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Some Perspectives on Monoamine-Opioid Peptide Interaction in Rat Central Nervous System[1]

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KHACHATURIAN, H. AND S. J. WATSON. *Some perspectives on monoamine-opioid peptide interaction in rat central nervous system.* BRAIN RES. BULL. 9(1–6) 441–462, 1982.—Light microscopic immunocytochemistry was employed to investigate possible sites of interaction between the endogenous opioid peptides and monoamines in the rat central nervous system. The opioid and related peptides examined included beta-endorphin (β-END), alpha-MSH (α-MSH) and leucine-enkephalin (Leu-ENK). The monoamines were examined using antisera generated against tyrosine hydroxylase, dopamine-β-hydroxylase as well as serotonin. Due to the long-tract nature of the central monoamine projections as well as β-END/α-MSH fiber systems, serial section analyses were performed utilizing parasagittal brain sections. Many areas rich in both the monoamines as well as opioid peptides were investigated. These included several thalamic and hypothalamic nuclei, several limbic structures, mesencephalic periaqueductal gray, brain stem noradrenergic cell groups and their rostral projections, the dopaminergic nigrostriatal system, and the serotonergic raphe nuclei and their projections. The results suggest a more intimate linkage between the monoamines and the opioid peptides than previously realized. Some of the intricacies of monoamine-opioid peptide interaction, in particular those pertaining to their possible role in pain and analgesia, catalepsy, and neuroendocrine effects are also discussed.

Opioid peptides Monoamines Hypothalamus Limbic system Periaqueductal gray Locus coeruleus Neuron interactions

EVER since the discovery of endogenous opioid peptides and their subsequent localization in specific central nervous system loci, it has become increasingly apparent that there exists a potential for an intimate interaction between these peptides and the monoaminergic systems. In fact, many investigators have noted the possibility of such an interaction while studying the distributional patterns of the opioid peptides (cf. [151]). In this chapter, we shall present data from our recent immunocytochemical studies suggesting a much more intimate linkage between the opioid peptides and monoamines than previously realized. Additionally, we will analyze some of the more salient features of monoamine-opioid peptide interaction in the rat central nervous system. Heavier emphasis will be placed upon the anatomical aspects of these interactions, along with the discussion of possible physiological implications where such is applicable. This article then is not an exhaustive review of the literature, rather a specialized, and we hope useful treatise of this subject. For those who have further interests in the topic, there will be references made to some of the more thorough reviews of the literature.

ENDOGENOUS OPIOID SYSTEMS

It is now clear that there exists within the central nervous system three distinct opioid peptidergic systems, namely those containing methionine- and leucine-enkephalin (met- and leu-ENK), beta-endorphin (β-END), and dynorphin (DYN) (and possibly α- and β-neo-endorphin). The opioid peptides demonstrate a high affinity for opiate receptors, and can induce narcotic agonist effects much like those produced by morphine administration. These effects have been demonstrated for met- and leu-ENK [23, 55, 56, 69, 106, 136] for β-END [19, 22, 74, 75, 83, 84, 138, 139, 154], and for DYN [41]. Furthermore, immunocytochemical studies have revealed a separate and distinct distributional pattern for each of the opioid peptides.

Enkephalins

The enkephalins were discovered in the mammalian brain by Hughes *et al.* [56], and were shown to exist in entirely separate neuronal systems from that containing β-END and its related peptides [16,149]. Met- and leu-ENK containing perikarya and fibers have a wide distribution throughout the neuraxis, and can be found in certain limbic structures, neostriatum, hypothalamus, periventricular and periaqueductal gray areas, locus coeruleus, as well as many lower brain stem structures and the spinal cord [32, 48, 50, 60, 125, 131, 141, 142, 147, 155]. Although these two peptides appear to

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TABLE 1
ANTIBODIES USED IN THIS STUDY

Antibody	Source	Specificity	Key Cross Reactivities
β-END (affinity)	Watson and Akil	C-terminus	6–8% XR-β_h-LPH
β-END (#5)	Watson and Akil	LPH_{77-91}	100% XR-β_h-LPH
α-MSH	Watson and Akil	COOH terminus	<0.1% XR-ACTH
leu-ENK	Watson and Akil	COOH terminus	<6% XR-Met-enkephalin
Tyrosine hydroxylase	T. Joh	Not DBH or PNMT	
Dopamine-β-hydroxylase	Watson, Akil, and Ciaranello	Single IEP band and single PAGE	
Serotonin	Watson and Akil	Not Melatonin, Tryptophan or 5-OHTRP, L-DOPA, etc.	Seems to see 5HT only

have similar anatomical distributions, Larsson *et al.* [68] have presented evidence for separate neuronal systems for met- and leu-ENK.

β-Endorphin

On the other hand, β-END [19, 43, 74], appears to exist in the anterior pituitary corticotrophs and intermediate lobe melanotrophs along with adrenocorticotrophic (ACTH) and melanocyte stimulating (α-MSH) hormones, respectively [15,108]. It has also been shown to exist in the brain in neuronal perikarya located in the arcuate and peri-arcuate regions of the hypothalamus. Fibers from this single system which also stores ACTH and α-MSH, project to hypothalamus, several limbic areas, periventricular thalamus, periaqueductal gray, the locus coeruleus, and medullary reticular formation [5, 6, 14, 16, 17, 148, 149, 150, 162]. Recently, other β-END containing neuronal perikarya have been localized in the brain stem of colchicine pre-treated rats, in the vicinity of the A2 noradrenergic cell group [128] and within nucleus reticularis gigantocellularis and motor trigeminal nucleus [36]. However, it remains to be seen whether or not these cells contain "true" β-END.

Dynorphin

Lastly, DYN, discovered by Goldstein *et al.* [41] appears to contain the leu-ENK molecule as its N-terminus, just as β-END contains met-ENK at its N-terminus. However, the COOH-terminus of DYN is unique to that peptide only. This peptide has been found to exist in the same hypothalamic magnocellular neurons that produce vasopressin in both the supraoptic and paraventricular nuclei. It has also been localized to medullary and spinal cord neurons [152,153].

MONOAMINERGIC SYSTEMS

Norepinephrine (NE)

Noradrenergic systems arise from several brain stem loci and have extensive and widespread projections throughout the neuraxis [26, 77, 143]. The nucleus locus coeruleus is the largest NE producing nucleus and is the primary source of central noradrenergic innervation, giving rise to fibers that course through the dorsal NE bundle in the midbrain periaqueductal gray to higher structures [7, 77, 81]. Other NE-producing cell groups collectively known as the lateral tegmental noradrenergic system, give rise to both descending and ascending (ventral NE-bundle) projections. NE terminals can be found in the spinal cord, brain stem, thalamus and hypothalamus, as well as many limbic and other telencephalic structures.

Dopamine (DA)

The dopaminergic cell groups of the mesencephalon [26], the most prominent of which is located in the substantia nigra give rise to ascending projections to the neostriatum (Caudate-putamen) via the nigrostriatal pathway, as well as other limbic and forebrain structures via the mesolimbic and mesocortical DA pathways which project to the nucleus accumbens, lateral septum and several cortical areas [77, 79, 80, 143]. Additionally the tuberoinfundibular DA neurons in the arcuate-periventricular region and the incertohypothalamic DA neurons in the zona incerta seem to exert their action upon the endocrine hypothalamus [13,49].

Serotonin (5HT)

5HT neuronal systems originate from the nuclei of the raphe which are midline structures in the regions of the mesencephalon, pons, and medulla [26]. Two of the most prominent 5HT nuclei are the raphe dorsalis and raphe magnus. These and other raphe neurons give rise to several pathways which include: ventral and dorsal ascending as well as other descending pathways to the spinal cord [26, 27, 39, 143]. Raphe dorsalis projections ascend periventricularly to innervate the locus coeruleus, mesencephalic periaqueductal gray as well as hypothalamus. Raphe magnus 5HT neurons project to the spinal cord, brainstem, locus coeruleus, substantia nigra, thalamus, hypothalamus, striatum, habenula, septum, amygdala, hippocampus and neocortex.

METHOD

Immunocytochemistry

A total of 6 normal male Sprague-Dawley rats (Charles

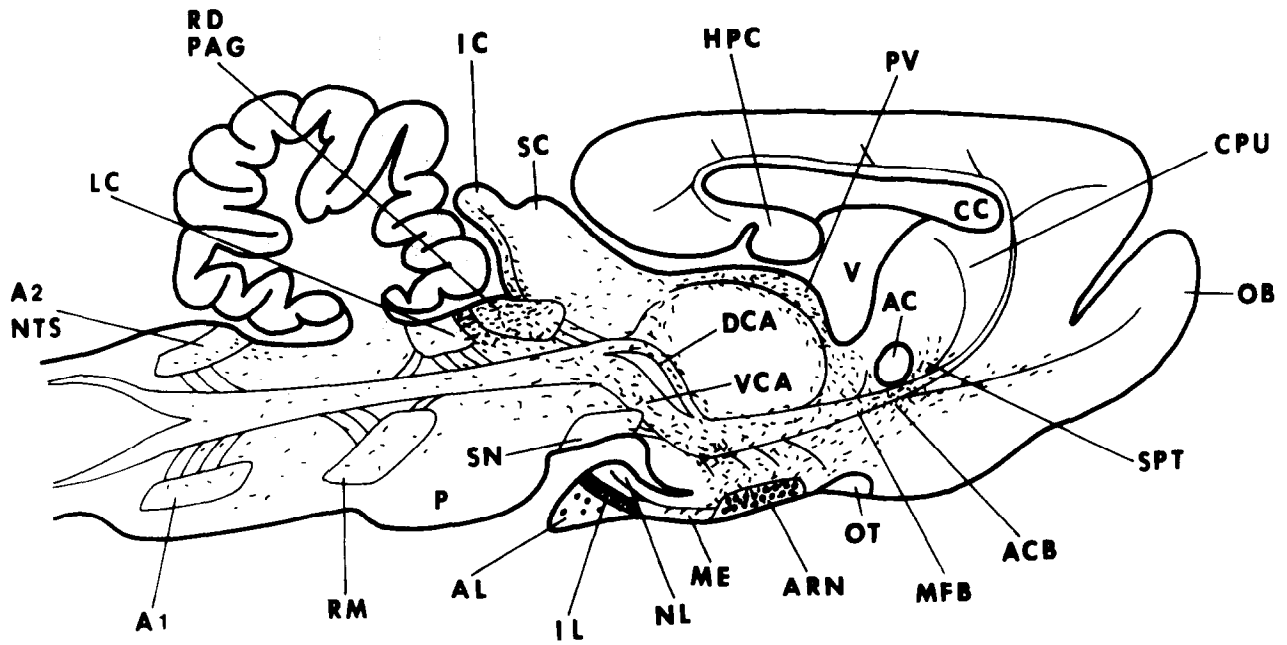

FIG. 1. Schematic representation of monoaminergic nuclei and projections (solid lines) in a parasagittal view of the rat brain, modified from Pellegrino *et al.* [107]. The locations of noradrenrgic, dopaminergic, and serotonergic nuclei and pathways were approximated using the works of Dahlström and Fuxe [26], Fuxe [38], Ungerstedt [143], and Lindvall and Björklund [78]. No monoamine cell bodies are shown. Superimposed upon the monoaminergic systems are the projections of β-END/α-MSH fibers (dashes) which originate from cell bodies in the hypothalamic arcuate nucleus. Also shown are β-END/α-MSH cell bodies in the intermediate and anterior lobes of the pituitary. Note the close association between the projections of these systems. The projections of the α-2 (α-MSH) system to the cortex and hippocampus are not shown. The reader is cautioned that due to the wealth of data, this schematic drawing is necessarily oversimplified. Also, many monoamine nuclei were omitted. Abbreviations: A1, A2, (noradrenergic cell groups). AC (anterior commissure). ACB (nucleus accumbens). AL (anterior lobe of pituitary). ARN (arcuate nucleus). CC (corpus callosum). CPU (caudate-putamen). DCA (dorsal catecholamine bundle). HPC (hippocampus). IC (inferior colliculus). IL (intermediate lobe of pituitary). LC (locus coeruleus). ME (median eminence). MFB (medial forebrain bundle). NL (neural lobe of pituitary). NTS (nucleus tractus solitarius). OB (olfactory bulb). OT (optic tract). P (pons). PAG (periaqueductal gray). PV (periventricular thalamus). RD (raphe dorsalis). RM (raphe magnus). SC (superior colliculus). SN (substantia nigra). SPT (septum). V (ventricle). VCA (ventral catecholamine bundle).

River) were used in this study. The immunocytochemical technique used here is described elsewhere [148,149]. Briefly, each animal is anesthetized by an intraperitoneal injection of sodium pentobarbital. The animal is then immersed in ice-water and the chest cavity is opened. The right atrium is cut open and the cardiovascular system is flushed with 50 ml cold saline administered through the left ventricle. The ventricles are cut away and a perfusion tube is inserted into the aorta and tied in place. The animal is perfused with 4% buffered formaldehyde at 4°C for 30 minutes. The brain is then excised, blocked and placed in the perfusion mixture for one hour at 4°C. Next, the blocks are transferred into 15% sucrose in phosphate buffered saline (PBS) and stored overnight at 4°C. The following day, the blocks are immersed in Tissue Tek (O.C.T.) and are frozen by immersion into liquid nitrogen. For sectioning, the O.C.T. blocks are frozen onto brass cryostat chucks and sectioned at −20°C. In this study, the brains were sectioned at the sagittal plane. The sections are picked up onto gelatin-coated slides and are stored at −90°C until further processing.

For immunocytochemistry, the sections are air-dried and placed in an incubator, set at 37°C. Primary rabbit antisera against particular antigens (see Table 1) are diluted with 0.3% Triton-PBS and applied to the sections and allowed to incubate for one hour. These are then incubated overnight at 4°C. The following day, the slides are washed in PBS and then incubated with goat anti-rabbit immunoglobulin (GAR-IgG) for 1 hour at 37°C, and then overnight at 4°C. On the third day, the slides are once more washed in PBS and the sections are incubated with peroxidase-antiperoxidase (PAP) complex for one hour at 37°C. Alternatively, some sections are incubated in successive steps with antiperoxidase antibody followed by peroxidase enzyme, each for 40 minutes. The slides are washed in PBS and are placed in a PBS solution of 12.5% diaminobenzidine tetrahydrochloride (DAB) and 0.03% H_2O_2 for 15 minutes. The sections are then washed, osmicated, washed, dehydrated and mounted in permount. See Table 1 for a list of antisera used and their characteristics.

Analyses

Since the monoaminergic neurons form long tract systems, it was advantageous to evaluate possible areas of interaction between these neurotransmitters and opioid peptides (β-END/α-MSH neurons also have long tract projections) in brains that were cut in the sagittal plane. The thickness of the sections varied from animal to animal and was in the range of 10–20 μm. In each animal the right side of the brain was examined. Serial parasagittal sections were ob-

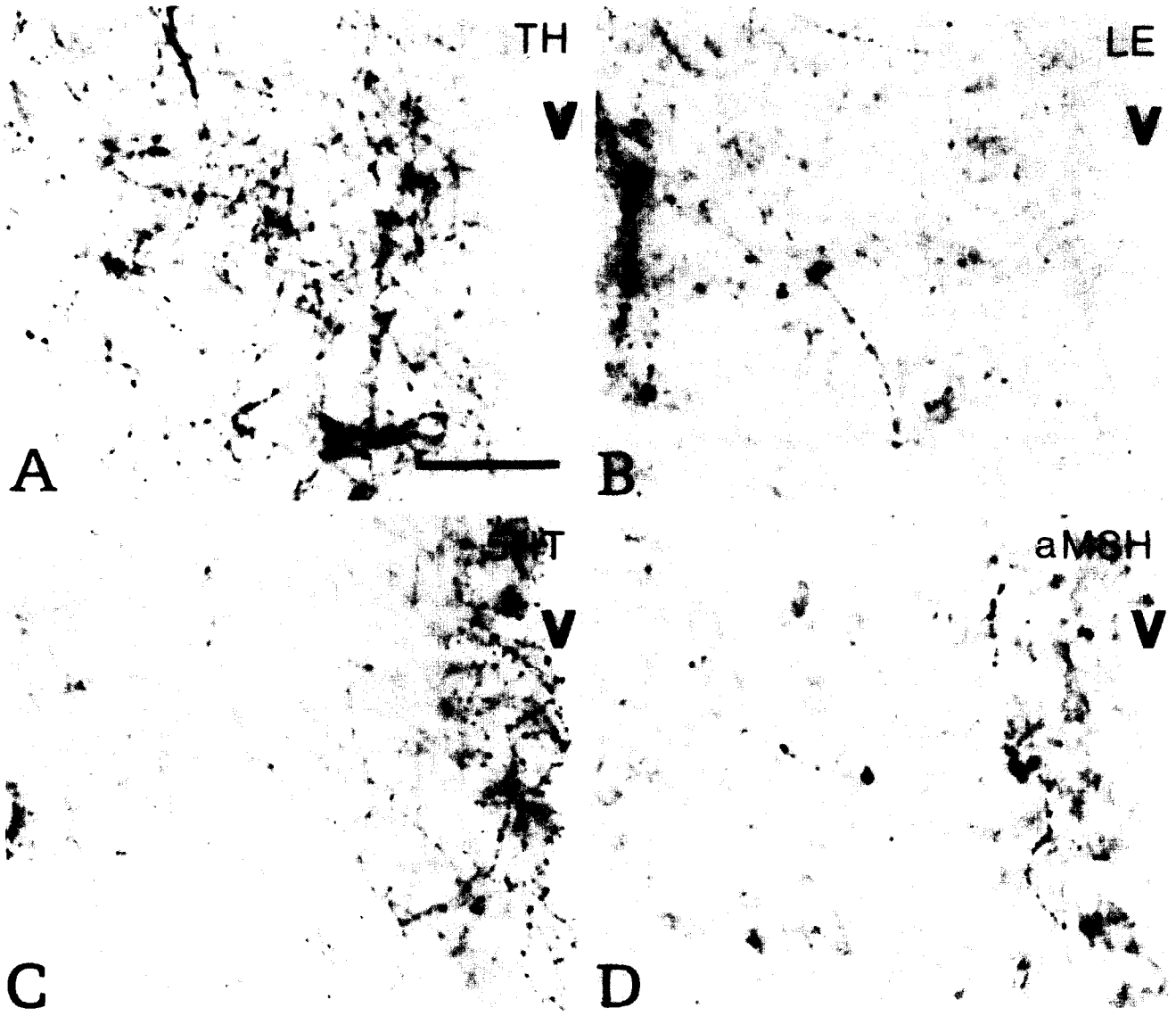

FIG. 2. Lateral septum: In these panels the common distribution of tyrosine hydroxylase (TH), leu-ENK (LE), 5HT, and α-MSH (also represents β-END) immunoreactive fibers are demonstrated in serial parasagittal sections (A through D) through the lateral septum (see Fig. 1 for orientation). Note the fine 5HT innervation of the ependyma. V (third ventricle). Bar=50 μm.

tained and every 10th section was stained with a Nissl stain for orientation purposes. The subsequent sections were stained immunocytochemically with antisera against the following substances (Table 1): β-END, α-MSH, leu-ENK, serotonin (5HT), tyrosine hydroxylase, and dopamine-β-hydroxylase. Observations and photographic analyses were made using a Leitz Orthoplan microscope equipped with an automatic camera system.

RESULTS AND DISCUSSION

Since data presented in this section are in the form of high-power photomicrographs through several regions of the brain in parasagittal sections, the reader is referred to Fig. 1 for a brief introduction as well as for orientation in the subsequent figures.

Norepinephrine-Opioid Peptide Interactions

The results of this study confirm and further extend the evidence for an extensive interaction between the noradrenergic and opioid peptide systems (references below). Many brain areas that contain opioid peptides are also rich in tyrosine hydroxylase and dopamine-β-hydroxylase immunoreactivities (these enzymes are responsible for the synthesis of DA and NE). This is especially true in the case of β-END/α-MSH projections which appear to coincide with NE projections. Thus, β-END/α-MSH fibers and terminals were noted in many brain loci rich in NE, such as anterior hypothalamic and preoptic areas (particularly the medial forebrain bundle), dorsomedial nucleus of the hypothalamus, septal nuclei (Fig. 2), nucleus accumbens, periventricular nucleus of thalamus (Fig. 3), periaqueductal gray (Fig. 4),

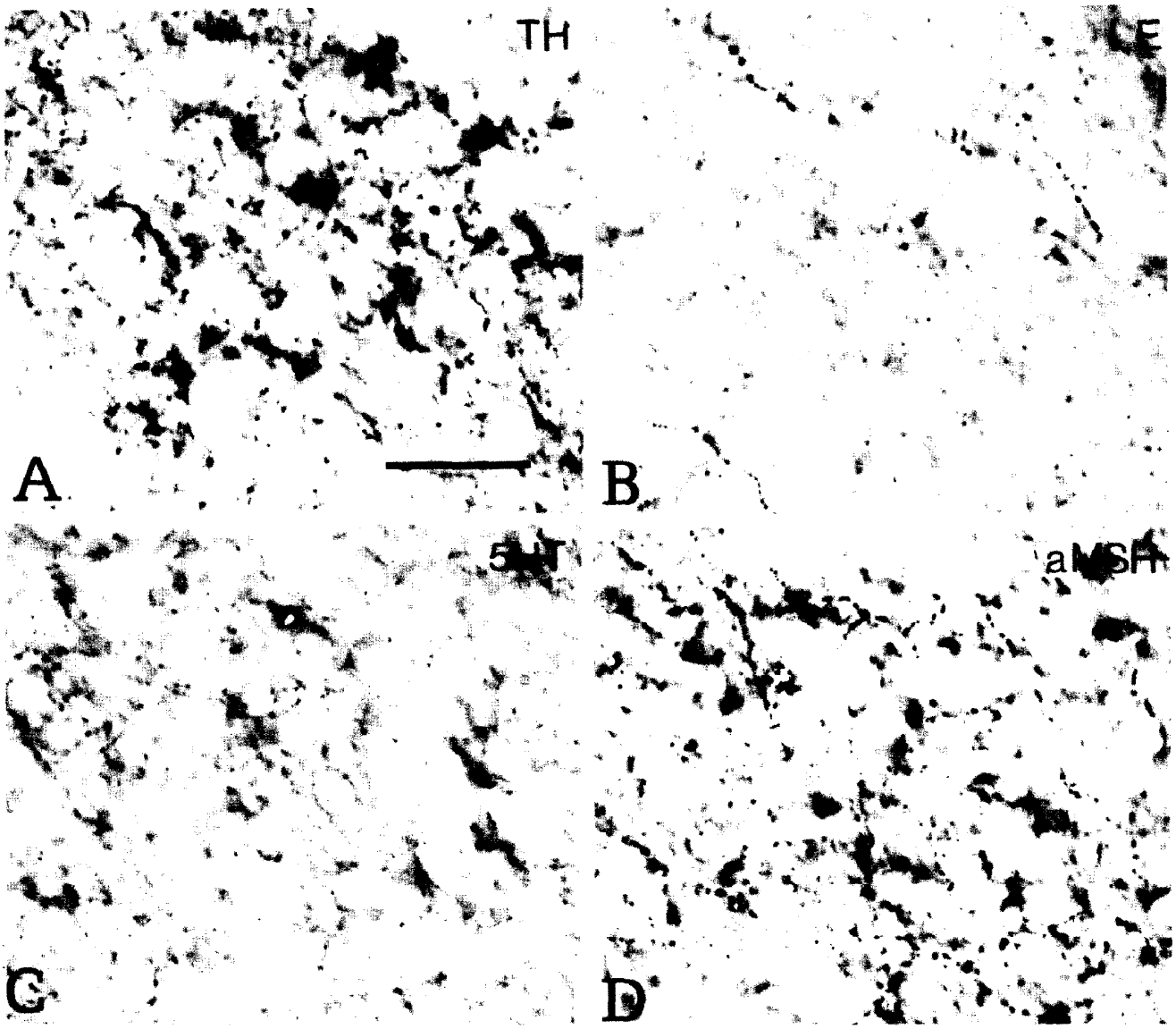

FIG. 3. Periventricular thalamus: Serial parasagittal sections (A through D) through the periventricular nucleus of the thalamus (see Fig. 1 for orientation) showing the proximity of tyrosine hydroxylase (TH), leu-ENK (LE), 5HT and α-MSH (also represents β-END) immunoreactivities. Bar=50 μm.

several raphe nuclei (Figs. 13, 14) as well as other noradrenergic cell groups including the locus coeruleus (Figs. 5, 6). Interestingly, we have noted many β-END/α-MSH immunoreactive fibers in both the "dorsal and ventral noradrenergic bundles" (Fig. 7), giving the impression that these peptidergic fibers follow the classical monoamine pathways in a retrograde fashion. Additionally, α-MSH (from a separate α-MSH neuronal system which does not contain β-END; [144,145]) fibers were also detected in the cortex and hippocampus, reaching these sites via the monoamine-rich cingulum bundle. Furthermore, many of the areas mentioned above also contained leu-ENK immunoreactive fibers and terminals (Figs. 2–7, 13, 14).

Similarly, other investigators, who have mapped the endorphins and enkephalins, have noticed the anatomical proximity of these peptides to the ascending NE fibers (cf. [151]). A potential for an extensive contact between β-END system of fibers and those of the locus coeruleus system was noted by Watson *et al.* [148]. Similarly, β-END neurons of the arcuate nucleus appear to be surrounded by terminals containing dopamine-β-hydroxylase [151]. Watson *et al.* [151] also have noted the presence of β-END-positive immunoreactive fibers in close association with locus coeruleus neurons, as well as dopamine-β-hydroxylase-containing fibers in the periaqueductal gray, medial forebrain bundle, periventricular thalamus, lateral septum, and certain hypothalamic nuclei. In addition to β-END innervation of the locus coeruleus, an enkephalinergic input to this nucleus has also been demonstrated by both immunocytochemical [64, 125, 141, 151] and iontophoretic [158] techniques.

Opiate receptors have been demonstrated on neurons of the locus coeruleus [11, 12, 111]. Locus coeruleus neurons

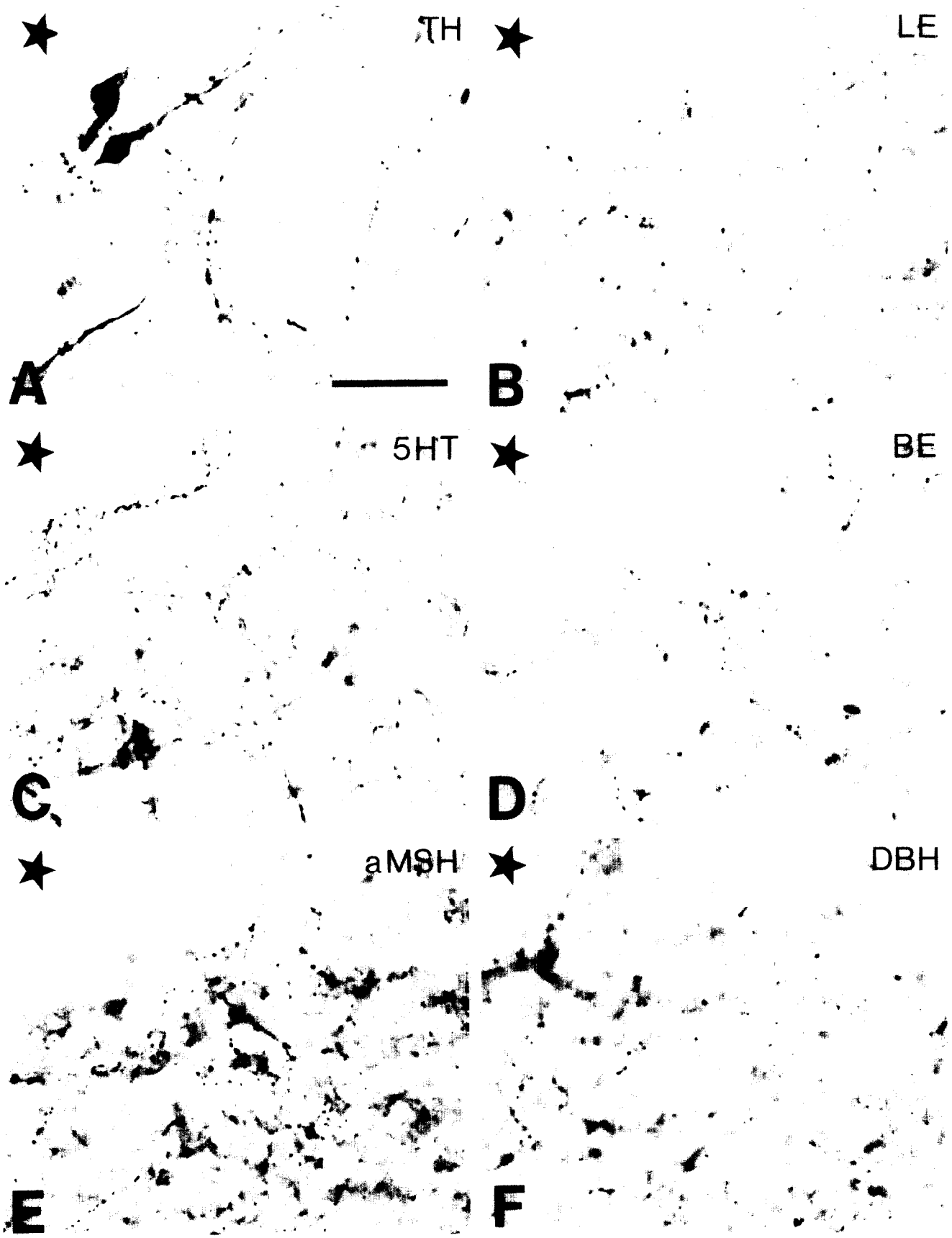
TH
LE
A
B
5HT
BE
C
D
aMSH
DBH
E
F

exhibit a depression in their spontaneous activity in response to iontophoretic [11] or parenteral [67] administrations of morphine. The latter effect was naloxone reversible. Likewise, morphine can reduce the locus coeruleus inhibitory influence upon the spinal trigeminal nucleus [126,127] and further can block the increase in the firing rate of midbrain reticular neurons caused by nociceptive stimuli [45]. Interestingly, Gold *et al.* [40] have shown that alpha-noradrenergic blockade inhibits signs of opiate withdrawal.

Opioid peptides are also capable of altering neuronal activity in certain areas of the brain. Microiontophoretic applications of met-/leu-ENK or β-END were shown to cause depression in firing rate of cells in certain catecholamine rich regions such as locus coeruleus, periaqueductal gray, thalamus, caudate nucleus and frontal cortex [37, 101, 158, 161]. Conversely, the opioid peptides produced an excitation of hippocampal cells [101,129].

The mesencephalic periaqueductal gray plays a primary role in the mediation of opiate-induced analgesia [47, 61, 73, 110, 140, 157], as well as stimulation-produced analgesia [73, 86, 88]. Stimulation-produced analgesia is partially antagonized by naloxone [4]. Akil and Liebeskind [2] were able to demonstrate a reciprocal relationship between NE mechanisms and stimulation-produced analgesia thought to involve endogenous opioid systems (i.e., depression of NE activity potentiated the analgesia). Segal and Sandberg [130] have also been able to elicit stimulation-produced analgesia from sites in the locus coeruleus and substantia nigra. In agreement with this reciprocal relationship [2] and the reciprocal innervation of locus coeruleus and arcuate nucleus alluded to above [151], is the observation that stimulation of the arcuate nucleus resulted in the inhibition of locus coeruleus firing, which was also reversible by naloxone [133]. Thus, the β-END system is apparently inhibitory to the locus coeruleus-noradrenergic system, although it is not as yet clear whether NE is also inhibitory to arcuate β-END neurons. Watson *et al.* [146], have implicated the dorsal periventricular NE bundle as a possible major neuroanatomic substrate for stimulation-produced analgesia. These investigators further noted that the production of analgesia was correlated with the proximity of the stimulating electrode to this NE-containing bundle. Thus, it is apparent that the locus coeruleus-noradrenergic system is involved in the production of analgesia, be it stimulation-produced or morphine-induced. Furthermore, an interaction between this system and the β-END projection system in the region of the periaqueductal gray is a strong possibility.

Other areas of possible interaction between an opioid peptide and ascending NE systems are the hypothalamic magnocellular nuclei. These nuclei, the supraoptic and paraventricular, are the sites of synthesis of vasopressin and oxytocin (cf. [28,30]). Several investigators have demonstrated ENK immunoreactivity within magnocellular neurons of both the rat and cat [32, 48, 93, 119, 125]. ENK-containing neurons apparently form a separate but parallel system to those of the vasopressin and oxytocin [93]. More recently, another opioid peptide, DYN, has been localized to the supraoptic and paraventricular nuclei. DYN appears to be uniquely located within the arginine-vasopressin neurons [152,153].

It has long been known that the release of vasopressin is at least partially under central noradrenergic control. Both the supraoptic and paraventricular nuclei are areas of particularly heavy NE innervation, as revealed by numerous fluorescence-histochemical studies [38, 51, 77, 89, 143]. There is evidence to suggest the existence of synaptic contacts of NE terminals upon magnocellular neurons, demonstrated by ultrastructural [72, 134, 159, 160], microiontophoretic [8, 9, 24, 96, 97] and organ-culture studies [132]. Furthermore, one of us (H. K.) has recently performed quantitative studies of NE input to supraoptic neurons. We have found the vasopressin-perikarya to be more heavily innervated by NE-varicosities than the oxytocin-perikarya, thus suggesting a differential control of hormone release by NE [66]. These results, when taken in light of the existence of DYN in vasopressin neurons, would suggest a possible site of interaction between NE and DYN. As interesting a possibility as this seems to be, it should be pointed out that further work remains to be done before one can speculate on possible implications of such interactions.

Dopamine-Opioid Peptide Interactions

In addition to β-END/α-MSH projections to noradrenergic cell groups already mentioned, we have also noted β-END/α-MSH immunoreactive fibers in the vicinity of substantia nigra as well as ventral tegmental dopaminergic nucleus. However, these are scattered fibers and it is not as yet clear whether they innervate these nuclei, or rather are a part of, and represent more ventrally situated β-END/α-MSH fibers which project to the brain stem (pons and medulla). In addition, many leu-ENK immunoreactive fibers were seen within the substantia nigra (Fig. 8) as well as along the nigrostriatal projections to the caudate-putamen (Fig. 9). Both leu-ENK and tyrosine hydroxylase immunoreactivities were particularly heavy in the globus pallidus (Fig. 10). Other DA-containing cell groups such as the A12 within the arcuate nucleus (Fig. 11) and the A13 within the zona incerta also contained both leu-ENK and β-END/α-MSH fibers and terminals. We have also noted the existence of tyrosine hydroxylase and leu-ENK immunoreactivities in the external zone of the median eminence, surrounding the portal capillaries (Fig. 11). Lastly, in the pituitary, tyrosine hydroxylase-immunoreactive terminals were seen in the intermediate and posterior lobes, but not the anterior lobe (all intermediate lobe cells produce β-END and α-MSH) (Fig. 12).

The behavioral effects of the endorphins and enkephalins has led many investigators to suggest that at least some of the actions of these peptides may be mediated through dopaminergic systems, such as catalepsy [18], certain narcotic-induced functions [94] and neuroendocrine effects [90]. Met-ENK neuronal elements have been demonstrated in the corpus striatum, in an experiment showing that sep-

FACING PAGE

FIG. 4. Periaqueductal gray: These photomicrographs represent serial parasagittal sections (A through F) through the mesencephalic periaqueductal gray (see Fig. 1 for orientation), showing the common distribution of tyrosine hydroxylase (TH), leu-ENK (LE), 5HT, β-END (BE), α-MSH and dopamine-β-hydroxylase (DBH) immunoreactive fibers. Note the position of two TH-containing cells (A) and their absence with DBH stain (F) representing dopamine neurons. Note also the fine innervation of the ependyma by 5HT fibers (C) and its lack of innervation by the other substances. Asterisk is in the cerebral aqueduct. Bar=50 μm.

FIG. 5. Locus coeruleus: Interaction between the opioid peptides and monoamines in the locus coeruleus (see Fig. 1 for orientation). Note the outline of the nucleus (dashed lines) in serial parasagittal sections: A through D (dark field), stained immunocytochemically with tyrosine hydroxylase (TH), leu-ENK (LE), 5HT, and α-MSH (also represents β-END), respectively. Also note that the bulk of leu-ENK and α-MSH terminals are outside the boundaries of locus coeruleus. Bar = 100 μm.

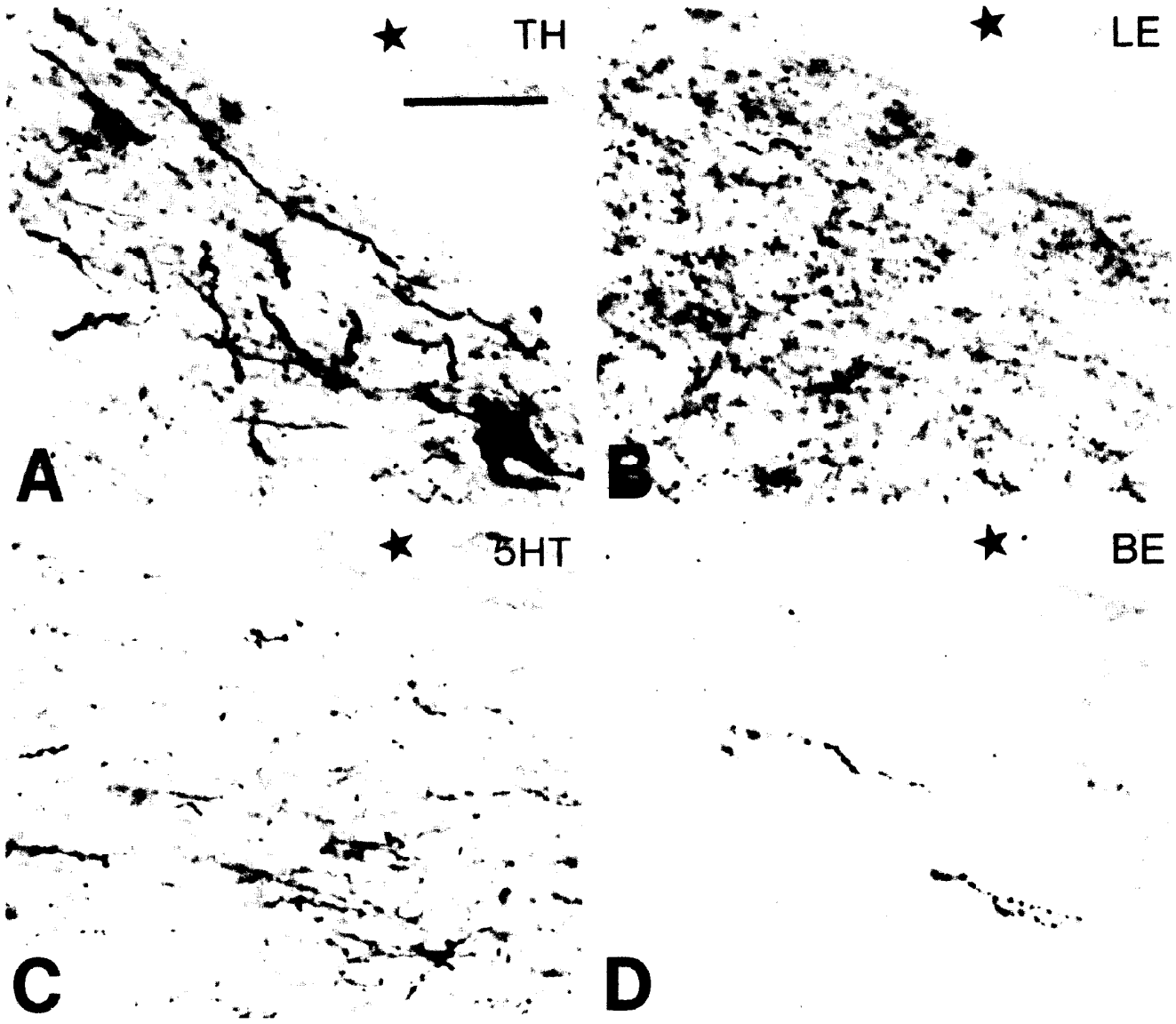

FIG. 6. Nucleus tractus solitarius: Comparative distribution of tyrosine hydroxylase (TH), leu-ENK (LE), 5HT, and β-END (BE) in serial parasagittal sections (A through D) through the nucleus tractus solitarius (see Fig. 1 for orientation). Note two TH-containing cells in panel A (belonging to the A2 noradrenergic cell group). Also note asterisk in the same capillary. Bar=50 μm.

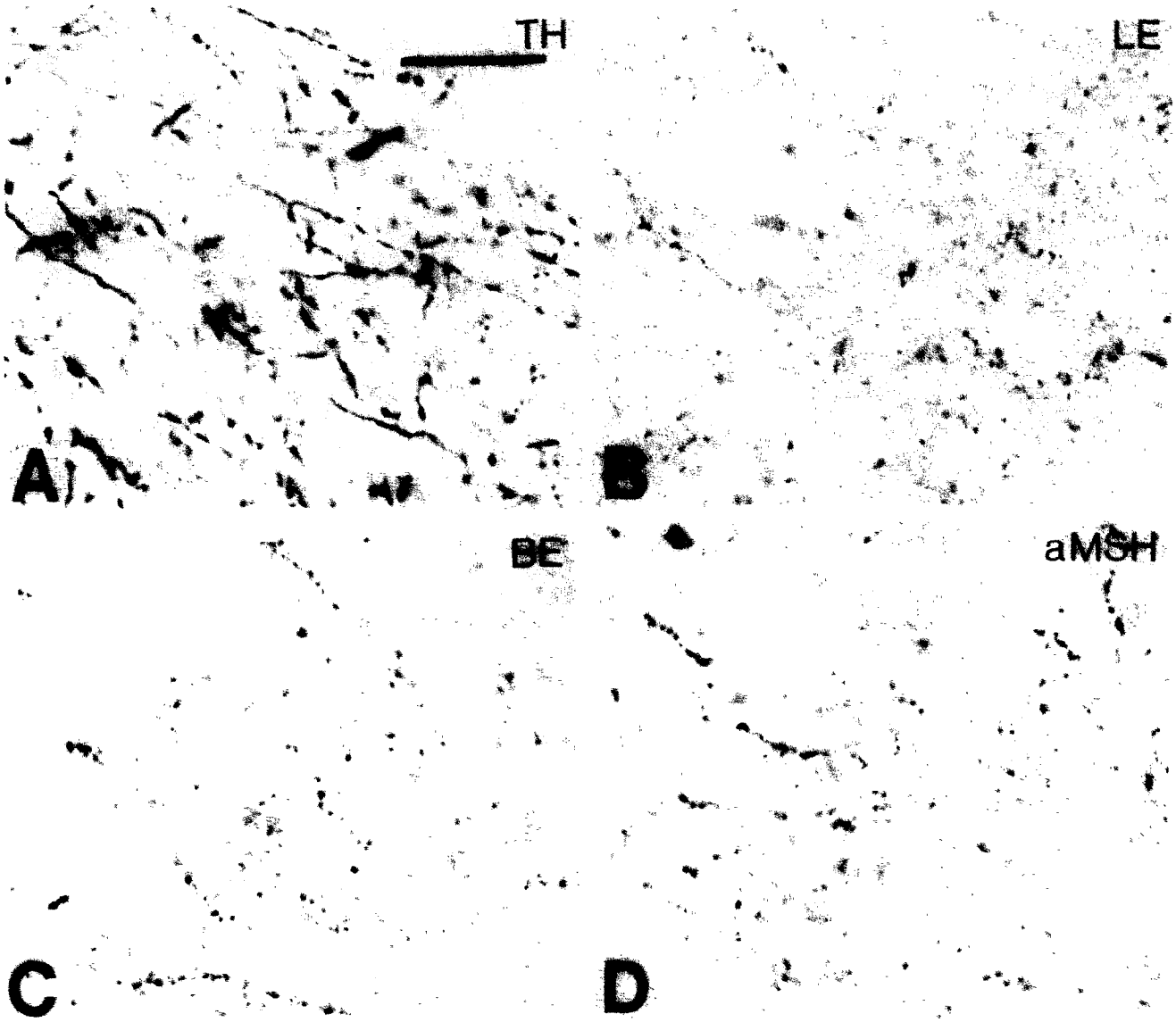

FIG. 7. Dorsal catecholamine bundle: These serial parasagittal sections show the common distribution of immunoreactive tyrosine hydroxylase (TH), leu-ENK (LE), β-END (BE) and α-MSH in panels A through D, in the "dorsal catecholamine bundle" (see Fig. 1 for orientation). Similar results were obtained from the analysis of the ventral catecholamine bundle. Bar=50 μm.

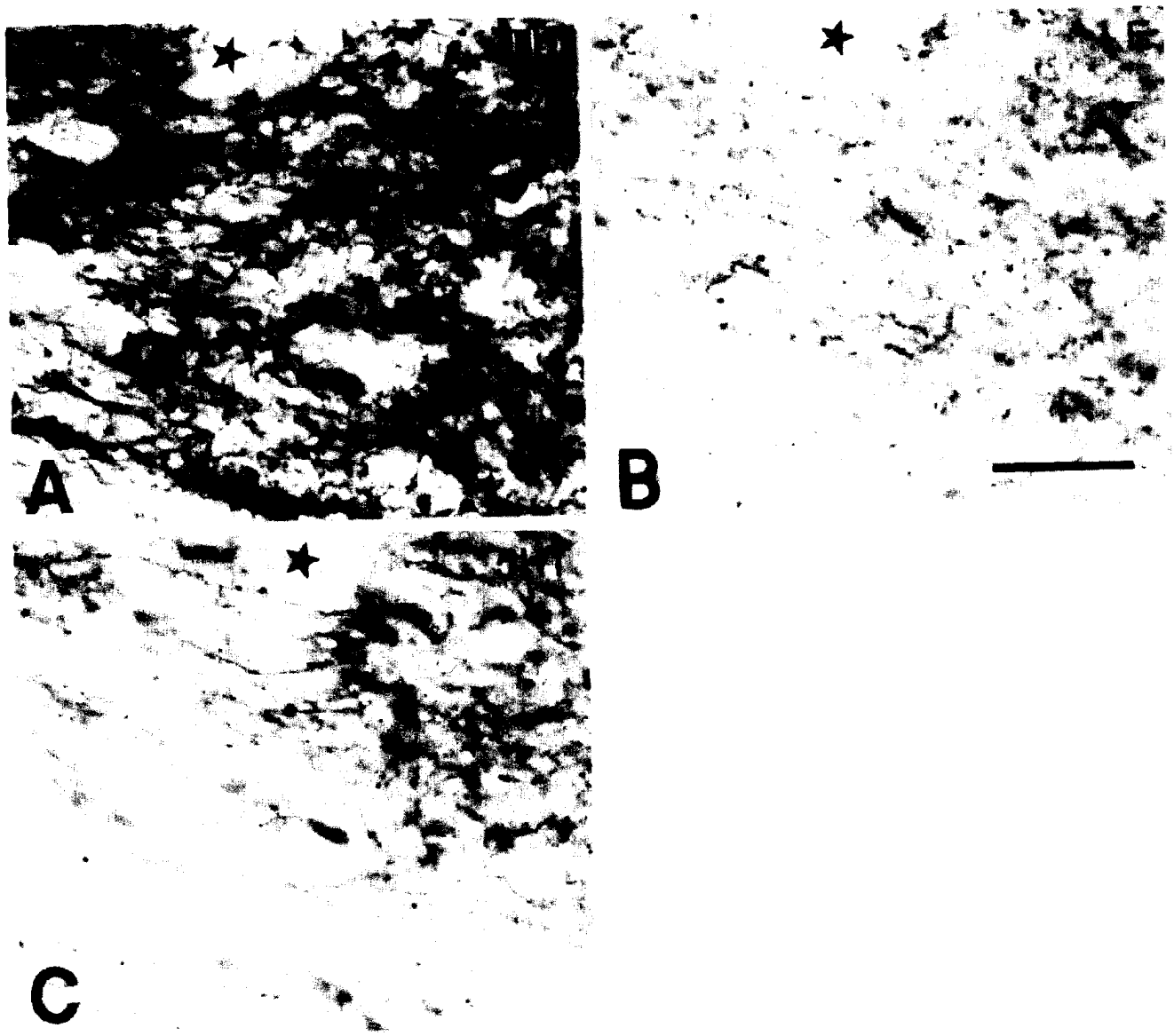

FIG. 8. Substantia nigra: The dopaminergic nucleus, substantia nigra (see Fig. 1 for orientation) is shown in serial parasagittal sections A through C. Note tyrosine hydroxylase (TH)-containing cells (dopaminergic), that are in an area innervated by leu-ENK (LE) and 5HT fibers. Asterisk shows the same region (non-staining fibers). Bar=50 μm.

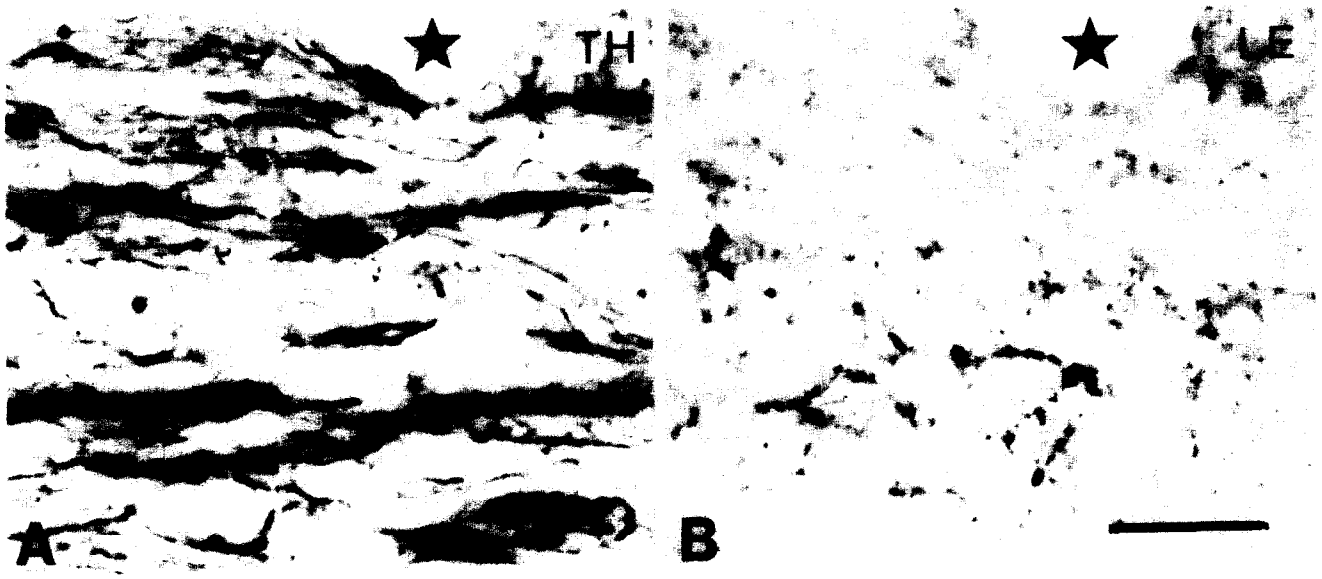

FIG. 9. Medial forebrain bundle: Comparative distributions of tyrosine hydroxylase (TH) and leu-ENK (LE) in serial parasagittal sections (A and B) through the medial forebrain bundle (see Fig. 1 for orientation). Note asterisk in the same capillary. Bar=50 μm.

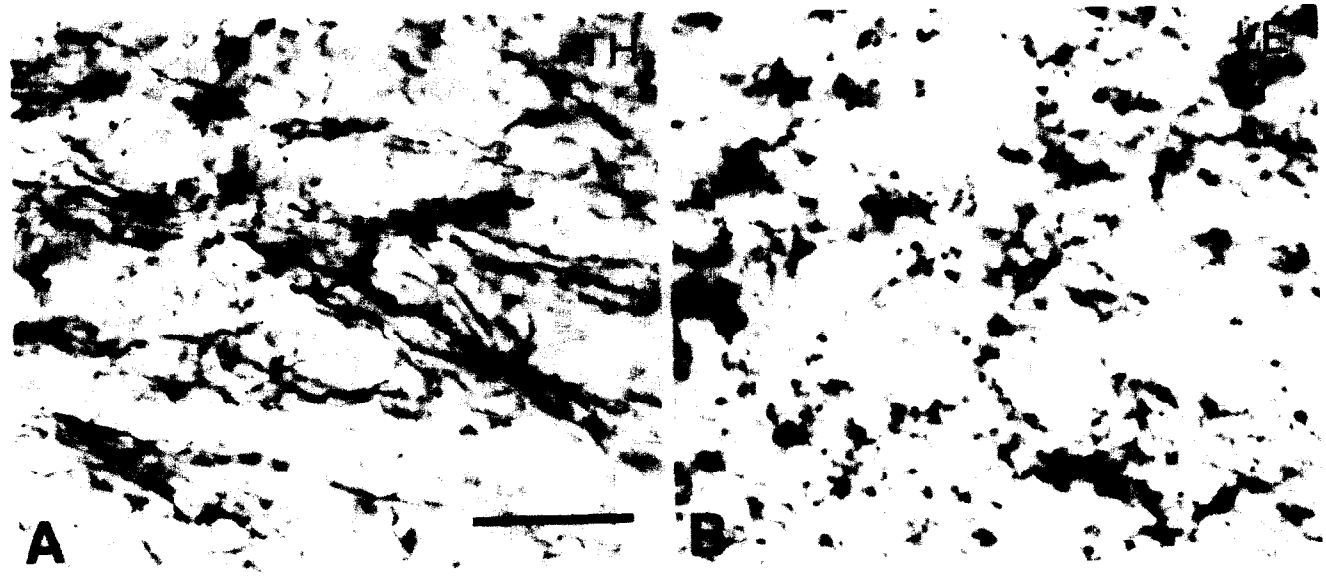

FIG. 10. Globus pallidus: Panels A and B represent serial parasagittal sections through the globus pallidus (see Fig. 1 for orientation). Note heavy tyrosine hydroxylase (TH) and leu-ENK (LE) immunoreactivities in this region. Bar=50 μm.

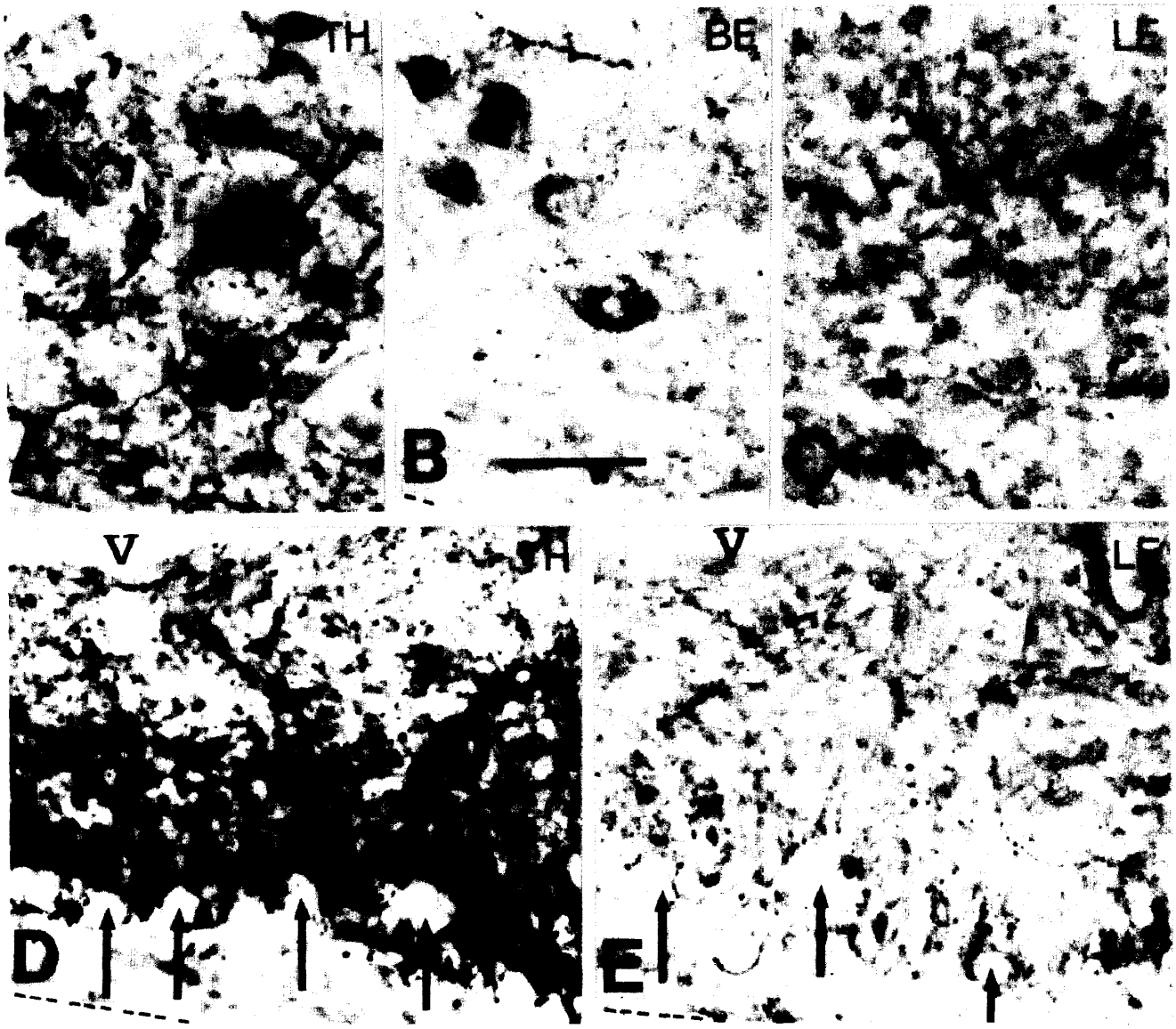

FIG. 11. Arcuate nucleus/median eminence: Serial parasagittal sections A, B, and C through the hypothalamic arcuate nucleus (see Fig. 1 for orientation), show tyrosine hydroxylase (TH) cells (dopaminergic), β-END (BE) cells and leu-ENK (LE) fibers in a similar location. Panels D and E are photomicrographs of the median eminence from the same sections as A and C showing TH and LE immunoreactivities in the external zone of the median eminence in contact with the portal capillaries (arrows). Note the ventral surface of the brain (dashed lines), as well as the third ventricle (V).

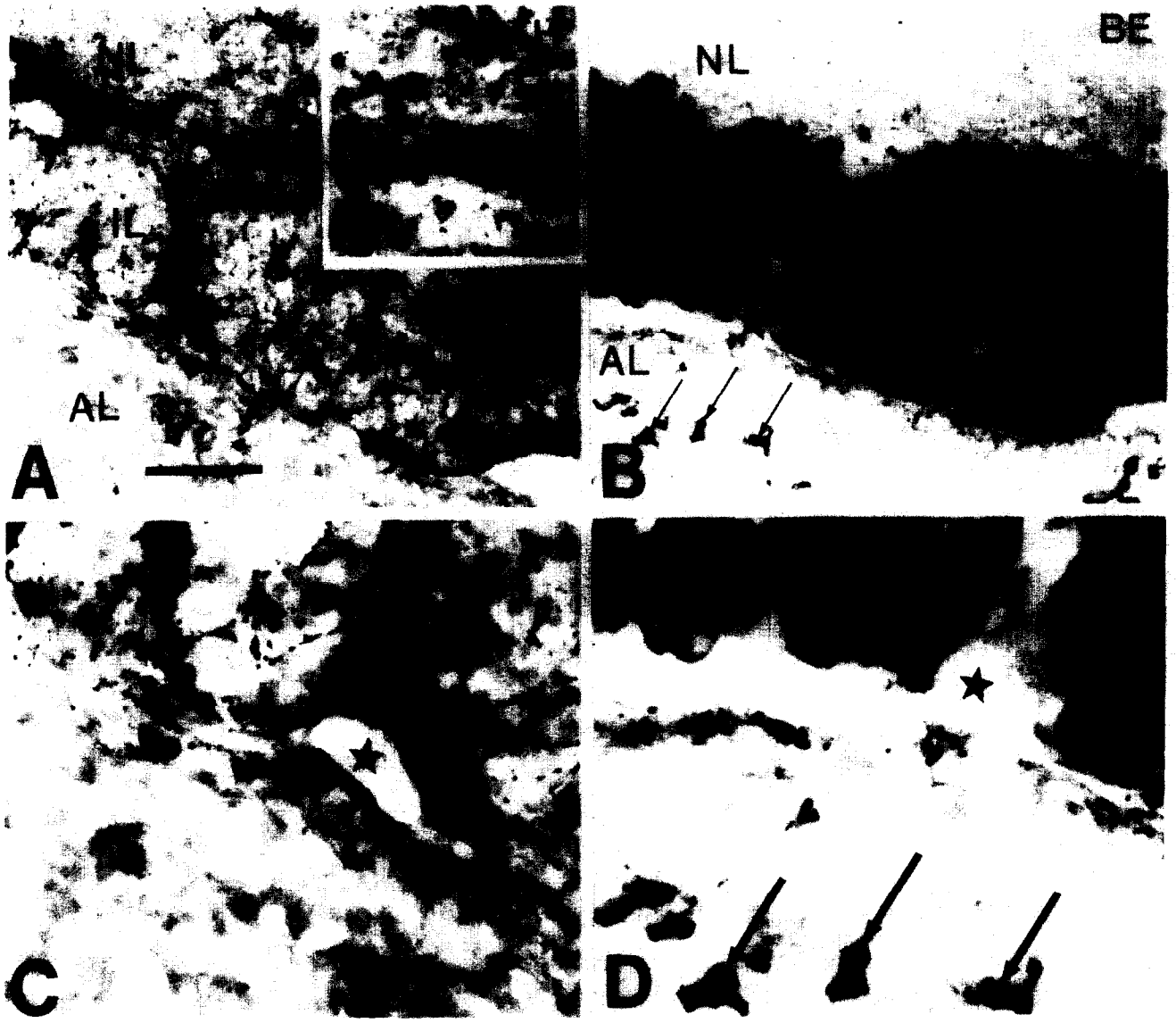

FIG. 12. Pituitary: Panels A and B represent serial parasagittal sections through the pituitary (see Fig. 1 for orientation). Panels C and D are higher power photomicrographs of A and B, respectively. Note asterisk in the same vessel in all panels. Tyrosine hydroxylase (TH) is seen to innervate the neural (NL) and intermediate (IL) lobes, but not the anterior lobe (AL). All IL cells and the AL corticotrophs (arrows) are immunoreactive for β-END (BE). The inset in Panel A shows the fine TH innervation of the NL (X is in the same vessel in Panel A and inset). Bar (panels A and B)=100 μm. Bar (panels C, D, and inset)=50 μm.

aration of striatum from substantia nigra by hemisections does not alter the striatal met-ENK levels, but that kainic acid induced lesions of the striatum does result in a marked depletion of caudate met-ENK levels [52]. It has also been suggested that at least some, but probably not all ENK-binding sites within the striatum are located on presynaptic DA terminals, since substantia nigra lesions in rats reduced the numbers of ^{3}H-leu-ENK binding sites in the corpus striatum [115]. The latter study, however, did not separate the caudate-putamen from the globus pallidus, site of the highest ENK levels in the brain. Soon thereafter, a striatopallidal leu-ENK pathway was shown to exist in the rat by Cuello and Paxions [25] in lesion studies that separated the caudate-putamen from the globus pallidus.

Immunocytochemical studies have shown both ENK-perikarya [48] and tyrosine hydroxylase axon terminals [114] in the rat neostriatum. In a recent ultrastructural study, Pickel *et al.* [113] have demonstrated that the majority of tyrosine hydroxylase-labeled (i.e., DA) axon terminals within the rat neostriatum lack synaptic specializations. They did, however, observe a few axo-dendritic (but no axo-axonic) synapses, arguing that DA axons could exert some effect on the ENK neurons via such axo-dendritic junctions. Therefore, it seems likely that DA might exert a modulatory influence on striatal ENK neurons.

Enkephalins are also present in the substantia nigra as revealed by both radioimmunoassay [53] and immunocytochemistry [32, 125, 141]. Likewise, opiate recep-

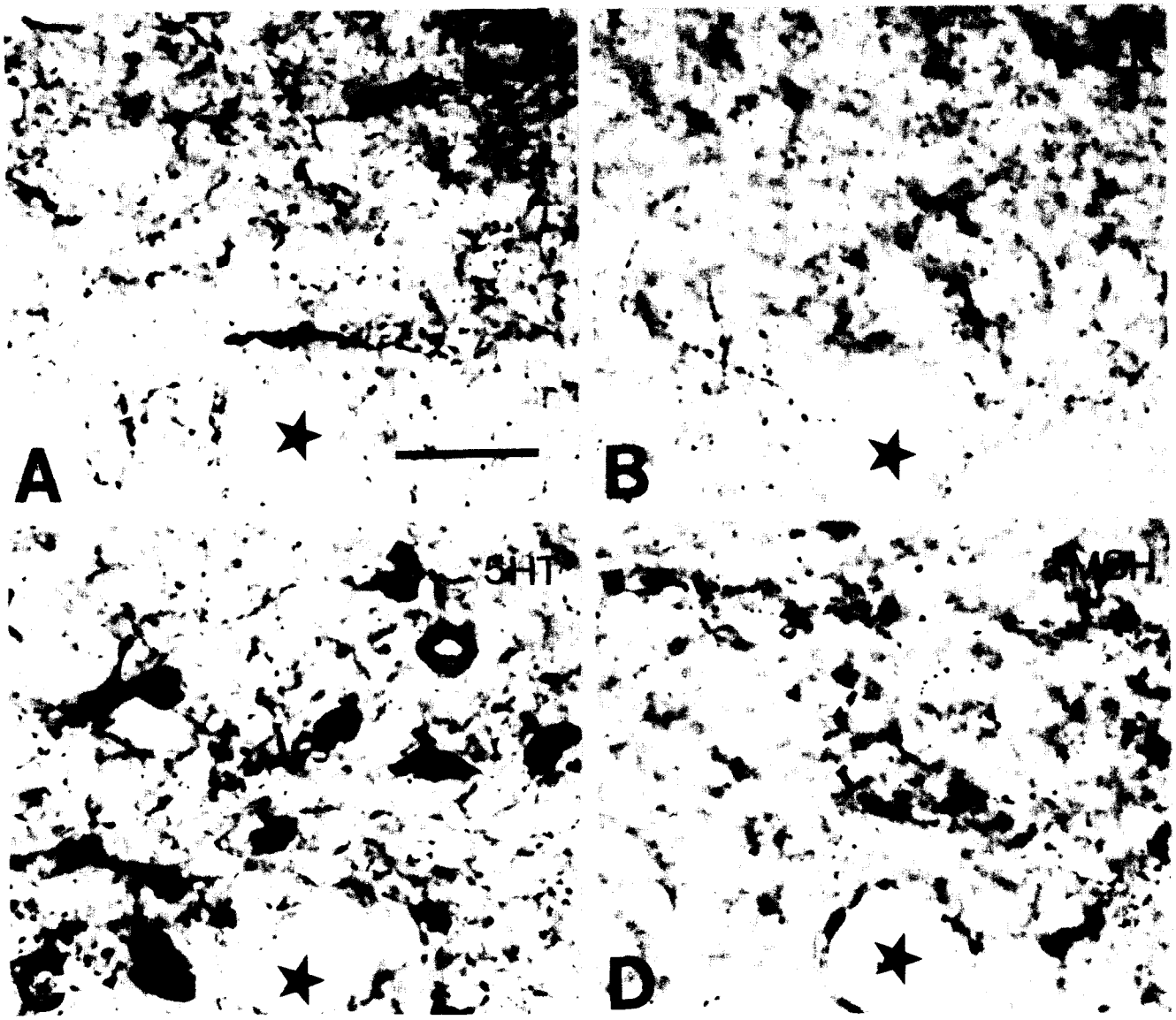

FIG. 13. Raphe dorsalis: This large serotonergic nucleus (see Fig. 1 for orientation) is depicted in serial parasagittal sections (A through D). Tyrosine hydroxylase (TH), leu-ENK (LE), and α-MSH (also representing β-END) fibers are seen in proximity to 5HT neurons (panel C) of this nucleus. Note asterisk in the same capillary through these sections. Bar=50 μm.

tors have been shown to exist in substantia nigra by autoradiographic [112] and lesion studies [82,116]. Furthermore, evidence in support of a direct interaction between ENK and DA neurons has been provided by a combined fluorescence-immunocytochemical study showing ENK terminals and fibers in close relation to the DA neurons of the substantia nigra and ventral tegmental area [65].

Parenteral administration of morphine resulted in an increase in the spontaneous firing rate of substantia nigra neurons [58, 70, 71, 102], but induced the suppression of neurons in the caudate nucleus [70,71]. The latter investigators noted that morphine might be stimulatory to substantia nigra-DA neurons which in turn innervate the caudate nucleus. In support of these experiments, an increase in DA-fluorescence intensity was demonstrated in the rat and mouse substantia nigra after morphine administration [46]. Thus narcotic analgesics (i.e., morphine and perhaps other opioid peptides) may exert an inhibitory effect at DA termination areas which would result in sedation and catalepsy [57], and an excitatory influence on the DA neuronal perikarya which might be due in turn to a presynaptic inhibition of an inhibitory neuronal influence upon those same DA perikarya [29].

It is becoming increasingly clear that β-END and the enkephalins may play a neurotransmitter or neuromodulatory role in conjunction with the catecholamines in many brain areas (cf. [57]). β-END injected intracerebroventricularly or in the region of the periaqueductal gray of rats was shown to induce analgesia as well as akinesia [18, 44, 62, 83], effects that were both reversed by the administration of L-DOPA or apomorphine [59,98]. Enkephalins may play a regulatory role upon the DA neurons of the substantia nigra

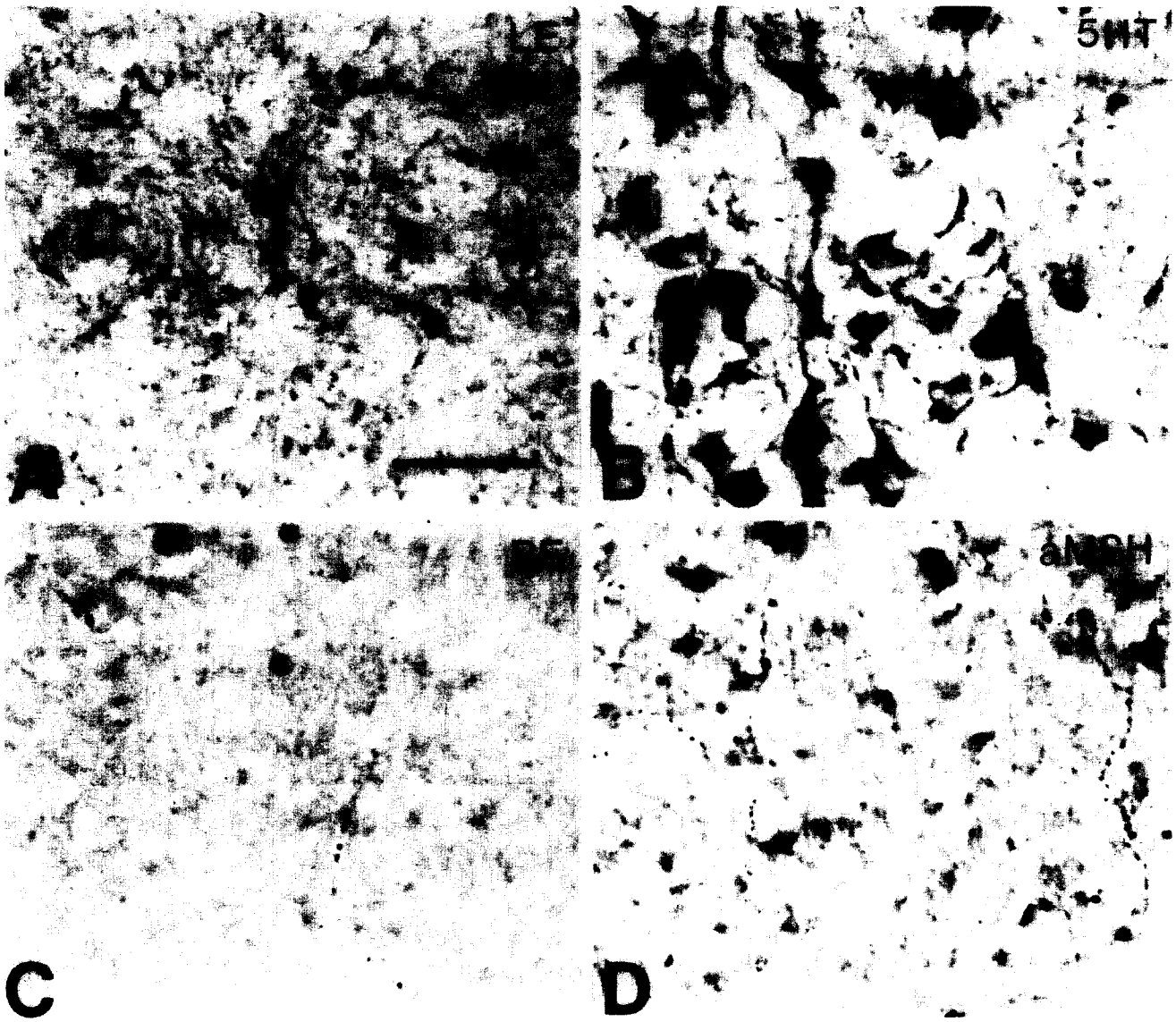

FIG. 14. Raphe magnus: The serotonergic nucleus raphe magnus (see Fig. 1 for orientation) is seen in these serial sections (A through D) to be innervated with leu-ENK (LE), β-END (BE), and α-MSH fibers. Note the 5HT neurons in panel B. Bar=50 μm.

and the ventral tegmental area, since motor activity was enhanced following the injection of opiates into these areas [21,109]. Furthermore, both β-END and met-ENK seem to influence the tuberoinfundibular DA system, since they were stimulatory to growth hormone and prolactin secretion [31, 33, 42, 76, 118].

Serotonin (5HT)—Opioid Peptide Interactions

In the case of 5HT, we have also noted many central nervous system loci of possible interaction of this monoamine with the opioid peptides. We have already mentioned the brain stem projections of the β-END/α-MSH neuronal system, particularly those to noradrenergic and serotonergic raphe nuclei. We have noted β-END/α-MSH fibers and terminals within the nucleus raphe dorsalis situated in and adjacent to periaqueductal gray (Fig. 13). Many leu-ENK immunoreactive fibers were also seen in the raphe dorsalis (Fig. 13). Of the other serotonergic nuclei examined that are innervated by both β-END/α-MSH as well as leu-ENK fibers and terminals, we should also mention the nucleus raphe magnus (Fig. 14), raphe medianus (nucleus centralis superior), and raphe pontis. Of the several serotonergic pathways examined, one of the most striking in terms of a possible interaction with an opioid peptide, is the serotonergic projection to the dorsal horn of the spinal cord. Serial section examination revealed the existence of dense leu-ENK immunoreactivity occupying the same position in the dorsal horn as the 5HT terminals (Fig. 15).

It seems likely that a 5HT stimulatory mechanism might be involved in the release of pituitary ACTH and β-END, perhaps via corticotropin releasing factor. In support of this notion is the evidence that various stressful stimuli that can enhance brain 5HT turnover [100,137] can also increase the release of ACTH and β-END [99,121]. Furthermore, direct evidence was provided by Sapun *et al.* [124] for a serotoner-

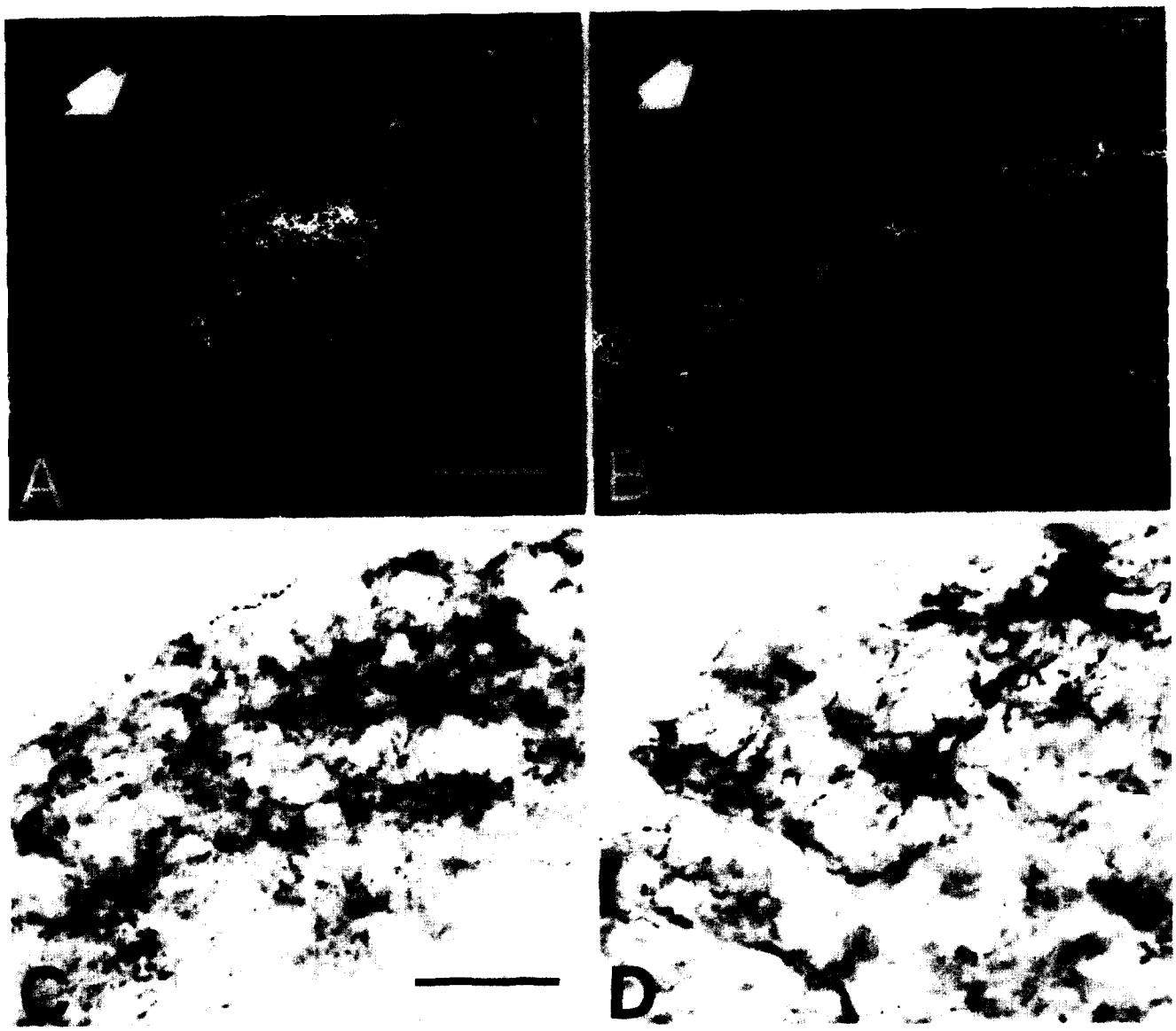

FIG. 15. Dorsal horn of spinal cord: The serotonergic (5HT) projection to the dorsal horn (see Fig. 1 for orientation) is shown in panel B (low power, dark field). The adjacent section (A) demonstrates the leu-ENK (LE) fibers occupying an identical position to that of 5HT. Panels C and D show high power, bright field photomicrographs of A and B, respectively. Arrows point to the dorsal surface of the spinal cord. Light bar=100 μm. Dark bar=50 μm.

gic stimulatory influence over the basal, and in part, the stress-induced release of pituitary β-END in the rat.

Serotonergic systems may also play an important role in brain mechanisms of pain and analgesia [91]. Experimental manipulations, pharmacological or otherwise, that elevate brain 5HT levels, also produce analgesia in both animals and man [54, 63, 85, 92, 120, 122, 123], while those manipulations which reduce 5HT levels induce hyperalgesia reversible by 5HT precursors [34,85].

It has been shown that the electrical stimulation of some brain regions such as the mesencephalic periaqueductal gray in the vicinity of the nucleus raphe dorsalis results in the production of analgesia (cf. [87]). In the rat, pretreatment with p-chlorophenylalanine (a 5HT synthesis inhibitor) appears to diminish the tail-flick latency normally caused by periaqueductal gray stimulation [3]. Furthermore, a reversal of this latter effect (of p-chlorophenylalanine) was shown to occur with the administration of 5-hydroxytryptophan, in itself a potentiator of electrical stimulation-produced analgesia [2]. Thus, the possibility exists for an involvement of a serotonergic neuronal system in the mechanism of stimulation-produced analgesia, at least in the periaqueductal gray. Accordingly, experimental evidence points to a positive correlation between high levels of brain 5HT and increased anti-nociception following narcotic administration (cf. [20]).

As was alluded to above, stimulation of periaqueductal gray results in an inhibition of pain transmission through the spinal cord, thus producing analgesia [1, 88, 103]. The nucleus raphe magnus seems to be involved in this mechanism of analgesia produced by the stimulation of periaqueductal gray [35]. The activity of raphe magnus neurons can be al-

tered by periaqueductal gray stimulation [117]. Furthermore, cells of the lamina I and V of the spinal cord (involved in pain transmission) can be inhibited by the stimulation of raphe magnus [35]. However, it should be pointed out that this 5HT system is only a part of a rather complex descending inhibitory system involved in the stimulation-produced or morphine-induced analgesia, including the nucleus reticularis paragigantocellularis [135], and encompassing several neurotransmitter substances, such as acetylcholine, NE, substance P, and ENK [50,105].

The nucleus raphe dorsalis is another 5HT nucleus involved in the mechanism of analgesia which can be induced by electrical stimulation or by opiates [10, 87, 104]. It has been shown that stimulation of raphe dorsalis can potentiate morphine-induced analgesia by increasing forebrain 5HT levels [123]. On the other hand, raphe dorsalis lesions have the opposite effect on morphine analgesia [156]. In the cat, the raphe dorsalis appears to be even more effective a site than periaqueductal gray for stimulation-produced analgesia [104]. Recently, Moss *et al.* [95] have demonstrated ENK immunoreactive perikarya in the cat raphe dorsalis, thus arguing that the effectiveness of electrical stimulation of the cat raphe dorsalis might be due to the excitation of both ENK-containing and 5HT neurons. In the rat also, ENK-perikarya have been shown to exist in the raphe dorsalis [141], but it appears that there are considerably fewer ENK neurons in the rat, especially along the midline [95]. Just how raphe dorsalis fits into the overall pain pathway system, particularly the descending system involving raphe magnus, is not yet well understood. Moreover, through its projections to the limbic structures, raphe dorsalis may be involved in the affective side of pain perception.

CONCLUSIONS

It is now clear that there exists the possibility of an extensive and intimate interaction between the endogenous opioid peptides and the classical monoamine neurotransmitters. Although we can only talk about the possibility of such an interaction due to the limitations of light microscopic immunocytochemistry, still there exist in many cases an unmistakable anatomical proximity between these peptidergic systems and the monoaminergic nuclei and projections. Electron microscopic analyses utilizing both pre- and postsynaptic markers will no doubt provide answers to some of the crucial questions regarding the synaptic or otherwise synaptoid connectivity between such systems.

It is becoming increasingly apparent that the opioid peptides play a neurotransmitter or neuromodulatory role in many brain regions. It is equally apparent that at least a part of this role is played in conjunction with the monoamines. Particularly, the profound behavioral effects of the opioid peptides are a good indication that some of their pharmacological actions must be mediated through the monoamines (i.e., catalepsy, analgesia, neuroendocrine effects).

Further combined pharmacological-morphological studies using manipulative techniques whereby one can alter the pharmacology of one or the other system, could prove valuable in assessing the more subtle influences and interactions of the opioids upon the monoamines and vice versa.

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