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Distribution of Oxytocin- and Vasopressin-Binding Sites in the Rat Extended Amygdala: A Histoautoradiographic Study

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ABSTRACT

Radioligand receptor autoradiography has shown that oxytocin- and vasopressin-binding sites exist in numerous rat brain regions, among which the amygdala and the bed nucleus of the stria terminalis (BST) are especially prominent. However, these descriptions did not take into account the numerous subdivisions of the amygdala and the BST. Thus, we have reinvestigated the distribution of these sites in the rat extended amygdala, which is formed by a continuum of structures stretching from the BST to the centromedial amygdala, including parts of the accumbens nucleus, substantia innominata, and transition areas between the amygdala and the striatum. For this purpose, histoautoradiography was used to detect binding sites at the cellular level, and anatomical boundaries were defined on the basis of acetylcholinesterase histochemistry and tyrosine-hydroxylase immunohistochemistry. Oxytocin- and vasopressin-binding sites were detected in well-defined subdivisions of both medial and central parts of the extended amygdala, but they almost never coexisted in the same region. Compared with previously reported distributions, our reinvestigation describes novel oxytocin- and vasopressin-binding sites in the lateral and supracapsular BST, in the sublenticular extended amygdala, in the interstitial nucleus of the posterior limb of the anterior commissure, in the marginal zone, in the central amygdaloid nucleus, and in the anterior amygdaloid area. These results indicate that oxytocin- and vasopressin-binding sites represent an important feature of the extended amygdala and may participate in the large variety of functions that characterize this area, including reproductive and ingestive behaviors, conditioned fear and autonomic regulation. J. Comp. Neurol. 383:305–325, 1997.

Indexing terms: neurohypophyseal peptides; receptors; autoradiography; bed nucleus of the stria terminalis; limbic system

Oxytocin (OT) and vasopressin (VP), in addition to their well-known neuroendocrine effects, seem to play a role in neuromodulation within the central nervous system. Indeed, central injections of these peptides can affect autonomic function and a variety of behaviors, ranging from thermoregulation to various aspects of the reproductive cycle, behavioral homeostasis, and learning (for reviews, see Meisenberg and Simmons, 1983; Richard et al., 1991). Numerous OT- and VP-immunoreactive fibers have been detected in several brain structures (Sofroniew, 1985), and binding sites for the two neuropeptides have been described by film autoradiography in various brain regions (De Kloet et al., 1985; Freund-Mercier et al., 1987, 1988; Tribollet et al., 1988). The most recent histoautoradiographic techniques allow precise determination of OT- and VP-binding sites with almost microscopic accuracy (Krémarik et al., 1993). Quantitative autoradiography has demonstrated that these binding sites present pharmacological characteristics of receptors (Freund-Mercier et al., 1987, 1991). Finally, both in vivo and in vitro electrophysiological results suggest

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the presence of functional OT and VP receptors in different regions of the brain (for review, see Richard et al., 1991).

The amygdala and the bed nucleus of the stria terminalis (BST) exhibit OT- and VP-binding sites in several subnuclei (Krémarik et al., 1993). The presence of OT-binding sites in the central amygdaloid nucleus has been correlated with a neuronal sensitivity to iontophoretically applied OT (Condés-Lara et al., 1994), and the OT-binding sites detected in the medial BST have been implicated in the regulation of the milk-ejection reflex in lactating rats (Moos et al., 1991) and may play a role in more complex aspects of reproduction, including parental behavior (Insel, 1992). In addition, various studies have indicated that application of OT and VP in the amygdala and the BST can induce cardiovascular and other behavioral changes (Roozendaal et al., 1993).

The similarity between the centromedial amygdala and the BST with respect to cellular morphology, immunocytochemistry, and hodology (McDonald, 1983; de Olmos et al., 1985; Holstege et al., 1985; Grove, 1988a,b; Moga et al., 1989; Schmued, 1994; Alheid et al., 1995) has progressively led to the notion that the centromedial amygdala and the BST belong to an important basal forebrain continuum (de Olmos et al., 1985), which has been termed the extended amygdala (Alheid and Heimer, 1988). The extended amygdala is usually divided on the basis of cytoarchitectural, histochemical, and hodological differences into two corridors: the central corridor, which is related to the central amygdaloid nucleus, and the medial corridor one, which is related to the medial amygdaloid nucleus. The extended amygdala, together with the shell of the accumbens nucleus, forms a superstructure that is thought to be an important center of integration involved in several higher functions of the brain, such as learning, emotion, ingestive behavior, regulation of autonomic functions during stress, and reproductive functions (for reviews, see Alheid and Heimer, 1988; Davis, 1992; Galaverna et al., 1993; Schulkin et al., 1994; Zardetto-Smith et al., 1994; Alheid et al., 1995).

The high density of OT- and VP-binding sites described in the amygdala and the BST suggests that binding sites for the two peptides may represent an important feature of the extended amygdala, like angiotensin II immunoreactivity, which typically labels the extended amygdala but not adjacent telencephalic areas (Lind et al., 1985; Alheid and Heimer, 1988). However, previous descriptions of OT- and VP-binding sites in the forebrain rarely take into account the high number of subdivisions in the amygdala and the BST, and the localization of these sites has never been explored in the context of the extended amygdala. Thus, the aim of this study was to determine precisely the localization of specific OT- and VP-binding sites in the extended amygdala of the male rat using histoautoradiographic techniques combined with acetylcholinesterase (AChE) histochemistry and tyrosine-hydroxylase (TH) immunohistochemistry to assess nuclei boundaries.

MATERIALS AND METHODS

Animals

Twelve Wistar male rats weighing from 250 to 350 g were killed by decapitation, and the brains were rapidly

Abbreviations

AAA	anterior amygdaloid area
AAAd	dorsal AAA
AAAv	ventral AAA
ac	anterior commissure
Acb	accumbens nucleus
AcbC	core area of the Acb
AcbSh	shell area of the Acb
AChE	acetylcholinesterase
AStr	amygdalostriatal area
av	anteroventral part of the medial subdivision of the central amygdaloid nucleus
BL	basolateral amygdaloid nucleus
BMP	posterior basomedial amygdaloid nucleus
BST	bed nucleus of the stria terminalis
BSTIA	intraamygdaloid BST
BSTL	lateral BST
BSTLD	dorsal BSTL
BSTLDc	capsular BSTLD
BSTLDcn	central BSTLD
BSTLI	intermediate BSTL
BSTLJ	juxtacapsular BSTL
BSTLP	posterior BSTL
BSTLV	ventral BSTL
BSTM	medial BST
BSTMA	anterior medial BST
BSTMP	posterior medial BST
BSTMPi	intermediate BSTMP
BSTMPl	lateral BSTMP
BSTMPm	medial BSTMP
BSTMV	ventral BSTM
BSTS	supracapsular BST
BSTSl	lateral BSTS
BSTSm	medial BSTS
c	capsular
CeL	lateral subdivision of the central amygdaloid nucleus
CeLc	capsular CeL
CeLcn	central CeL
CeM	medial subdivision of the central amygdaloid nucleus
CeMad	anterodorsal CeM
CeMav	anteroventral CeM
CeMpv	posteroventral CeM
cn	central
CPu	caudate putamen
CV	cresyl violet
GP	globus pallidus
I	intermediate
IMG	intramedullary gray
Ip	paracapsular, intercalated cell clusters
IPAC	interstitial nucleus of the posterior limb of the anterior commissure
IPACl	lateral IPAC
IPACm	medial IPAC
l	lateral
L1	layer 1 of the medial nucleus of the amygdala
m	medial
MeAD	anterodorsal part of the medial amygdaloid nucleus
MeAV	anteroventral part of the medial amygdaloid nucleus
MePD	posterodorsal part of the medial amygdaloid nucleus
MePDi	intermediate subnucleus of the MePD
MePDl	lateral subnucleus of the MePD
MePDm	medial subnucleus of the MePD
MePV	posteroventral part of the medial amygdaloid nucleus
MZp	marginal zone, posterior
OT	oxytocin
ot	optic tract
SLEA	sublenticular extended amygdala
SLEAc	central division of the SLEA
SLEAm	medial division of the SLEA
Sz	sparse cell zone of the BSTMP
Tu	olfactory tubercle
TH	tyrosine hydroxylase
VP	vasopressin
ZL	zona limitans of the accumbens

removed, frozen in isopentane at −40°C, and stored at −20°C until sectioning. Sixteen-micrometer-thick serial sections following frontal, sagittal, or horizontal planes through the telencephalon (interaural level A10.2–A5.2 mm for frontal sections, six animals; lateral level 0.9–4.2 mm for sagittal sections, three animals; and interaural level 0.9–4.4 mm for horizontal sections, three animals, according to the atlas of Paxinos and Watson, 1986) were cut on a cryostat at −15°C, thaw mounted on gelatin-coated slides, and stored at −20°C until use. The protocol has been approved by the French Ministère de l'Agriculture et de la Forêt.

Preparation of ligands and binding procedure

The OT antagonist d(CH_2)$_5$[Tyr(Me)^2,Thr^4, Tyr-NH_2^{9}]ornithine vasotocin (OTA), which is specific for the OT receptor, and the linear VP antagonist Phaa,D-Tyr(Me),Phe,Gln,Asn,Arg,Pro,Arg,Tyr-NH_2 (VPA), which is highly selective for VP receptors of the V_{1a} type (Johnson et al., 1993), were iodinated by using chloramine T according to a procedure described by Stoeckel and Freund-Mercier (1989). The monoiodinated compounds were purified on a reverse-phase column by high-performance liquid chromatography.

After incubation for 20 minutes at room temperature in Tris buffer (Tris-HCl 85 mM, $MgCl_2$ 5 mM, bovine serum albumin 0.1%, pH 7.4), the sections were incubated at 4°C for 24 hours in the same buffer containing either 30 pM [^{125}I]OTA or 50 pM [^{125}I]VPA, which was added with OT 0.1 µM to displace [^{125}I]VPA from OT receptors. Nonspecific [^{125}I]OTA and [^{125}I]VPA binding was determined in the presence of 1 µM OT or 1 µM VP, respectively (Bachem, CH-4416 Bubendorf, Switzerland). The incubation was followed by two 5-minute washes in cold buffer (4°C), and the sections were rapidly rinsed in cold distilled water and dried with a cold air stream.

Autoradiography

Film autoradiographs were generated by apposing the slide-mounted labelled sections to a [^{3}H] Hyperfilm (Amersham) in Kodak x-ray film cassettes. After 3–5 days of exposure at 4°C, the films were developed for 5 minutes in Kodak D19 at 18°C.

After exposure, the sections were postfixed with paraformaldehyde vapors at 80°C for 3 hours according to Herkenham and Pert (1982). The slides were then demyelinated through aqueous alcohol and xylene and were coated with Ilford K5 emulsion for histoautoradiography. After 20 or 30 days of exposure, the slides were developed for 5 minutes in Kodak D19 at 18°C, stained with thionine, mounted with Eukitt, and observed in darkfield and brightfield light microscopy. The darkfield micrographs presented in Figures 2–12 have been manually retouched with a black pen in order to erase the white spots provoked by the dust on the slides.

AChE histochemistry and TH immunohistochemistry

Sections adjacent to those treated for histoautoradiography were processed for histochemical detection of AChE activity following the method described by Karnovsky and Roots (1964). Other adjacent sections were processed for TH immunohistochemistry. These two histochemical techniques are robust stains that are easy to reproduce and that serve to delineate most of the subdivisions in the basal forebrain (Alheid et al., 1995). Briefly, slides were postfixed for 20 minutes in paraformaldehyde 4% and rinsed in 0.1 M phosphate-buffered saline (PBS). They were then irradiated in a microwave oven at 700 W three times for 5 minutes in citrate buffer (0.01 M). After saturation of nonspecific sites by skimmed milk powder (2% in PBS, 0.5% Triton X-100 detergent), slides were incubated in monoclonal anti-TH antibody (1:1,000; Chemicon) in PBS-Triton X-100 for 24 hours at room temperature; after three 10-minute rinses, slides were incubated for 1 hour in biotinylated sheep anti-mouse antibody (1:200; Vector), rinsed three times for 10 minutes with PBS, and incubated with avidin-biotin-peroxidase complex (ABC Elite Kit, Vectastain; Vector) for 1 hour. The peroxidase reaction was then visualized by using Peroxidase Substrate Kit DAB (Vector) and was stopped by rinsing in PBS, and the sections were dehydrated in graded alcohol, cleared in xylene, and mounted with Eukitt.

Image analysis and semiquantification of labelling

Image-analysis software (IPS; Alcatel, Lyon, France) was used to correlate labelling on adjacent sections and to quantify the relative densities of binding sites in different regions. Briefly, images were obtained with a black-and-white camera attached to a personal computer under the control of the photodensitometric image-analysis software. The boundaries of anatomical structures were drawn from AChE-stained and TH-immunoprocessed sections and were superimposed onto the immediately adjacent sections on films to assess anatomical localization of the labelling.

Semiquantitative analysis was conducted to compare labelling density of different regions and was performed on films from a single animal (frontal sections). For each [^{125}I]OTA- and [^{125}I]VPA-labeled structure, the optical density was measured in four to eight consecutive sections, and the lowest value for each structure was subtracted from the others before averaging. The obtained values (optical density ×100) were then divided into four equal groups in order to categorize the labelling density (Table 1). The densities of [^{125}I]OTA and [^{125}I]VPA labelling are not compared, because iodinated ligands differ in their specific activity, and the duration of film exposure was also different.

RESULTS

High concentrations of OT- and VP-binding sites were demonstrated in the different regions of the extended amygdala (BST, interconnecting sublenticular, supracapsular, and lateral forebrain cell corridors, and centromedial amygdala), where distribution will be successively described. In addition, medial (shell) areas of the accumbens nucleus (Acb) and medial portions of the olfactory tubercle were also labelled; these zones are closely related to the extended amygdala and may represent transitional areas between ventral basal ganglia and amygdala. The nomenclature used was that of Alheid et al. (1995). All the data resulted from successive observations of conventional film autoradiographs (Fig. 1) and histoautoradiographs (Figs. 2–12). Table 1 gives the mean optical density of each labelled structure

TABLE 1. Optical Densities of Oxytocin (OT)- and Vasopressin (VP)-Binding Sites in the Extended Amygdala and Related Nuclei

OT-binding sites				VP-binding sites			
Description	Nucleus	Mean (OD × 100)	Standard error of the mean	Description	Nucleus	Mean (OD × 100)	Standard error of the mean
Low	IPAC[1]	12.66	0.27	Low	SLEAc	5.6	0.20
Moderate (>16)	MePDi	18.97	1.57	Moderate (>6)	MZp	8.81	0.44
	BMP	21.31	0.43		BSTLV	8.55	0.09
	BSTMPi	23.53	0.80		AAAd	8.75	0.25
	Ip/IMG	23.86	1.18		CeMpv, av	9.57	0.57
Dense (>24)	IPAC[2]	24.10	0.33		AStr	9.76	0.23
	Acb	24.34	1.07		BSTLP	10.81	0.25
	IPAC[3]	26.16	1.28	Dense (>12)	AAAv	13.21	0.51
	MePDm,l	28.47	0.66		IPAC[4]	14.92	0.29
	BSTLDc,cn	30.20	0.83		Tu	16.58	0.26
Very dense (>32)	BSTMPm	32.15	0.41		BSTSl	17.13	0.81
	CeLc,cn	32.69	0.53	Very dense (>18)	IPAC[1]	20.51	0.68
	Tu	38.18	0.51		Acb	22.03	0.50
					BSTLJ	22.15	0.48

[1]This part of the IPAC corresponds to the portion apposed to the ventrolateral edge of the pallidum below the posterior limb of the anterior commissure, in which OT- and VP-binding sites overlap (see Fig. 8). See Materials and Methods for protocol of measure. For abbreviations, see list.
[2]This part of the IPAC corresponds to the [^{125}I]OTA-labelled cluster at the ventral border of the [^{125}I]VPA-labelled area (see Fig. 9).
[3]This part of the IPAC corresponds to the [^{125}I]OTA labelling observed at posterior levels above the central amygdaloid nucleus (see Fig. 10).
[4]This part of the IPAC corresponds to the [^{125}I]VPA-labelled area above the posterior limb of the anterior commissure (see Fig. 5).

Olfactory tubercle

In the olfactory tubercle, OT and VP-binding sites were present in its medial part. OT-binding sites appeared with very high density on clusters of medium cells (Fig. 2A,B) but obviously avoided the islands of Calleja. VP-binding sites appeared roughly in the same region as OT-binding sites (Fig. 2C,D), but the labelled areas never overlapped.

Acb

In the Acb, histoautoradiography demonstrated differential OT- and VP-binding site localization. OT-binding sites were restricted to the posteromedial portion of the shell of the Acb (AcbSh), where the density of labelling increased in a ventromedial-to-dorsolateral gradient and abruptly disappeared at the surface of the core of the Acb (AcbC; Figs. 2A,B, 3C,D).

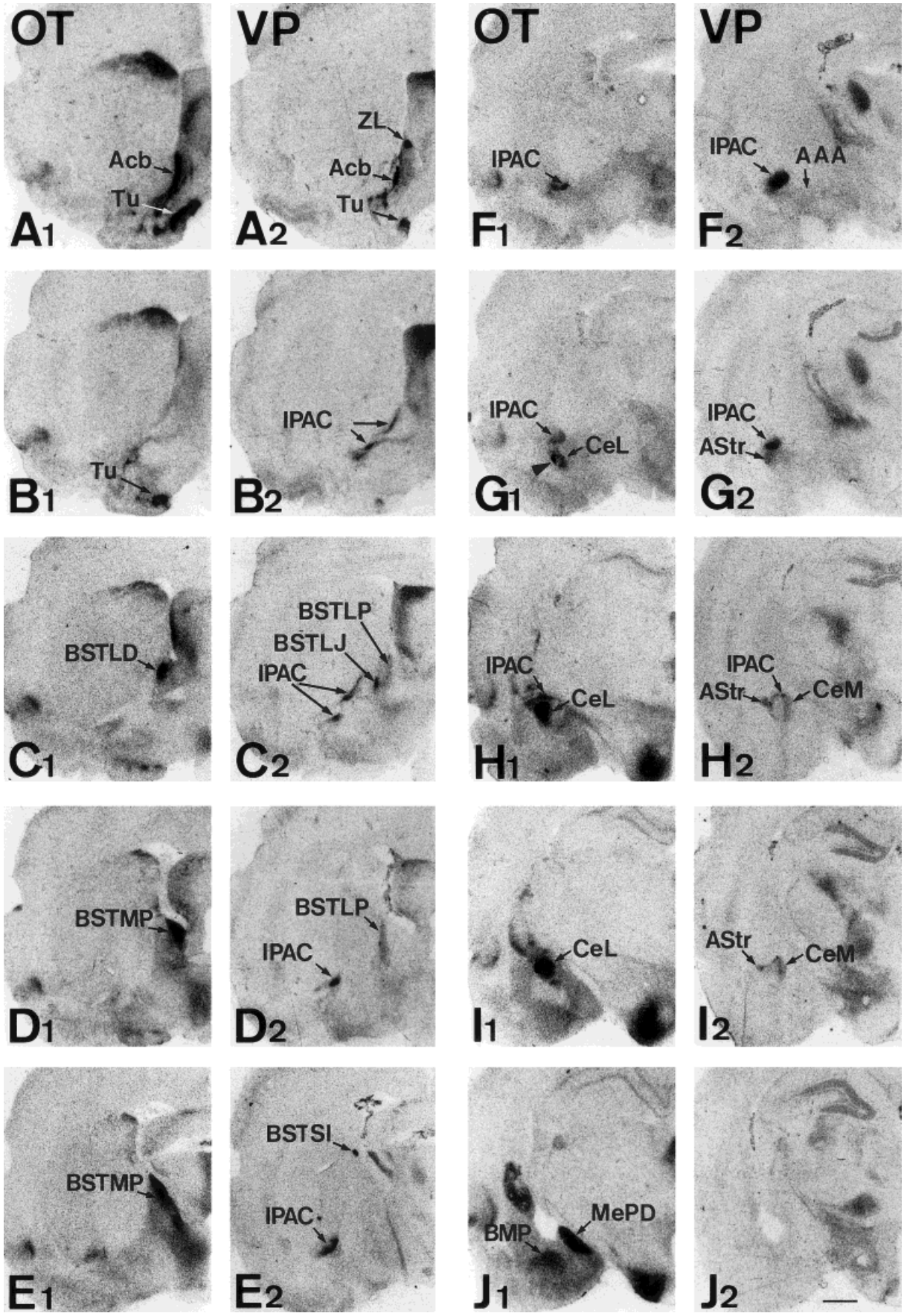
VP-binding sites were found at the periphery of the shell, where thin bands of labelling occurred at the ventrolateral boundary between the shell and the core, around the anterior limb of the anterior commissure, and at the dorsomedial boundary between accumbens shell and septum, that is, the zona limitans of the accumbens (ZL; Phillips et al., 1988; Figs. 2C,D, 3E,F, 4C,D). The dense labelling between shell and core appeared to cover medium-sized cell clusters (Figs. 2E, 4E), some of which displayed low TH immunoreactivity with respect to the rest of the Acb (Fig. 2F), and became more diffuse at the caudal end of the AcbSh, where it merged with the rostral BST without clear-cut boundaries (Fig. 4C,D).

BST

In the BST, OT-binding sites occurred in both the lateral (BSTL) and the medial (BSTM) subdivisions, whereas VP-binding sites occurred only in the lateral subdivision. [^{125}I]OTA heavily labelled both capsular (BSTLDc) and central (BSTLDcn) divisions of the dorsal lateral BST (Figs. 3C,D, 5C,D, 6C), which could be differentiated by the higher density of TH-immunoreactive terminals in the central rather than the capsular division (Fig. 6F). This labelling sharply ended against the internal capsule laterally and progressively disappeared medially and caudally at the boundaries with the posterior lateral BST (BSTLP). At more posterior levels, a second high concentration of OT-binding sites was detected in the posterior part of the medial BST (BSTMP; Figs. 3C,D, 6C, 7C,D). The labelling remained intense in the total extent of the medial dense-celled column (BSTMPm) and the associated cell-sparse zone (Sz; see, e.g., Krémarik et al., 1993), whereas it decreased laterally in the intermediate column (BSTMPi), to disappear in the lateral column (BSTMPl; Figs. 6C,E, 7C–E).

A high density of VP-binding sites occurred in the juxtacapsular part of the lateral BST (BSTLJ), which runs along the internal capsule at the dorsal face of the anterior commissure (Figs. 5E,F, 6D). This spindle-shaped nucleus was defined by strong AChE activity (Figs. 5B, 6B) and dense TH terminals (Fig. 6F). The labelling was continuous with that of the Acb, as shown by horizontal section (Fig. 4C,D). Another small area that was densely labelled by [^{125}I]VPA medial to the internal capsule appeared just ventral to the tip of the lateral ventricle and may have been a dorsal extension of the juxtacapsular nucleus, on the basis of cell morphology and AChE histochemistry (Fig. 5B,E,F). Medial to the juxtacapsular nucleus, moderate labelling was found in the posterior subnucleus of the

Fig. 1. Film autoradiographs of frontal sections through the telencephalon of a male rat showing the distribution of oxytocin (OT)- and vasopressin (VP)-binding sites in the extended amygdala. Adjacent sections were incubated in the presence of 30 pM [^{125}I] $d(CH_2)_5$[Tyr(Me)^2,Thr^4, Tyr-NH_2^9]ornithine vasotocin (OTA; A1-J1; OT) or 50 pM [^{125}I]Phaa,D-Tyr(Me),Phe,Gln,Asn,Arg,Pro,Arg,Tyr-NH_2 (VPA) + 0.1 μM OT (A2-J2; VP). **A1–J1:** OT-binding sites in the accumbens nucleus (Acb), the olfactory tubercle (Tu), the dorsal part of the lateral bed nucleus of the stria terminalis (BSTLD), the posterior part of the medial bed nucleus of the stria terminalis (BSTMP), the interstitial nucleus of the posterior limb of the anterior commissure (IPAC), a small spot-like zone in the anterior amygdala (arrowhead in G1), the lateral subdivision of the central amygdaloid nucleus (CeL), the posterodorsal part of the medial amygdaloid nucleus (MePD), and the posterior basomedial amygdaloid nucleus (BMP). **A2–J2:** VP-binding sites in the accumbens nucleus, the zona limitans of the accumbens (ZL), the olfactory tubercle, the interstitial nucleus of the posterior limb of the anterior commissure, the posterior (BSTLP) and juxtacapsular (BSTLJ) parts of the lateral bed nucleus of the stria terminalis, the lateral parts of the supracapsular bed nucleus of the stria terminalis (BSTSl), the anterior amygdaloid area (AAA), the amygdalostriatal area (AStr), and the medial subdivisions of the central amygdaloid nucleus (CeM). Scale bar = 1 mm.


OT
Acb
Tu
A1
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ZL
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Figure 1

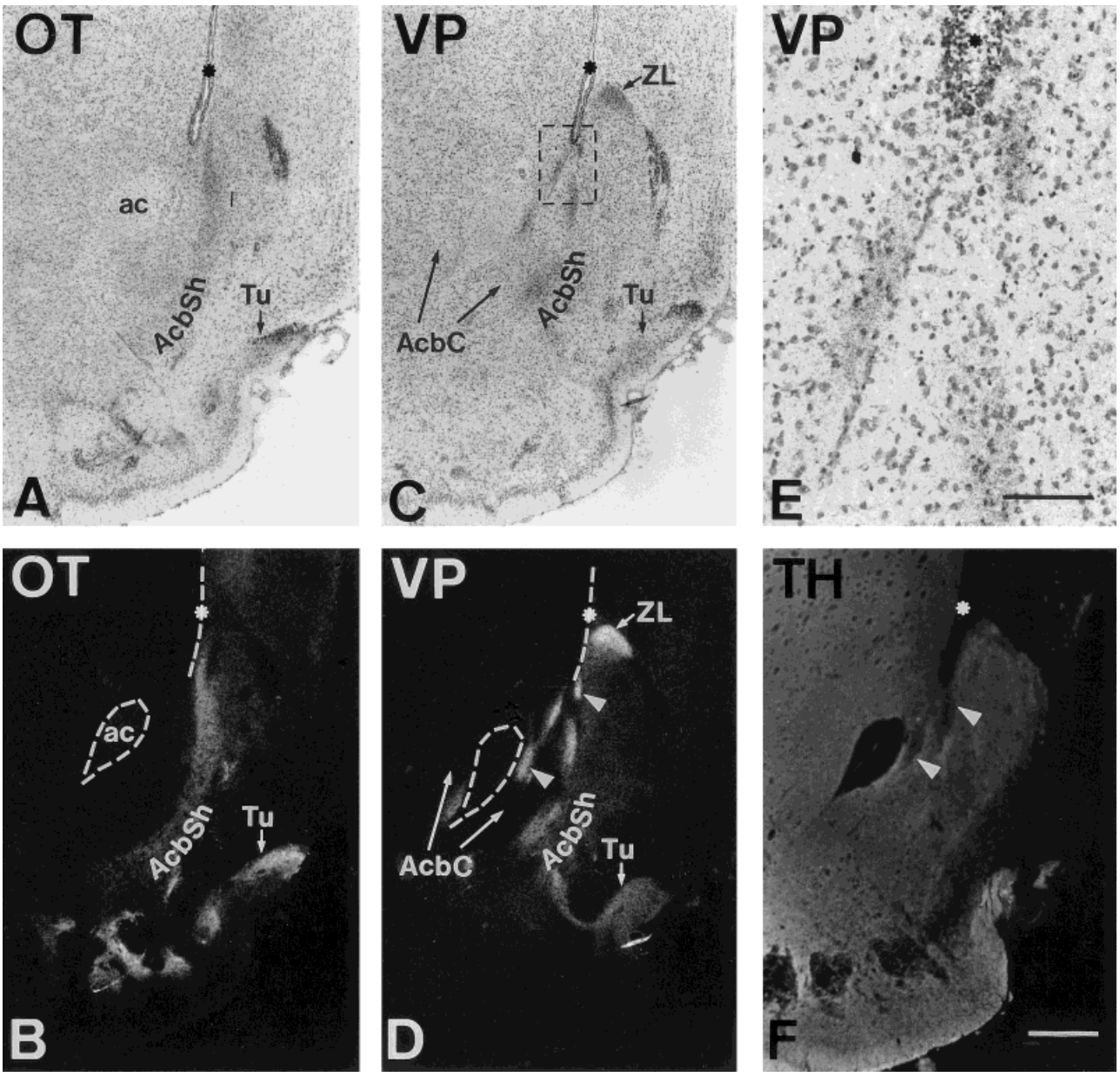

Fig. 2. Frontal sections through the accumbens nucleus. The left hemisphere is shown with the medial direction on the right side (same orientation for Figs. 4–12). **A,B:** OT-binding sites in the shell of the accumbens nucleus (AcbSh) and the olfactory tubercle (Tu). **C,D:** VP-binding sites in the zona limitans of the accumbens (ZL) and in clusters situated in regions between the core (AcbC) and the AcbSh accumbens nucleus and around the anterior commissure (ac) in the olfactory tubercle. **E:** Higher power photomicrograph of the boxed area in C showing VP-binding sites between the core and shell of the accumbens nucleus. **F:** Reversed photomicrograph of the same area in a section adjacent to the histoautoradiograph shown in C and D processed for tyrosine-hydroxylase (TH) immunodetection; note the low TH immunoreactivity (arrowheads) in the same location where [^{125}I]VPA labelling is visible in C and D. A,C, brightfield; B,D, darkfield photomicrographs of the same area on adjacent sections. The darkfield photomicrographs presented in the figures have been retouched manually with a black pen in order to erase the white spots provoked by the dust on the slides (same in following figures). Asterisks indicate the lateral ventricle (same in following figures). Scale bars = 500 µm in A–D,F, 100 µm in E.

lateral BST, which extended ventrally (Fig. 4C,D), surrounding the ventromedial face of the dorsal subnucleus of the lateral BST (Figs. 5E,F, 6D). Caudally, the labelling of the posterior lateral BST remained confined between the internal capsule and the medial BST. Low-to-moderate labelling was also detected in the ventral part of the lateral BST (BSTLV) ventrolateral to the anterior commissure (Fig. 5E,F).

Sublenticular, including the interstitial nucleus of the posterior limb of the anterior commissure, and supracapsular portions of the extended amygdala

In the interstitial nucleus of the posterior limb of the anterior commissure (IPAC), OT-binding sites occurred in a few well-circumscribed areas, whereas VP-binding sites

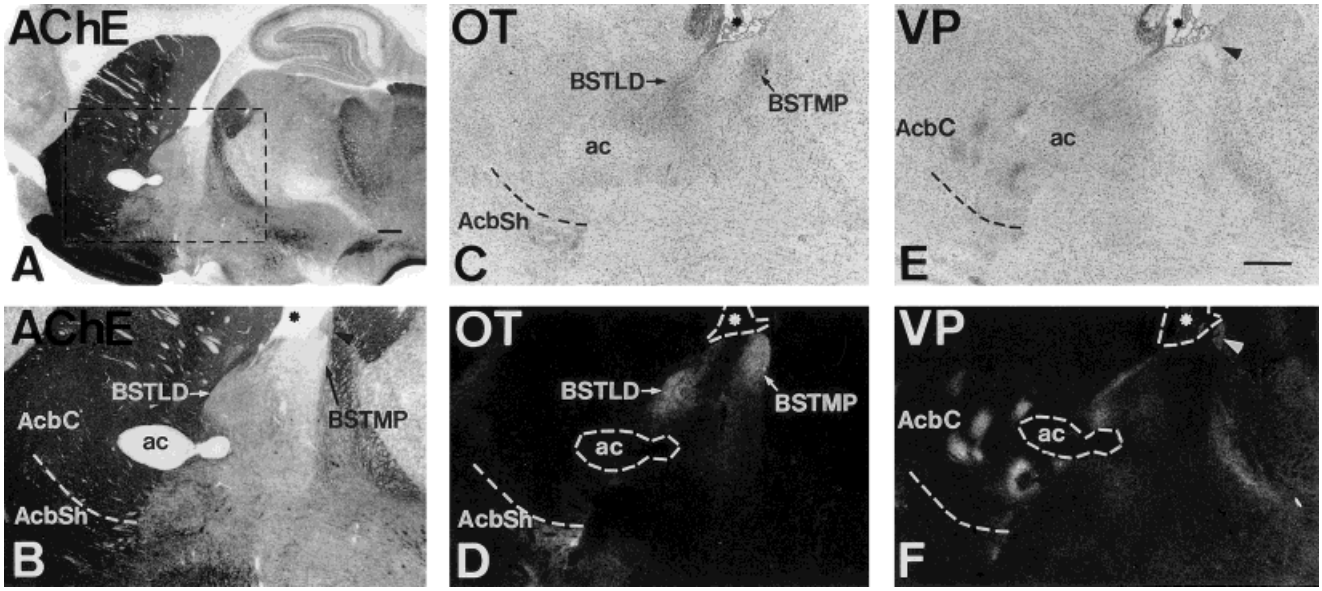

Fig. 3. Sagittal sections through the Acb and the bed nucleus of the stria terminalis (BST). **A:** Acetylcholinesterase (AChE)-stained section interposed between the histoautoradiographs shown in C,D and E,F. **B:** Higher power photomicrograph of the boxed area in A. **C,D:** Same field as in B. OT-binding sites in the AcbSh, in the dorsal part of the lateral BST (BSTLD), and in the posterior part of the medial BST (BSTMP). **E,F:** Same field as in B. VP-binding sites in the Acb close to the anterior commissure (ac) and in a small area at the tip of the stria terminalis (arrowheads). C,E, brightfield; D,F, darkfield photomicrographs of the same area. Scale bars = 500 µm (E also applies to B–D,F).

were found in high concentrations in several locations. The interstitial nucleus corresponds to a population of cells adjacent to and sometimes surrounding the posterior limb of the anterior commissure. On the basis of connectional data (Alheid et al., 1995), it has been subdivided into two more or less continuous columns, one ventromedial and the other dorsolateral to the first, although, in Nissl and most histochemical stains, the border between these two is indistinct. Similarly, OT- and VP-binding sites overlapped diffusely in the interstitial nucleus, although, in a few areas, distinct cell clusters embedded within or adjacent to the IPAC are labelled in a complementary fashion by [^{125}I]OTA or [^{125}I]VPA.

The interstitial nucleus begins as a band of cells crossing the rostroventral face of the pallidum; at this level, it is generally included within the caudal surface of the Acb or the ventral caudate putamen. As it follows the posterior limb of the anterior commissure caudoventrally, it occupies a site adjacent to the ventrolateral corner of the globus pallidus, just above and below the anterior commissure, until the latter merges with the external capsule. The ventral part of this rostral part of the IPAC occupies the medial portions of what has been termed the fundus striati in the atlas of Paxinos and Watson (1986). Caudal to this level, the IPAC continues alongside the pallidum but in a position just dorsal to the central amygdaloid nucleus.

Diffuse [^{125}I]OTA binding was observed in the rostral portions of the IPAC that was generally confined to the ventrolateral edge of the pallidum, corresponding somewhat to the medial column of the IPAC but with an indistinct lateral border from the binding sites (Figs. 8B,C, 9C,D). A dense concentration of OT-binding sites was observed in a small AChE$^-$ area just ventral to the border of the IPAC (Fig. 8B,C,E). Moderate-to-high [^{125}I]OTA labelling also occurred in the posterior part of the IPAC above the lateral subdivision of the central amygdaloid nucleus (CeL; Fig. 10D–F).

Strong [^{125}I]VPA labelling also occurred in the IPAC, emerging from the labelling of the Acb and the rostral BST and extending laterally as a narrow band across the face of the pallidum just above the posterior limb of the anterior commissure, until the latter disappeared (Figs. 5E,F, 11D). High densities of VP-binding sites were also detected in the portion of the IPAC just below the posterior limb of the anterior commissure (Figs. 8D, 9E,F). In the posterior part of the IPAC, the [^{125}I]VPA labelling decreased in size as the IPAC rode over the central amygdaloid nucleus. This was eventually confined in a strong TH-immunoreactive and AChE-dense spot in the medialmost portion of the AChE$^+$ area adjacent to the caudal ventrolateral pallidum (Fig. 10B,C,G–I). This labelling appeared just ventromedial to the [^{125}I]OTA-labelled area (Fig. 10F). Lateral to the posterior IPAC, the amygdalostriatal area (AStr) displayed moderate VP-binding site density (Figs. 10G,H, 11D). This labelling extended ventrally between the basolateral and central amygdaloid nuclei (Fig. 9E,F). Some elements of the posterior marginal zone were moderately labelled by [^{125}I]VPA at the medial contact between the globus pallidus and the caudate putamen (Fig. 8D,F). At the rostral face of the globus pallidus, VP-binding sites in the anterior marginal zone described by Shu and coworkers (1988) were found only in the band of cells related to the IPAC (Fig. 5E,F).

VP-binding sites were also located in the lateral part of the supracapsular BST (BSTSl) (Fig. 7F), which is a column of gray matter found along the lateral edge of the stria terminalis as it traverses the dorsal part of the internal capsule. At the medial edge of the stria terminalis, some VP-binding sites were also noted (Fig. 3E,F). This might represent elements of the medial supracapsular

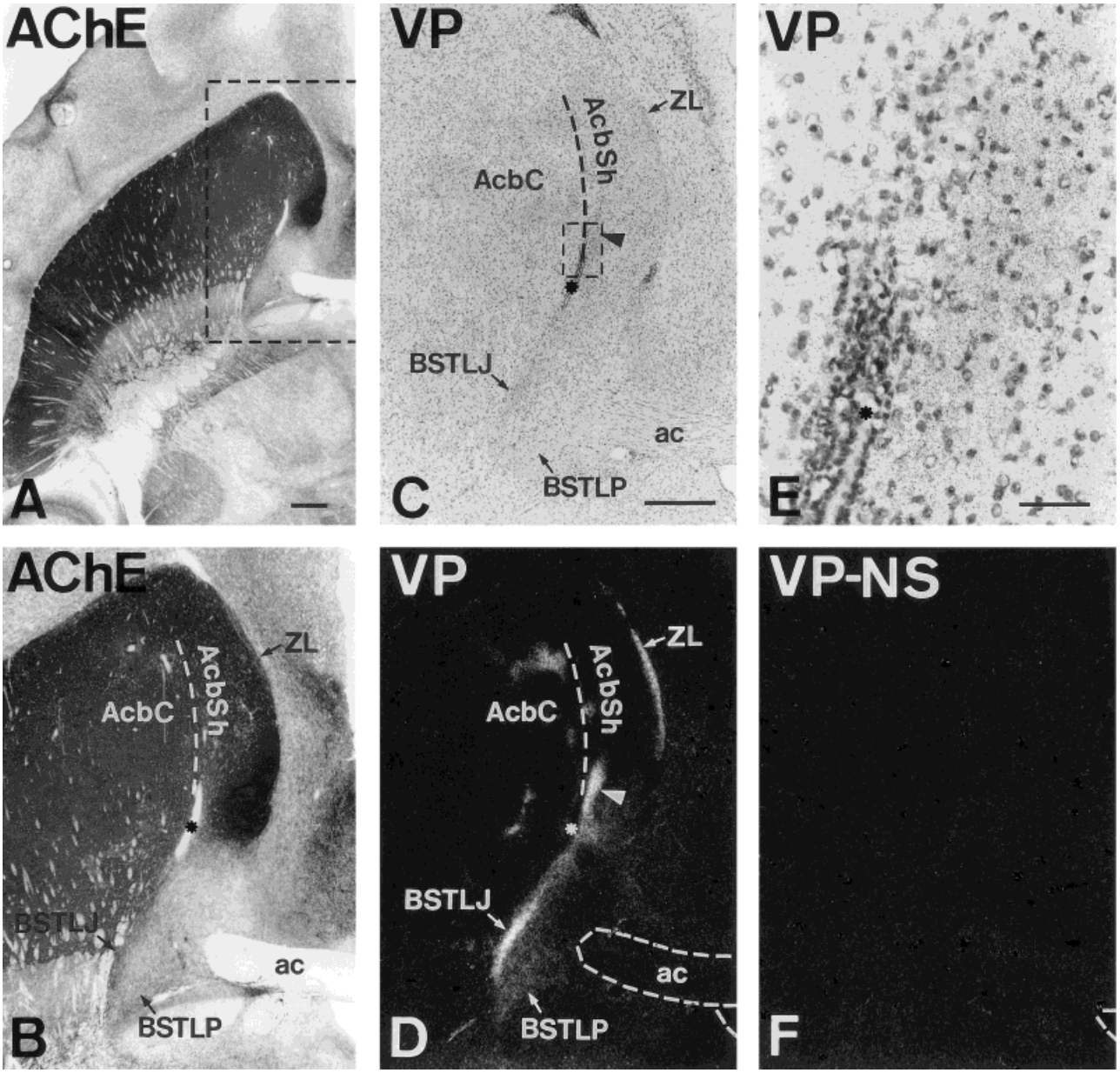

Fig. 4. Horizontal sections through the Acb and the BST at the level of the crossing of the ac. **A:** AChE-stained section adjacent to the histoautoradiographs shown in C,D, and F. **B:** Higher power photomicrograph of the boxed area in A. **C,D:** Same field as in B. VP-binding sites in the ZL of the accumbens at the periphery of the AcbSh and the AcbC, at the junction between the Acb and rostral BST (arrowhead), and in the juxtacapsular (BSTLJ) and posterior (BSTLP) parts of the lateral BST. **E:** Higher power micrograph of the boxed area in C showing VP-binding sites at the caudal end of the AcbSh close to the lateral ventricle (asterisk). **F:** Same field as in B. Darkfield micrograph of nonspecific [^{125}I]VPA binding (VP-NS) in a section adjacent to the histoautoradiograph shown in C and D incubated with [^{125}I]VPA + OT + VP. C, brightfield; D, darkfield photomicrographs of the same area. Scale bars = 500 µm in A, C (C also applies to B, D, F), 50 µm in E.

BST, which is represented by scattered neurons on the surface of and embedded within the medial tip of the stria terminalis. However, this VP binding is also contiguous with the labelling in the reticular and anteroventral nuclei of the thalamus, and we lack evidence to determine the exact identify of the observed labelling.

In the sublenticular area, very slight [^{125}I]VPA labelling was occasionally observed ventromedial to the IPAC and dorsomedial to the anterior amygdaloid area (Fig. 8D) and may correspond to the central division of the sublenticular extended amygdala. No OT-binding sites were evident in the supracapsular BST or the sublenticular extended amygdala..

Amygdala

In the amygdala sensu stricto, OT-binding sites were found in high density in three nuclei. The lateral subdivision of the central nucleus was heavily labelled by [^{125}I]OTA in the caudal two-thirds of its capsular and central parts

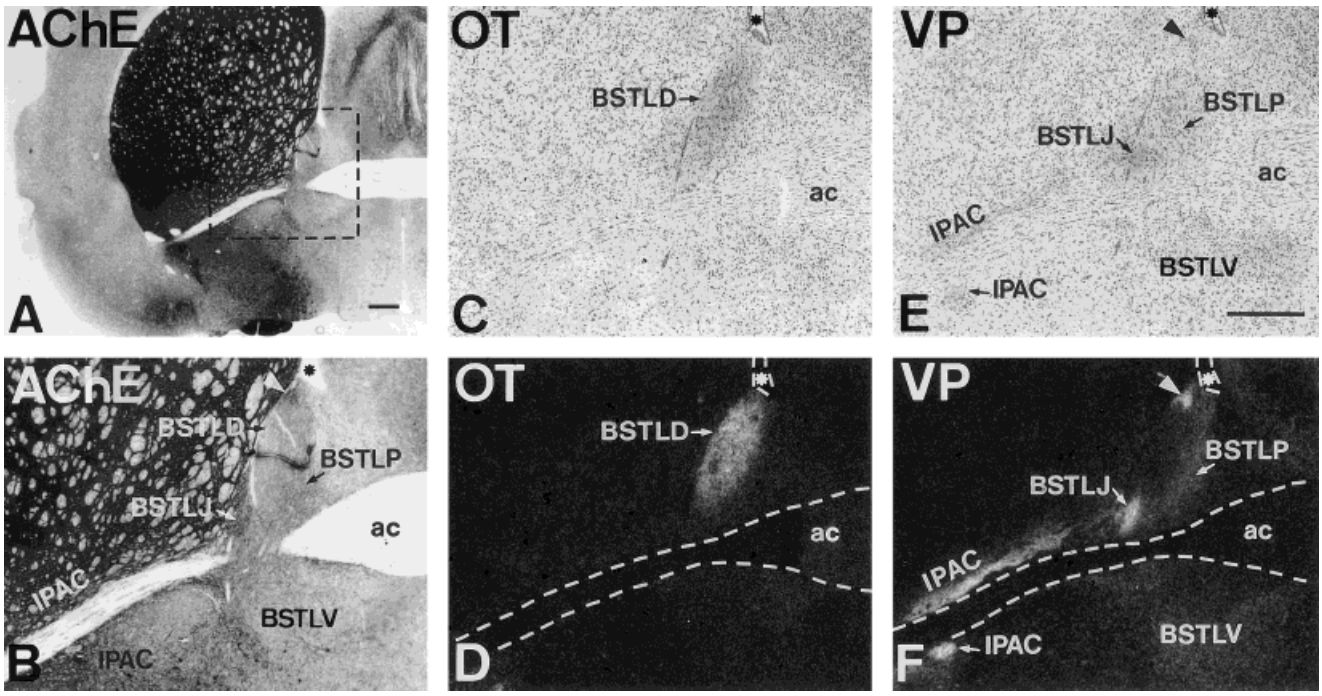

Fig. 5. Frontal sections through the anterior part of the BST. **A:** AChE-stained section interposed between histoautoradiographs shown in C,D and E,F. **B:** Higher power photomicrograph of the boxed area in A. **C,D:** Same field as in B. OT-binding sites the in dorsal part of the lateral BST (BSTLD). **E,F:** Same field as in B. VP-binding sites in the juxtacapsular (BSTLJ), posterior (BSTLP), and ventral (BSTLV) parts of the lateral BST; in a small area ventral to the tip of the lateral ventricle (arrowheads, in B, E, and F); and in the interstitial nucleus of the posterior limb of the anterior commissure (IPAC) above and below the ac. C,E, brightfield; D,F, darkfield photomicrographs of the same area. Scale bars = 500 µm (E also applies to B–F).

(Figs. 10D,E, 11C), which can be differentiated by the higher density of TH-immunoreactive terminals in the central part (Figs. 10C, 11F). Caudally, high densities of OT-binding sites were detected in the posterior basomedial nucleus and in the posterodorsal part of the medial nucleus (Figs. 11D, 12C,D). This latter structure exhibited numerous OT-binding sites in the medial, intermediate, and lateral subnuclei and also over layer 1 of the molecular layer (L1) between the optic tract and the posterodorsal part of the medial amygdaloid nucleus (Fig. 12E). In addition to these nuclei, a small cell cluster devoid of AChE that displayed strong [^{125}I]OTA labelling was found along the medial face of the rostral basolateral nucleus (Fig. 9B–D). These cells may be related either to a subgroup of the paracapsular intercalated cell masses or possibly to elements of the intramedullary gray.

The anterior amygdaloid area was moderately labelled by [^{125}I]VPA, with a higher density in the ventral part than in the dorsal part (Fig. 8D). The medial subdivision of the central nucleus displayed moderate density of VP-binding sites, especially in its anteroventral and posteroventral parts, the anterodorsal part being almost devoid of VP-binding sites (Figs. 10G,H, 11D). In Figures 6 and 11, [^{125}I]VPA- and [^{125}I]OTA-labelled nuclei of the amygdala and the BST are compared on horizontal sections.

DISCUSSION

The distribution of OT- and VP-binding sites in the brain has been described previously using film autoradiography and histoautoradiography (De Kloet et al., 1985; Freund-Mercier et al., 1987, 1988; Tribollet et al., 1988; Krémarik et al., 1991, 1993; Johnson et al., 1993). Nevertheless, the fine anatomy of the amygdala and the BST was rarely taken into account in the previous studies. Here, the use of histoautoradiography combined with histochemical delineation of anatomical subdivisions enabled us to reinvestigate the localization of OT- and VP-binding sites in these regions with respect to the concept of the extended amygdala. The present study confirms the presence of OT- and VP-binding sites in different regions of the extended amygdala, determines their precise localization, and also describes new sites of binding for these peptides. The presence of the two types of binding sites in the two subdivisions of the extended amygdala (medial and central) suggests that OT may be involved in reproductive functions associated with the medial portion of extended amygdala, and both OT and VP may be involved in autonomic regulation associated with the central portion of the extended amygdala.

Nomenclature

The amygdala sensu stricto may be divided into four groups, namely, the olfactory, basolateral, medial, and central groups, on the basis of cytoarchitectural and hodological grounds (de Olmos et al., 1985). The close homology between the BST and parts of the substantia innominata with medial and central groups of the amygdala has led to the notion of an "extended amygdala" (de Olmos et al., 1985; Alheid and Heimer, 1988; Alheid et al., 1995), a superstructure in which a striking symmetry can be observed between rostral and caudal poles, with a virtually symmetrical axis crossing the telencephalon at the level of substantia innominata. The posteromedial

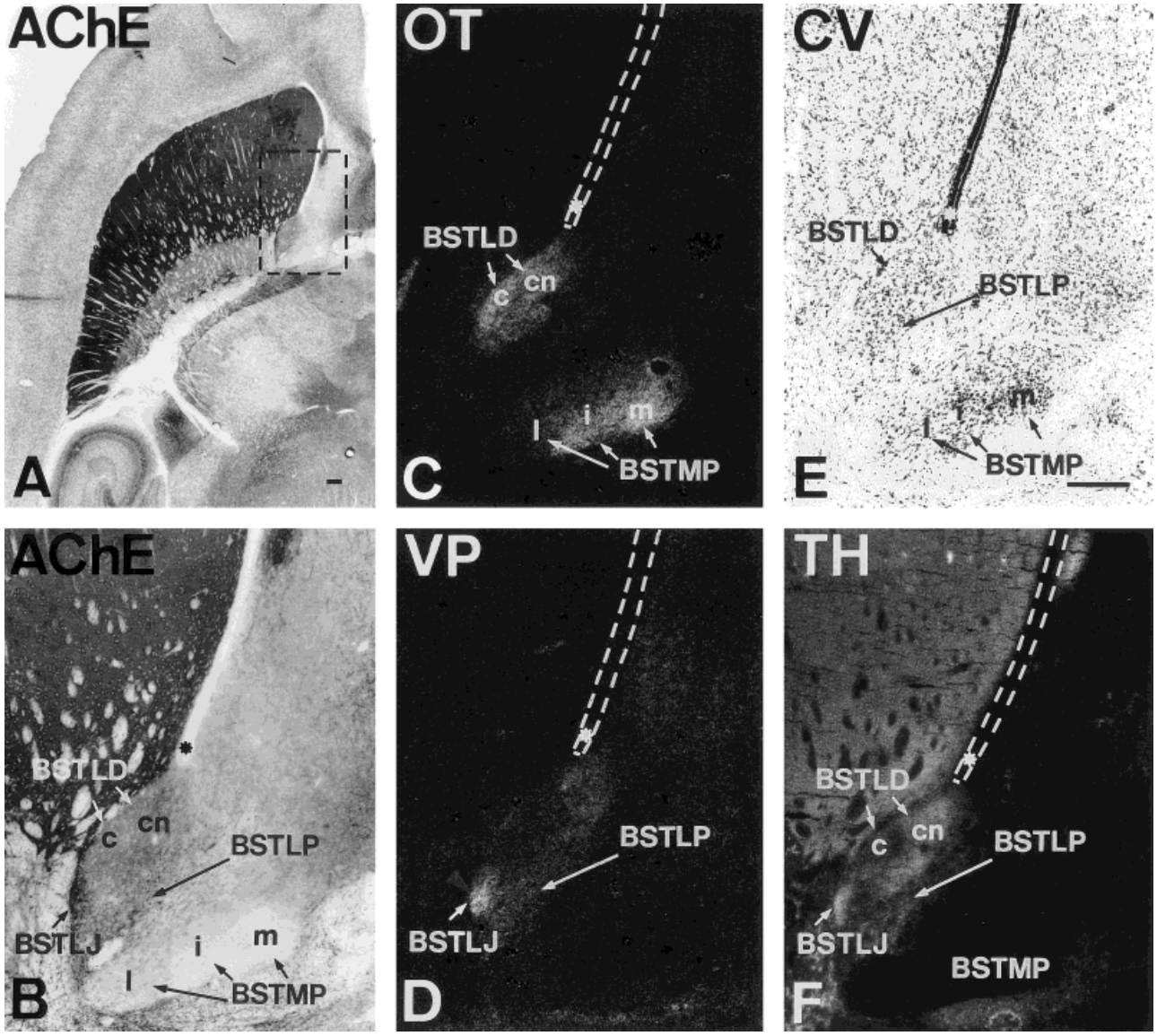

Fig. 6. Horizontal sections through the BST at a level just dorsal to the crossing of the ac. **A:** AChE-stained section interposed between the histoautoradiographs shown in C and D. **B:** Higher power photomicrograph of the boxed area in A. **C:** Same field as in B. Darkfield photomicrograph of a histoautoradiograph showing OT-binding sites in capsular (c) and central (cn) parts of the dorsal lateral BST (BSTLD) and in medial (m) and intermediate (i) parts of the posterior medial BST (BSTMP), the lateral part (l) being devoid of OT-binding sites. **D:** Same field as in B. Darkfield photomicrograph of a histoautoradiograph showing VP-binding sites in the juxtacapsular (BSTLJ) and posterior (BSTLP) parts of the lateral BST. **E:** Same field as in B. Brightfield photomicrograph of a cresyl violet (CV)-stained section adjacent to the histoautoradiograph shown in C. **F:** Same field as in B. Reverse photomicrograph of a section adjacent to the histoautoradiograph shown in D processed for TH immunodetection (TH); note that the density of TH immunoreactivity is higher in the central than in the capsular part of the dorsal lateral BST. Scale bars = 250 μm (E also applies to B–D,F).

part of the AcbSh was also considered to be closely related to this structure due to its unusual histochemistry and "nonstriatal" connections with the lateral hypothalamus and the brainstem (Heimer et al., 1991). This portion of the accumbens can be considered as a transitional area between the basal ganglia and the striatum. This may also be true for the most medial portions of the olfactory tubercle, although its connections are less known. Alheid et al. (1995) recently reexamined the anatomy of amygdala and the extended amygdala and proposed the nomenclature we used in this report. The main compartments described by de Olmos et al. (1985) are retained, but some structures have been withdrawn from the defined groups and left unclassified (e.g., the anterior amygdaloid area, amygdalostriatal area, intramedullary gray).

OT- and VP-binding site distribution in the extended amygdala

Table 2 indicates the "symmetrical subdivisions" of the medial and central extended amygdala exhibiting VP- or OT-binding sites along with their relative densities.

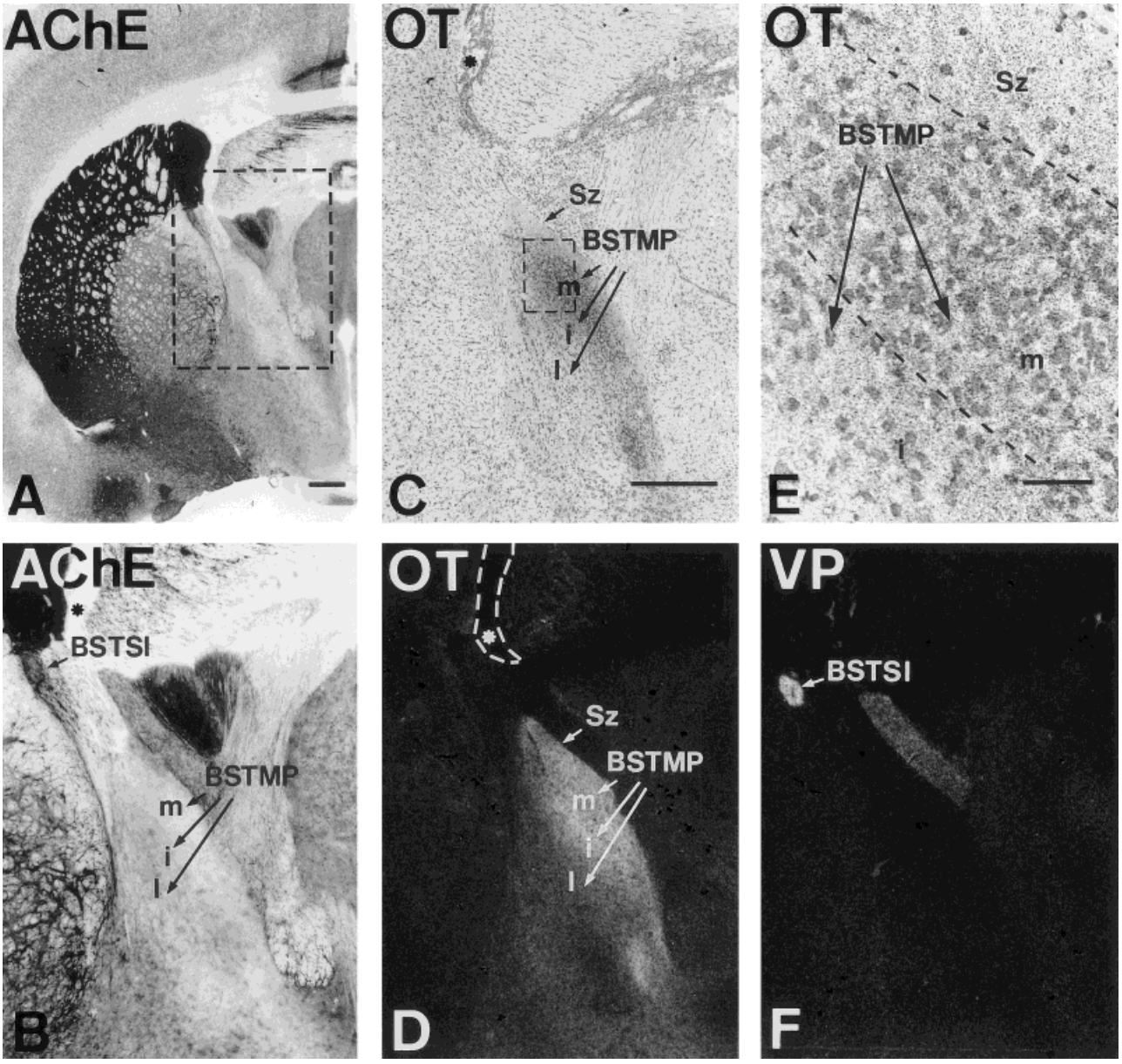

Fig. 7. Frontal sections through the posterior part of the BST. **A:** AChE-stained section interposed between the histoautoradiographs shown in C, D, and F. **B:** Higher power photomicrograph of the boxed area in A. **C,D:** Same field in as B. OT-binding sites in the medial (m) and intermediate (i) parts of the posterior medial BST (BSTMP) and its associated sparse cell zone (Sz); note the absence of labelling in the lateral part (l) of the posterior medial BST. **E:** Higher power micrograph of the boxed area in C showing silver grains over the Sz, which is almost devoid of neurons, and the medial dense-celled and intermediate more loosely arranged cell columns of the posterior medial BST. Dashed lines, from the top to the bottom, demarcate the boundaries between the sparse cell zone (Sz) and the medial (m) and intermediate (i) parts of the posterior medial BST. **F:** Same field as in B. VP-binding sites in the lateral part of the supracapsular BST (BSTSl). C, brightfield; D,F, darkfield photomicrographs. Scale bars = 500 µm in A,C (C also applies to B,D,F), 50 µm in E.

Medial portion of the extended amygdala. The medial portion of the extended amygdala is formed by the medial BST (five subdivisions), the medial division of the sublenticular extended amygdala, the medial part of the supracapsular BST, the intraamygdaloid portion of the BST, and the medial amygdaloid nucleus (six subdivisions).

The OT-binding sites were previously described in the principal encapsulated nucleus of the BST, which corresponds roughly to the medial and intermediate parts of the posterior medial BST and the posterior medial amygdaloid nucleus in the rat brain (Freund-Mercier et al., 1987, 1988; Krémarik et al., 1991, 1993). Our results confirm this OT-binding site localization and indicate that the density of binding sites is high in the medial part and moderate in the intermediate part of the posterior medial BST. The labelling in the medial amygdaloid nucleus is intense in the medial and lateral subnuclei of the posterodorsal part of this structure and is slightly less intense in the intermediate subnucleus. VP-binding sites occur solely in locations

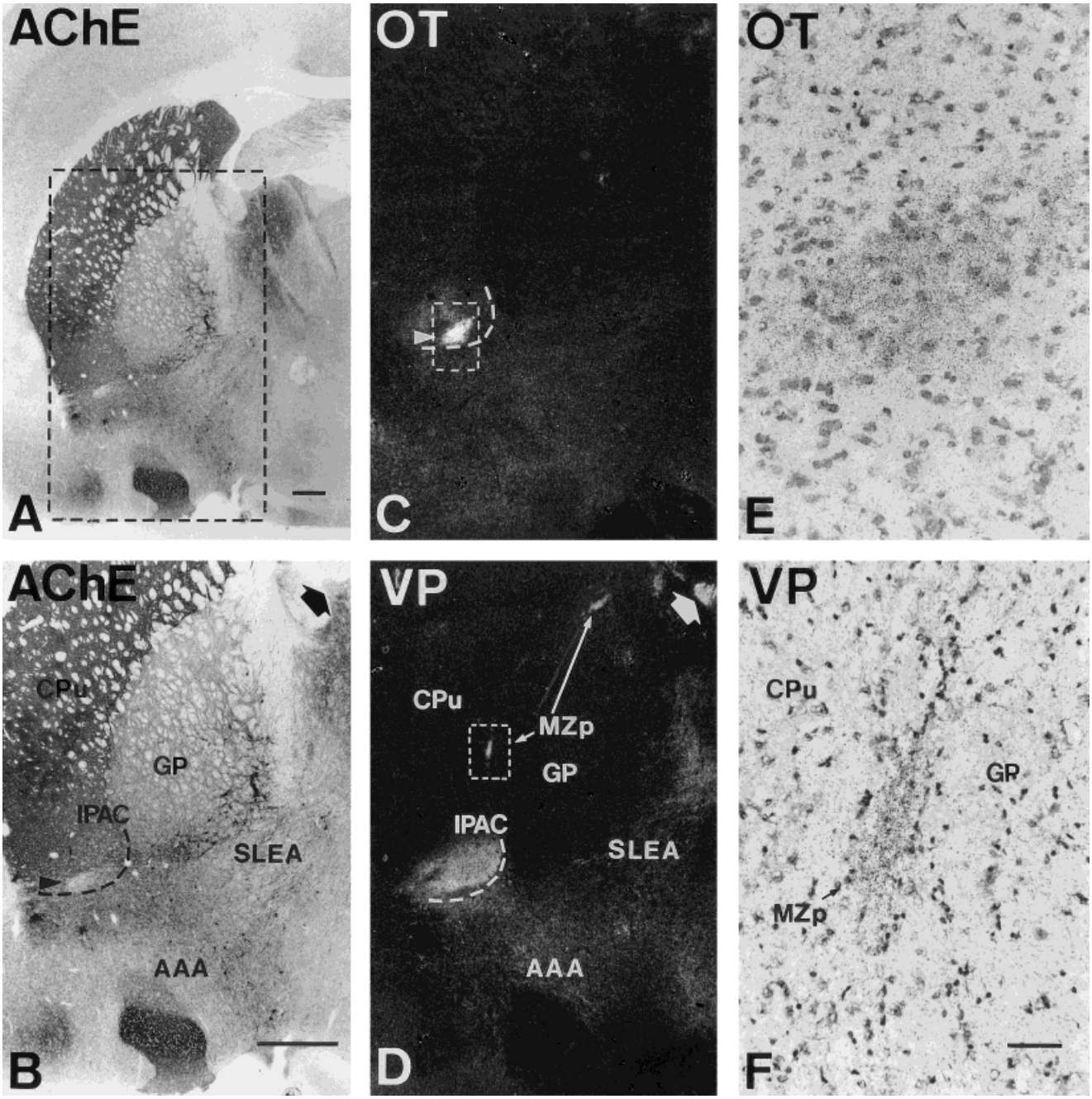

Fig. 8. Frontal sections through the anterior amygdala. **A:** AChE-stained section interposed between the histoautoradiographs shown in C and D. **B:** Higher power photomicrograph of the boxed area in A. **C:** Same field as in B. OT-binding sites in a small cluster (arrowheads in B and C) just ventral to the border of the IPAC. **D:** Same field as in B. VP-binding sites in the IPAC, the posterior marginal zone (MZp) interposed between the caudate putamen (CPu) and the globus pallidus (GP), the anterior amygdaloid area (AAA), and on the medial surface of the stria terminalis (thick arrow in B and D). **E:** Higher power photomicrograph of the boxed area in C showing silver grains over the cell cluster close to the interstitial nucleus. **F:** Higher power photomicrograph of the boxed area in D showing silver grains over the MZ. C,D, darkfield; E,F, brightfield photomicrographs of the same sections. Scale bars = 500 μm in A,B (B also applies to C,D), 50 μm in F (also applies to E).

that could correspond to the medial part of the supracapsular BST over cell pockets at the medial edge of the supracapsular stria terminalis. However, additional data are necessary to determine whether the labelled area is in the supracapsular BST. Moreover, it would be the only portion of the medial extended amygdala that contains VP-binding sites, because no VP-binding sites were detected in other portions of the medial extended amygdala.

Central portion of the extended amygdala. The central portion of the extended amygdala is formed by the lateral BST (five subdivisions), the central division of the sublenticular extended amygdala, the lateral part of the

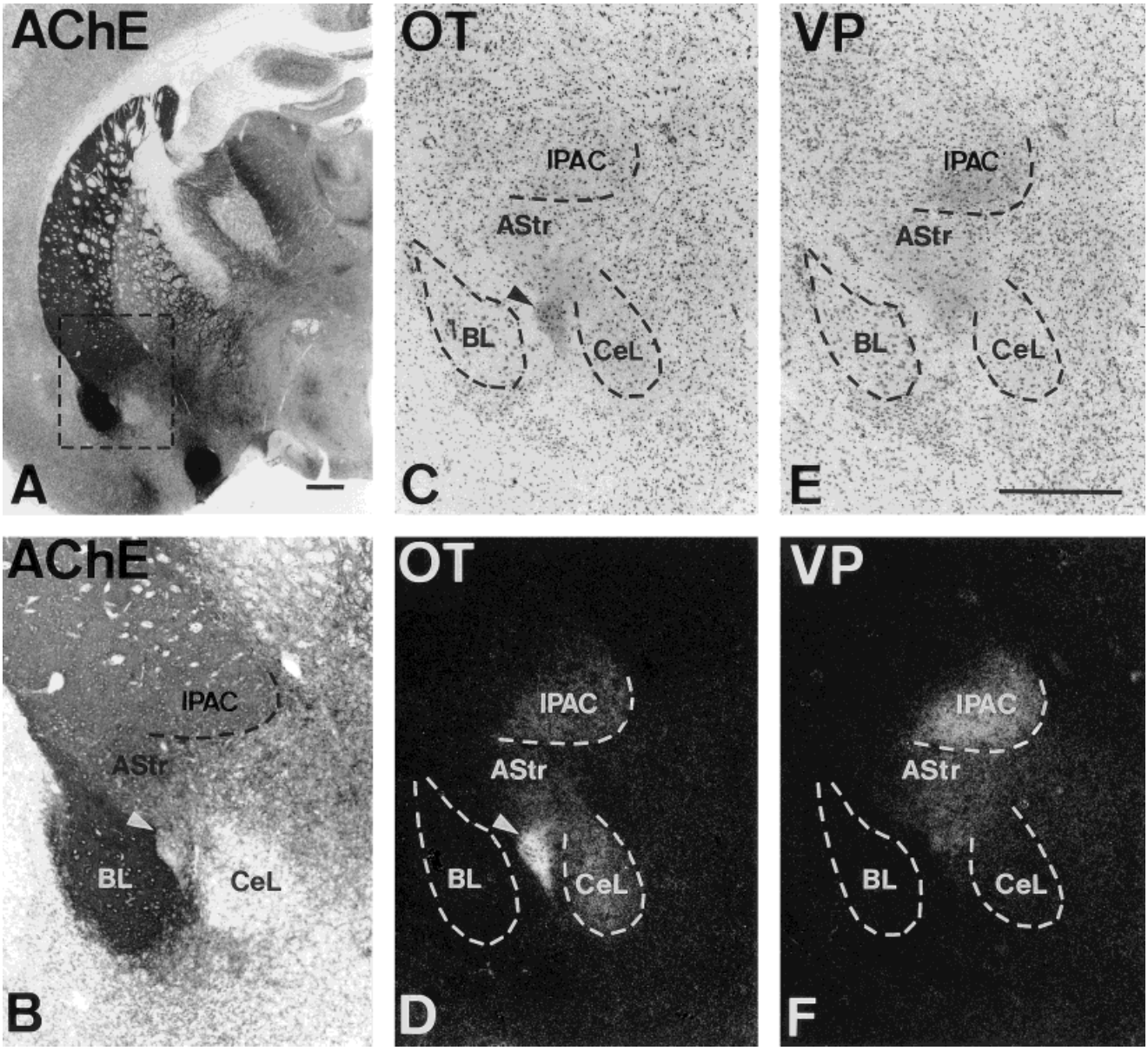

Fig. 9. Frontal sections through the anterior amygdala, posterior to the level shown in Figure 8. **A:** AChE-stained section interposed between the histoautoradiographs shown in C,D and E,F. **B:** Higher power photomicrograph of the boxed area in A. **C,D:** Same field as in B. OT-binding sites in the IPAC, the lateral subdivision of the central amygdaloid nucleus (CeL), and a cluster (arrowheads in B–D) situated between the central and basolateral (BL) amygdaloid nuclei. **E,F:** Same field as in B. VP-binding sites in the IPAC and the amygdalostriatal area (AStr). C,E, brightfield; D,F, darkfield photomicrographs of the same area. Scale bars = 500 μm (E also applies to B–D,F).

supracapsular BST, the IPAC (two subdivisions), and the central amygdaloid nucleus (five subdivisions). The posteromedial Acb and the medial olfactory tubercle may be included as a transition between the ventral striatum and the extended amygdala.

Binding sites for both OT and VP have been described in the Acb, mainly in posteromedial portions corresponding to the AcbSh (Freund-Mercier et al., 1987; Phillips et al., 1988; Tribollet et al., 1988; Krémarik et al., 1993). VP-binding sites display a patchy distribution, in accordance with previous studies (Phillips et al., 1988; Krémarik et al., 1993), whereas [^{125}I]OTA labelling is distributed more homogeneously, with no colocalization of the two ligands in the same area.

In the lateral BST, OT-binding sites were described in the oval nucleus (Krémarik et al., 1991, 1993), which corresponds to both the capsular and the central parts of the dorsal part of the lateral BST in our description. VP-binding sites were reported in the lateral BSTL of the rat, especially in its juxtacapsular part (Phillips et al., 1988; Tribollet et al., 1988; Krémarik et al., 1993). Our results state precisely their distribution in the posterior and ventral parts of the lateral BST as well as in the lateral part of supracapsular BST.

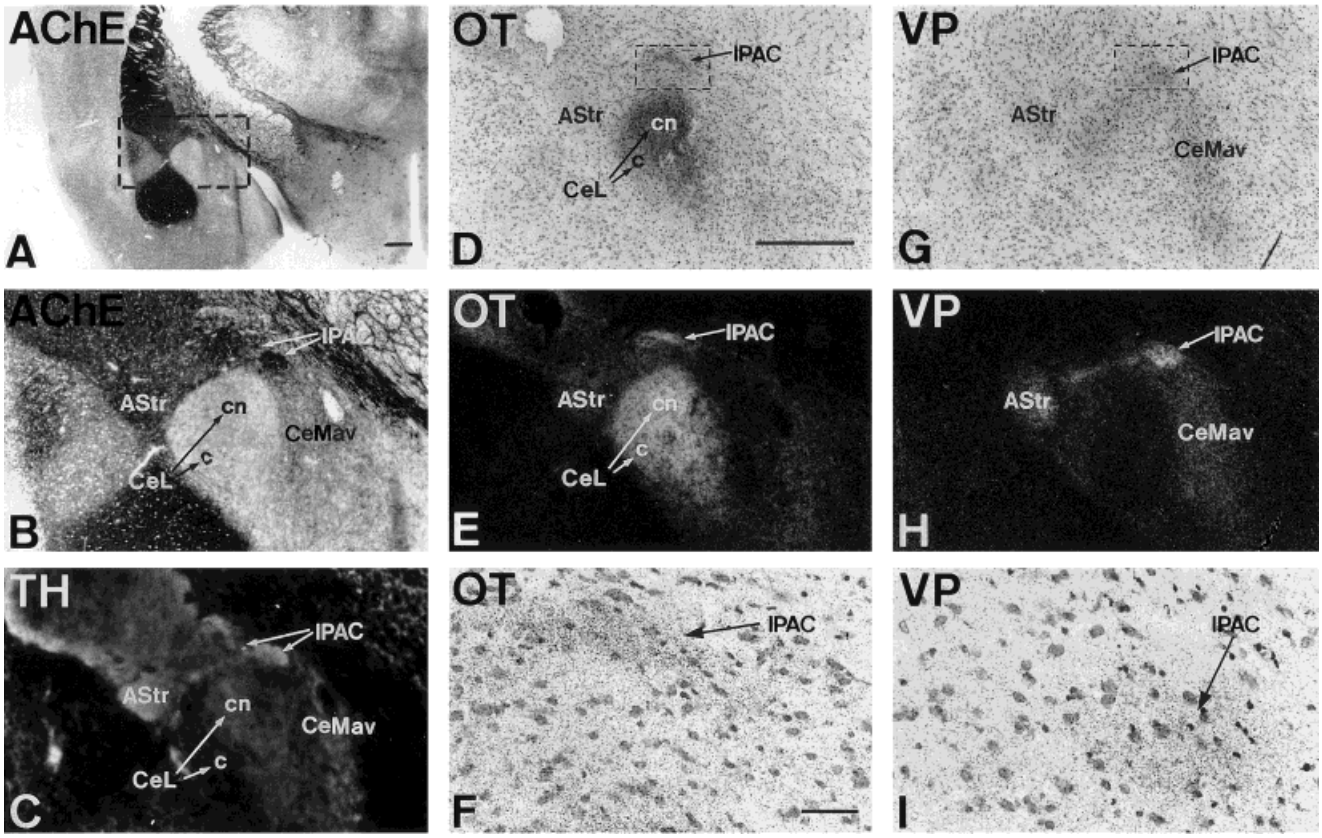

Fig. 10. Frontal sections through the central amygdaloid nucleus. **A:** AChE-stained section interposed between histoautoradiographs shown in D,E,F and G,H,I. **B:** Higher power photomicrograph of the boxed area in A. **C:** Same field as B: Reversed photomicrograph of a section adjacent to the histoautoradiograph shown in G,H,I processed for TH immunodetection (TH); note that the density of TH immunoreactivity is higher in the central (cn) than in the capsular (c) part of the lateral central nucleus (CeL) and the high density in a spot-like area possibly belonging to the posterior part of the IPAC. **D,E:** Same field as in B. OT-binding sites in the part of the IPAC and in capsular (c) and central (cn) parts of the lateral subdivision of the central amygdaloid nucleus (CeL). **F:** Higher power photomicrograph of the boxed area in D showing the silver grain accumulation over a small region of the posterior part of the IPAC. **G,H:** Same field as in B. VP-binding sites in the posterior part of the IPAC, the AStr, and the anteroventral part of the medial subdivision of the central amygdaloid nucleus (CeMav). **I:** Same field as in F. Higher power photomicrograph of the boxed area in G showing the silver grain accumulation over a spot-like region of the posterior part of the IPAC. D,G, brightfield; E,H, darkfield photomicrographs of the same area. Scale bars = 500 μm in A,D (D also applies to B,C,E,G,H), 50 μm in F (also applies to I).

In the central division of the sublenticular extended amygdala, which corresponds roughly to the dorsal substantia innominata described by Grove (1988a,b), neither OT- nor VP-binding sites have been described. Our results indicate slight [^{125}I]VPA labelling emerging from the dorsal anterior amygdaloid area. This labelling may appear ambiguous due to its low density, but, because this structure is an interconnecting cell corridor between the posterior lateral BST and the anteroventral part of the medial central amygdaloid nucleus (Alheid et al., 1995), structures with VP-binding sites, it is possible that the [^{125}I]VPA labelling in the central portion of the sublenticular extended amygdala is specific.

In the IPAC, both OT- and VP-binding sites occur in complementary elements of the structure, with little overlapping. [^{125}I]VPA strongly labels the part of the IPAC accompanying the posterior limb of the anterior commissure across the rostral face of the globus pallidus. The labelling continues caudally in the portion of the IPAC below the anterior commissure in the area termed the fundus striati by Paxinos and Watson (1986), where VP-binding sites were previously described (Freund-Mercier et al., 1988; Phillips et al., 1988; Johnson et al., 1993; Krémarik et al., 1993). OT-binding sites appear over a small area just below the [^{125}I]VPA-labelled portion of the IPAC, as reported by Krémarik et al. (1993). More caudally, both VP- and OT-binding sites were reported dorsal to the central amygdaloid nucleus (Freund-Mercier et al., 1987; Phillips et al., 1988; Krémarik et al., 1993) but previously were attributed to the amygdalostriatal transition area, which is located more laterally and caudally, according to the more recent analysis by Alheid et al. (1995). We suggest that this labelling is in the posterior part of the IPAC, given its apparent continuity with binding sites in the more rostral parts of the same structure.

In the amygdala, OT-binding sites were described previously in the central nucleus, mainly in its posterior half, whereas VP-binding sites were located in its anterior half (Dorsa et al., 1984; De Kloet et al., 1985; Freund-Mercier et al., 1987, 1988; Phillips et al., 1988; Tribollet et al., 1988). Histoautoradiographic study showed that [^{125}I]OTA

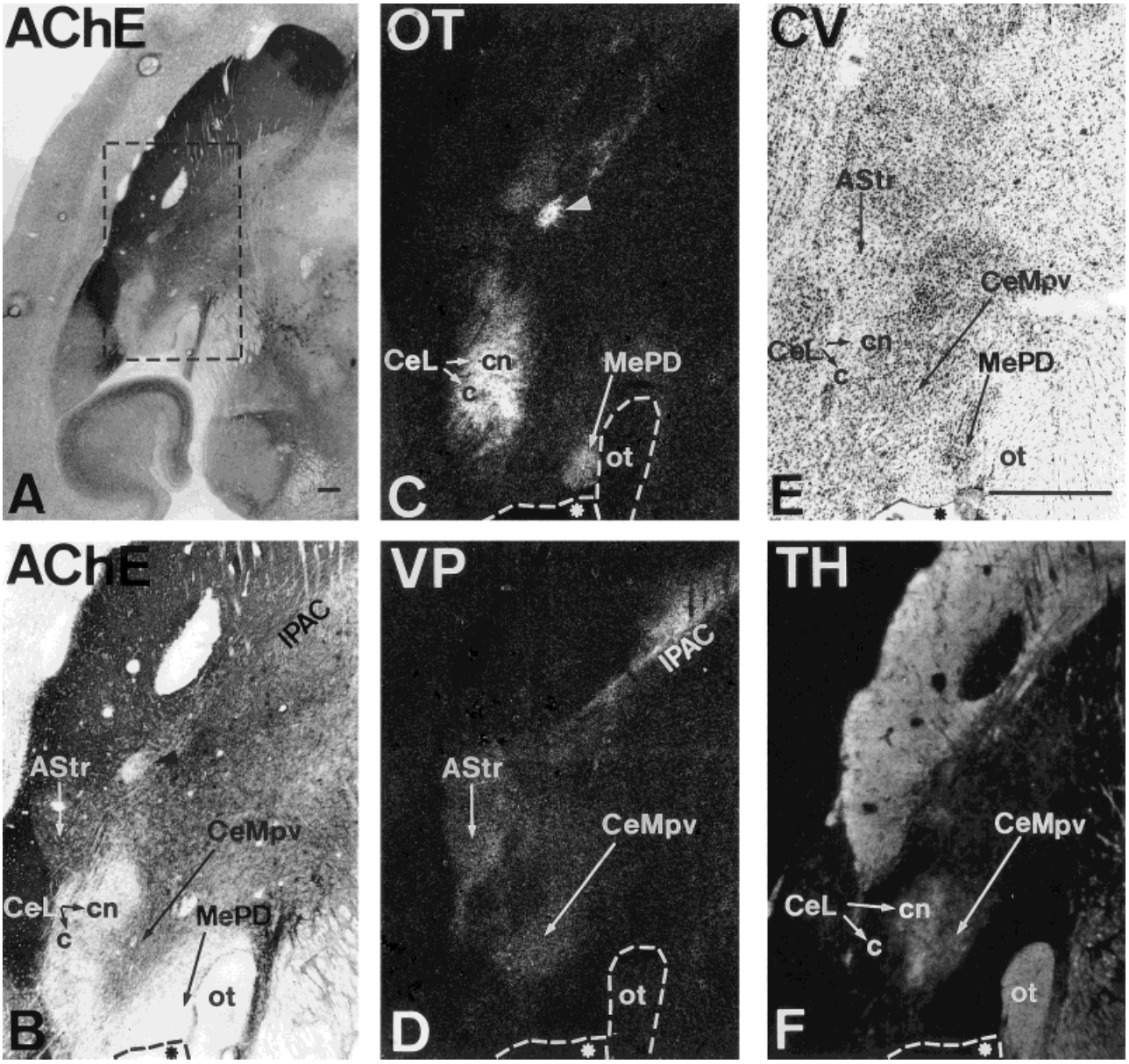

Fig. 11. Horizontal sections through the amygdala. **A:** AChE-stained section interposed between the histoautoradiographs shown in C and D. **B:** Higher power photomicrograph of the boxed area in A. **C:** Same field as in B. Darkfield photomicrograph of a histoautoradiograph showing OT-binding sites in capsular (c) and central (cn) parts of the lateral subdivision of the central amygdaloid nucleus (CeL), the posteromedial part of the medial amygdaloid nucleus (MePD), and a small cluster in the IPAC (arrowheads in B and C); this cluster is located in the same position as the labelled cluster shown in Figure 8C,E. **D:** Same field as in B. Darkfield photomicrograph of a histoautoradiograph showing VP-binding sites in the IPAC, in the AStr, and in the posteroventral part of the medial subdivision of the central amygdaloid nucleus (CeMpv). **E:** Same field as in B. Brightfield photomicrograph of a CV-stained section adjacent to the histoautoradiograph shown in C. **F:** Same field as in B. Reverse photomicrograph of an adjacent section to the histoautoradiograph shown in D processed for TH immunodetection; note that the density of TH immunoreactivity is higher in the central (cn) than in the capsular (c) parts of the lateral subdivision of the CeM. ot, Optic tract. Scale bars = 500 μm (E also applies to B–D,F).

labelling occurred exclusively in the lateral subdivision of the nucleus and that [^{125}I]VPA labelling targeted the fundus striati and the amygdalostriatal transition area rather than the central nucleus (Krémarik et al., 1993). Our results confirm the presence of OT-binding sites in both capsular and central parts of the lateral subdivision of the central amygdaloid nucleus and describe for the first time the presence of VP-binding sites in the medial subdivision of this nucleus. This labelling, which is more restricted to anteroventral and posteroventral parts, is detected due to the high affinity of the linear ligand for the V_{1a} receptor.

It is interesting to note that, among the six groups of "symmetrical" structures in the central extended amygdala described by Alheid et al. (1995), five display OT- and/or VP-binding sites, and the same type of binding site

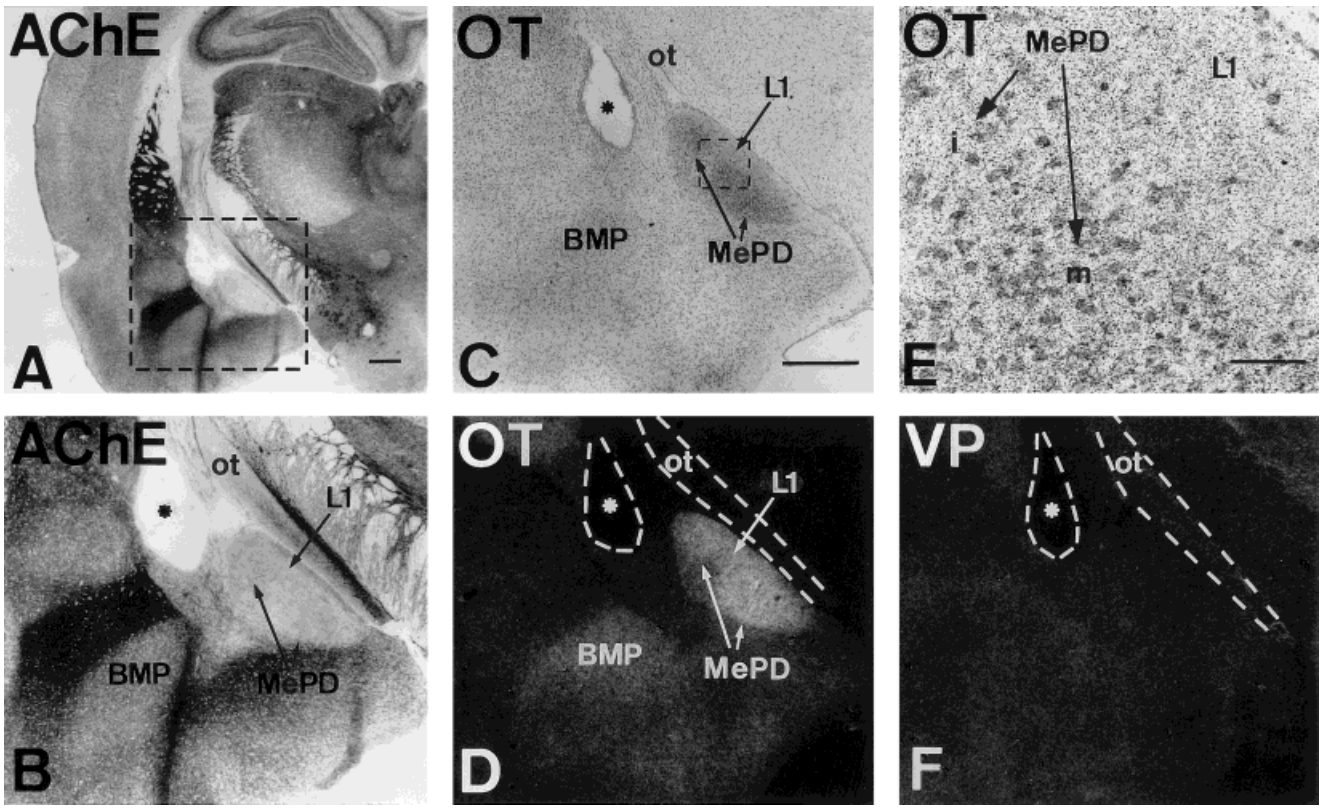

Fig. 12. Frontal sections through the posterior amygdala. **A:** AChE-stained section interposed between the histoautoradiographs shown in C, D, and F. **B:** Higher power photomicrograph of the boxed area in A. **C,D:** Same field as in B. OT-binding sites in the posterodorsal part of the medial amygdaloid nucleus (MePD) and in the associated layer 1 (L1) and posterior basomedial nucleus (BMP). **E:** Higher power photomicrograph of the boxed area in C showing silver grains over L1 almost devoid of neurons and the medial dense-celled (m) and the intermediate medium-sized cell (i) columns of the posterodorsal part of the medial amygdaloid nucleus. **F:** Same field as in B. No VP-binding sites were present at this level. C, brightfield; D,F, darkfield photomicrographs of the same area. ot, Optic tract. Scale bars = 500 µm in A,C (C also applies to B,D,F), 50 µm in E.

TABLE 2. Distribution of OT- and VP-Binding Sites in Symmetrical Structures of Medial and Central Divisions of the Extended Amygdala[1]

Rostral forebrain[2]		Sublenticular, supracapsular, and lateral forebrain (IPAC)[2]		Amygdala[2]	
Medial division of the extended amygdala					
BSTMPm	○○○○			MePDm,l	○○○
				MePV	None
BSTMPi	○○			MePDi	○○
BSTMPl	None	SLEAm and BSTSm	None	MeAD	None
BSTMA	None			BSTIA	None
BSTMV	None			MeAV	None
Central division of the extended amygdala					
BSTLI	None	BSTSl[3]	●●●	CeMad	None
BSTLP	●●	SLEAc and BSTSl[3]	●	CeMav	●●
			●●●		
BSTLV	●●	IPACm[3]	○ to ○○○	CeMpv	●●
			●●● to ●●●●		
BSTLDcn	○○○	BSTSl[3]	●●●	CeLcn	○○○○
BSTLDc	○○○			CeLc	○○○○
Acb posterior[3]	○○○	IPACl (ant)[3]	○ to ○○○	IPACl (post)[3]	○○○
	●●●●		●●● to ●●●●		●●●●

[1]Open and solid symbols represent the relative density of OT- and VP-binding sites, respectively, following the grouping shown in Table 1, with one symbol indicating low labelling, two symbols indicating moderate labelling, three symbols indicating dense labelling, and four symbols indicating very dense labelling. For abbreviations, see list.
[2]The table is organized in order to display on the same line "symmetrical" structures in rostral and caudal forebrain and intervening columns, following the parcellation of Alheid et al. (1995).
[3]Regions where OT- or VP-binding sites occurred only in parts of the structure and not in the whole structure.

is found in a given rostral-caudal pair of nuclei with roughly the same density (see Tables 1, 2). This reinforces the heuristic value of this proposition of symmetry in the analysis of the rat amygdala. In addition, the sixth group of symmetrical structures of the central extended amygdala, which is constituted by the Acb and the IPAC, is the only one in which both OT- and VP-binding sites are found (see Table 2) in a complementary fashion and even overlap-

ping in parts of the IPAC. This may reflect the presence of striatal elements mixed with neurons related to the extended amygdala and may confirm the position of the IPAC, posteromedial Acb, and also the medial olfactory tubercle as transition areas between the basal ganglia and the amygdala.

Other parts of the amygdala and unclassified cell groups. In their earlier descriptions of the amygdala, de Olmos et al. (1985) included the posterior basomedial nucleus in both the medial group and the basolateral group; included the anterior amygdaloid area in the olfactory group; and included the amygdalostriatal area, intercalated cell masses, and intramedullary gray in the central group. Alheid et al. (1995) placed the posterior basomedial nucleus in the basolateral group and placed the other structures in a category of "unclassified nuclei" that share only some common features with extended amygdala. Our results show the presence of OT-binding sites in the posterior basomedial nucleus and possibly in some paracapsular intercalated cell masses and the presence of VP-binding sites in the anterior amygdaloid area, the amygdalostriatal area, and the intramedullary gray. [^{125}I]OTA labelling was previously described in the posterior basomedial nucleus (Freund-Mercier et al., 1988; Krémarik et al., 1993). The term "amygdalostriatal area" was used to described the area above the central nucleus and between basolateral and central nuclei that displayed OT- and VP-binding sites (Phillips et al., 1988; Krémarik et al., 1993), but the most medial part of this amygdalostriatal area, rather, is related to the posterior portion of the IPAC, and the most ventrolateral part could encompass elements of the intramedullary gray; however, this latter group has been expanded from its earlier definition (de Olmos et al., 1985) to include a more widespread network of neurons occurring between many of the larger amygdaloid nuclei (de Olmos, 1990; Alheid et al., 1995). The [^{125}I]VPA labelling in the anterior amygdaloid area and the [^{125}I]OTA labelling of the small cell clusters along the basolateral nucleus have never been described. Finally, we describe for the first time VP-binding sites in parts of the posterior marginal zone, a structure that was described by Shu et al. (1988) and that seems to be a transitional zone between the striatum and the extended amygdala (Alheid et al., 1995). From this point of view, the presence of strong [^{125}I]VPA labelling continuous with that of the IPAC is relevant.

Although faint and diffuse [^{125}I]OTA labelling is found to cover the quasitotality of the amygdala, it is of particular interest that well-circumscribed [^{125}I]OTA- or [^{125}I]VPA-labelled nuclei belong to medial or central groups and an "unclassified group." This particular distribution suggests that OT and VP neuromodulation may represent an important functional feature of the extended amygdala.

In a general manner, some of the [^{125}I]OTA- or [^{125}I]VPA-labelled areas, especially small clusters, are still intriguing. The AChE levels observed in these areas ranged from poor to very dense, and examination of thionine-stained sections is still insufficient to attribute these clusters to a larger subdivision of the amygdala or basal ganglia except on the criterion of proximity. However, the fact that this labelling seemed to occupy a rather stable position within the forebrain strengthens the hypothesis that it does not represent an isolated case but, rather, some specialized structures of the forebrain, possibly related to the IPAC. The examination of neurochemical markers, such as peptide-like immunoreactivity, in the extended amygdala, along with OT- and VP-binding site detection, would be a useful follow-up to our study. Recently, Chen et al. (1996), using antibodies raised against Lyn protein, the product of the sarcoma protooncogene *c-lyn*, labelled preferentially the IPAC, including the same [^{125}I]VPA-enriched cluster; also, stripes of Lyn immunoreactivity were found in the accumbens nucleus and its zona limitans almost in the same position as VP-binding sites that occur at this level.

Taken together with our demonstration that OT- and VP-binding sites are found in the majority of extended amygdala subdivisions, these observations suggest that OT and VP receptors may be present on a coherent neuronal network within the basal forebrain, and their possible functional significance will be discussed in the context of medial and central portions of the extended amygdala.

Functional implications

OT in the medial extended amygdala. The medial extended amygdala is characterized by its extensive and reciprocal connections with the medial hypothalamus, especially with nuclei related to reproductive functions (Conrad and Pfaff, 1976a,b; Swanson and Cowan, 1979; Canteras et al., 1994, 1995). In addition, terminals from the accessory olfactory bulb are found in the molecular layer dorsomedial to the posterodorsal part of the medial amygdaloid nucleus and in the cell-sparse zone of the posterior medial BST (Scalia and Winans, 1975). The posterodorsal part of the medial amygdaloid nucleus and posterior medial BST may also be interconnected (Swanson and Cowan, 1979; Canteras et al., 1995).

Several neuropeptides have been described in fibers and cells in the medial extended amygdala (Roberts et al., 1982; Woodhams et al., 1983). Among them, VP was found in the lateral part of the posterior medial BST and the anterodorsal part of the medial nucleus amygdala (Caffé and Van Leeuwen, 1983; Van Leeuwen and Caffé, 1983). VP- and OT-immunoreactive fibers have been described in the posterior medial BST and the medial amygdaloid nucleus (Sofroniew, 1985); however, the origin of these fibers still remains unclear.

An number of important studies suggest that the medial extended amygdala may be involved in several aspects of reproductive functions. The posterior medial BST and the posterodorsal part of the medial amygdaloid nucleus were shown to be sexually dimorphic (Van Leeuwen et al., 1985; Simerly et al., 1989; Hines et al., 1992). Several studies showed that OT-binding sites in the posterior medial BST and in the posterodorsal part of the medial amygdaloid nucleus are steroid-sensitive in both males and females (Krémarik et al., 1991, 1995b).

The medial BST has been implicated in the regulation of the milk-ejection reflex in the female rat. Moos et al. (1991) demonstrated that OT injections in the BST facilitated the milk-ejection reflex by increasing either the frequency and/or the amplitude of OT neuron bursts recorded in the contralateral supraoptic nucleus. This effect may be mediated by OT receptors in the BST, namely, the posterior medial BST and the dorsal part of the lateral BST, because in vitro electrophysiological recording in slices showed that OT application increased the firing rate of BST neurons receiving a direct projection from the paraventricular hypothalamic nucleus (Ingram and Moos, 1992). Because the OT-binding sites in the dorsal part of the lateral

BST are steroid-insensitive (Krémarik et al., 1991), it is more likely that this effect is mediated by posterior medial BST OT receptors. Moreover, it has been shown that the posterior part of the BST is one of the rare forebrain regions that projects to both ipsi- and contralateral OT magnocellular hypothalamic nuclei (Sawchenko and Swanson, 1983).

In addition to the milk-ejection reflex, the posterior medial BST and the posterodorsal part of the medial amygdaloid nucleus have been implicated in several other aspects of reproductive behavior in both sexes, such as sexual and parental activities (Claro et al., 1995; Flanagan-Cato and McEwen, 1995; Numan and Numan, 1995). Finally, comparative studies have revealed that OT may play a role in social attachment in different vole species and that the medial BST may be a site of OT action (Insel, 1992; Williams et al., 1994). Thus, it seems that OT-binding sites in the medial extended amygdala may represent functional receptors that are largely implicated in reproductive functions, even if their role in males remains unclear.

In addition, several studies have suggested that the medial amygdaloid nucleus and the medial BST could be involved in core temperature regulation during fever and that endogenous VP synthesized in the medial extended amygdala may act as an antipyretic. A population of BST neurons increased their firing rate during fever (Mathieson et al., 1989), and VP injection into the medial amygdaloid nucleus suppressed fever induced by intracerebroventricular injection of prostaglandin E_1 (Federico et al., 1992). However, because we did not find any VP-binding sites in the medial amygdaloid nucleus, it is more likely that the antipyretic effect of VP in this case could be mediated by an interaction of VP with OT receptors.

OT/VP in the central extended amygdala. The central extended amygdala receives extensive projections, carrying sensory and autonomic-related information from numerous brain structures, including telencephalic areas and the brainstem (Krettek and Price, 1978; Beckstead et al., 1979; Ottersen, 1980, 1981; Saper and Loewy, 1980; Turner and Zimmer, 1984; Grove, 1988a; Turner and Herkenham, 1991). Efferent pathways from the central extended amygdala are more specific and mainly reach autonomic-related areas in the hypothalamus (Krettek and Price, 1978; Grove, 1988b; Gray et al., 1989; Heimer et al., 1991) and the brainstem (Gray and Magnuson, 1987; Grove, 1988b; Moga et al., 1989; Rizvi et al., 1991). In addition to extrinsic connections, numerous associative connections occur in the central extended amygdala (Swanson and Cowan, 1979; Grove, 1988a,b; Heimer et al., 1991; Sun and Cassel, 1993).

The central amygdaloid nucleus and the lateral BST display a characteristic organization of connections. The majority of cortical and thalamic afferents reach primarily the lateral subdivision of the central amygdaloid nucleus and the lateral BST (Sun et al., 1994; Moga et al., 1995), whereas efferents to the lower brainstem originate mainly from the medial subdivision of the central amygdaloid nucleus and its rostral counterparts, the posterior, intermediate, and ventral parts of the lateral BST (Danielsen et al., 1989). Neurochemical studies revealed the presence of numerous γ-aminobutyric acid (GABA)ergic neurons in the lateral subdivision of the central amygdaloid nucleus and in the dorsal part of the lateral BST, which projected exclusively into the central extended amygdala, to the medial subdivision of the central amygdaloid nucleus and to the posterior part of the lateral BST (Sun and Cassel, 1993).

Cells in the central extended amygdala synthesize numerous neuropeptides, especially in the lateral subdivision of the central amygdaloid nucleus and in the dorsal part of the lateral BST, whereas other parts of the central extended amygdala display fibers with fewer neurons (Roberts et al., 1982; Swanson et al., 1983; Woodhams et al., 1983; Lind et al., 1985; Ju et al., 1989). A moderate VP and OT innervation has been described in the different regions of the extended amygdala, with VP fibers arising mainly from the medial BST (De Vries et al., 1985; Sofroniew, 1985). The origin of OT fibers remains unknown, because the paraventricular hypothalamic nucleus seems to be the only origin of the brain OT innervation (De Vries and Buijs, 1983), and no projections from this nucleus to the central extended amygdala could be demonstrated.

Electrophysiological results indicated that OT was able to alter electrical activity of amygdala neurons. Condés-Lara et al. (1994) showed that, in vivo, nearly 50% of recorded neurons in the amygdala, mostly in the central nucleus, responded to iontophoretic application of OT by an increase of their firing rate discharge, suggesting the presence of functional receptors.

An impressive number of studies have implicated the amygdala, particularly the central nucleus, and the BST in the regulation of behavior and autonomous/endocrine functions (for review, see Davis, 1992). Increasing evidence suggests that the central nucleus may function as the main output of the amygdala to initiate autonomic responses to conditioning (Davis et al., 1994) and that the GABAergic neurons in the lateral subdivision of the central amygdaloid nucleus may play a crucial role by filtering the inputs (Sun and Cassel, 1993). Several neuropeptides may act at this level, including OT and VP, which can modify autonomic and behavioral functions after central injections (for review, see Richard et al., 1991). Roozendaal et al. (1993) showed that VP (20 pg) injected onto the central nucleus induced bradychardia, whereas OT (2 ng) induced tachychardia. Thus, it seems that OT and VP had opposite effects in the central amygdaloid nucleus, consistent with our finding that OT- and VP-binding sites exist in different subdivisions of this nucleus.

OT was shown to affect several aspects of drug addiction (for review, see Sarnyai and Kovács, 1994) that are situations in which the central amygdaloid nucleus and the Acb are suggested as participants. OT injection in the Acb inhibited morphine tolerance (Kovács et al., 1984), and OT receptor antagonist injection in the central nucleus facilitated it (Sarnyai et al., 1988), suggesting an inhibitory role for endogenous OT. It is also argued that OT effects related to drugs could be due to an interaction with dopaminergic neurotransmission in the mesolimbic pathway (Sarnyai and Kovács, 1994), which heavily innervates the central extended amygdala (Freedman and Cassel, 1994).

The role of intervening areas of the central extended amygdala (sublenticular extended amygdala, IPAC, and supracapsular BST) as well as associated areas (amygdalostriatal area, intercalated cell masses, anterior amygdaloid area, and marginal zone) still remains unclear. However, it is likely that, with respect to the similarity of

connections, they may share some of the functions suggested for the central amygdala and the lateral BST, with similar roles for OT and VP receptors. Interestingly, Krémarik et al. (1995a) showed that a VP injection in the fundus striati of anesthetized rats increased the mean arterial pressure, suggesting an involvement of IPAC VP receptors in the regulation of cardiovascular functions.

CONCLUSIONS

The present study reveals the presence of OT- and VP-binding sites in the extended amygdala of the rat with an organized topography. The approach used, which combined radioligand receptor autoradiography with a precise histochemical delineation of structural boundaries, indicated that OT- and VP-binding sites exist throughout the medial and central groups of the amygdala but not in the basolateral and cortical groups, which do not belong to the extended amygdala. This distribution within symmetrical structures of the amygdala, the BST, the IPAC, and the Acb reinforces the concept of the extended amygdala and suggests that OT- and VP-binding sites constitute an important feature of this anatomical entity. OT- and VP-binding sites are distributed in a complementary fashion on different elements, where OT and VP may influence reproductive (medial extended amygdala), autonomic (central extended amygdala), and probably other functions via functional receptors.

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