers projecting to the portal capillaries of the median eminence (ME). In Fig. 3a, dense VP fibers terminate in the internal and external layers of the ME. While in Fig. 3b, OT fibers are only observed in the internal layer of the ME.

**Figs. 4a-b.** Fig. 4a shows several VP neurons in the dorsal hypothalamic area (DH), ventromedial nucleus (VM) and the dorsomedial nucleus (DM) intermingle in the lateral hypothalamic area (LH). No OT cell bodies are found at or behind this level. Fig. 4b is more caudal than 4a. Note VP neurons assemble in the dorsal capsule of the VM.

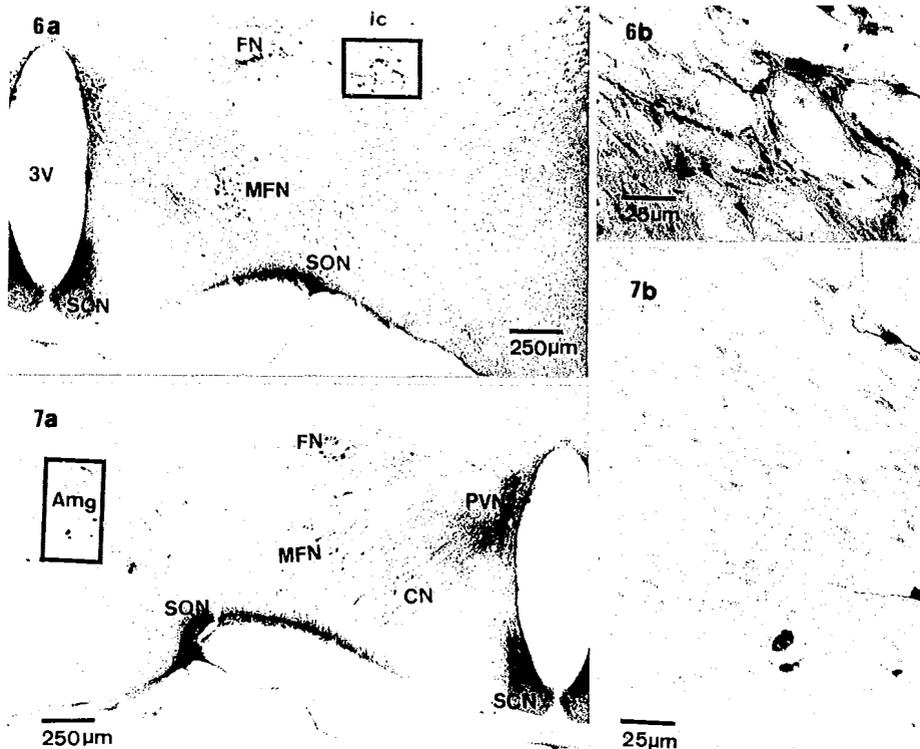
**Table 2.** The Density of Vasopressin (VP) and Oxytocin (OT) Nerve Fibers in the CNS of the Gerbil

	VP	OT
<b>Telencephalon</b>		
Frontal cortex	(+)	(+)
Diagonal tract of Broca	+++	(+)
Accumbens nucleus	+	+
Lateral septum	++	(+)
Medial septum	+	(+)
Medial amygdala	+	(+)
Bed nucleus of stria terminalis	++	+
Hippocampus	+	+
Subfornical organ	(+)	(+)
<b>Diencephalon</b>		
Neural lobe	++++	++++
Median eminence		
internal zone	++++	++++
external zone	+++	
Organum vasculosum laminae terminalis	+++	
Lateral habenular nucleus	++	(+)
Mediodorsal thalamus	++	(+)
Periventricular zone	++	(+)
Dorsomedial nucleus	++	+
Posterior nucleus	++	(+)
Supramammillary nucleus	++	+
Mammillary body	+	
<b>Mesencephalon</b>		
Substantia nigra	+	+
Dorsal raphe nucleus	++	++
Ventral tegmental area	+	+
Interpeduncular nucleus	+	+
Subcommissural organ	(+)	(+)
<b>Rhombencephalon</b>		
Area postrema	(+)	(+)
Periventricular gray	+	+
Parabrachial nucleus	++	++
Locus coeruleus	+	+
Raphe pontis nucleus	+	+
Nucleus solitarius/vagus complex	++	++
Commissural nucleus	+	+
Lateralis reticularis nucleus	+	+
Raphe magnus nucleus	++	++
Raphe obscurus nucleus	+	+
Nucleus of spinotrigeminal tract	+	+
<b>Spinal cord</b>		
Dorsal horn	(+)	(+)
Central gray	(+)	+
Lateral horn	(+)	(+)

“+” indicate positive reacted fibers: (+) observed sometimes; + very low density; ++ low density; +++ moderate density; ++++ high density; +++++ very high density.



**Figs. 5a-b.** Fig. 5a shows the relation of four VP containing nuclei in the hypothalamus. Fig. 5b is an enlarged photomicrograph with details displayed in Fig. 5a. In Fig. 5b, numerous immunoreacted cell bodies are aggregated within the paraventricular nucleus, supraoptic nucleus and fornical nucleus (FN), and is scant in the nucleus of medial forebrain bundle (MFN). Obviously, the immunoreacted cells of the paraventricular nucleus ventrolaterally extend their long processes out toward the supraoptic nucleus and fornical nucleus. Note the immunoreacted cell processes adjacent to the border of the optic tract (ot) and ventral surface of the forebrain.



**Figs. 6a-b.** Fig. 6a shows VP immunoreactivities in the anterior hypothalamus. Note some VP neurons diffuse in the inferior part of the internal capsule. Fig. 6b is enlarged from the rectangle of Fig. 6a.

**Figs. 7a-b.** Fig. 7a shows VP immunoreactivities in the amygdala. Fig. 7b is enlarged from the rectangle of the Fig. 7a.

OT neuron was observed there.

Bed nucleus of stria terminalis

Small VP and OT neurons scattered around the anterior commissure; Some oriented in the direction of the stria terminalis.

Other parts of the brain

In addition to the distribution of reacted neurons described above, a number of VP and OT neurons appeared in the rostral forebrain and caudal diencephalon. At the most rostral part, both VP and OT neurons were found in the rostral periventricular zone, but only large OT neurons were detected in the medial preoptic nucleus (Fig. 1). At the caudal diencephalon, numerous small pure VP neurons were encountered in the dorsal hypothalamic area, dorsomedial nucleus, lateral hypothalamic area and dorsal capsule of ventromedial nucleus (Figs. 4a, b). There were 786 out of a total 8164 VP neurons in these nuclei (Table 1). Moreover, some VP neurons were sparsely distributed in the inferior part of the internal capsule (Fig. 6b) and in the medial amygdala (Fig. 7b).

### Immunoreacted fibers

Almost all VP and OT fibers projected to the same fields. OT fiber density was much less in the forebrain, particularly in the limbic system. In the brainstem, OT fibers as well as VP fibers were moderately distributed within the dorsal raphe nucleus, raphe magnus nucleus and nucleus solitarius/vagus complex. Further, VP fibers dispersed in both internal and external zones of the median eminence, while OT fibers were only observed in the internal zone of the median eminence (Figs. 5a, b). Only VP fibers could be found in the organum vasculosum laminae terminalis.

### Telencephalon

OT fibers were slightly dispersed, while various densities of VP fibers were found in most areas of the telencephalon. Moderately VP labeled fibers were observed in the diagonal band of Broca (Fig. 8). VP fiber density was low in the lateral septum (Fig. 9) and bed nucleus of striae terminalis. The accumbens nucleus, medial septum, medial amyg-

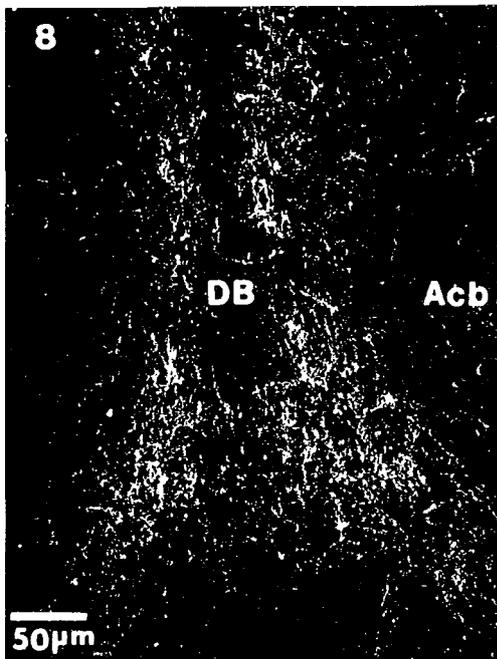


Fig. 8. Dark field photomicrographs show no immunoreacted neurons but dense and conspicuous fibers in the diagonal bank of Broca.

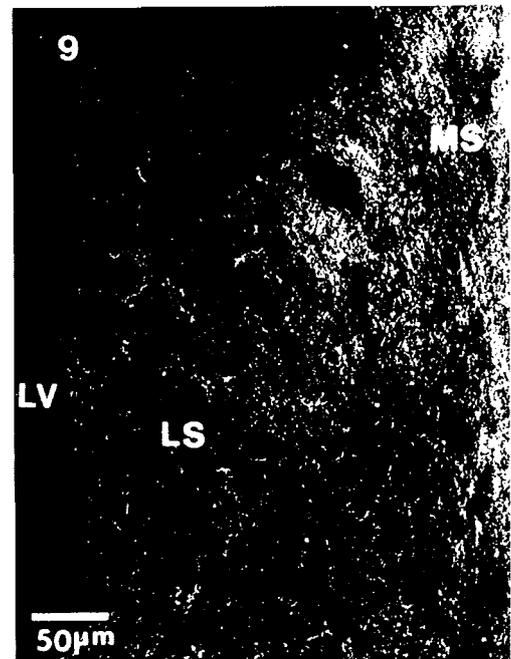


Fig. 9. Dark field photomicrographs show no immunoreacted neurons but dense and conspicuous fibers in the lateral septum.

dala and hippocampus were labeled with a very low VP fiber density. Only few VP fibers were found in the cerebral cortex and subfornical organ.

#### Diencephalon

This part had much more VP and OT fibers than other parts of the central nervous system. Distinctively, the highest reacted fiber density was in the neural lobe and median eminence. Lowly labeled fibers were found in the lateral habenular nucleus, mediodorsal thalamus (Fig. 11), periventricular zone, dorsomedial nucleus, posterior nucleus, suprachiasmatic nucleus (Figs. 6a, 7a) and supramammillary nucleus. A very low density of reacted fibers distributed in the ventral and lateral of the mammillary body (Fig. 10).

#### Mesencephalon

Excluding lowly labeled VP and OT fibers in the dorsal raphe nucleus, reacted fibers scantily terminated in the central gray, substantia nigra, ventral tegmental area and interpeduncular nucleus. Reacted fibers in the subcommissural organ were observed occasionally.

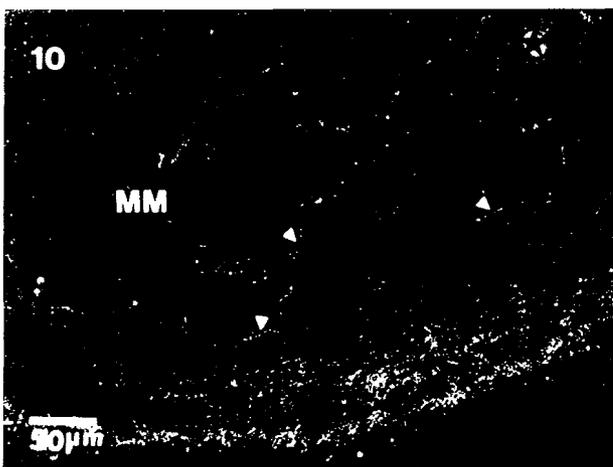
#### Rhombencephalon and spinal cord

Both VP and OT fibers were sparsely distributed in the parabrachial nucleus, raphe magnus (Fig. 12), nucleus solitarius/vagus complex (Fig. 11). In the locus coeruleus, raphe pontis nucleus, lateral reticular nucleus, raphe obscurus nucleus and nucleus of spinotrigeminal tract, a very low reacted fiber density was observed. Slightly reacted fibers

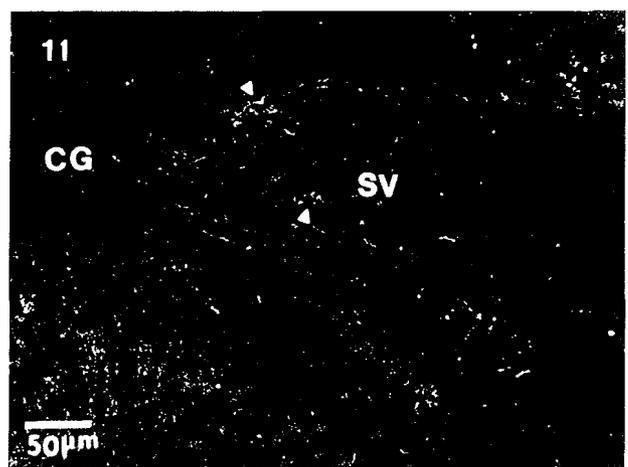
were observed in the area postrema, and the dorsal horn, central gray and lateral horn of the spinal cord.

### DISCUSSION

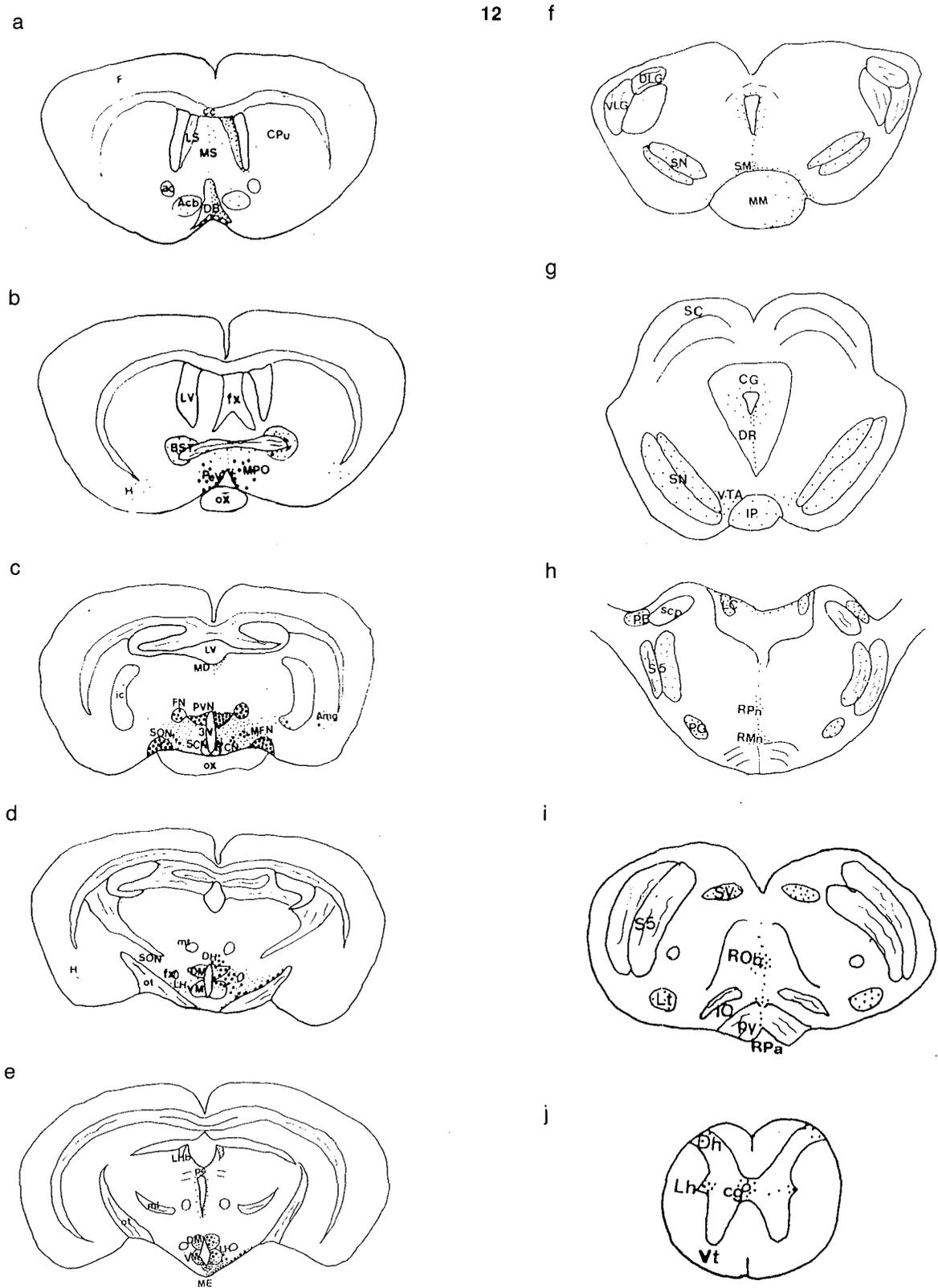
This is the first paper to describe the distribution of vasopressin and oxytocin neurons in the CNS of the Mongolian gerbil. Both neuropeptidergic neuron arrangement patterns of the gerbil are similar to those of other mammals (Silverman and Zimmerman 1983). Both VP and OT neurons are mainly associated with the paraventricular nucleus, supraoptic nucleus and accessory nuclei of the gerbil. The arrangement of both neuropeptidergic neurons within the paraventricular nucleus and supraoptic nucleus have spatial differences; VP neurons are situated more caudally and ventrally, while OT neurons are located more rostrally and dorsally. However, the distribution of these neurons in other nuclei is different between the gerbil and other mammals (Silverman and Zimmerman 1983). Numerous VP neurons occur in the internal capsule and in the nuclei of the caudal diencephalon, including the dorsal hypothalamic area, dorsomedial nucleus, lateral hypothalamic area and the dorsal capsule of the ventromedial nucleus of the gerbil, but not in those of other species. For example, only the lateral hypothalamic nucleus of the cat (Reaves and Hayward 1979) and the dorsomedial nucleus of the rat (Caffe and Van Leeuwen 1983) have been reported to contain



**Fig. 10.** Dark field photomicrographs show no immunoreacted neurons but dense and conspicuous fibers in the mammillary body.



**Fig. 11.** Dark field photomicrographs show no immunoreacted neurons but dense and conspicuous fibers in the solitarius/vagus complex.



**Fig. 12.** Schematic drawings show the distribution of VP immunostaining neurons (large dots) and fibers (small dots) from the forebrain to the spinal cord of the gerbil. OT neurons only distribute before level e and no VP neurons are found under caudal diencephalon (level f). The thoracic level of the spinal cord is presented in j.

VP neurons. Similarly, a group of OT neurons in the gerbil medial preoptic area have been described only in those of the pig (Van Eedenburg 1992), horse (Melrose and Knigge 1989) and guinea pig (Dubois-Dauphin 1989a, b). VP and OT neurons have not been found in the diagonal band of Broca of the gerbil, guinea pig (Dubois-Dauphin 1989a, b) and pig (Van Eerdenburg 1992). Such results contrast with reports of VP neurons in the diagonal band of Broca of the rat (Sofroniew 1985), cat (Caverson et al. 1987), monkey (Caffe et al. 1989) and man (Ulfig et al. 1990), as well as with a report of OT neurons in the diagonal tract of Broca of the cat (Caverson et al. 1987). That VP neurons located in the locus coeruleus of the rat (Caffe and Van Leeuwen 1983) is also not found in the gerbil and other species (Silverman and Zimmerman 1983).

VP and OT containing fibers and terminals with various densities are located throughout the gerbil CNS. The majority VP and OT fibers stem from the paraventricular nucleus and supraoptic nucleus and project to the neurohypophysis and median eminence. In the gerbil forebrain, VP fibers are higher in density than OT fibers, especially in the limbic system including: the lateral septum, lateral habenular nuclei, amygdala, mammillary body and some hypothalamic nuclei. While the densities of both VP and OT fibers are light in the most areas of the brainstem and spinal cord of the gerbil. Comparing the gerbil to other mammals (Swanson and Kuypers 1980, De Vires and Buijs 1983), various data on both reacted fibers exists. Among these VP projection targets, reports regarding the mammillary body are scant and have only been revealed by anterograde autoradiographic tracing method (Alonso et al. 1984).

The roles of VP and OT as hormones, when transported to vascular targets, have been confirmed by many investigators (Gash and Boer 1987). Other reports concerning their extrahypophyseal projections as neurotransmitters, when transported to the effector neurons in axons, are abundant (Cooper et al. 1979, De Wied 1983, Van Wimersma Greidanus 1982). The VP neurons spreading in the lateral hypothalamic area and VP fibers diffusing in the subfornical organ are deeply thought to involve in drinking behavior and body water balance (Emmers 1973, Milton and Paterson 1974, Miselis 1982). Dominant distribution of VP neurons in the lateral hypothalamic area and sparse disposition of VP fibers in the subfornical organ might explain the excellent drought-resisting ability of the gerbil. VP and OT fibers, related with the

limbic system, imply a wide variety of behavioral effects on the gerbil and other species (De Wied 1977, De Wied and Versteeg 1979).

Although VP and OT fibers are sparse in the most areas of the gerbil brainstem and spinal cord, medium densities of both reacted fibers in some brainstem regions of the gerbil have been encountered, such as the dorsal raphe nucleus, nucleus vagus/solitarius complex and parabrachial nucleus. Additionally, VP and OT projections located in the autonomic centers of the brainstem including the locus coeruleus, parabrachial nucleus, nucleus tractus solitarius, dorsal motor nucleus of vagus and the lateral horn of the spinal cord. They are thought to participate in the processes of autonomic regulation (Swanson 1977). The nucleus solitarius, involved in cardiovascular regulation, has been shown to receive VP fibers from the supra-chiasmatic nucleus and paraventricular nucleus (Swanson 1977, Sofroniew and Weidl 1978b). Our observations also concur with their views.

## REFERENCES

- Alonso G, A Szafarczyk, J Assenmacher. 1984. Radioautographic evidence of extrahypothalamic differences from the supraoptic nuclei in the rat. *Proceed VIIth Int. Congr. Endocrinol. Quebec. July 1-7: 331.*
- Bargmann W, E Scharrer. 1954. The site of origin of the hormones of the posterior pituitary. *Am. Sci.* **39**: 255-259.
- Berk ML, TA Reaves Jr., JN Hayward, JA Finkelstein. 1982. The localization of vasotocin and neurophysin neurons in the diencephalon of the pigeon *Columbia livia*. *J. Comp. Neurol.* **204**: 392-406.
- Caffe AR, FW Van Leeuwen. 1983. Vasopressin-immunoreactive cells in the dorsomedial hypothalamic region and medial amygdaloid nucleus of the rat. *Cell Tissue Res.* **233**: 23-33.
- Caffe M, PC Van Ryen, TP Van Der Woude, FW Van Leeuwen. 1989. Vasopressin and oxytocin systems in the brain and upper spinal cord of *Macaca fascicularis*. *J. Comp. Neurol.* **287**: 302-325.
- Caverson MM, J Ciriello, FR Calaresu, TL Krukoff. 1987. Distribution and morphology of vasopressin-, neurophysin II-, and oxytocin-immunoreactive cell bodies in the forebrain of the cat. *J. Comp. Neurol.* **259**: 211-236.
- Cooper KE, NW Kasting, WL Veale. 1979. Evidence supporting a role for endogenous vasopressin in natural suppression of fever in the sleep. *J. Physiol. Lond.* **295**: 33-45.
- Cross BA. 1958. On the mechanism of labor in the rabbit. *J. Endocrinol.* **16**: 261-276.
- Cross BA, G Leng. 1982. The neurohypophysis: structure, function and control. *Prog. Brain Res.* **60**: 3-24.
- Dawson AB. 1953. Evidence for the termination of neurosecretory fibers within the pars intermedia of the hypophysis of the frog. *Anat. Rec.* **115**: 63-69.
- De Vires GJ, RM Buijs. 1983. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain, with special reference to the lateral septum. *Brain Res.* **273**: 303-317.

- De Wied D. 1977. Peptides and behavior. *Life Sci.* **20**: 195-204.
- De Wied D, DHG Versteeg. 1979. Neurophyseal principles and memory. *Fed. Proc.* **38**: 2348-2354.
- De Wied D. 1983. Central actions of neurohypophyseal hormones. *Prog. Brain Res.* **60**: 155-169.
- Dierickx K, F Vandesande. 1977. Immunocytochemical localization of the vasopressinergic and oxytocinergic neurons in the human hypothalamus. *Cell Tissue Res.* **184**: 15-31.
- Dubois-Dauphin M, E Tribollet, JJ Dreifuss. 1989a. Distribution of neurohypophyseal peptides in the guinea pig brain. I. An immunocytochemical study of the vasopressin related glycopeptide. *Brain Res.* **496**: 45-65.
- Dubois-Dauphin M, E Tribollet, JJ Dreifuss. 1989b. Distribution of neurohypophyseal peptides in the guinea pig brain. II. An immunocytochemical study of the oxytocin. *Brain Res.* **496**: 66-81.
- Emmers R. 1973. Interaction of neural systems which control body water. *Brain Res.* **49**: 323-347.
- Goossens N, K Dierickx, F Vanfesinde. 1977. Immunocytochemical demonstration of the hypothalamo-hypophyseal vasotocinergic system of *Lampetra fluviatilis*. *Cell Tissue Res.* **177**: 317-323.
- Gash DM, GJ Boer. 1987. Vasopressin: principles and properties. New York: Plenum Press, pp. 435-475.
- Martin G, G Baverek. 1981. Vasopressin promotes the metabolism of near-physiological concentration of glutamine in isolated rat liver cells. *Biosci. Rep.* **4**: 171.
- Melrose PA, KM Knigge. 1989. Topography of oxytocin and vasopressin neurons in the forebrain of equus caballus: Further support of proposed evolutionary relationships for propiomelanocortin, oxytocin and vasopressin neurons. *Brain Behav. Evol.* **33**: 193-204.
- Milton AS, AT Peterson. 1974. A microinjection study of the control of antidiuretic hormone release by the supraoptic nucleus of the hypothalamus in the cat. *J. Physiol.* **241**: 607-628.
- Miselis R. 1982. The subfornical organ's neural connections and their roles in water balance. *Peptides* **3**: 501-502.
- Mohring J, J Kintz, J Schoun, McNeill Jr. 1981. Pressor responsiveness and cardiovascular reflex activity in spontaneously hypertensive and normotensive rats during vasopressin infusion. *J. Cardiovasc. Pharmacol.* **3**: 948-957.
- Munsick RA. 1960. Effect of magnesium ion on the response of rat uterus to neurohypophyseal hormones and analogues. *Endocrinol.* **66**: 451-457.
- Peeters G, H Stormorken, F Vanschoubroek. 1960. The effect of different stimuli on milk ejection and diuresis in the lactating cow. *J. Endocrinol.* **20**: 163-172.
- Peterson RP. 1966. Large neurosecretory centers in the rat hypothalamus. *J. Comp. Neurol.* **128**: 181-190.
- Reaves Jr. TA, JN Hayward. 1979. Immunocytochemical identification of vasopressinergic and oxytocinergic neurons in the hypothalamus of the cat. *Cell Tissue Res.* **196**: 117-122.
- Sawyer WH. 1971. Neurohypophyseal hormones. *Pharmacol. Rev.* **13**: 225-227.
- Scharrer E, B Scharrer. 1954. Hormone produced by neurosecretory cells. *Recent. Prog. Horm. Res.* **10**: 183-240.
- Silverman AJ, EA Zimmerman. 1983. Large neurosecretory system. *Annu. Rev. Neurosci.* **6**: 357-380.
- Sofroniew MV, A Weindl. 1978b. Projections from the parvocellular vasopressin- and neurophysin-containing neurons of the suprachiasmatic nucleus. *Am. J. Anat.* **153**: 391-401.
- Sofroniew MV, A Weindl, I Schinko, R Wetzstein. 1979. The distribution of vasopressin-, oxytocin- and neurophysin in the guinea pig brain. *Cell Tissue Res.* **196**: 367-384.
- Sofroniew MV. 1985. Vasopressin, oxytocin and their related neurophysin. In *Hand-book of Chemical Neuroanatomy*, Vol. 4: GABA and Neuropeptides in the CNS, Part 1, eds. A Bjorklund, T Hokfelt. Amsterdam: Elsevier, pp. 93-165.
- Sokol HW, EA Zimmerman, WH Sawyer, AG Robinson. 1976. The hypothalamoneurohypophyseal system of the rat: Localization and qualification of neurophysin by light microscopic immunocytochemistry in normal rats and in Brattleboro rats deficient in vasopressin and a neurophysin. *J. Endocrinol.* **98**: 1176-1188.
- Sternberger LA, PH Hardy Jr., JH Cuculis, HG Meyer. 1970. The unlabeled antibody enzyme method of soluble antigen-antibody complex (horseradish peroxidase antihorseradish peroxidase) and its use in identifications of spirochetes. *J. Histochem. Cytochem.* **18**: 315-333.
- Swanson LW. 1977. Immunohistochemical evidence for a neurophysin-containing autonomic pathway arising in the paraventricular nucleus of the hypothalamus. *Brain Res.* **128**: 346-353.
- Swanson LW, HG Kuypers. 1980. The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double labelling methods. *J. Comp. Neurol.* **194**: 555-570.
- Ulfing N, E Braak, TG Ohm, CW Pool. 1990. Vasopressinergic neurons in the magnocellular nuclei of the human basal forebrain. *Brain Res.* **530**: 176-180.
- Vandesande F, K Dierickx. 1975. Identification of the vasopressin producing and of the oxytocin producing neurons in the hypothalamic large neurosecretory system of the rat. *Cell Tissue Res.* **164**: 153-162.
- Vandesande F, K Dierickx, J De Mey. 1975. Identification of the vasopressin-neurophysin II and oxytocin-neurophysin I producing neurons in the bovine hypothalamus. *Cell Tissue Res.* **156**: 189-200.
- Van Eerdenburg FJCM, DF Swaab, FW Van Leeuwen. 1992. Distribution of vasopressin and oxytocin cells and fibres in the hypothalamus of the domestic pig (*Sus scrofa*). *J. Comp. Neurol.* **318**: 138-146.
- Van Wimersma Greidanus TB. 1982. Disturbed behavior and memory of the Brattleboro rat. *Ann. N.Y. Acad. Sci.* **394**: 622-635.
- Zimmerman EA. 1976. Localization of hypothalamic hormones by immunocytochemical techniques. Raven press, New York 4: 25-62.
- Zimmerman EA, JL Antunes, PW Carmel, R Defendini, M Ferin. 1977. Large neurosecretory pathways in the monkey. Immunohistochemical studies of the normal and lesioned hypothalamus using antibodies to oxytocin, vasopressin and neurophysin. *Trans. Am. Neurol. Assoc.* **101**: 1-4.

## ABBREVIATIONS

3V	Third ventricle
ac	Anterior commissure
Acb	Accumbens nucleus
Amg	Amygdala
BST	Bed nucleus of stria terminalis
cc	Corpus callosum
CN	Circular nucleus

CG	Central gray (cg: lamina X)	MS	Medial septum
CPu	Caudate putamen	mt	Mammillothalamic tract
DB	Diagonal tract of Broca	ot	Optic tract
DLG	Dorsal lateral geniculate nucleus	ox	Optic chiasm
Dh	Dorsal horn (lamina I-III)	PB	Parabrachial nucleus
DH	Dorsal hypothalamic area	pc	Posterior commissure
DM	Dorsomedial nucleus	PeV	Periventricular zone
DR	Dorsal raphe nucleus	PG	Paragigantoreticular nucleus
F	Frontal cortex	PVN	Paraventricular nucleus
FN	Fornical nucleus	py	Pyramidal tract
fx	Fornix	RMn	Raphe magnus nucleus
H	Hippocampus	ROb	Raphe obscurus nucleus
ic	Internal capsule	RPa	Raphe pallidus nucleus
IPN	Interpeduncular nucleus	RPn	Raphe pontis nucleus
IO	Inferior olivary	S5	Nucleus of spinotrigeminal tract
LC	Locus coeruleus	SC	Superior colliculus
LHb	Lateral habenula	scp	Superior cerebellar peduncle
Lh	Lateral horn	SM	Supramammillary nucleus
LS	Lateral septum	SN	Substantia nigra
Lt	Lateralis reticularis nucleus	SON	Supraoptic nucleus
LV	Lateral ventricle	ST	Stria terminalis
MD	Mediodorsal thalamus	SV	Solitarius/vagus complex
MEe	Median eminence (external zone)	Vh	Ventral horn
MEi	Median eminence(internal zone)	VLG	Ventral lateral geniculate nucleus
MFN	Nucleus of medial forebrain bundle	VM	Ventromedial nucleus
MM	Mamillary body	VTA	Ventral tegmental area
MPO	Medial preoptic nucleus		

## 含血管加壓素及催產素的神經元在沙鼠之中樞神經系統的分佈

吳慶明 沈清良

本實驗是首次以免疫組織化學法來探討含血管加壓素(vasopressin, VP)及催產素(oxytocin, OT)的神經元和其突起在蒙古種沙鼠中樞神經系統中的分佈情形。結果顯示這兩類神經元主要分佈於腦室旁核、視徑上核以及一些附屬神經分泌核中。這些神經元可分為大型多角形和小型梭形兩類，且共存於一些神經核中。含血管加壓素的神經元比含催產素的神經元分佈廣；在視交叉上核、內側杏仁核、下視丘的背側下視丘區、背內側核、外側下視丘區、腹內側核的背側中，只有含血管加壓素的神經元，且這些皆為小型神經元。相反的，在內側視丘前區則僅有含催產素的神經元。含血管加壓素和含催產素的神經纖維，廣泛的分佈於沙鼠的中樞神經系統中。這些纖維大部分投射到腦下垂體和正中隆突，另有一些則屬下視丘外的投射。沙鼠含血管加壓素的神經元及其纖維的分佈比其它哺乳類的來得廣，如在內囊、背側下視丘區及腹內側核的背側等，均有含血管加壓素的神經元存在，而這些區域在其它的動物，尚未有含血管加壓素神經元的發現。而沙鼠含血管加壓素的神經纖維，投射至乳頭體的情形，在其它哺乳類動物的腦中相當少見。基於血管加壓素在抗利尿功能上的角色，沙鼠如此發達的血管加壓素系統，可能足以反映其卓越的耐渴能力。

關鍵詞：免疫組織化學法，血管加壓素，催產素。