

# Distribution of Vasopressin and Vasoactive Intestinal Polypeptide (VIP) Fibers in the Human Hypothalamus With Special Emphasis on Suprachiasmatic Nucleus Efferent Projections

JIAPEI DAI, DICK F. SWAAB, AND RUUD M. BUIJS\*

Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research,  
1105 AZ Amsterdam ZO, The Netherlands



## ABSTRACT

The human suprachiasmatic nucleus (SCN) is located in the basal part of the anterior hypothalamus and is considered as the biological clock that generates circadian rhythms and synchronizes the daily activity pattern with the environmental light-dark cycle. However, the mechanisms and pathways by which the SCN transmits its information to the other brain areas are unknown. Therefore, in the present study, we investigated the efferent projections of the SCN by the immunocytochemical staining of two major peptidergic SCN neurotransmitters: vasopressin (VP) and vasoactive intestinal polypeptide (VIP). It confirmed that these peptides are present in different subdivisions of the SCN. The results of this investigation show that VP and VIP fibers arising from the SCN were detected to branch extensively and hence seem to innervate the SCN itself and the central and medial part of the anteroventral hypothalamic area (AVH), the area below the paraventricular nucleus (sub-PVN), the ventral part of the paraventricular nucleus (PVN), and the dorsomedial nucleus of the hypothalamus (DMH). There appeared to be substantial congruity between the presumptive human SCN projections and those as observed by tracing in rat or hamster. Regarding the anatomical organization of the human SCN projections, the main projection areas appeared to be the AVH, the sub-PVN, the ventral part of the PVN, and the DMH. The observation that VIP and in particular VP fibers pass between the SCN and the PVN suggests that the human SCN and the PVN may have a direct anatomical connection. In addition, VP and VIP fibers were detected in several other hypothalamic areas that are not known to have clear direct connections to the SCN. The possible origin of these VP and VIP fibers is discussed. *J. Comp. Neurol.* 383:397-414, 1997. © 1997 Wiley-Liss, Inc.

**Indexing terms:** paraventricular nucleus; sub-paraventricular zone; dorsomedial nucleus of hypothalamus; anteroventral hypothalamic area; CRH

The suprachiasmatic nucleus (SCN) is responsible for maintaining circadian rhythms in sleep, wakefulness, hormone levels, body temperature, and blood pressure in mammals (Rusak and Zucker, 1979). To understand how the SCN communicates its circadian information to the rest of the brain, knowledge of the organization of SCN projections is essential. Studies using immunocytochemical staining for SCN neurotransmitters (Sofroniew and Weindl, 1978; Buijs et al., 1978; Buijs, 1978; Card et al., 1981; Card and Moore, 1984; Smale et al., 1991), SCN lesions (Hoorneman and Buijs, 1982), retrograde transport of horseradish peroxidase (HRP), fluorescent dyes (Stephan et al., 1981; Watts and Swanson, 1987), anterograde

transport of <sup>3</sup>H-labeled amino acids (Swanson and Cowan, 1975; Berk and Finkelstein, 1981; Stephan et al., 1981), and, more recently, Phaseolus vulgaris-leucoagglutinin (PHA-L) tracing (Watts et al., 1987; Buijs et al., 1993; Kalsbeek et al., 1993; Orpen and Steiner, 1994; Morin et

Grant sponsor: Netherlands Institute for Brain Research; Grant number: 94-11.

\*Correspondence to: Ruud M. Buijs, Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands. E-mail: R.Buijs@nih.knaw.nl

Received 30 August 1996; Revised 21 January 1997; Accepted 28 January 1997

TABLE 1. Clinical Diagnosis and Pathology<sup>1</sup>

NBBn	Sex	Age (y)	Side	BW (g)	CSF pH	PMD (h)	CTD	MA	Clinical diagnosis, neuropathology, cause of death
94-098	M	79	R	1297	6.83	7:20	6:10	Sep	Bronchus carcinoma, septic shock
94-116	F	72	R	1031	6.07	6:40	7:30	Nov	AD, respiratory insufficiency
94-117	F	71	L	1150	6.23	4:20	7:15	Nov	AD, cachexia and dehydration
94-123	F	76	L	1095	6.50	9:20	8:30	Dec	Pneumonia, respiratory insufficiency
94-124	M	63	R	967	6.60	5:30	2:00	Dec	Pneumonia, respiratory insufficiency
94-125	M	51	R	1518	5.50	6:00	17:15	Dec	Progressive liposarcoma and ileus
95-096	F	95	R	1016	6.87	2:50	17:20	Oct	AD, pneumonia
96-010	M	63	R	1250	6.37	10:20	5:00	Jan	Diabetes mellitus, pneumonia

<sup>1</sup>NBBn, Netherlands Brain Bank number; M, male; F, female; y, years; R, right; L, left; BW, brain weight; g, gram; CSF, cerebrospinal fluid; PMD, postmortem delay; CTD, clock time of death; MA, month of autopsy; h, hours:mins; AD, Alzheimer's disease.

al., 1994) have identified the SCN projection areas in rodents. This identification has provided insight into which hypothalamic areas in the rodent brain may serve to integrate the circadian signal. In the rat and hamster, the subparaventricular zone and dorsomedial nucleus of the hypothalamus (DMH) were identified as major projection areas. Only a limited number of direct projections to the paraventricular nucleus (PVN) where neurosecretory neurons are located were found, suggesting that the SCN may influence PVN functions mainly by means of multisynaptic connections (Watts and Swanson, 1987; Buijs et al., 1993).

Also, the human SCN forms an essential component in the adaptation to environmental changes and is able to generate circadian rhythms. It was found that day-night behavioral rhythms are disturbed when the SCN is affected, as is the case in Alzheimer's disease (Swaab et al., 1985). Although insight into SCN projections was extended from rodent to primate, e.g., macaque monkey (Moore, 1993), so far, nothing is known about human SCN projections. In the present study, the putative projections of the SCN were examined by immunocytochemical staining for SCN neurotransmitters in the human hypothalamus.

The human SCN is composed of a small heterogeneous group of parvicellular neurons (Sofroniew and Weindl, 1980; Braak and Braak, 1992; Moore, 1992) located in the basal part of the anterior hypothalamus containing a

number of neuroactive compounds, e.g., vasopressin (VP), vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY), somatostatin (SOM), peptide histidine methionine (PHM, which is homologous to the peptide histidine isoleucine [PHI] in rat), gastrin-releasing peptide (GRP), neurotensin (NT), and galanin (Dierickx and Vandesande, 1977; Sofroniew and Weindl, 1980; Itoh et al., 1983; Stopa et al., 1984; Swaab et al., 1985; Mai et al., 1987; Cassone et al., 1988; Gai et al., 1990; Walter et al., 1990; Moore, 1992; Zhou et al., 1995a). Studies of the intrinsic organization of the human SCN have demonstrated that, as in the rodent (Vandesande et al., 1975; Card et al., 1981; Cassone et al., 1988; Mikkelsen et al., 1991; Kalsbeek and Buijs, 1992), the human SCN is also segregated into anatomical subdivisions in which the different neurotransmitters are preferentially located (Stopa et al., 1984; Mai et al., 1991; Moore, 1992). To examine which SCN neurotransmitter is suitable for determining SCN projections, a number of SCN neuropeptides, i.e., VP, VIP, NPY, SOM, PHM, GRP, NT, and galanin were studied. It was found, however, that most neuropeptide staining could not be used for this purpose due to the massive presence of the same peptidergic fibers derived from other sources. Only VP and VIP staining revealed clear projections from the SCN without a too disturbing interference of staining of the same peptides from other sources. Consequently, the course of efferent SCN VP and VIP fibers and the site of innervation of their terminals in the human hypothalamus will be described in the present study.

#### Abbreviations

AVH	anteroventral hypothalamic area
BST	bed nucleus of the stria terminalis
CRH	corticotrophin-releasing hormone
CSF	cerebrospinal fluid
DMH	dorsomedial nucleus of the hypothalamus
FO	fornix
INF	infundibular nucleus
MB	mamillary body
NBM	nucleus basalis of Meynert
NTL	lateral tuberal nucleus
OC	optic chiasm
OT	optic tract
POA	preoptic area
PVN	paraventricular nucleus
PH	posterior hypothalamic nucleus
PHI	peptide histidine isoleucine
PEN	periventricular nucleus
PHA-L	phaseolus vulgaris leucoagglutinin
SCN	suprachiasmatic nucleus
SDN	sexually dimorphic nucleus of preoptic area
SON	supraoptic nucleus
sub-PVN	the area below paraventricular nucleus
TBS	Tris-buffered saline
TMN	tuberomamillary nucleus
VMN	ventromedial nucleus
VP	vasopressin
VIP	vasoactive intestinal polypeptide
3V	third ventricle

## MATERIALS AND METHODS

### Brain material

For the present study, the brains of eight subjects were obtained from the Netherlands Brain Bank (NBB; coordinator Dr. R. Ravid). The required permission for brain autopsy was obtained either from the patients themselves or from partners or relatives. Autopsy was performed according to the protocols of the NBB, which includes measurement of pH of the cerebrospinal fluid to estimate agonal state (Hardy et al., 1985; Ravid et al., 1992). Neuropathology was performed on all subjects in a systematic way by Dr. W. Kamphorst (Free University, Amsterdam, The Netherlands) or Prof. F. C. Stam (NBB). Details of age, postmortem delay, brain weight, clinical diagnosis, and cause of death of the subjects are given in Table 1. None of the controls or patients had died of raised intracranial pressure.

### Tissue treatment

The hypothalamus was dissected at autopsy and immersed in a fixative containing 4% paraformaldehyde in

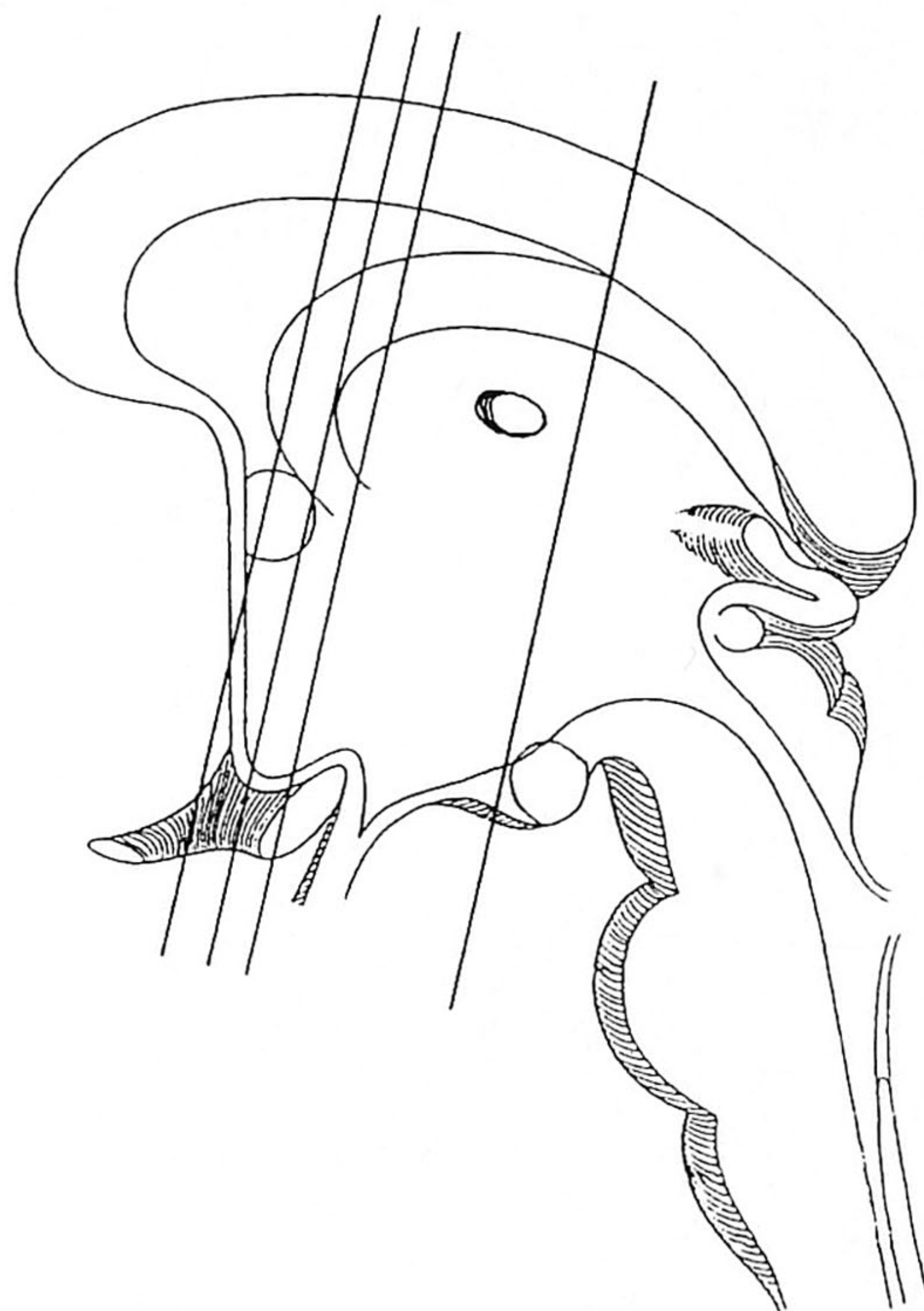


Fig. 1. Schematic illustration of human hypothalamus. Four fine solid lines indicate the plane of the frontal sections.

0.1 M phosphate buffer (pH 7.4). To facilitate the penetration of the fixative, an additional treatment was applied to the tissue within 4 hours. The tissue was put in a small container with the same fixative, placed in a beaker filled with ice cubes and water, and put in the microwave at low setting (80 W) for 15 minutes (Buijs et al., 1993). Then the tissue was kept in the same fresh fixative at room temperature for 8–10 days. After fixation, the tissue was stored in 30% sucrose-phosphate buffer (pH 7.4) for 2–3 days. Sections, 25 to 35  $\mu$ m, were cut on a cryostat in the frontal (Fig. 1, seven cases) or sagittal plane (one case, NBBn 95-096) and collected in a series of vials in sequential order. Subsequently, free-floating sections were rinsed in Tris-buffered saline (TBS, pH 7.4) at 4°C (2 days to 2 weeks) until immunocytochemical staining was performed.

### Immunocytochemistry

Immunocytochemical staining for VP, VIP was performed with the ABC (avidin-biotin complex) ELITE detection method (Vector Laboratories, Burlingame, CA) and consisted of the following steps at room temperature unless stated otherwise: (1) pretreatment with 100% methanol (10 minutes) and 3%  $H_2O_2$  in 0.05 M Tris, 0.9% NaCl (TBS; 10 minutes); (2) rinse in TBS ( $3 \times 10$  minutes); (3) incubation with the first antibody (1 hour at room temperature, and overnight at 4°C; incubation buffer consisted of 0.05 M Tris, 0.9% NaCl, 0.25% gelatin, and 0.5% Triton X-100, pH 7.6). The first antibodies were (a) rabbit VP antiserum (Truus, 29-01-89, Netherlands Institute for Brain Research [NIBR]), 1:1000–4000 and (b) rabbit VIP antiserum (Viper, 18-09-86, NIBR), 1:2000–6000; (4) rinse in TBS ( $3 \times 10$  minutes); (5) incubation with

biotinylated goat anti-rabbit IgG (H+L, Vector) 1:400 (1.5 hours); (6) rinse in TBS ( $3 \times 10$  minutes); (7) incubation with ABC (Vector), 1:800 (1.5 hours); (8) rinse in TBS ( $3 \times 10$  minutes); (9) 0.05% 3,3'-diaminobenzidine tetrachloride (DAB) plus 0.2% nickel-ammoniumsulphate, activated with 0.001%  $H_2O_2$  (10–15 minutes); (10) rinse in TBS ( $2 \times 10$  minutes).

The sections were mounted on chrome alum-coated glass slides, air-dried, dehydrated in graded ethanol series, and mounted in Entellan (Merck, Darmstadt, Germany).

## RESULTS

We analyzed the hypothalamus in serial sections from the lamina terminalis to the level of the mamillary body (MB). Counterstaining with haematoxylin-eosin or thionine was performed in some sections for orientation. In these sections, anatomical structures such as PVN, supra-optic nucleus (SON), periventricular nucleus (PEN), sexually dimorphic nucleus (SDN) of preoptic area, lateral tuberal nucleus (NTL), and tuberomamillary nucleus (TMN) were clearly visible. Little variation was found in the pattern of innervation of VP or VIP fibers between the studied subjects. One representative case (NBBn 96-010) who did not die of a neurological or psychiatric disease will be described in detail, with reference to others where relevant. A series of drawings (Fig. 2 A–V) were made from consecutive sections of the hypothalamus of this subject to illustrate the distribution of VP and VIP staining.

### VP and VIP neurons in the SCN

Neurons with immunoreactivity for VIP or VP were located in topographically distinct regions of the SCN with some overlap (Fig. 3A,B). VP cells were present in the dorsal and medial part of the SCN throughout its anterior-posterior extent (Figs. 3B, 4B). In addition, a much smaller group of VP cell bodies was present in the ventral part of the SCN, particularly at the anterior level. VIP neurons were concentrated in the ventral part of this nucleus (Fig. 3A). Some densely packed VIP neurons were also detected in the centromedial part of the SCN (Fig. 4A), and these VIP neurons were usually surrounded by VP neurons (Figs. 2C,D, 4A,B). In most cases, only a few VIP cells were evident in the dorsal part of the SCN. Some VIP positive neurons were observed in the SCN immediately adjacent to the optic chiasm (OC), whereas a few VIP positive neurons were found to be embedded in the OC (Fig. 3A).

### Projections and distribution of VP and VIP immunoreactive fibers

**The chiasmatic region.** VIP positive fibers from the ventral and central part of the SCN leave the SCN and run anteriorly, dorsally, and posterodorsally. The anteriorly directed VIP fibers reach the anterior part of the medial preoptic area (POA). A small number of VIP and some VP fibers innervate diffusely in these areas. The dorsal-running VIP fibers from the SCN are very dense. Most of these VIP fibers reach the central and medial part of the anteroventral hypothalamic area (AVH) at anterior SCN level (Figs. 2C, 5A,C) and tend to innervate the medial part of the AVH at posterior SCN level (Figs. 2E,G, 4A,C). In frontal sections, VIP fibers in this pathway were seen to branch and give rise to terminal boutons, and to continue

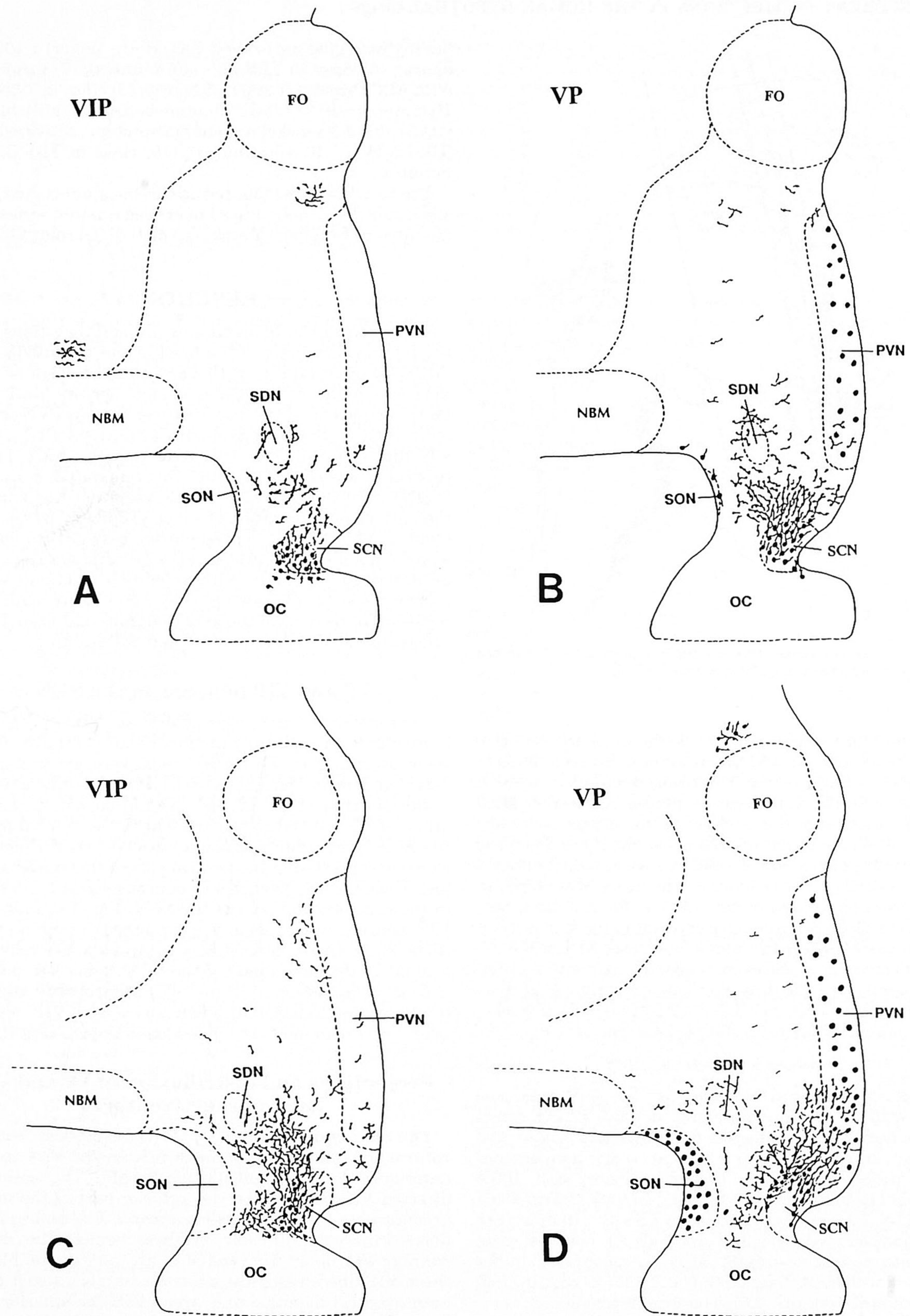


Fig. 2. A series of line drawings arranged from rostral to caudal (A-V) to illustrate schematically the location of vasoactive intestinal polypeptide (VIP) and vasopressin (VP) cell bodies and fibers in a human hypothalamus (NBBn 96-010). The dots correspond to the

position and density of the cell bodies. Short lines (in J, L, N) illustrate the area through which the fibers of VP magnocellular cell bodies pass and their axonal morphology also can be seen in Figure 6B. For explanation of abbreviations, see Abbreviations list.

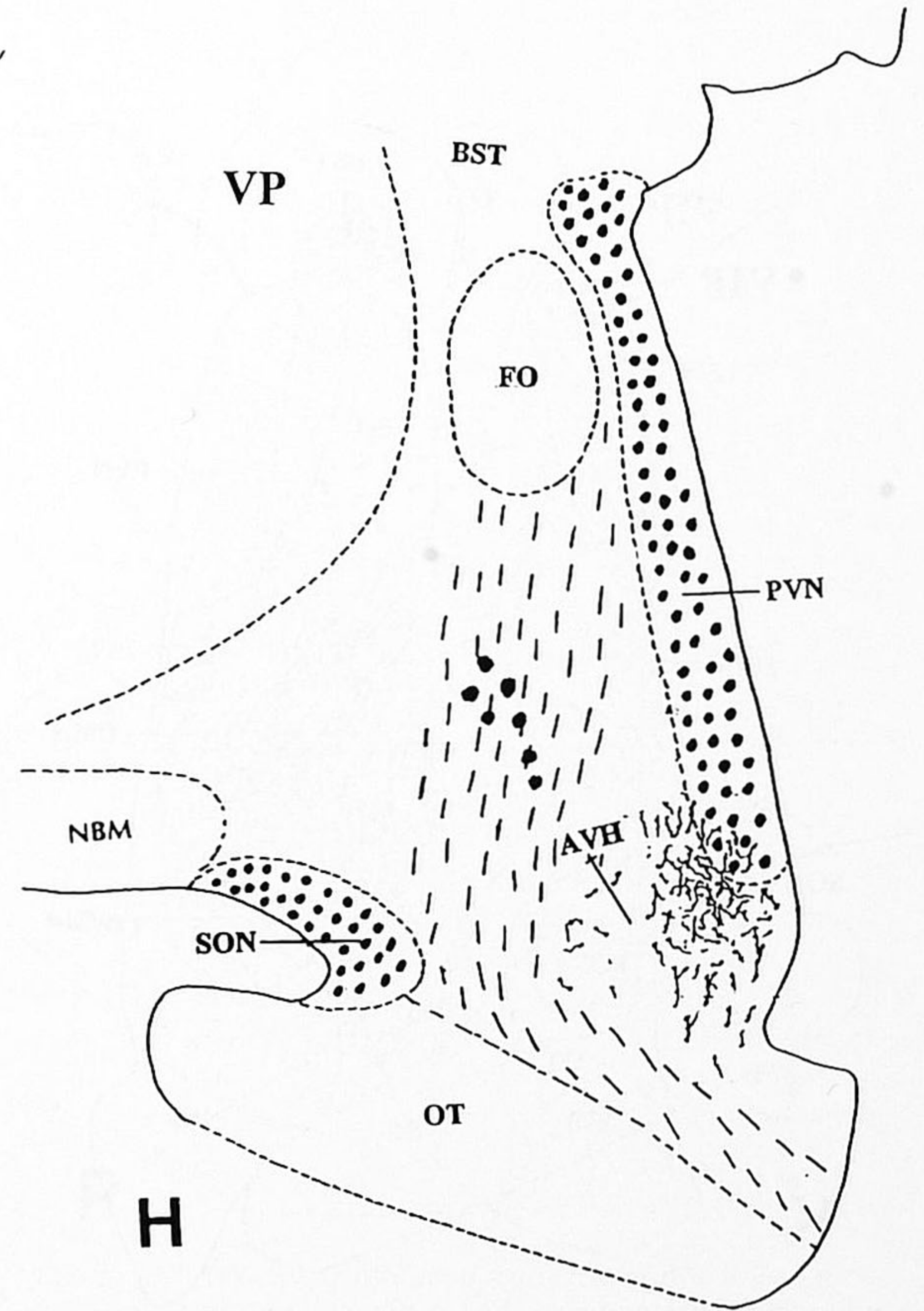
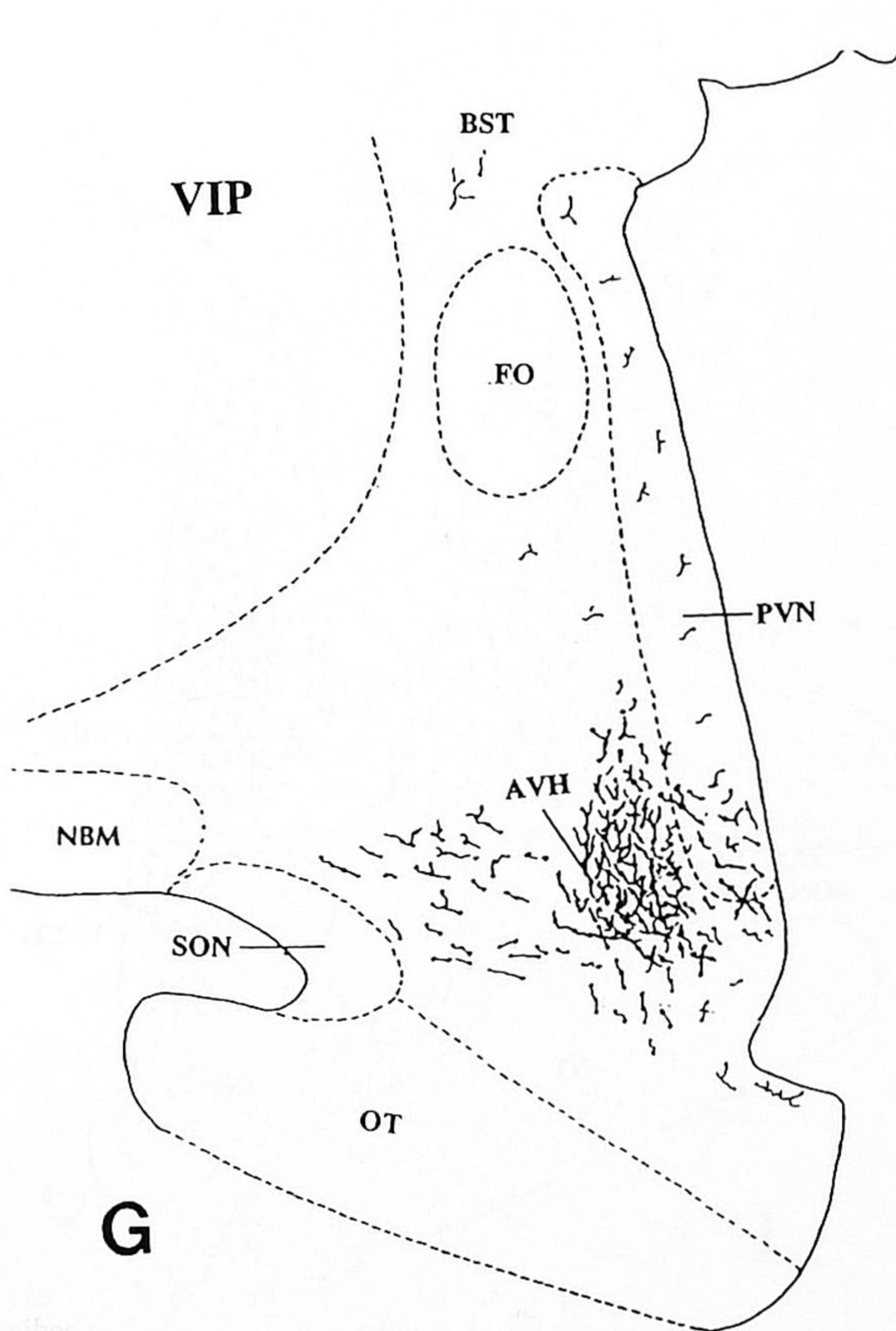
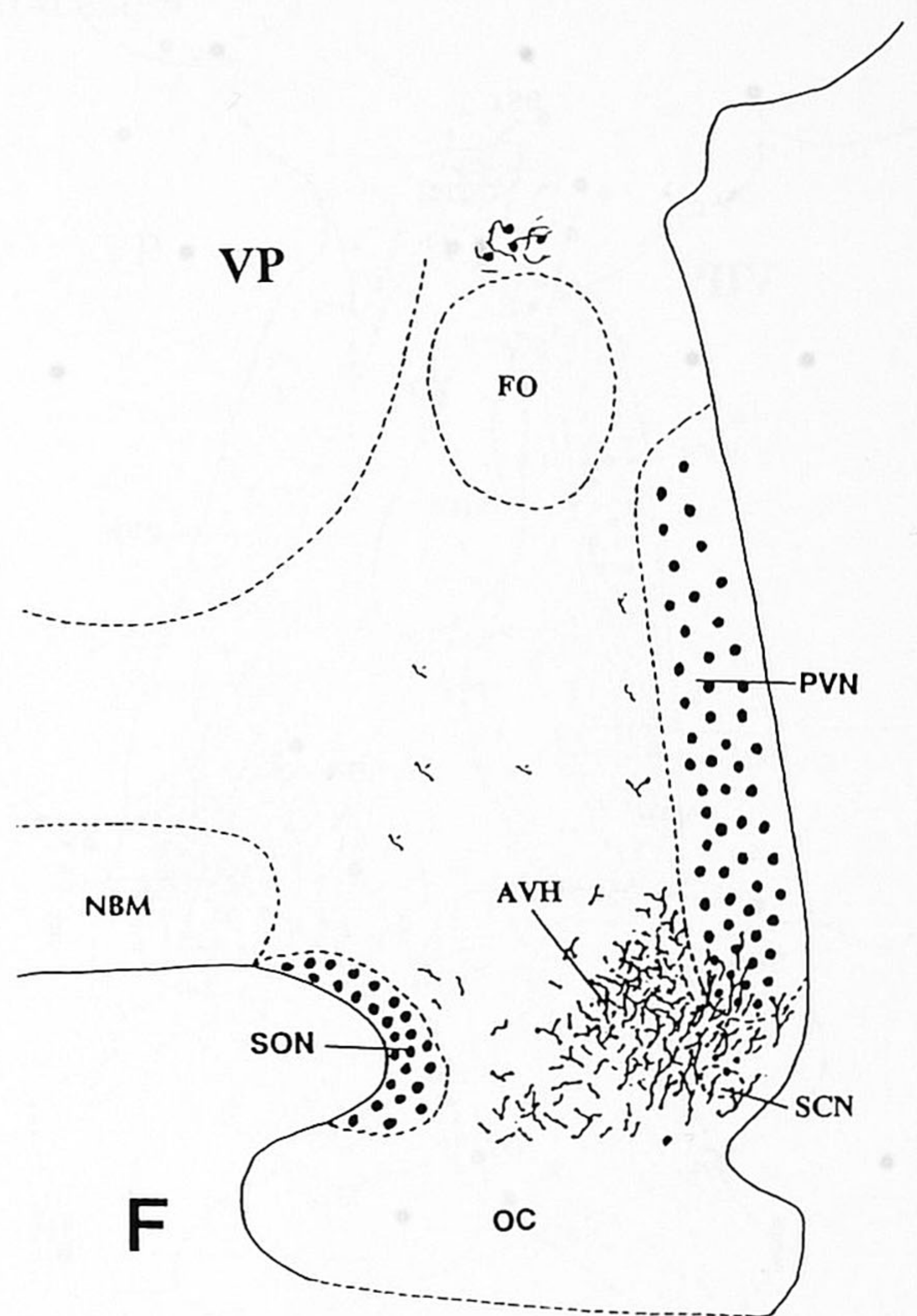
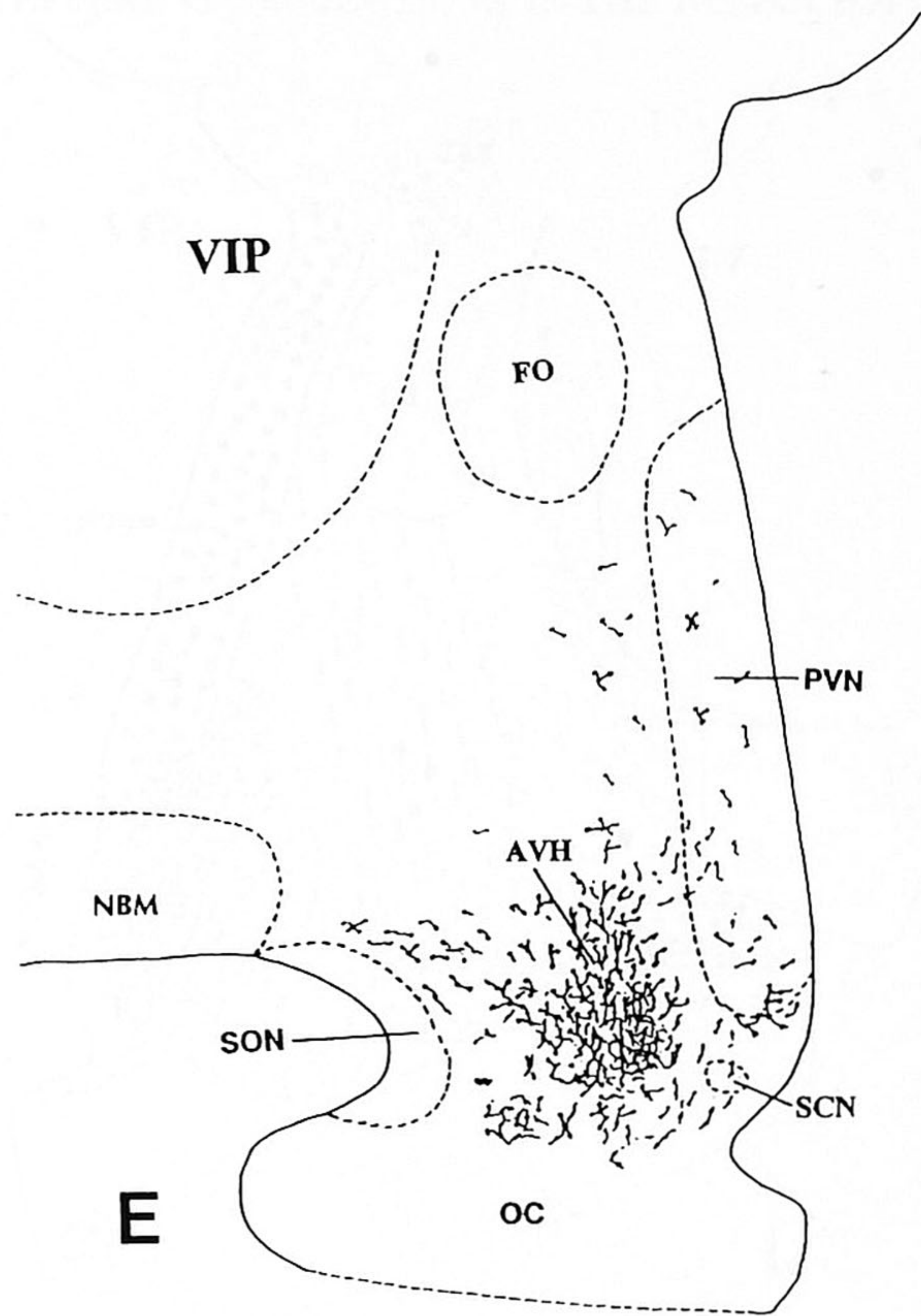


Figure 2 (Continued.)

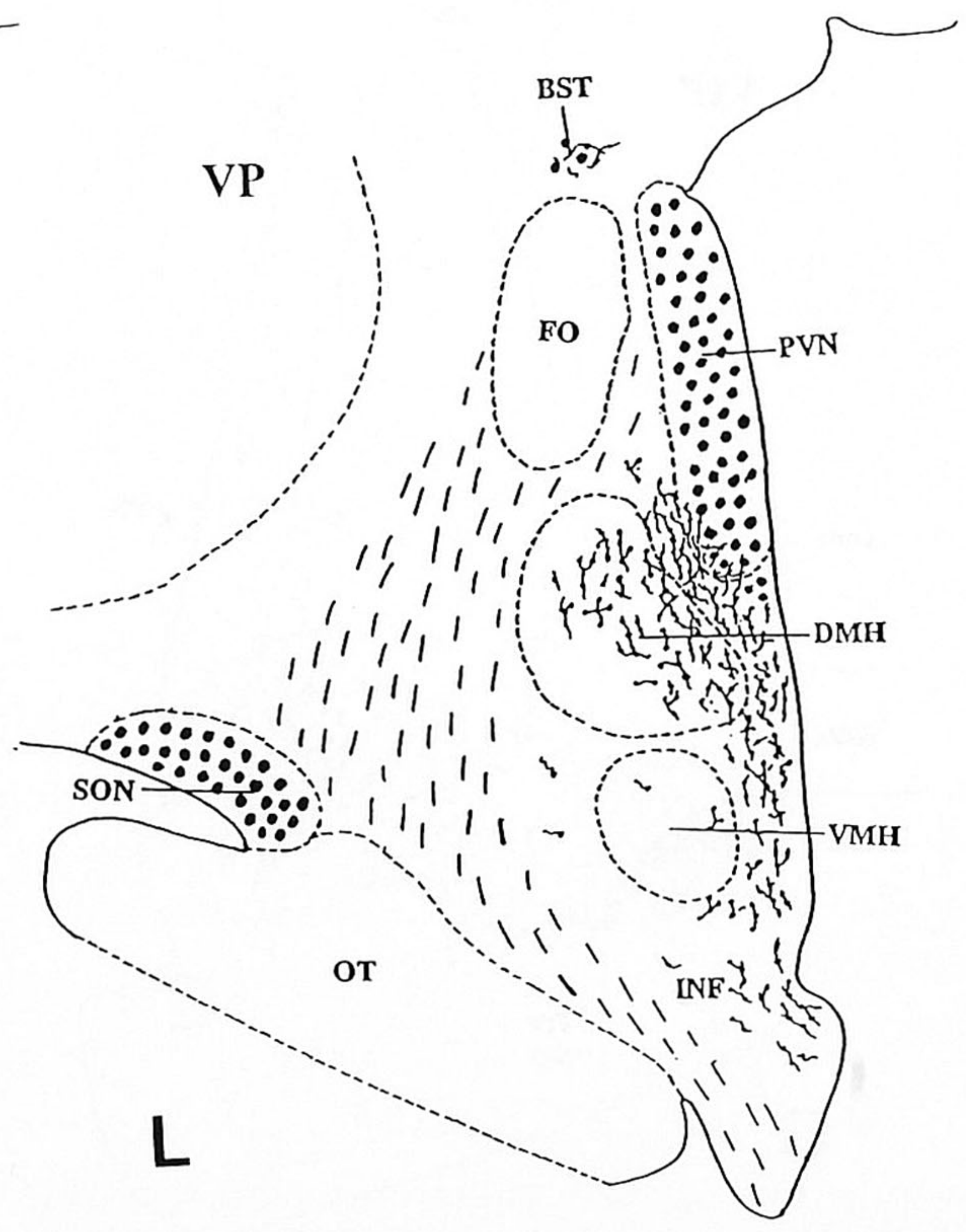
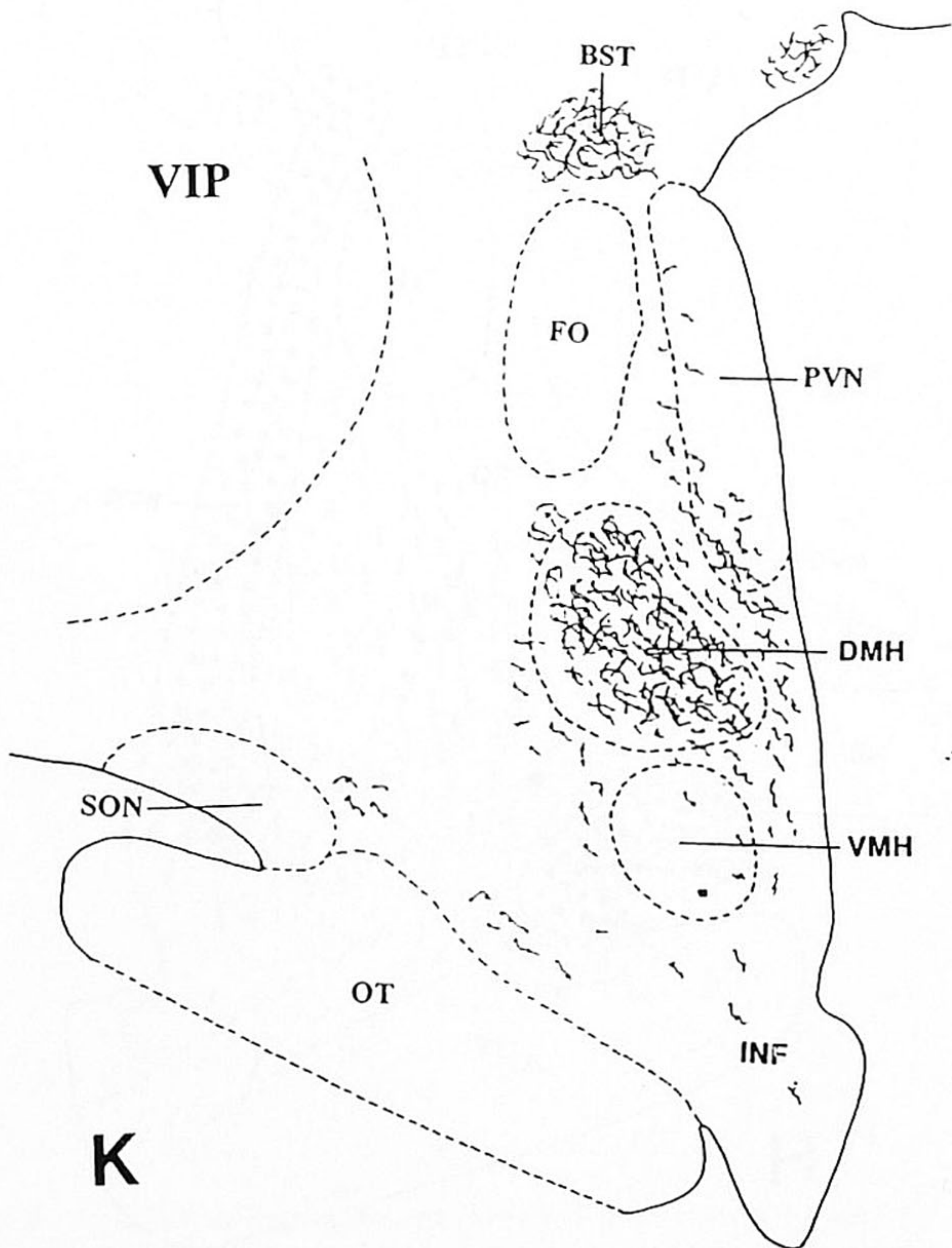
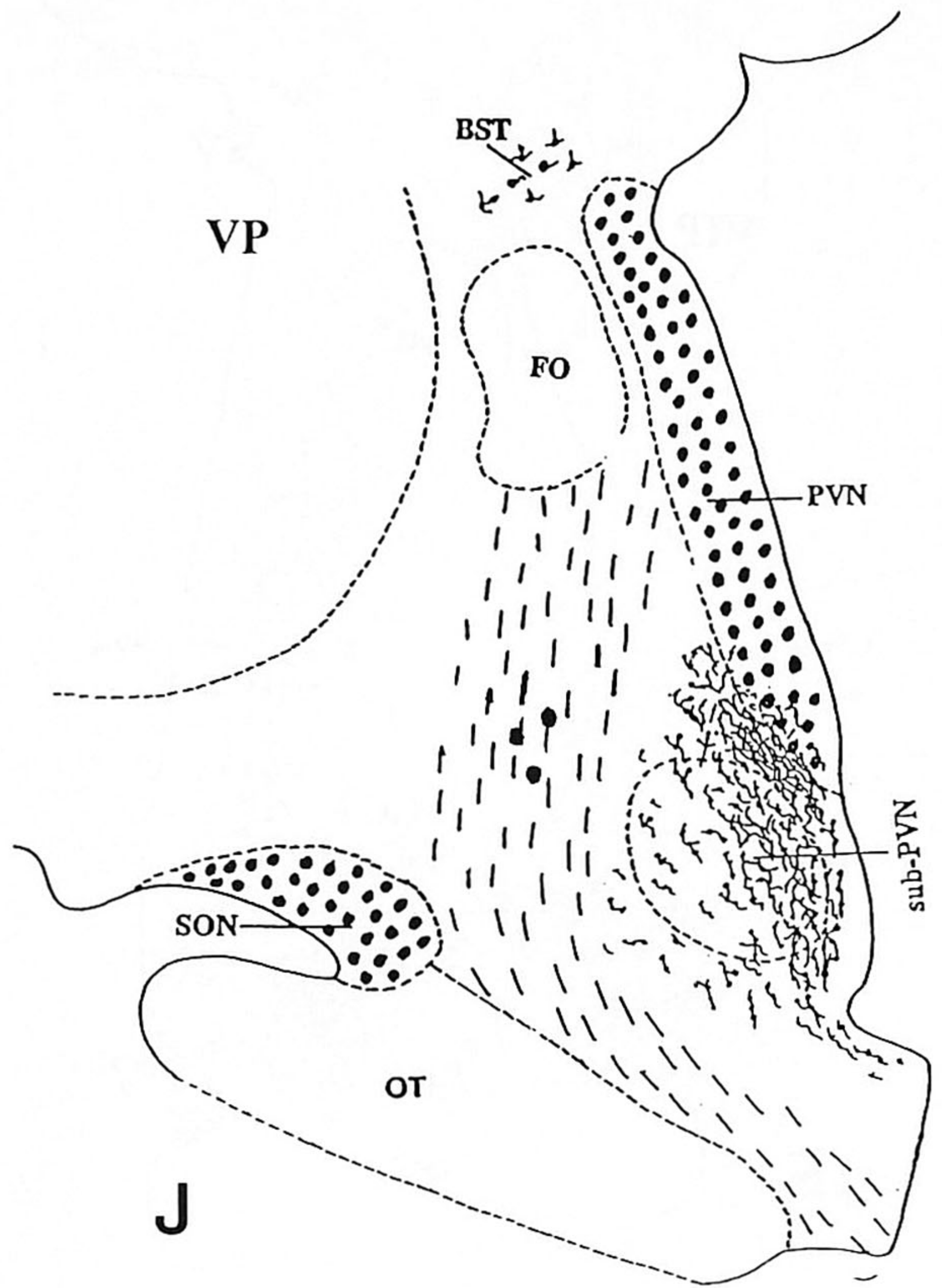
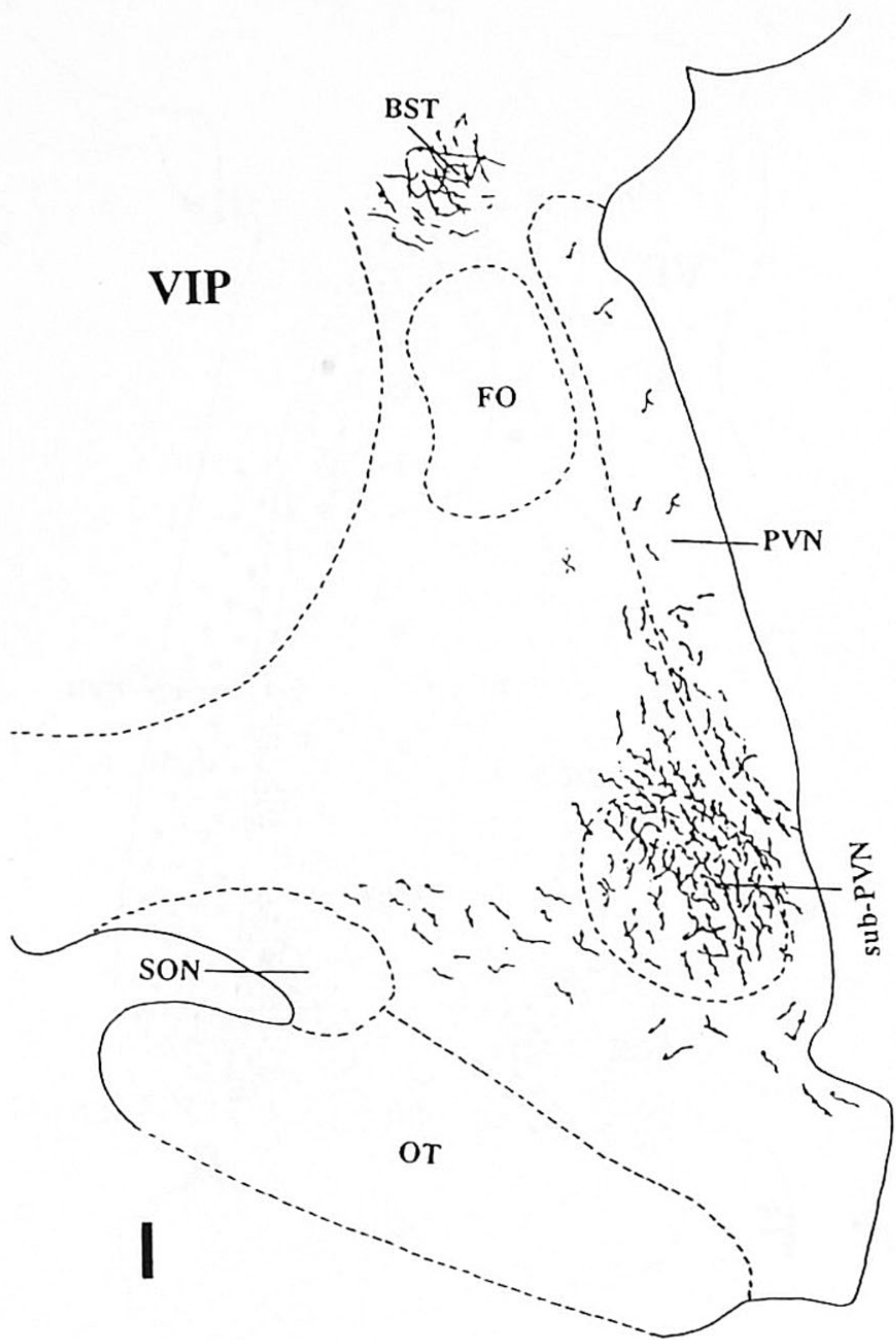


Figure 2 (Continued.)

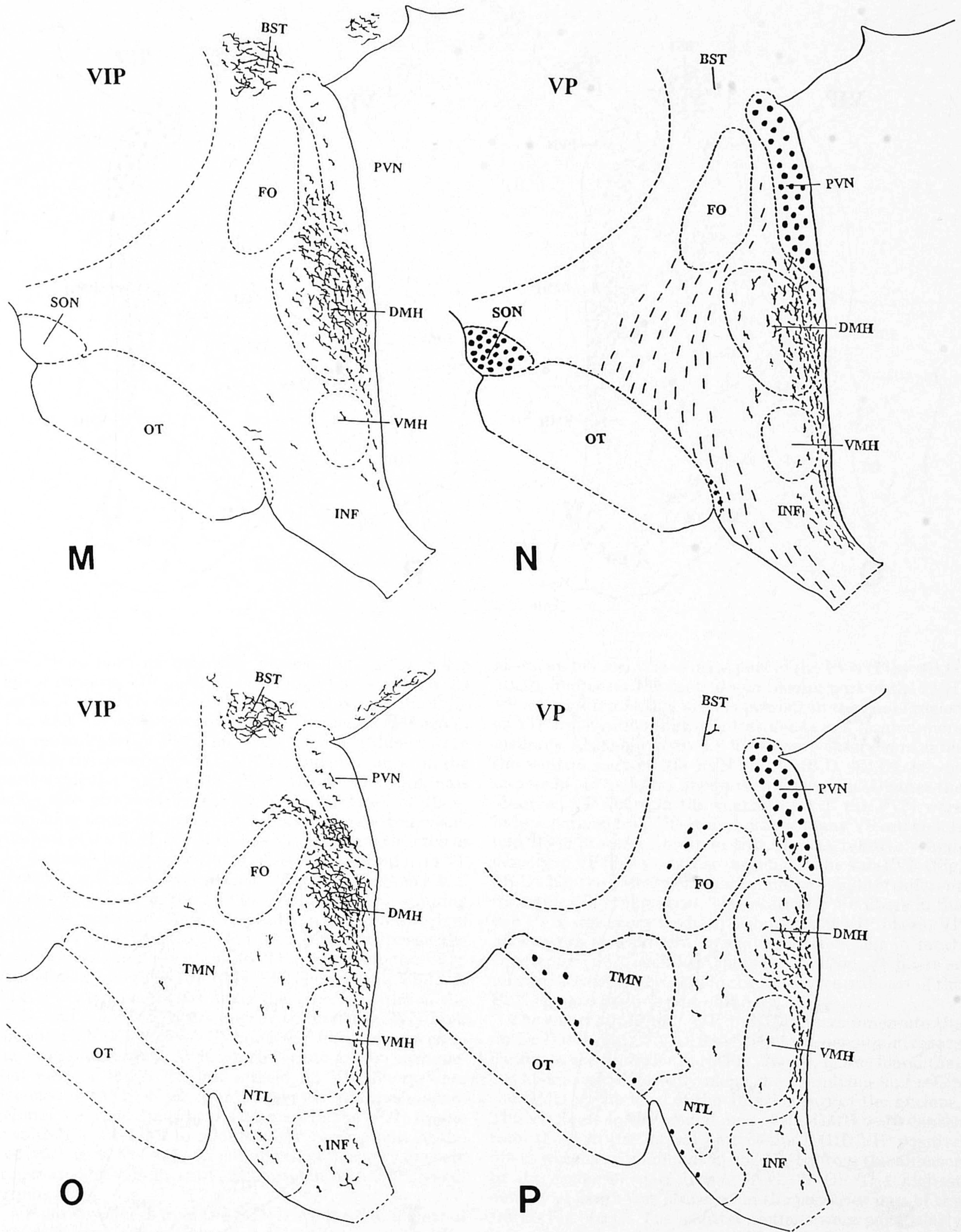


Figure 2 (Continued.)

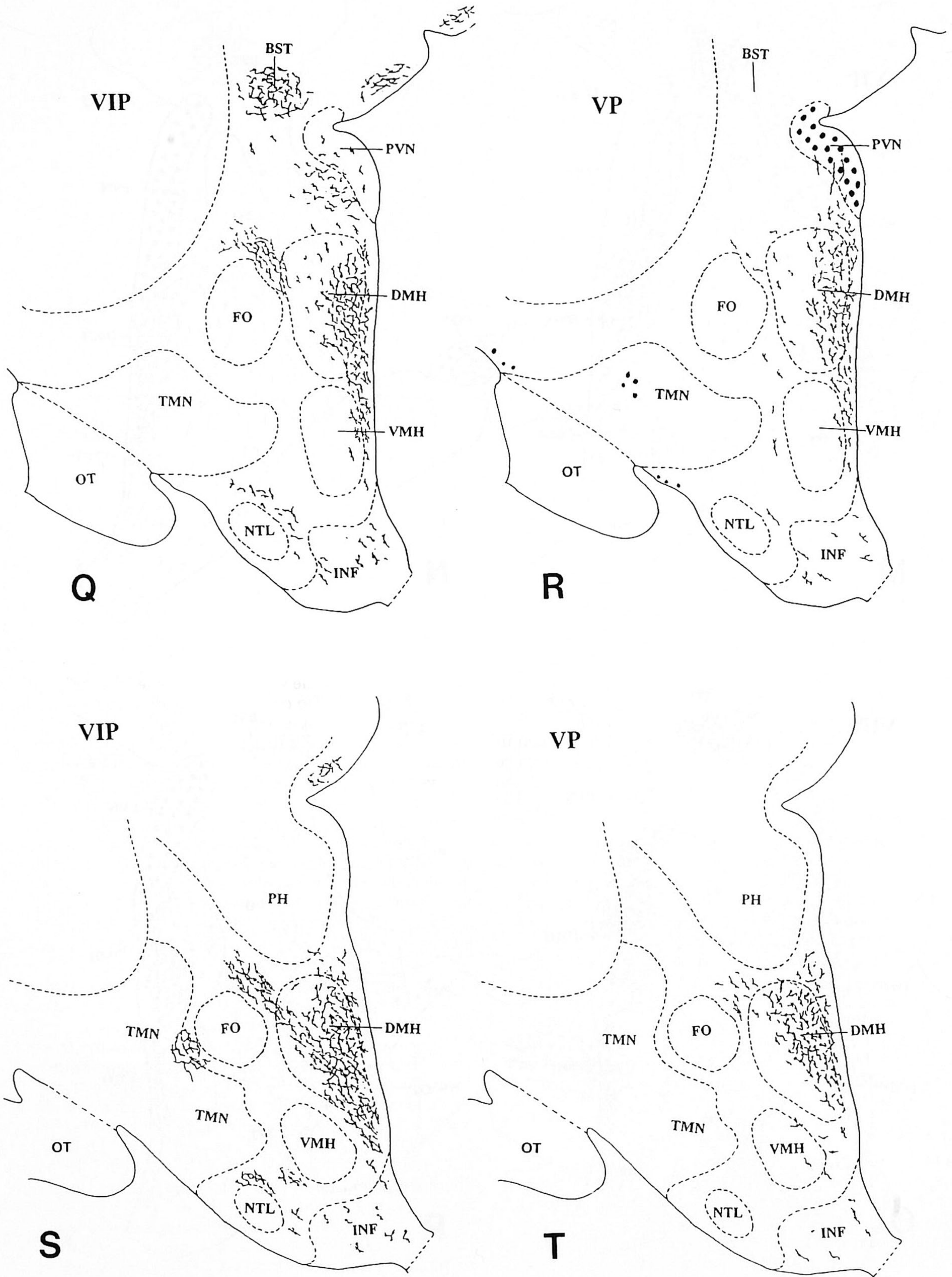


Figure 2 (Continued.)



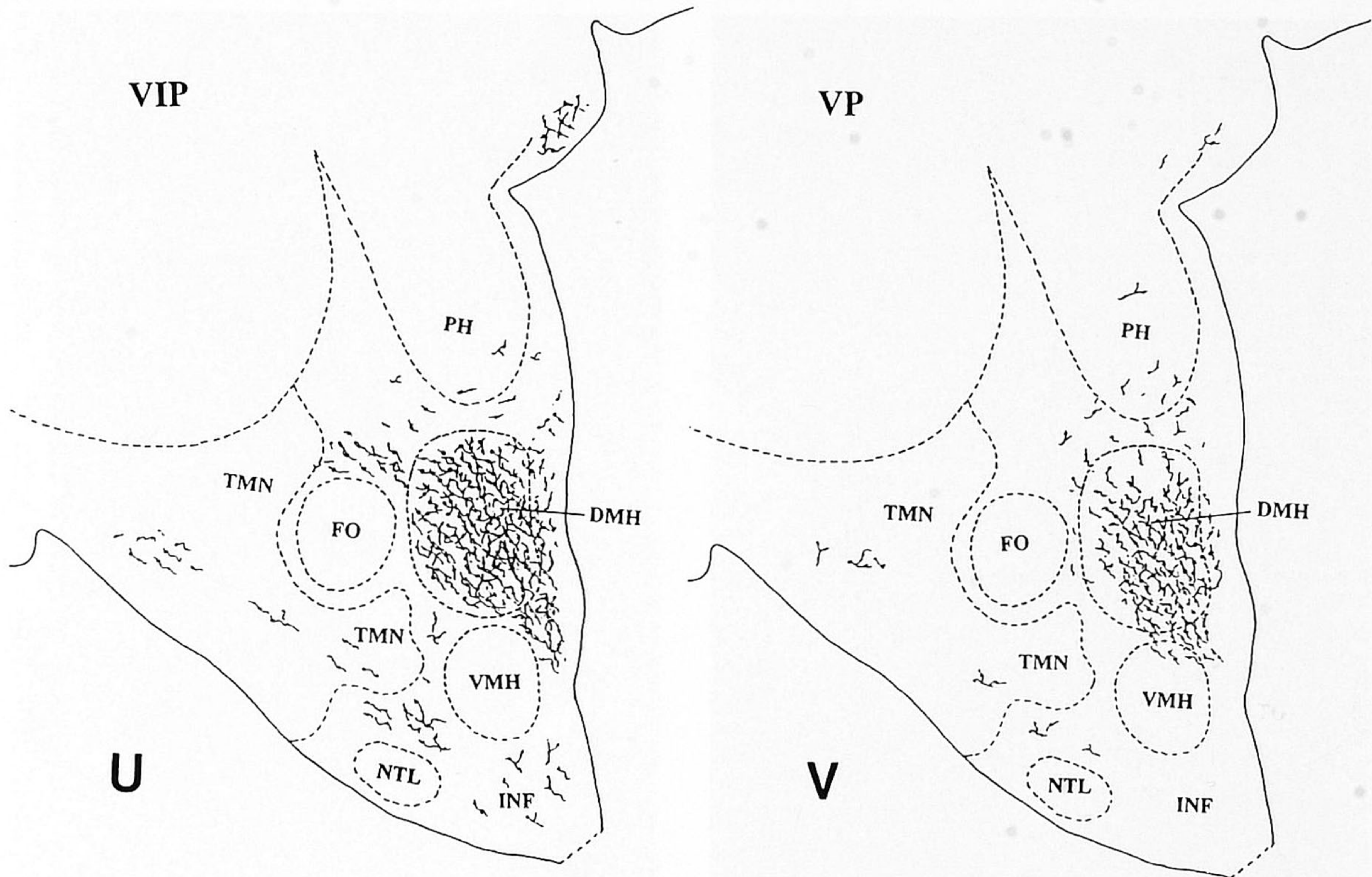


Figure 2 (Continued.)

dorsally to form an extensive plexus (Fig. 5A,C). Some dorsal-running VIP fibers pass through the area between the SCN and PVN and reach the ventral part of the PVN (Fig. 4A,C). A moderate number of VIP fibers were seen in the ventral part of PVN, but only a few VIP fibers were found in the dorsal part of the PVN, and even fewer in the periventricular part of the PVN. Dense VIP terminals were also observed in the SCN itself. Most of these branching fibers are concentrated in the dorsal and posterior part of the SCN (Fig. 4A). The VIP terminals located in the posterodorsal part of the SCN seem to overlap with VP neurons in consecutive sections (Fig. 4A,B). Some VIP fibers are present in the midline part of the OC, running along the ventricle. More posteriorly, a dense network of VIP fibers was detected in the area located between the posterior SCN and the anterior VMH, ventral to the PVN. We define this area as sub-PVN. VIP fibers in the sub-PVN form an extensive plexus along the ventral border of the PVN, and a few VIP fibers appear to enter the PVN from the sub-PVN (Figs. 2I, 6A,C). Some VIP fibers were seen in the lateral part of the AVH, which is close to the ventromedial part of the SON, but almost no VIP fibers were detected in the SON except for a few VIP positive magnocellular neurons and fibers scattered in the SON in one case (NBBn 94-124). In addition, the central part of the bed nucleus of the stria terminalis (BSTc) is very densely innervated by VIP fibers of unknown but probably diverse origin.

VP positive fibers from the SCN leave the dorsal part of the SCN and run mainly dorsally and posterodorsally. Most dorsal-running VP fibers pass through the area between the SCN and the ventral part of the PVN but are

also seen to reach the ventral part of the PVN (Figs. 4B,D, 5B,D), and enter the central and medial part of the AVH. VP innervation in this area is present in the same region as VIP innervation, but it is less dense and located more medially. Although many VP fibers were observed to enter the ventral part of the PVN (Figs. 4B,D, 5B,D), it was impossible to make an unequivocal judgment whether the observed VP fibers in the ventral part of the PVN were indeed derived from the SCN, because many VP cell bodies and their processes exist in this area. A relatively high density of VP fibers was also detected in the sub-PVN (Fig. 6B,D), but the pattern of innervation was slightly different from the VIP innervation. The location of VP fibers in the sub-PVN was more medially than that of VIP fibers. VP fibers were also present in the SCN itself, but in much smaller amounts than VIP fibers. In addition, VP fibers or terminals can also be seen in the anteroventral part of the PEN and the midline part of the OC.

**The tuberal region.** VP and VIP fibers innervate the entire DMH (Figs. 7A-D, 8A-D), but the density increases clearly in the posterior part (Fig. 2Q-V). It was found that VP fibers course dorsally along the ependyma and reach the DMH at the level of the anterior part of the nucleus. The VP fibers in the medial part of the DMH were denser than those in the lateral part of the DMH. VIP positive fibers were very abundant in the DMH, from the anterior to the posterior part (Figs. 7A,C, 8A,C). The highest density of fibers was observed in the posterior part of the DMH (Fig. 8A,C). The sagittal sections were particularly helpful to follow the VP and VIP fibers from the SCN in anterior-posterior direction. In these sections, VIP fibers from the SCN were indeed seen to reach the ventral part of

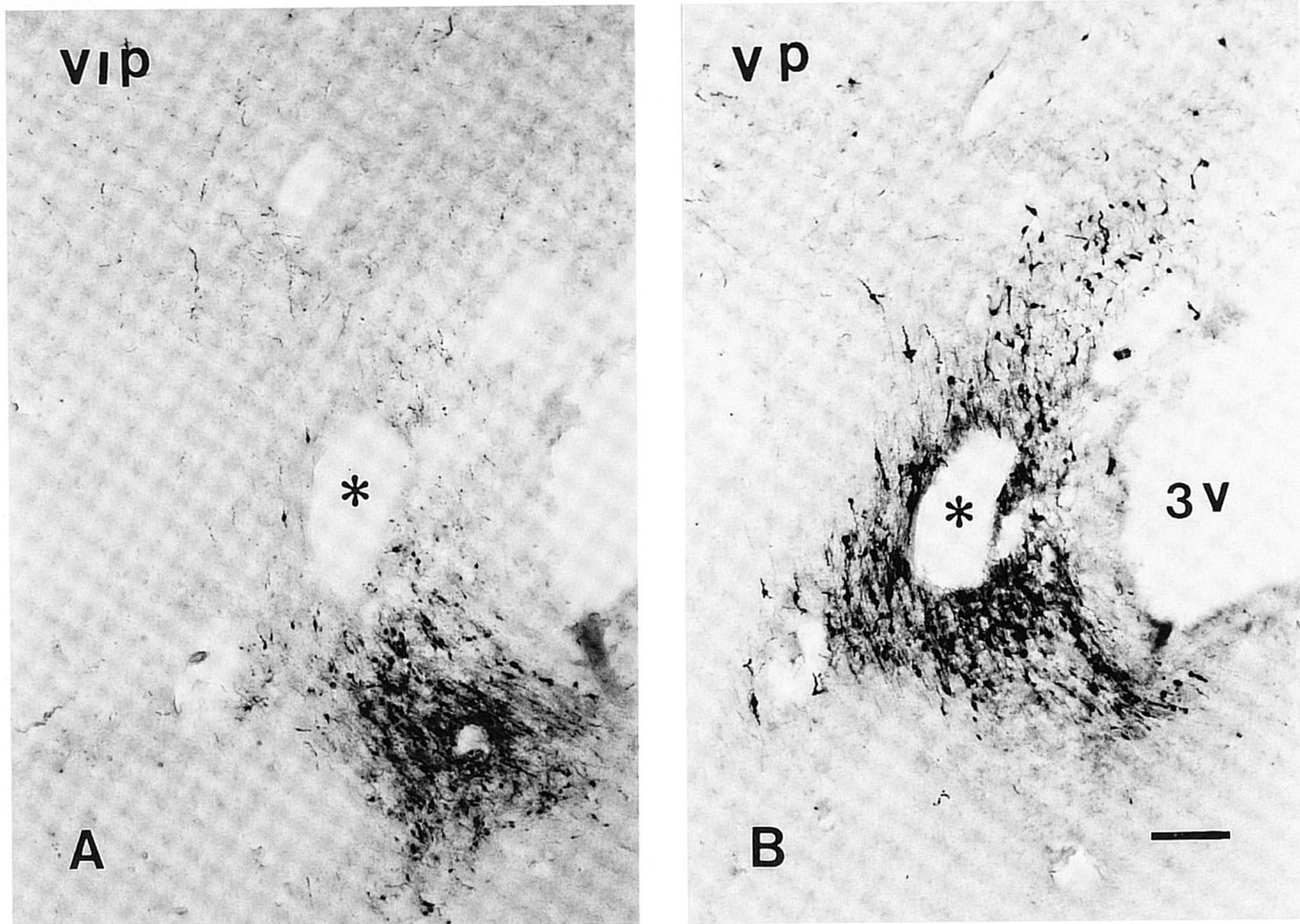


Fig. 3. Consecutive sections showing distribution of VIP (A) and VP (B) cell bodies in the human suprachiasmatic nucleus (SCN). Please note that the VP cell bodies are located more dorsally than the VIP cell bodies. Dorsal of the SCN VP and VIP terminals are present. Asterisk, blood vessel. 3V, third ventricle. Scale bar = 200  $\mu$ m.

the DMH directly. A small difference between VP and VIP innervation in the DMH was detected. VIP positive fibers seem to be denser than the VP fibers. In some cases VIP fibers tend to innervate the ventrolateral part of the DMH, or to show an inferolateral direction innervation, whereas VP fibers are mainly located in the medial part of the DMH close to the ventricle. In addition, VIP fibers can also be seen in the posterior part of the BST, infundibular nucleus (INF), some areas of the TMN and the NTL. Nevertheless, VP fibers are very sparse in these areas.

## DISCUSSION

With respect to the distribution of VIP and VP neurons in the human SCN, our present results are in agreement with previous observations (Stopa et al., 1984; Swaab et al., 1985; Mai et al., 1991). Up until now, the putative projections of VP and VIP fibers originating from the SCN have not been described in detail in the human hypothalamus. It will be clear that, based only on the present immunocytochemical staining for VP and VIP, no definitive conclusions can be drawn with respect to their site of origin. However, the use of the combination of frontal and sagittal sections makes it possible to follow VP and VIP fibers over some distance and draw some conclusions with reasonable certainty about the origin of VP and VIP fibers

in the hypothalamus. The detailed knowledge on SCN projections by means of PHA-L tracing and VP and VIP immunocytochemistry in the rat and hamster brain helped us to evaluate the present data (Buijs et al., 1978; Buijs, 1978; Sofroniew and Weindl, 1978; Stephan et al., 1981; Watts et al., 1987; Mikkelsen and Moller, 1988; Buijs et al., 1993; Kalsbeek et al., 1993; Orpen and Steiner, 1994; Mikkelsen and Vrang, 1994; Morin et al., 1994). The present study shows that many VIP fibers branch extensively and presumably from terminals in the following areas: (1) the central and medial part of the AVH, (2) the SCN, (3) the sub-PV, (4) the DMH, and (5) the BSTc. Many VP fibers do likewise in same areas as VIP fibers with some variability in the density with the exception of the large thick fibers from the magnocellular system which show a typical projection pattern. Branching of VIP fibers in the SCN are present throughout the entire extent of the nucleus but are most prevalent in its dorsal and posterior portions. It is difficult to determine where these presumptive VIP terminals in the SCN come from. The possibilities are that they originate from the ipsilateral and contralateral SCN. There are far fewer VP fibers in the SCN compared with VIP fibers. VIP and VP fibers were found to run along the dorsal surface of the OC but not in the optic tract (OT), suggesting that commissural fibers running between the left and right SCN exist (Swaab et al., 1985).

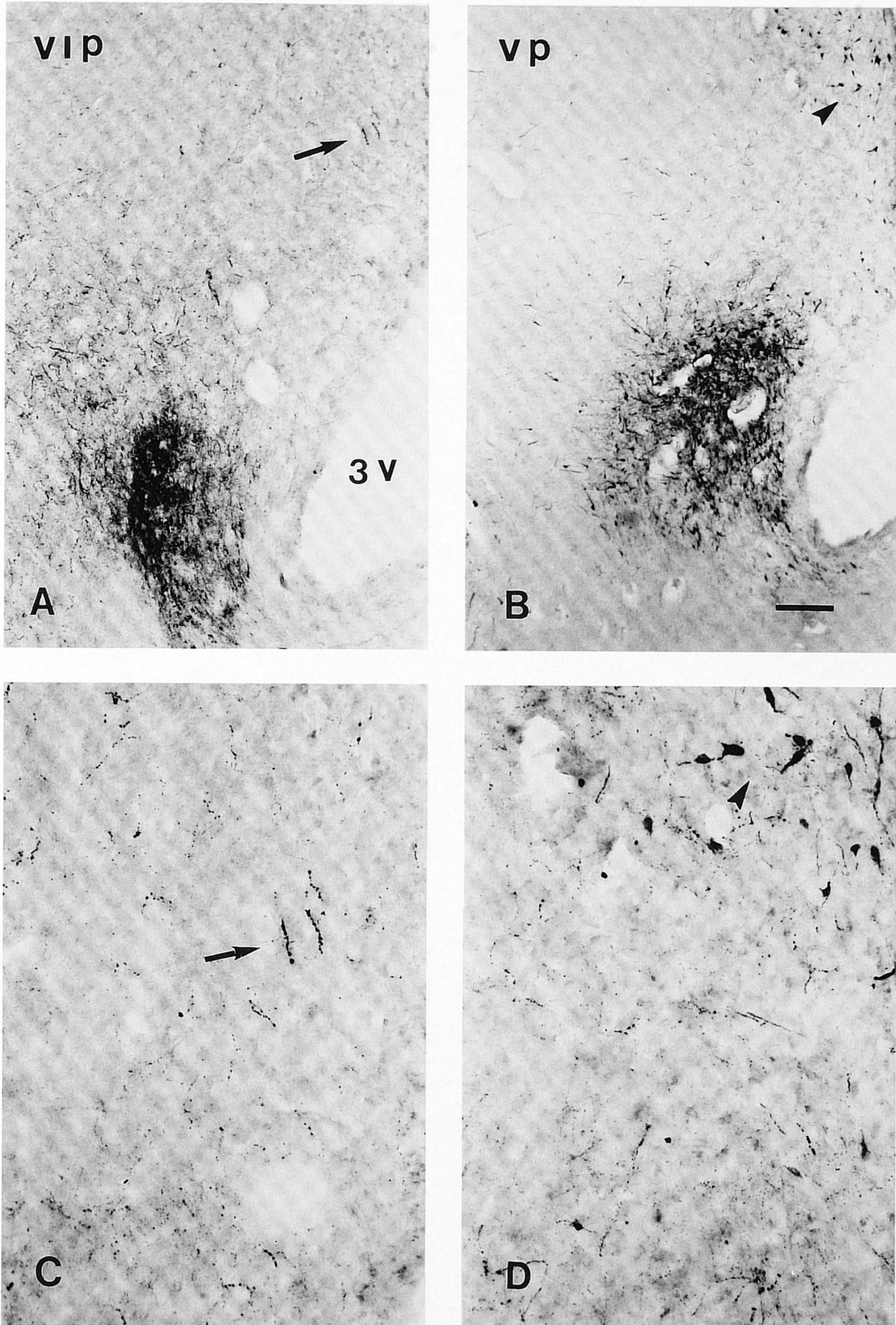


Fig. 4. Caudal level of the SCN, showing distribution of VIP and VP cell bodies and fibers. **C** and **D** are the magnification of the area of the AVH (arrow and arrowhead) in **A** and **B**, respectively. VIP and VP

fibers and terminals are visible in the ventral part of PVN (arrowheads). 3V, third ventricle. For abbreviations, see list. Scale bar = 200  $\mu$ m for A,B, 500  $\mu$ m for C,D.

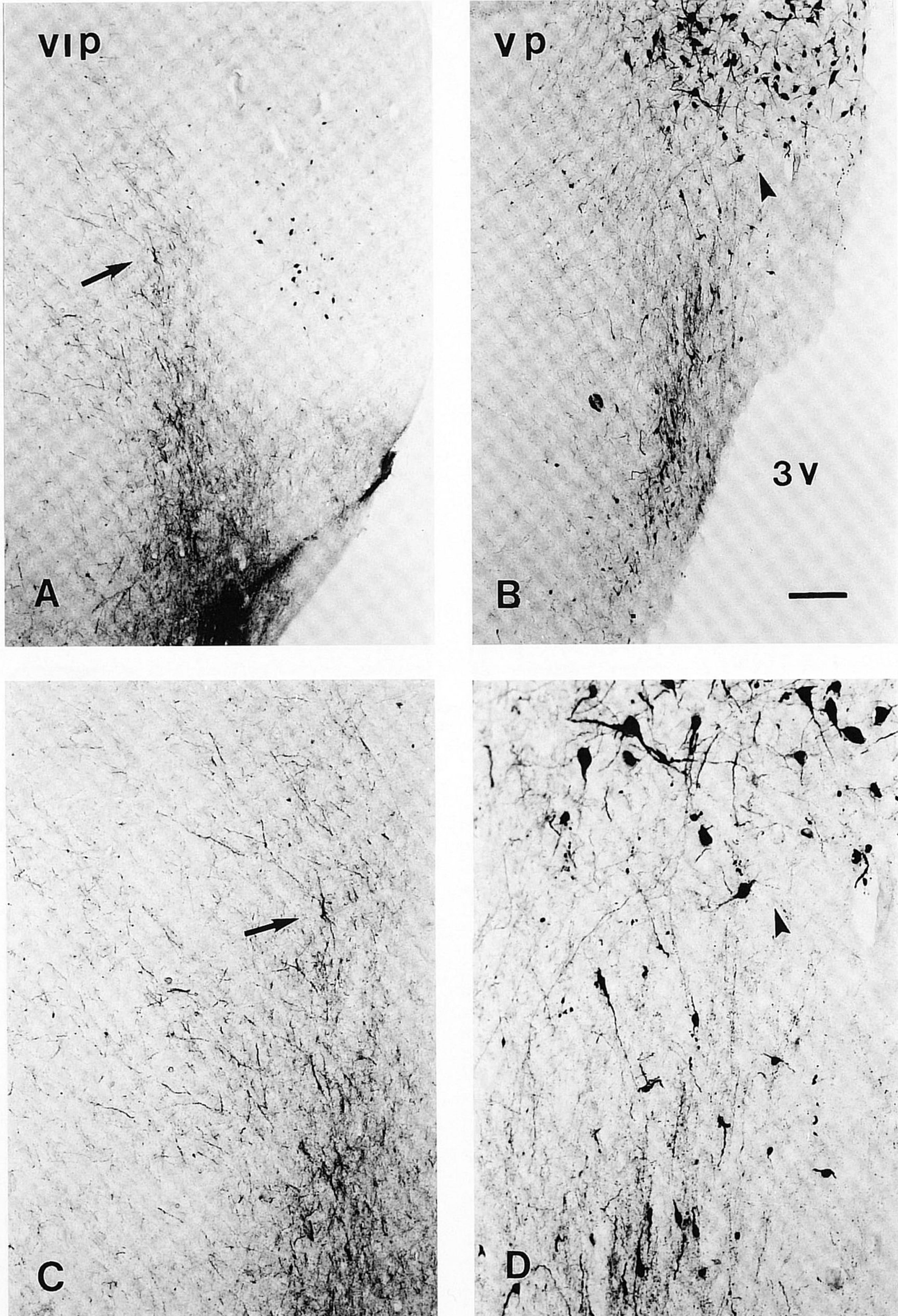


Fig. 5. Illustrating innervation of VIP (A, C) and VP (B, D) fibers in the anterior hypothalamic areas. **A:** Many VIP fibers from the SCN project directly to the central part of the anteroventral hypothalamic area (AVH) and form an extensive plexus just lateral and ventral of the paraventricular nucleus (PVN). Some VIP neurons are visible in the

ventral part of the PVN. **B:** VP fibers from the SCN project to the medial part of the AVH. Many fibers seem to project into the ventral part of the PVN. **C** and **D** are the magnification of the area (arrow and arrowhead) in A and B, respectively. 3V, third ventricle. For abbreviations, see list. Scale bar = 200  $\mu$ m for A,B, 500  $\mu$ m for C,D.

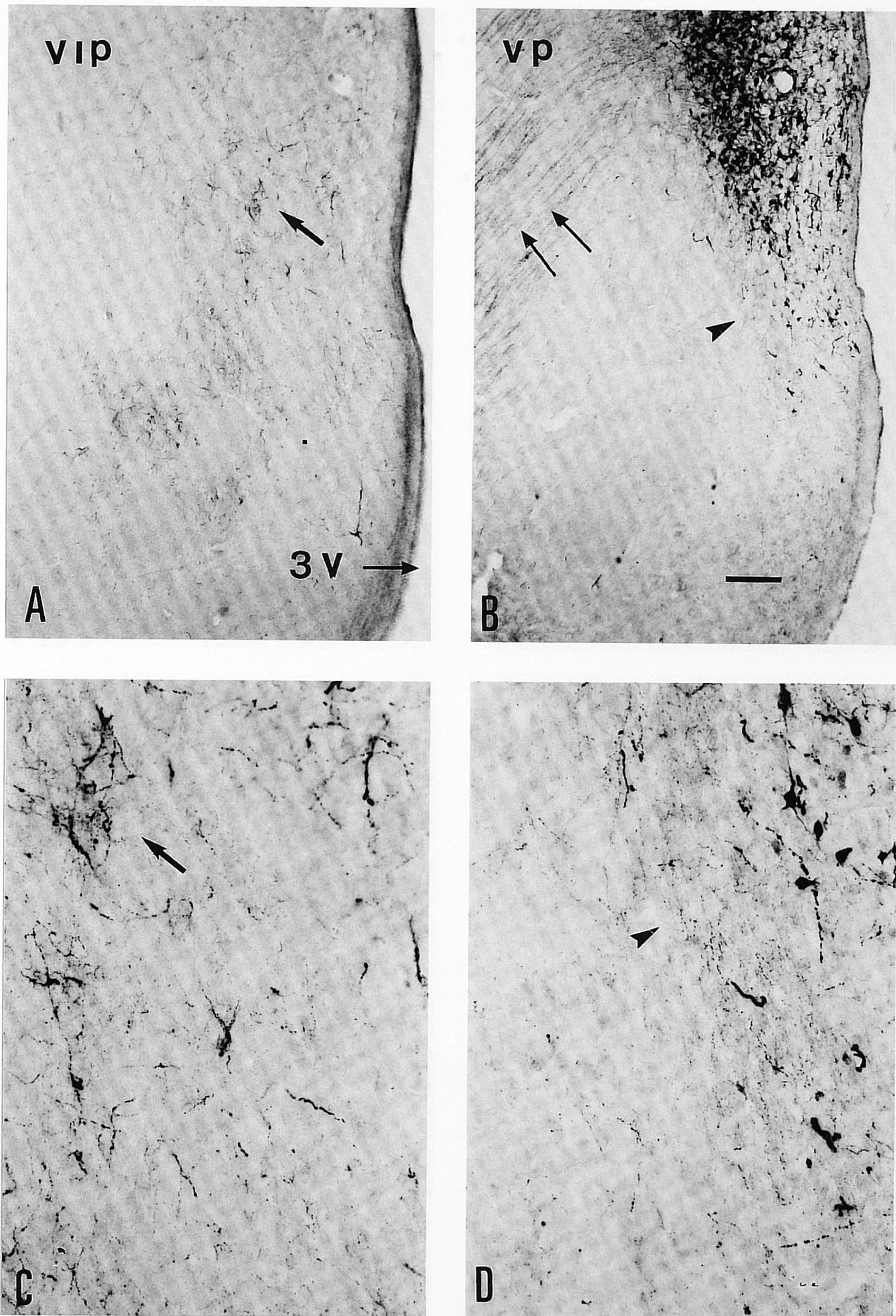


Fig. 6. Distribution of dense VIP and VP fibers in the sub-PVN. C is a high magnification of a part of the ventral PVN in A; VIP fibers are visible in this area (arrow). Many VP fibers along the ventral border of

the PVN are shown in B, and are presented in more detail in the high magnification (D). Some of them seem to pass into the PVN. For abbreviations, see list. Scale bar = 200  $\mu$ m for A,B, 500  $\mu$ m for C,D.

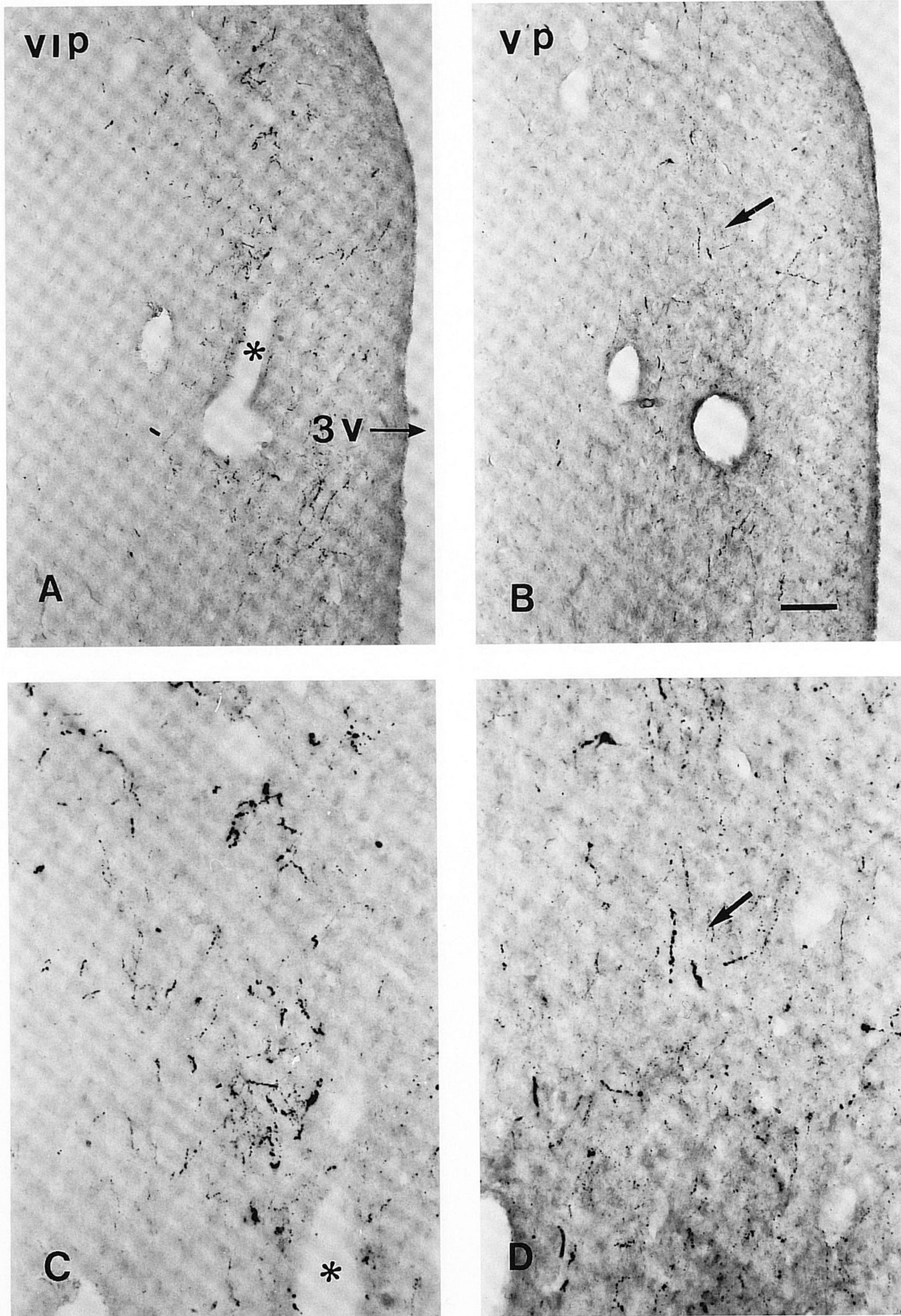


Fig. 7. Distribution of VIP (A, C) and VP (B, D) fibers in the medial (Fig. 7) and posterior (Fig. 8) level of the dorsomedial nucleus of the hypothalamus (DMH) in consecutive sections. VIP fibers are denser than VP fibers. C and D are the magnifications of A and B, respectively. 3V, third ventricle. Asterisk, blood vessel. Scale bar = 200  $\mu$ m for A,B, 500  $\mu$ m for C,D.

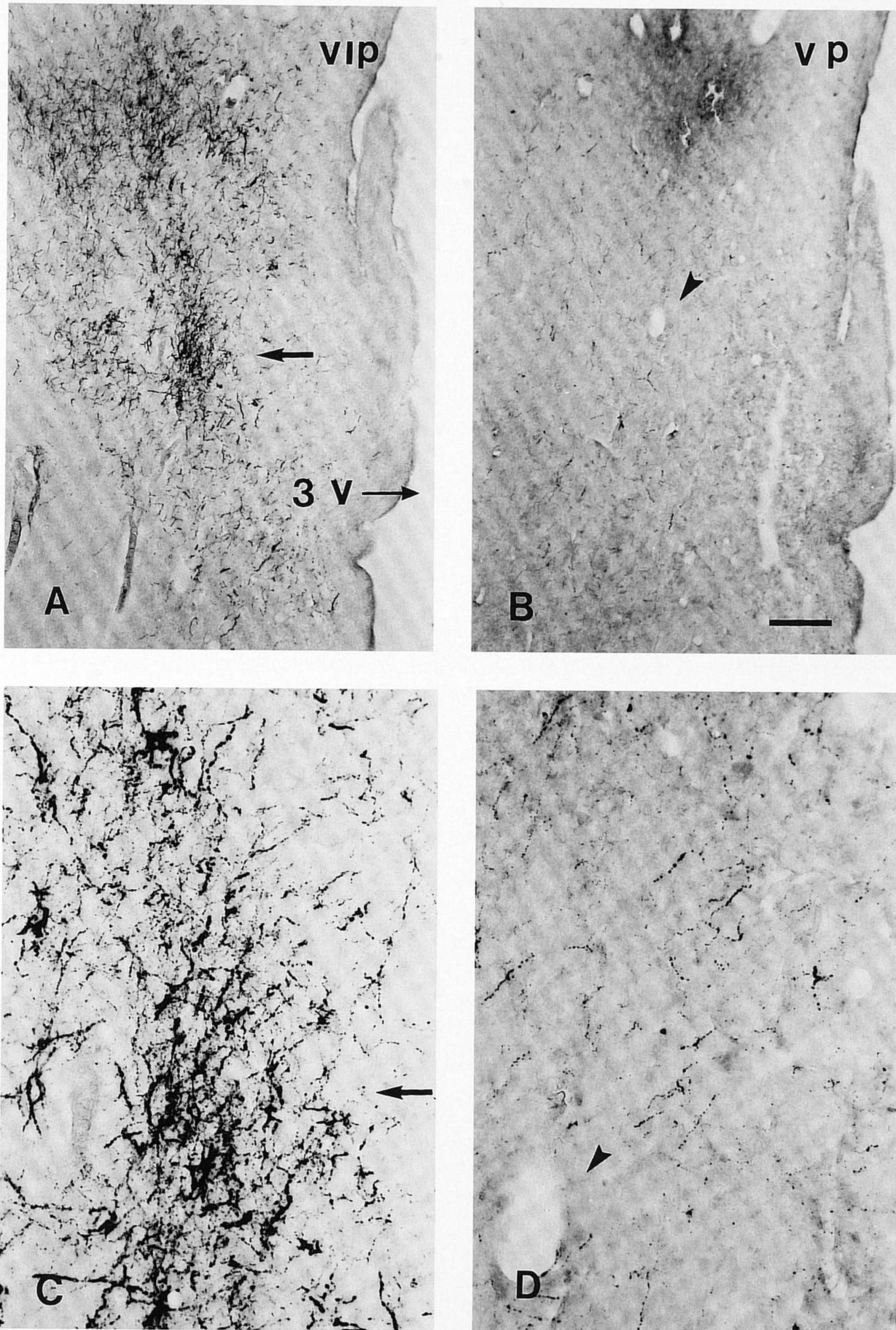


Fig. 8. Distribution of VIP (A, C) and VP (B, D) fibers at the level of the posterior part of the dorsomedial nucleus of the hypothalamus (DMH) in consecutive section. VIP fibers are denser than VP fibers. C

and D are the magnifications of A and B, respectively. Arrows in A and C, and arrowheads in B and D point to the same area. 3V, third ventricle. Scale bars = 200  $\mu$ m for A,B, 500  $\mu$ m for C,D.

Animal tracing studies showed that indeed one side of the SCN can project to another one (Stephan et al., 1981; Buijs et al., 1994). The human SCN may thus also have contralateral VP and VIP projections that can synchronize the two SCNs. The intense VIP immunocytochemical staining fibers and terminals in the human BSTc have been described in the previous studies (Walter et al., 1991; Zhou et al., 1995b). Very sparsely labeled fibers were found in the BST from the animal SCN tracing studies. We did not get any indication for fibers running from the human SCN to the BSTc. In addition, no VP fibers that can match with the density of VIP fibers are present in this nucleus. Therefore, it is probable that VIP fibers in the human BSTc are derived from other brain regions, such as the amygdala, rather than from the SCN.

Four areas that contain a combination of VP and VIP fibers are most probably SCN terminal fields. The first area is the central and medial part of the AVH. In frontal and sagittal sections throughout the SCN, it was shown that many VP and VIP positive fibers leave the SCN and directly reach this area. The fibers in this pathway branch and give rise to terminal boutons to form an extensive plexus. All these observations strongly suggest that the SCN has direct efferent projections to this area. This innervation pattern is similar to that found in primates (Cassone et al., 1988; Moore, 1993). In addition, compared with rat and hamster hypothalamic topographical organization, the present evidence suggests that the human central and medial part of the AVH may be homologous with the rat or hamster medial preoptic nucleus (MPN) (Simerly and Swanson, 1986). It has been demonstrated in rodents that the MPN receives inputs from widely distributed areas in both the forebrain and the brainstem, and sends its efferent projections to other brain regions (Renaud, 1979; Berk and Finkelstein, 1981; Simerly and Swanson, 1986). This area has been implicated in the regulation of sleep, thirst, reproductive behavior, sexual behavior, and body temperature (Larsson, 1979; Simerly and Swanson, 1986). Whether the human AVH has similar functions is unknown. The human SDN, also called the intermediate nucleus, is located in the central part of the AVH between the SON and the PVN at the same anteroposterior level as the SCN (Swaab and Fliers, 1985; Hofman and Swaab, 1988; Braak and Braak, 1992). Some VP and VIP fibers and terminals innervate in the SDN, suggesting that the SCN may influence SDN function possibly related to sexual behavior.

The second area is the sub-PVN. The distribution of VP fibers is slightly more medial and dorsal than that of VIP fibers. In the human hypothalamus, the sub-PVN has not been described before. This zone, according to its cytoarchitecture, could not clearly be distinguished from the surrounding hypothalamic area. The dense VP and VIP fibers innervating this area suggest a particular function that is different from the surrounding hypothalamic area. It should be emphasized that the sub-PVN is anteriorly continuous with the medial part of the AVH which is also innervated by dense VP and VIP fibers and terminals, and it is also impossible to distinguish these two structures on the basis of their cytoarchitecture. The question arises, therefore, whether sub-PVN and AVH could be considered as one structure that receives SCN input and shares the same functions. In the rat and hamster, SCN projections to the sub-PVN have been firmly established using immunocytochemical staining for SCN neurotransmitters, SCN

lesions, and tracing studies (Hoorneman and Buijs, 1982; Watts et al., 1987; Buijs et al., 1993; Kalsbeek et al., 1993; Orpen and Steiner, 1994; Morin, et al., 1994; Vrang et al., 1995). On the basis of these studies it has been suggested that the sub-PVN plays an important role in conveying SCN signals to the PVN (Watts, 1991). In addition, a recent study by Boudaba et al., (1996) suggest the areas, including the sub-PVN, around the PVN feeding inhibitory information into the PVN, which may reinforce the idea. However, this possibility still needs proof both in rodents and in humans.

The third area is the ventral part of the PVN. Substantive VP and some VIP fibers from the SCN seem to reach the ventral part of the PVN directly. VP neurons from the SCN seem to have denser projections to the ventral part of the PVN than VIP neurons. So far, direct projections from the SCN to the neurosecretory neurons have been shown to be very limited in other species (Watts et al., 1987; Buijs et al., 1993; Vrang et al., 1995). However, it should be added that it is extremely difficult to determine with certainty the pattern of innervation of VP fibers which come from the SCN in the ventral part of the PVN. This difficulty is due to the large number of VP neurons and their processes in this nucleus. There are possibly direct projections from the SCN to the PVN in the present observation, suggesting that the SCN and the PVN may have direct anatomical connections. The periventricular part of the PVN is a narrow structure along the third ventricle. The clear features is that this area contains medium-sized and small nerve cells, and immunocytochemical staining for SOM shows many positive neurons. Almost no VIP fibers are present in this area, suggesting that VIP neurons in the SCN may have no projections to this area. However, it is not known whether the presently observed VP fibers represent a direct projections from VP neurons of the SCN. The functional role of this area also needs to be investigated.

The fourth area is the DMH. In previous studies with conventional staining it was suggested that the DMH is poorly differentiated from the surrounding hypothalamic area in the human hypothalamus (Braak and Braak, 1992). Until now, no immunocytochemical staining for neuropeptidergic transmitters or histochemical staining for classical neurotransmitters has been found to mark specifically DMH neurons. However, in the present study, the DMH borders, with the exception of the ventral part that borders the VMH, could be easily recognized in the Nissl stained sections. At the anterior level of the DMH, the paraventriculo-hypophyseal tract (containing VP fibers) passes through the lateral part of the hypothalamus and projects to the pituitary; the tract forms the anterolateral border of the DMH (Figs. 2L,N, 6B). At the posterior level, the lateral border connects with the TMN, which is characterized by the presence of neurons containing a large cell body with lipofuscin granules. The medial border of the DMH is the third ventricle. The anterodorsal part of the DMH often borders with the medial side of the fornix (FO) and the ventrolateral border of the PVN (Fig. 2N,P) and merges gradually with the posterior hypothalamic nucleus (PH) at the posterodorsal part of the DMH (Fig. 2R). It is usually difficult to distinguish the ventral border of the DMH from the VMH with conventional Nissl staining, although in some cases the medium-sized nerve cells of the DMH are markedly richer in lipofuscin deposits than the neurons of the VMH. During our search for



immunocytochemical markers for the DMH, a dense network of SOM fibers was found to innervate the VMH from the anterior to the posterior part. Using SOM immunocytochemical staining we could thus indirectly distinguish the ventral border of the DMH from the VMH. VP and VIP fibers show extensive branching and densely innervate the DMH from the anterior to the posterior part. In the present study, although some direct projections from the SCN to the DMH were detected in sagittal sections, it is not known with certainty whether all the VP and VIP fibers that terminate in the DMH come from the SCN. On the other hand, the fact that in animal experiments VP and VIP fibers in the DMH are derived from the SCN (Hoorneman and Buijs, 1982; Kalsbeek et al., 1993; Buijs et al., 1993) reinforces the idea that this may also be the case in human.

The present study illustrates the projections of the SCN in the human hypothalamus by the immunocytochemical staining of SCN neurotransmitters and provides a picture of which areas might receive an input from the SCN. Our results showed that these areas (such as the central and medial part of the AVH, the sub-PVN, and the DMH) may receive SCN projections. The results match those of the animal studies using immunocytochemical staining for SCN neurotransmitters and tracing technique, suggesting that hypothalamic topographical organization might be comparable for many species. In addition, the remarkable finding that there clearly are VIP and in particular VP fibers running between the SCN and the ventral part of the PVN in the human, suggests that the SCN may be responsible for the expression of circadian rhythms in hormone secretion (e.g., ACTH and corticosterone secretion) by direct pathways. In addition, the projections to the medial part of the AVH, sub-PVN and DMH may allow an indirect influence on PVN neurons by means of multisynaptic pathways. We also demonstrated in the human as in the rat (Buijs et al., 1993) a different pattern of innervation for VP and VIP in the AVH, sub-PV and DMH. It appears that the VP fibers and terminals tend to innervate more medially than VIP in these three areas. The possibility exists thus that VIP and VP neurons in the SCN may affect different neurons, which further supports the idea that VIP and VP neurons in the SCN may be responsible for different functions in expressing biological rhythms.

### ACKNOWLEDGMENTS

We are grateful to A. Holtrop, M. Kooreman, J. Wouda, and S. Pindak for their help for collecting and documenting brain material, G. van der Meulen for the photographs, and W.T.P. Verweij for secretarial help. The brain material was obtained from the Netherlands Brain Bank (Coordinator R. Ravid). This study was supported by Netherlands Institute for Brain Research grant 94-11.

### LITERATURE CITED

- Berk, A.L., and J.A. Finkelstein (1981) An autoradiographic determination of the efferent projections of the suprachiasmatic nucleus of the hypothalamus. *Brain Res.* 226:1-13.
- Boudaba, C., K. Szabó, and J.G. Tasker (1996) Physiological mapping of local inhibitory inputs to the hypothalamic paraventricular nucleus. *J. Neurosci.* 16:7151-7160.
- Braak, H., and E. Braak (1992) Anatomy of the human hypothalamus (chiasmatic and tuberal region). [Review]. In D.F. Swaab, M.A. Hofman, M. Mirmiran, R. Ravid, and F.W. van Leeuwen (eds): *The Human Hypothalamus in Health and Disease*. Progress in Brain Research, Vol 93. Amsterdam: Elsevier, pp. 3-14.
- 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.
- Buijs, R.M., D.F. Swaab, J. Dogterom, and F.W. Van Leeuwen (1978) Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell Tiss. Res.* 186:423-433.
- Buijs, R.M., M.M. Markman, Y.X. Hou, and S. Shinn (1993) Projections of the suprachiasmatic nucleus to stress-related areas in the rat hypothalamus: A light and electron microscopical study. *J. Comp. Neurol.* 335:42-54.
- Buijs, R.M., Y.X. Hou, S. Shinn, and L. P. Renaud (1994) Ultrastructure evidence for intra- and extranuclear projections of GABAergic neurons of the suprachiasmatic nucleus. *J. Comp. Neurol.* 340:381-391.
- Card, J.P., and R.Y. Moore (1984) The suprachiasmatic nucleus of the golden hamster: Immunohistochemical analysis of cell and fiber distribution. *Neuroscience* 13:415-431.
- Card, J.P., N. Brecha, H.J. Karten, and R.Y. Moore (1981) Immunocytochemical localization of vasoactive intestinal polypeptide-containing cells and processes in the suprachiasmatic nucleus of the rat: Light and electron microscope analysis. *J. Neurosci.* 1:1289-1303.
- Cassone, V.M., J.C. Speh, J.P. Card, and R.Y. Moore (1988) Comparative anatomy of mammalian suprachiasmatic nucleus. *J. Biol. Rhythms* 3:71-91.
- Dierickx, K., and F. Vandesande (1977) Immunocytochemical localization of the vasopressinergic and the oxytocinergic neurons in the human hypothalamus. *Cell Tissue Res.* 184:15-27.
- Gai, W.P., L.B. Geffen, and W.W. Blessing (1990) Galanin immunoreactive neurons in the human hypothalamus: Colocalization with vasopressin-containing neurons. *J. Comp. Neurol.* 298:265-280.
- Hardy, J.A., P. Wester, B. Winblad, C. Gezelius, G. Bring, and A. Eriksson (1985) The patients dying after long terminal phase have acidotic brain: Implications for biochemical measurements on autopsy tissue. *J. Neural. Transm.* 61:253-264.
- Hofman, M.A., and D.F. Swaab (1988) The sexually dimorphic nucleus of the preoptic area in the human brain: A comparative morphometric study. *J. Anat.* 164:55-72.
- Hoorneman, E.M.D., and R.M. Buijs (1982) Vasopressin fibers pathways in the rat brain following suprachiasmatic nucleus lesioning. *Brain Res.* 243:235-241.
- Itoh, N., K-I. Obata, N. Yanaihara, and H. Okamoto (1983) Human preprovasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. *Nature* 304:547-549.
- Kalsbeek, A., and R.M. Buijs (1992) Peptidergic transmitters of the suprachiasmatic nucleus and the control of circadian rhythmicity. *Progr. Brain Res.* 92:321-334.
- Kalsbeek, A., R. Teclemariam-Mesbah, and P. Pevet (1993) Efferent projections of the suprachiasmatic nucleus in the golden hamster (*Mesocricetus auratus*). *J. Comp. Neurol.* 332:293-314.
- Larsson, K (1979) Features of the neuroendocrine regulation of masculine sexual behavior. In C. Berger (ed): *Endocrine Control of Sexual Behavior*. New York: Raven Press, pp. 77-163.
- Lydic, R., and M.C. Moore-Ede (1980) Three dimensional structure of the suprachiasmatic nuclei in the diurnal squirrel monkey (*Saimiri sciureus*). *Neurosci. Lett.* 17:295-299.
- Mai, J.K., J. Triepel, and J. Metz (1987) Neurotensin in the human brain. *Neuroscience* 22:499-524.
- Mai, J.K., O. Kedziora, L. Teckhaus, and M.V. Sofroniew (1991) Evidence for subdivisions in the human suprachiasmatic nucleus. *J. Comp. Neurol.* 305:508-525.
- Mikkelsen, J.D., and M. Moller (1988) Vasoactive intestinal peptide in the hypothalamohypophysial system of the Mongolian gerbil. *J. Comp. Neurol.* 273:87-98.
- Mikkelsen, J.D., and N. Vrang (1994) A direct pretectosuprachiasmatic projection in the rat. *Neuroscience* 62:497-505.
- Mikkelsen, J.D., P.J. Larsen, M.M.T. Ohare, and S.J. Wiegand (1991) Gastrin releasing peptide in the rat suprachiasmatic nucleus: An immunohistochemical chromatographic and radioimmunological study. *Neuroscience* 40:55-66.
- Moore, R.Y (1992) The organization of the human circadian timing system. In D.F. Swaab, M.A. Hofman, M. Mirmiran, R. Ravid, and F.W. van Leeuwen (eds): *The Human Hypothalamus in Health and Disease*. Progress in Brain Research, Vol 93. Amsterdam: Elsevier, pp. 101-117.

- Moore, R.Y. (1993) Organization of the primate circadian system. *J. Biol. Rhythms* 8:s3-s9.
- Morin, L.P., N. Goodless-Sanchez, L. Smale, and R.Y. Moore (1994) Projections of the suprachiasmatic nuclei, subparaventricular zone and retrochiasmatic area in the golden hamster. *Neuroscience* 61:391-410.
- Orpen, B.G., and M. Steiner (1994) Neural connections of the suprachiasmatic nucleus and medial hypothalamus of the Syrian hamster (*Mesocricetus auratus*). *J. Anat.* 184:23-33.
- Ravid, R., E.J. van Zwieten, and D.F. Swaab (1992) Brain banking and the human hypothalamus-factors to match for, pitfalls and potentials. In D.F. Swaab, M.A. Hofman, M. Mirmiran, R. Ravid, and F.W. van Leeuwen (eds): *The Human Hypothalamus in Health and Disease*. Progress in Brain Research, Vol 93. Amsterdam: Elsevier, pp. 83-95.
- Renaud, L.P. (1979) Neurophysiology and neuropharmacology of medial hypothalamic neurons and their extrahypothalamic connections. In P.J. Morgane, and J. Panksepp (eds): *Handbook of the Hypothalamus, Anatomy of the Hypothalamus*, Vol I. New York: Marcel Dekker.
- Rusak, B., and I. Zucker (1979) Neural regulation of circadian rhythms. *Physiol. Rev.* 59:449-526.
- Simerly, R.B., and L.M. Swanson (1986) The organization of neural inputs to the medial preoptic nucleus of the rat. *J. Comp. Neurol.* 246:312-342.
- Smale, L., J. Blanchard, R.Y. Moore, and L.P. Morin (1991) Immunocytochemical characterization of the suprachiasmatic nucleus and the intergeniculate leaflet in the diurnal ground squirrel, *Spermophilus lateralis*. *Brain Res.* 563:77-86.
- Sofroniew, M.V., and A. Weindl (1978) Projections from the parvocellular vasopressin and neurophysin containing neurons of the suprachiasmatic nucleus. *Am. J. Anat.* 153:391-430.
- Sofroniew, M.V., and A. Weindl (1980) Identification of parvocellular vasopressin and neurophysin neurons in the suprachiasmatic nucleus of a variety of mammals including primates. *J. Comp. Neurol.* 193:659-675.
- Stephan, F.K., K.J. Berkly, and R.L. Moss (1981) Efferent connections of the rat suprachiasmatic nucleus. *Neuroscience* 6:2625-2641.
- Stopa, E.G., J.C. King, R. Lydic, and W.C. Schoene (1984) Human brain contains vasopressin and vasoactive intestinal polypeptide neuronal subpopulation in the suprachiasmatic region. *Brain Res.* 297:159-163.
- Swaab, D.F., and E. Fliers (1985) A sexually dimorphic nucleus in the human brain. *Science* 228:1112-1114.
- Swaab, D.F., E. Fliers, and T.S. Partiman (1985) The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res.* 342:37-44.
- Swanson, L.W., and W.M. Cowan (1975) The efferent connection of the suprachiasmatic nucleus of the hypothalamus. *J. Comp. Neurol.* 160:1-12.
- Vandesande, F., K. Dierickx, and J. Demey (1975) Identification of the vasopressin-neurophysin producing neurons of the rat suprachiasmatic nucleus. *Cell Tissue Res.* 156:377-380.
- Vrang, N., P.J. Larsen, M. Moller, and J.D. Mikkelsen (1995) Topographical organization of the rat suprachiasmatic-paraventricular projection. *J. Comp. Neurol.* 353:585-603.
- Walter, A., J.K. Mai, and W. Jimenez-Hartel (1990) Mapping of Neuropeptide Y-like immunoreactivity in the human forebrain. *Brain Res. Bull.* 24:297-311.
- Walter, A., J.K. Mai, L. Lanta, and T.J. Gorcs (1991) Differential distribution of immunohistochemical markers in the bed nucleus of the stria terminalis in the human brain. *J. Chem. Neuroanat* 4:281-298.
- Watts, A.G. (1991) The efferent projections of the suprachiasmatic nucleus: Anatomical insights into the control of circadian rhythms. In D.C. Klein, R.Y. Moore, and S.M. Reppert (eds): *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press, pp. 77-106.
- Watts, A.G., and L.W. Swanson (1987) Efferent projections of the suprachiasmatic nucleus: II. Studies using retrograde transport of fluorescent dyes and simultaneous peptide immunohistochemistry in the rat. *J. Comp. Neurol.* 258:230-252.
- Watts, A.G., L.W. Swanson, and G. Sanchez-Watts (1987) Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of *Phaseolus vulgaris* leucoagglutinin in the rat. *J. Comp. Neurol.* 258:204-229.
- Zhou, J.N., M.A. Hofman, L.J.D. Gooren, and D.F. Swaab (1995a) VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. *Neurobiol. Aging* 16:571-576.
- Zhou, J.N., M.A. Hofman, L.J.D. Gooren, and D.F. Swaab (1995b) A sex difference in the human brain and its relation to transsexuality. *Nature* 378:68-70.