

Basal Ganglia Organization in Amphibians: Chemoarchitecture

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ABSTRACT

Recent studies dealing with the investigation of the afferent and efferent connections of the basal ganglia of amphibians have revealed many similarities with basal ganglia structures of amniotes. In a further step, the chemoarchitecture of basal ganglia of the frog *Rana perezi* has been investigated. For use as main markers of amphibian basal ganglia structures, antibodies against tyrosine hydroxylase, substance P, and enkephalin were selected. Moreover, the distributions of nitric oxide synthase (nicotinamide adenine dinucleotide phosphate-diaphorase histochemistry), calretinin, dopamine- β -hydroxylase, choline acetyltransferase, mesotocin, vasotocin, somatostatin, neuropeptide Y, neuropeptide FF, and serotonin were studied to corroborate a comparison with both basal ganglia and amygdaloid structures of amniotes. On the basis of connections and chemoarchitecture, a striatum proper, nucleus accumbens, dorsal and ventral pallidum, bed nucleus of the stria terminalis, and amygdaloid complex have been identified. Accordingly, a new terminology is proposed that is in line with our current understanding of basal ganglia organization in amphibians. *J. Comp. Neurol.* 392:285-312, 1998. © 1998 Wiley-Liss, Inc.

Indexing terms: striatum; nucleus accumbens; pallidum; amygdala; bed nucleus of the stria terminalis

The organization of the basal ganglia (BG) in reptiles, birds, and mammals has been demonstrated to share many key features with respect to chemoarchitecture and connections (Reiner et al., 1984a; Parent, 1986; Smeets, 1992; Heimer et al., 1995; Medina and Reiner, 1995; Parent and Hazrati, 1995). Histochemical and tract-tracing studies have demonstrated the presence of two distinct dorsal and ventral striatal regions in all amniotes studied thus far. Moreover, dorsal and ventral pallidal structures that receive their major inputs from the dorsal and ventral striatum, respectively, have also been widely recognized in amniotes. Immunohistochemical techniques have been proven to be of particular value for comparing chemical properties of the dorsal and ventral striatopallidal systems among amniotes. Thus, the neurochemical characterization of BG inputs and outputs as well as intrinsic neurochemical markers allow the identification of subdivisions within the dorsal and ventral striatopallidal systems. For example, the striatum (Str) and the nucleus accumbens (Acc) exhibit a considerably stronger dopaminergic innervation than most surrounding cortical and

subcortical areas (Björklund and Lindvall, 1984; Reiner, 1994; Reiner et al., 1994; Smeets, 1994). On the other hand, enkephalin (ENK) and substance P (SP) are present in striatal projection neurons, and the patterns of distribution of these substances have been used to identify distinct pallidal compartments (Haber and Elde, 1981; Haber and Nauta, 1983; Reiner et al., 1983, 1984b; Reiner, 1987; Russchen et al., 1987).

Like in the case of amniotes, abundant catecholamines, opioid peptides, and tachykinins have been demonstrated in the basal telencephalon of amphibians (Inagaki et al., 1981a,b; Taban and Cathieni, 1983; Merchenthaler et al., 1989; González and Smeets, 1994). However, no detailed

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information has been provided to support compartmentalization within the amphibian basal telencephalon. Only the distinction between dorsal and ventral striatal regions (i.e., Str proper and Acc) was reported in previous immunohistochemical studies in amphibians (González and Smeets, 1991, 1992a,b, 1993, 1994; González et al., 1993; Maderdrut et al., 1996; Muñoz et al., 1996). Recent studies on the connectivity of the amphibian Str and Acc have further supported a functional subdivision of the basal forebrain of amphibians (Marín et al., 1997a,b,d). However, distinct pallidal structures have not been recognized in any cytoarchitectonic study of the amphibian telencephalon, although the demonstration of extensive, intratelencephalic striatal projections has suggested a further compartmentalization of the amphibian basal forebrain (Marín et al., 1997b).

In the present report, (immuno)histochemical techniques have been used in addition to classical cell-staining procedures to study the chemoarchitecture of the basal telencephalon in the frog, *Rana perezii*. Boundaries of cell groups and related fiber zones in the BG are defined by their chemical nature. Tyrosine hydroxylase (TH), ENK, and SP immunoreactivities were selected as main markers of the amphibian BG components. In addition, the distribution of nitric oxide synthase [nicotinamide adenine dinucleotide phosphate-diaphorase (NADPHd) histochemistry; see Muñoz et al., 1996; González et al., 1996] and immunoreactivities for calretinin (CR), vasotocin (AVT), mesotocin (MST), dopamine- β -hydroxylase (DBH), choline acetyltransferase (ChAT), somatostatin (SOM), neuropeptide Y (NPY), neuropeptide FF (NPFF), and serotonin (5-HT) are also reported and discussed. Evidence is presented to support compartmentalization in the basal telencephalon of the frog, in which striatal and pallidal components, together with the amygdaloid complex and the bed nucleus of the stria terminalis (BST), are tentatively recognized. It should be noted that the analysis of the purely septal regions (including the diagonal band of Broca) is not reported here, because it will be the subject of a separate study.

MATERIALS AND METHODS

In the present study, the brains of 110 adult frogs (*R. perezii*) were used. The animals were obtained from the laboratory stock of the Department of Cell Biology, University Complutense of Madrid. The original research reported herein was performed under animal care guidelines established by Spanish Royal Decree 223/1988.

For a cytoarchitectonic analysis of the basal telencephalon, cresyl violet-stained series were available that were cut either transversely, horizontally, or sagittally at a thickness of 30 μ m. The histochemical and immunohistochemical techniques used in this study are described below. For each marker, series of brain sections cut in the three main brain planes were available. Complete series of brain sections were stained either for one marker or, alternately, for two markers. Thus, full information about a certain chemical substance was obtained not only for all levels throughout the forebrain but also for its relative position to other neuromarkers. Finally, when the set of antibodies allowed it, double labeling was made on the same sections to study possible interactions in the BG. The nomenclature used here is largely the same as that of our previous studies on the amphibian BG (Marín et al., 1997a,b). However, some new terms and abbreviations are introduced and explained.

Immunohistochemical procedures

For the immunohistochemical procedures used, animals were anesthetized with an overdose of a 0.3% solution of tricaine methane sulphonate (MS222; Sandoz, Basel, Switzerland) and were transcardially perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains were removed and post-fixed for 2–3 hours in the same fixative. They were subsequently immersed in a 30% sucrose solution in PB at 4°C, embedded in a 15% gelatin and 30% sucrose solution in PB, and stored for 5 hours in a 4% formaldehyde solution at room temperature. Brains were cut on a freezing microtome at 40 μ m, and the sections were collected in Tris-buffered saline (TBS; 0.05 M, pH 7.6). All antibodies were diluted in 5–10% normal serum of the species in which the secondary antibody was raised in PB with 0.1% Triton X-100 (Sigma, St. Louis, MO) and 2% bovine serum albumin (BSA; Sigma). The sections were processed according to the peroxidase-antiperoxidase (PAP) technique (Sternberger, 1979) in a series of incubations with the antisera described below.

TH immunohistochemistry. For TH immunohistochemistry, mouse anti-TH (Inctar, Stillwater, MN) was used diluted 1:1,000, for 60 hours at 4°C; goat anti-mouse (Dakkopatts, Copenhagen, Denmark) was used diluted 1:100, for 1 hour; and mouse PAP complex (Chemicon, Temecula, CA) was used diluted 1:600, for 90 minutes.

ChAT immunohistochemistry. For ChAT immunohistochemistry, goat anti-ChAT serum (Chemicon) was used diluted 1:100, for 60 hours at 4°C; rabbit anti-goat serum

Abbreviations

AA	anterior amygdaloid area	Lpv	lateral pallium, ventral division
Acc	nucleus accumbens	Ls	lateral septum
aob	accessory olfactory bulb	MeA	medial amygdala
Apl	amygdala, pars lateralis	Mgd	magnocellular preoptic nucleus, dorsal part
Apm	amygdala, pars medialis	Mgv	magnocellular preoptic nucleus, ventral part
BST	bed nucleus of the stria terminalis	ml	mitral cell layer of the olfactory bulb
CeA	central amygdala	mob	main olfactory bulb
DB	nucleus of the diagonal band of Broca	Mp	medial pallium
DP	dorsal pallidum	Ms	medial septum
dStr	dorsal striatum	pe	postolfactory eminence
Ea	anterior entopeduncular nucleus	POa	anterior preoptic area
gl	glomerular layer of the olfactory bulb	Str	striatum
igl	internal granular layer of the olfactory bulb	v	ventricle
LA	lateral amygdala	VP	ventral pallidum
lfb	lateral forebrain bundle	vStr	ventral striatum
Lp	lateral pallium		

(Chemicon) was used diluted 1:50, for 1 hour, and goat PAP complex (Chemicon) was used diluted 1:600, for 2 hours.

CR, DBH, ENK, MST, NPFF, NPY, SOM, SP, AVT, and 5-HT immunohistochemistry. Immunohistochemical procedures for CR, DBH, ENK, MST, NPFF, NPY, SOM, SP, AVT, and 5-HT were carried out as follows: 1) incubation for 24–72 hours at 4°C in the following solution of each primary antibody: rabbit anti-CR (Chemicon) diluted 1:1,000, rabbit anti-DBH (provided by Dr. M. Goldstein, New York University, New York, NY) diluted 1:300, rabbit anti-Leu-ENK (Genosys Biotech, Cambridge, United Kingdom) diluted 1:1,000, rabbit antiisotocin (anti-IST; provided by Dr. J.M. Guerné, University of Strassbourg, Strassbourg, France) diluted 1:2,000, rabbit anti-NPFF (provided by Dr. Yang, NIMH, Bethesda, MD) diluted 1:1,000, rabbit anti-NPY (gift from Dr. J.D. Mikkelsen, University of Copenhagen, Copenhagen, Denmark) diluted 1:1,000, rabbit anti-SOM (Incstar) diluted 1:1,000; rabbit anti-SP (CRB, Cambridge, United Kingdom) diluted 1:1,000, rabbit anti-AVT (provided by Dr. R.M. Buijs; Netherlands Institute for Brain Research, Amsterdam, The Netherlands; Thepen et al., 1987) diluted 1:5,000, and rabbit anti-5-HT (Incstar) diluted 1:1,000; 2) incubation for 1 hour in swine anti-rabbit (Dakopatts) diluted 1:50; and 3) incubation for 90 minutes in rabbit PAP complex (Dakopatts) diluted 1:600.

In all cases, after rinsing, the sections were incubated with 0.5 mg/ml 3,3'-diaminobenzidine (DAB; Sigma) with 0.01% H₂O₂ in PB for 10–15 minutes. In most cases, visualization of the immunostaining was improved by processing the sections with nickel-enhanced DAB (0.05% DAB, 0.01% H₂O₂, 0.04% ammonium-nickel sulphate in PB; for details, see Adams et al., 1981). ChAT immunohistochemistry was visualized according to the glucose-oxidase method (Shu et al., 1988; for details, see Marín et al., 1997e). After rinsing, the sections were mounted on glass slides, dried overnight, and coverslipped with Entellan (Merk, Darmstadt, Germany).

Controls for the immunohistochemistry experiments included 1) staining some selected sections with preimmune mouse serum (1:1,000 for TH), goat serum (1:100 for ChAT), or rabbit serum (1:300 for DBH; 1:1,000 for CR, ENK, NPFF, NPY, SOM, and SP immunohistochemistry; 1:2,000 for 5-HT and IST immunohistochemistry; and 1:5,000 for AVT immunohistochemistry) instead of the primary antibody; and 2) controls in which either the primary antibody, the secondary antibody, or the PAP complex was omitted. Previous studies in amphibians, reptiles, birds, and mammals (see, e.g., González et al., 1993; Bailhache and Balthazart, 1993; Font et al., 1995; Smith et al., 1996) have shown that the monoclonal antibody against TH (Incstar) specifically labels catecholaminergic neurons and fibers. The antibody is believed to have wide species cross reactivity, because it recognizes an epitope in the midportion of the TH molecule where extensive species homology exists. The anti-DBH antiserum (Dr. M. Goldstein) was raised in rabbits against purified bovine adrenal DBH and has been fully characterized. No cross reaction with antibodies against other catecholamine-synthesizing enzymes was observed (Goldstein et al., 1972, 1973). The DBH antiserum has been applied successfully to the brains of several nonmammalian species (Smeets and Steinbusch, 1989, 1990; González and Smeets, 1993). The antiserum against CR (Chemicon) has been characterized in mammals (Winsky et al., 1989).

Moreover, the distribution of CR-immunoreactive (CRi) cells and fibers observed in the brain of *R. perezi* (present study) is essentially the same as that previously obtained with another CR antiserum (Puelles et al., 1995). The specificity of the antiserum against ChAT (Chemicon) has been tested (Shiromani et al., 1987; Medina and Reiner, 1994; Grosman et al., 1995), and the overall distribution of ChATi neurons and fibers in the brain of amphibians largely resembles that observed in amniotes (Marín et al., 1997e). The specificities of the antisera against [Leu⁵]ENK (Genosys Biotech) and SP (CRB) have been tested in the brains of nonmammalian and mammalian species (Russchen et al., 1987; Voorn et al., 1989; Font et al., 1995). In addition, the results obtained with these antisera are in agreement with those of previous studies in amphibians by means of fully characterized antisera (Inagaki et al., 1981a; Taban and Cathieni, 1983; Merchenthaler et al., 1989). However, the antiserum against [Leu⁵]enkephalin (Genosys Biotech) appears to cross react with [Met⁵]enkephalin, and the specificity of the SP antiserum against other tachykinins has not been completely characterized. The specificity of the antisera against arginine-AVT and IST was tested previously (Thepen et al., 1987). It was proven that the IST antiserum cross reacts with amphibian MST (Thepen et al., 1987; González and Smeets, 1992). The antiserum against NPFF raised in rabbit (Dr. Yang) has been characterized in mammals, in which it does not cross react with other neuropeptides (Majane and Yang, 1987). For details about the specificity of the antiserum against NPY, the reader is referred to the study of Mikkelsen and O'Hare (1991). The distribution of NPY and SOM immunoreactivities in the brain of *R. perezi*, as revealed in the present study, largely resembles that described previously in other amphibian species with different immunohistochemical procedures (Inagaki et al., 1981b; Danger et al., 1985; Lázár et al., 1993). The structure of SOM has been shown to be similar if not identical among different classes of vertebrates (King and Millar, 1979). Finally, the antiserum against 5-HT has been tested previously in several nonmammalian species and does not cross react with other monoamines (Vecino and Ekström, 1990; Stuesse et al., 1992; Liu and Debski, 1995).

NADPHd histochemistry

For NADPHd histochemistry, the animals were anesthetized in MS222 (Sandoz) and subsequently perfused transcardially with a 0.9% saline solution followed by a fixative containing 4% paraformaldehyde and 15% saturated picric acid in 0.1 M PB, pH 7.4. The brains were dissected out and further fixed in the same fixative for 6–8 hours at room temperature. They were subsequently immersed in a 30% sucrose solution in PB at 4°C, embedded in a 15% gelatin and 30% sucrose solution in PB, and stored for 5 hours in a 4% formaldehyde solution at room temperature. On a freezing microtome, 30- μ m or 40- μ m sections were cut and collected in PB. Free-floating sections were incubated in a medium containing 1 mM β -NADPH, 0.8 mM nitroblue tetrazolium, and 0.06% Triton X-100 (Sigma) in 0.1 M PB, pH 7.6, at 37°C for 1–2 hours. After incubation, the sections were rinsed thoroughly in PB, mounted on gelatin-coated glass slides, and, after drying overnight, were coverslipped. Selected sections were counterstained with 1% neutral red. In some cases, after rinsing, the sections

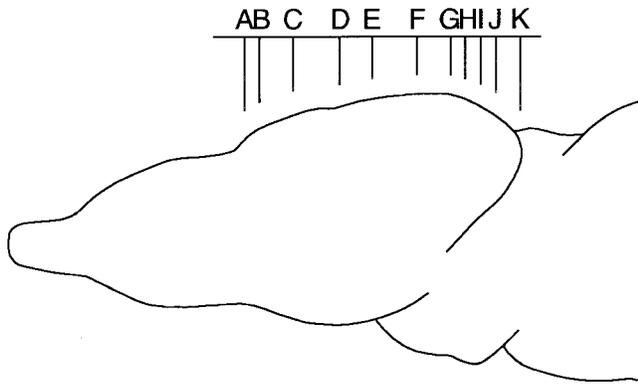


Fig. 1. Lateral view of the forebrain of *Rana perezi*. The letters on the top refer to the levels of transverse sections shown in Figure 2.

were also processed for TH, SP, ENK, ChAT, CR, or NPF immunohistochemistry as described above.

RESULTS

To study the main components of the telencephalic BG and adjacent structures, the proposed regions, as delineated in previous studies based on classical staining techniques (Northcutt and Kicliter, 1980; Northcutt, 1981; Neary, 1990), were studied in Nissl-stained brain sections of *R. perezi*. Because most cell bodies stay close to the ventricular lining, it is difficult to delineate nuclei or areas. The use of a variety of (immuno)histochemical stainings revealed chemoarchitectonic features in the basal telencephalon, which allowed us to interpret each main region as a complex set of cell populations and fiber fields that were not recognizable in Nissl-stained material. On the basis of these chemoarchitectonic features in combination with hodological data previously obtained by our group (Marín et al., 1997a–d), we have identified a set of structures in the anuran BG that are tentatively labeled according to their counterparts in amniotes. To what extent each subdivision has an equivalent structure in amniotes will be dealt with in detail in the Discussion.

The distribution of THi, SPi, ENKi, and NADPHdi cell bodies and fibers was charted in representative transverse sections through the forebrain (Figs. 1, 2). In Figure 2, the first vertical row of sections are photomicrographs of cresyl violet-stained sections in which the classically considered regions in the ventral aspect of the hemisphere are indicated. In the second row, the compartmentalized pattern, as proposed by the present study, is shown. The adjacent third through sixth rows depict the distribution of the main markers (TH, SP, ENK, and NADPHd).

To facilitate the flow of the results of our study, first, the components of the basal telencephalon, as defined by the set of markers used, are described. Subsequently, the specific distribution of each staining is dealt with separately, mostly attending to their distinct distribution within the striatal, pallidal, and amygdaloid regions as well as in the region of the BST.

Basal telencephalic subdivisions

Striatal complex. The striatal complex extends in the ventrolateral and ventromedial telencephalic regions throughout most of the length of the hemisphere and is

comprised of two distinct cell populations, i.e., the Acc and the Str (Fig. 2A–H).

Nucleus accumbens. The Acc is a quite compact, periventricular cell population located in the ventromedial telencephalic wall along the rostral one-third of the telencephalic hemisphere (Fig. 2A–D). Rostrally, it is separated from the internal granular layer of the main olfactory bulb by a thin, cell-sparse region that intervenes between them (Fig. 2A). At rostral telencephalic levels, the Acc borders on the postolfactory eminence, which is substituted more caudally by the ventral division of the lateral septal nucleus (Fig. 2A–D). The Acc is laterally continuous with the Str and ventromedially with the nucleus of the diagonal band of Broca (DB) and the ventral pallidum (VP; Fig. 2B–D). Caudally, the Acc is replaced by the BST, although the border between both nuclei cannot be defined clearly on Nissl-stained sections (Fig. 2E). Within the Acc, distinct dorsal and ventral portions are readily recognized, even in cresyl violet-stained sections (Fig. 2C,D), although immunohistochemical staining delineates them more clearly.

Striatum. The Str occupies a ventrolateral position within the telencephalic hemisphere, extending from a level just caudal to the olfactory bulb to the level of the lamina terminalis (Fig. 2B–H). It consists of a periventricular cell zone and a laterally located neuropil. Rostrolaterally, the Str is separated from the accessory olfactory bulb by the rostral aspect of the ventral division of the lateral pallium, i.e., the rostral aspect of the lateral amygdala (LA), which, at this level, expands ventrally. At intermediate-to-rostral telencephalic levels, the Str lies ventromedial to the anterior amygdaloid area (AA), whereas, at more caudal levels, it is capped dorsally by the medial amygdala (MeA; Fig. 2C–H). The Str merges medially with the Acc, the BST, and the pallidum (Fig. 2B–H). Remarkably, the degree of cell migration within the ventromedial aspect of the striatal cell plate increases as the Str expands caudally (Fig. 2C–F). The caudal pole of the Str reaches the level of the lamina terminalis, where it intervenes between the MeA and the pallidum (Fig. 2H).

Pallidum. The anuran pallidum consists of a large cell population with ill-defined boundaries that are not noticeable in Nissl-stained sections. It extends from levels slightly caudal to the rostral pole of the Acc to the level of the anterior commissure (Fig. 2C–K). Only by means of immunohistochemical stainings is a clear delineation of the pallidum observed, which may be further subdivided into ventral and dorsal parts (VP and DP, respectively).

Ventral pallidum. The VP occupies the most ventromedial aspect of the telencephalic hemisphere and constitutes the superficial part of the anuran pallidum. At rostral telencephalic levels, the VP is continuous dorsolaterally with the Acc and is bounded medially with the DB (Fig. 2C,D). At more caudal telencephalic levels, however, it lies ventral to the DP and the BST, whereas, medially, it is separated by a cell-sparse region from the DB (Fig. 2E,F). At caudal telencephalic levels, the VP occupies the territory between the DP and the BST without a clear separation (Fig. 2G–I). The VP, as presently defined, coincides with the region occupied by the terminal field formed by the majority of the intratelencephalic projections originating from the Acc (Marín et al., 1997b).

Dorsal pallidum. The DP consists of a large number of neurons extending from midtelencephalic levels rostrally to a territory just caudal to the anterior commissure caudally (Fig. 2F–J). Like the VP, the precise boundaries of

the DP could not be outlined in Nissl-stained sections, and only immunohistochemistry allowed its identification. Thus, at rostral levels, the DP intervenes between the ventromedial aspect of the Str and the lateralmost part of the BST without clear-cut limits (Fig. 2F). More caudally, the DP is located just medial to the lateral forebrain bundle and borders dorsally on the Str, medially on the BST, and ventrally on the VP (Fig. 2G–I). At levels just caudal to the anterior commissure, the DP lies ventrolateral to the MeA.

Amygdaloid complex. The complete amygdaloid complex consists of a tier of nuclei organized in several adjacent dorsoventral layers that are disposed in a parallel arrangement along the telencephalic hemisphere. It forms a bowl-like structure from the dorsal border of the Str at rostral telencephalic levels to a relatively medial region at the level of the preoptic area. Three main parts within this complex are distinguished: the LA, the olfactory amygdala, and the central amygdala (CeA).

Lateral amygdala. The LA consists of a rather large cell population that extends from levels just caudal to the olfactory bulb to the level of the anterior commissure, where it disappears between the lateral pallium and the MeA (Fig. 2C–I). Its rostral pole is interposed between the accessory olfactory bulb and the Str. Throughout its entire extent, the LA is continuous dorsally with the lateral pallium, whereas, ventrally, it is attached to the olfactory amygdala (Fig. 2C–I). The LA, as it is presently defined, largely resembles the ventral division of the lateral pallium described in anurans (Northcutt and Kicliter, 1980), although it does not include the most ventral portion considered in the latter structure (lateral prominence; after Herrick, 1948), which, in turn, is regarded as part of the olfactory amygdala.

Olfactory amygdala. The olfactory amygdala is characterized mainly by its relation with the main and accessory olfactory bulbs. Like the LA, the olfactory amygdala is constituted by a rostrocaudal, column-shaped cell population that extends from the level of the olfactory bulb to the level of the postcommissural preoptic area (Fig. 2C–K). In this view, the olfactory amygdala includes the striatopallial transition area (Marin et al., 1997a,b) and the pars lateralis of the amygdala, as identified classically in anuran amphibians (Northcutt and Kicliter, 1980). We distinguish two main divisions within the olfactory amygdala.

Anterior amygdaloid. This part of the olfactory amygdala corresponds with an area formerly included in the most ventral portion of the lateral pallium (lateral prominence; after Herrick, 1948) and its adjacent periventricular cell plate, which is continuous ventrally with the Str. The AA extends from the olfactory bulb to a level just rostral to the lamina terminalis (Fig. 2C–G), where it is replaced caudally by the MeA (Fig. 2H). Throughout its length, the AA is bounded dorsally to the LA (Fig. 2C–I).

Medial amygdala. This amygdaloid region represents the caudal part of the olfactory amygdala and would correspond to the cortical amygdaloid nucleus and the medial amygdaloid nucleus, as defined by Scalia et al. (1991). The MeA is continuous rostrally with the AA; dorsally with the LA and the most caudal aspect of the lateral pallium; and ventrally with the Str, CeA, and pallidum (Fig. 2H–J). The caudal pole of the MeA occupies the dorsolateral aspect of the medial preoptic area (Fig. 2K).

Central amygdala. This region is an elongated cell population located in the caudal part of the hemisphere in close apposition to the caudal aspect of the striatal cell plate, from which is distinguished by differences in cell-packing densities (Fig. 2G,H). The CeA is continuous dorsally and medially with the Str and ventromedially with the pallidum.

Bed nucleus of the stria terminalis. The BST occupies a periventricular position in the ventromedial telencephalic wall, just caudal to the Acc. The limit between both nuclei, as mentioned above, is not noticeable in Nissl-stained sections (Fig. 2D,E). Caudally, the BST extends up to the level of the anterior commissure (Fig. 2J). The BST lies ventral to the lateral septal nucleus and medial to the Str, pallidum, and MeA (Fig. 2E–J).

Chemoarchitectonic data

TH immunoreactivity. The Acc contains the densest accumulation of THi fibers and axon terminals in the basal telencephalon. A conspicuous plexus of THi boutons and fibers is located in a ventral, periventricular position along the rostral one-third of the medial telencephalic wall, thus outlining the limits of the Acc (Fig. 2A–D). Within the Acc, distinct dorsal and ventral regions are distinguished on the basis of THi. The innervation of the dorsal portion is less abundant than that of the ventral part, which resembles the differences observed in cell-packing densities between both regions (Figs. 2E, 3A). The amount of THi fibers decreases in the transition zone between the Acc and the BST (Fig. 3B). In contrast to the Acc, the BST is innervated only moderately by THi fibers (Fig. 3D).

The Str is also characterized by a conspicuous plexus of THi fibers and terminals, although the density of THi elements in the Str is considerably lower than in the Acc (Fig. 2C–H). The Str has a graded distribution of TH immunoreactivity along its rostrocaudal extent. Thus, the rostral part of the Str contains disperse, fine, THi fibers (Fig. 2C,D), whereas, in the caudal half of the Str, the density of THi fibers is notably higher (Figs. 2E–H, 3B). At intermediate-to-caudal hemispheric levels, the transition region between the Str and the pallidum is identified by a marked decrease in the number of THi fibers in the floor of the lateral ventricle (Fig. 2F). Differing from the adjacent striatal and amygdaloid cell groups, the pallidum contains only a moderate number of THi fibers (Fig. 2F–J). Nevertheless, the density of THi fibers is noticeably higher in the VP than in the DP (Figs. 2C–H, 3A,B,D).

The LA contains THi fibers throughout its rostrocaudal extent, although they are more abundant at caudal telencephalic levels (Figs. 2, 3C). The catecholaminergic innervation of the LA is in sharp contrast with that of the dorsally adjacent lateral pallium, which is almost devoid of THi fibers. The AA is strongly immunoreactive for TH throughout its rostrocaudal extent (Figs. 2, 3C). In the MeA, the numbers of THi fibers and terminals are remarkably high in the periventricular cell plate, which consists of a few layers of closely packed cells separated from the ependyma and the rest of the MeA by two narrow fiber zones (Figs. 2H–J, 4). Only a few, sparsely distributed THi fibers are found in other regions of the MeA. In contrast, the CeA contains a dense plexus of THi fibers and terminals compared with that observed in the Str (Figs. 2H,I, 3D). At the level of the anterior commissure, the BST contains a moderate-to-dense plexus of THi fibers and a few small, THi perikarya (Figs. 2J, 4A).

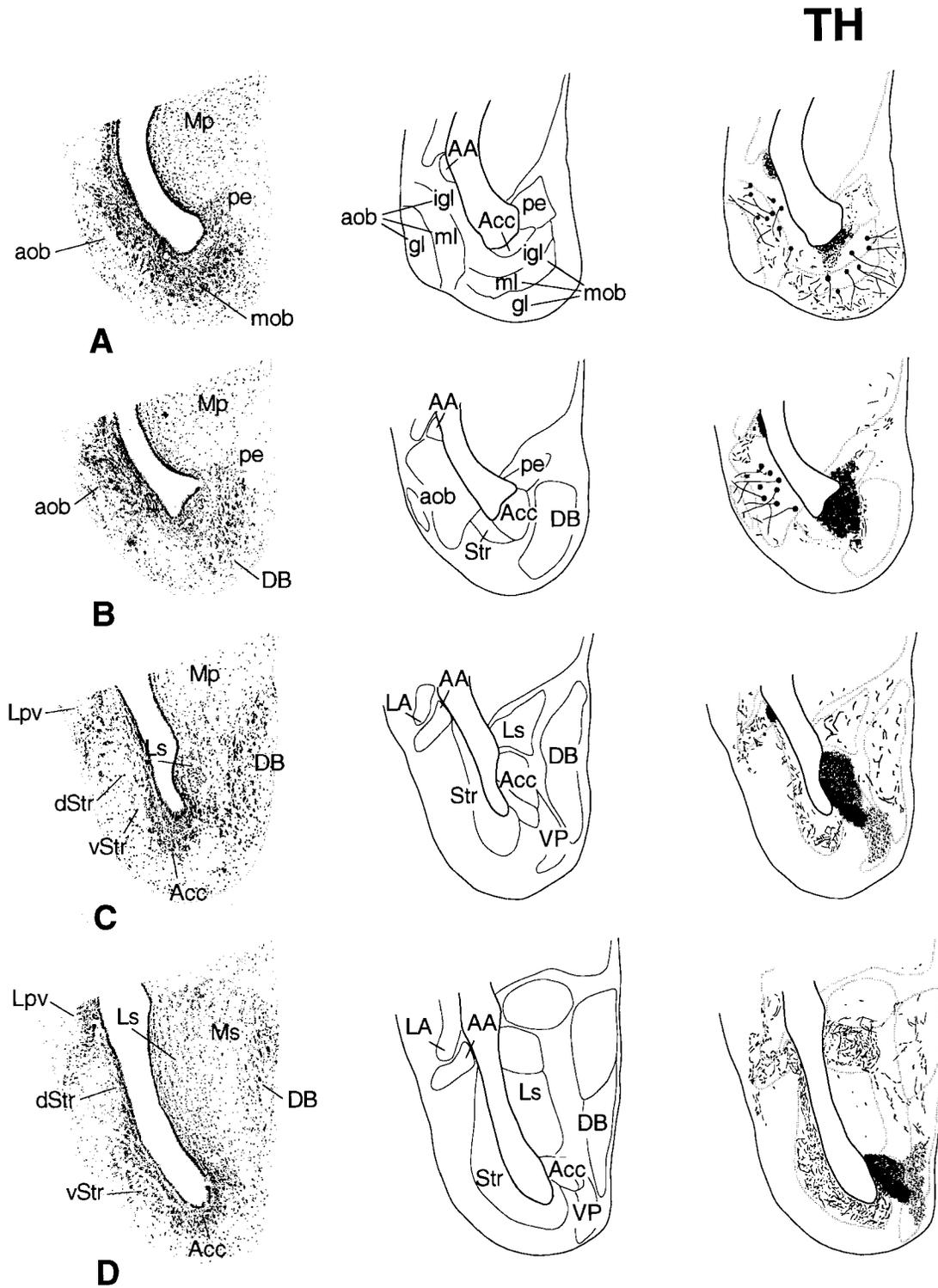


Fig. 2. **A-K**: Series of transverse sections through the forebrain of *R. perezii*. The far left vertical row presents high-contrast photomicrographs of Nissl-stained sections in which the previously proposed subdivisions of the basal telencephalon are indicated (after Northcutt and Kicliter, 1980). In the second vertical row, the new subdivision, as proposed on the basis of hodological (Marín et al., 1997a-d) and chemoarchitectonic (present study) criteria, is depicted. The third

through sixth rows are line drawings at comparable levels showing the distribution of tyrosine hydroxylase (TH)-, substance P (SP)-, enkephalin (ENK)-, and nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd)-(immuno)reactive cell bodies (large dots) and fibers/terminals (small dots, wavy lines). For abbreviations, see list.



Figure 2 (Continued)

SP immunoreactivity. In our experiments, colchicine treatment was not employed; therefore, neuropeptide immunoreactivity was confined mainly to fibers and axon terminals. Only occasionally was specific staining found in cell bodies. Like THi, SPi is very dense in the Acc, clearly delineating its boundaries (Figs. 2A–D, 5A,B, 6A). In

addition, numerous coarse, SPi fibers and terminals are present in the VP (Figs. 2C–I, 5B, 6B). In the Str, diffuse and homogeneous staining is found in the superficial neuropil, and well-labeled, sparse, SPi, varicose fibers and some weakly stained cell bodies are occasionally observed (Fig. 2B–G). The DP is innervated only moderately by SPi fibers.

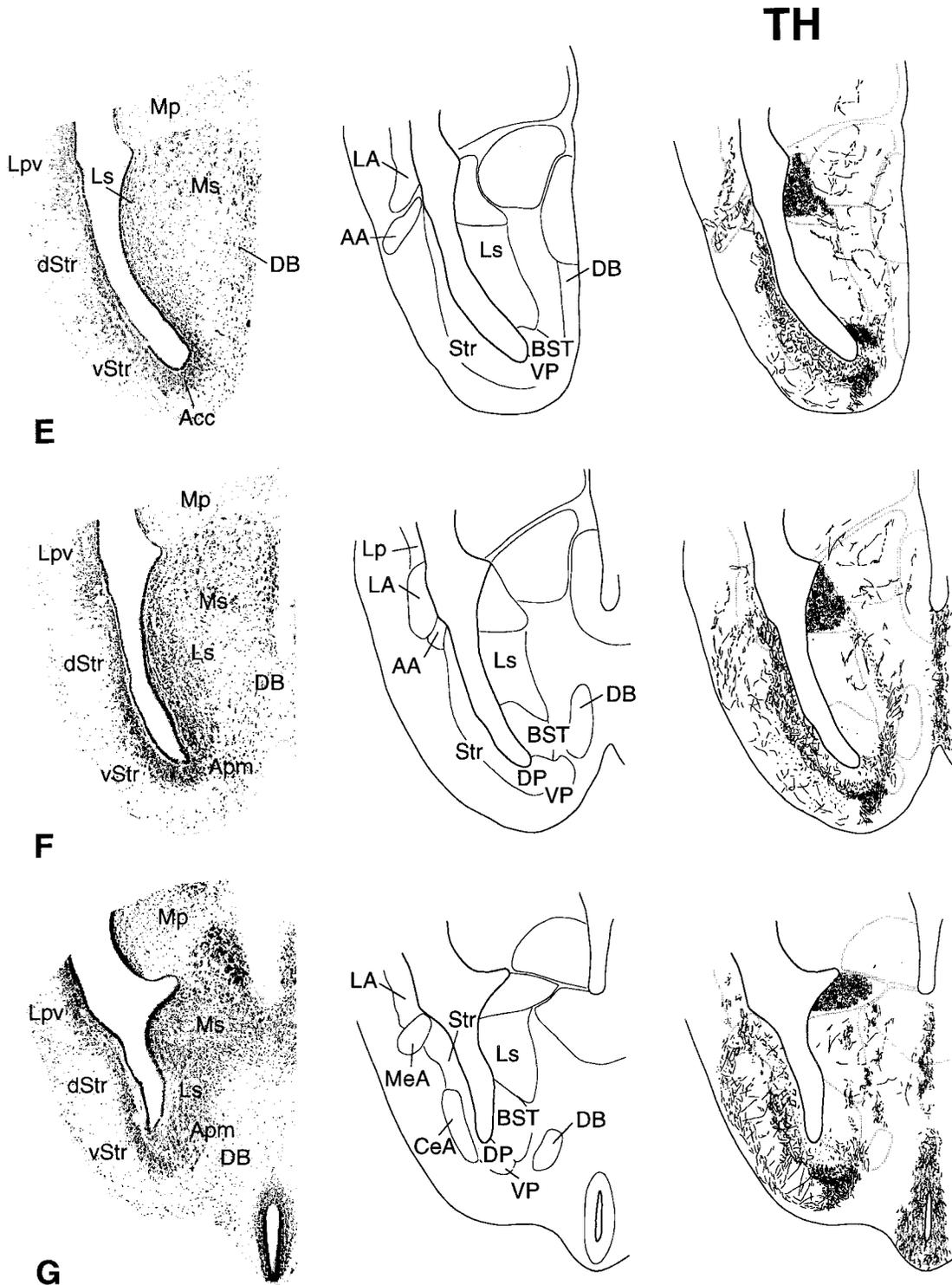


Figure 2 (Continued)

In the BST, SPi fibers are slightly less abundant than in the Acc (Figs. 2, 6A). The boundary between the BST and the ventral division of the lateral septum is indicated by a discontinuity in immunoreactivity for SP (Figs. 2, 5A). A few small, SPi perikarya are present in the BST at levels of the anterior commissure.

The LA contains a few SPi fibers at intermediate-to-rostral hemispheric levels, but numerous SPi fibers are present in the caudal one-third of this group in the neuropil immediately adjacent to the cell plate (Fig. 2H,I). In turn, the olfactory amygdala is invested prominently with SPi fibers throughout its entire extent (Figs. 2, 5C).

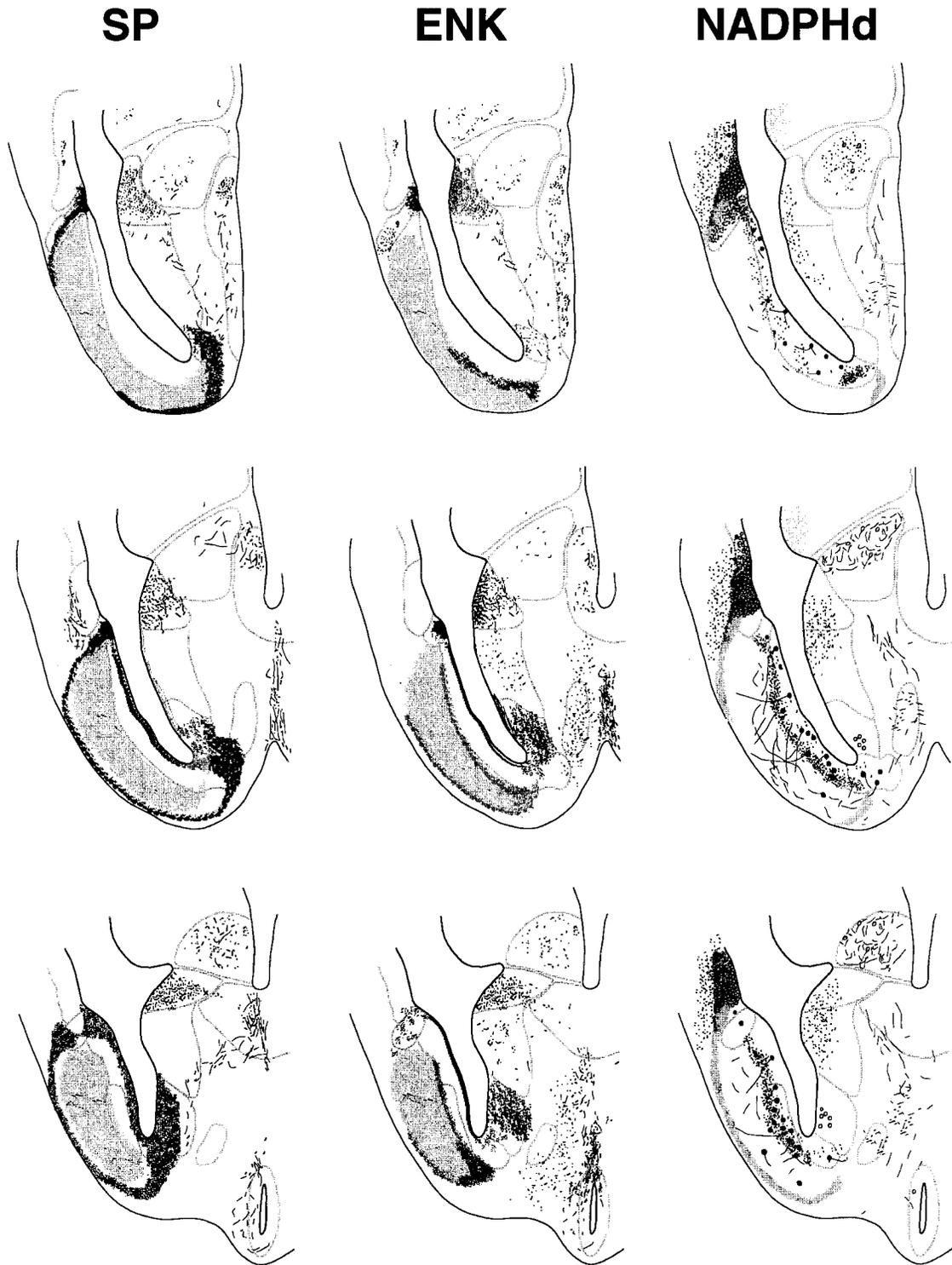


Figure 2 (Continued)

On SP-stained sagittal sections, it is observed clearly that the AA and the MeA form a cellular continuum along the telencephalic hemisphere (Fig. 5D). In the AA, the periventricular cell plate possesses a dense innervation of SPi fibers; whereas, in the laterally migrated component of

this area, SPi fibers are distributed sparsely (Figs. 2C-F, 5C). Although it is directly continuous with the AA, the MeA contains the densest plexus of SPi fibers in the amygdala. Thus, SP immunohistochemistry clearly outlines the entire MeA, including the periventricular cell

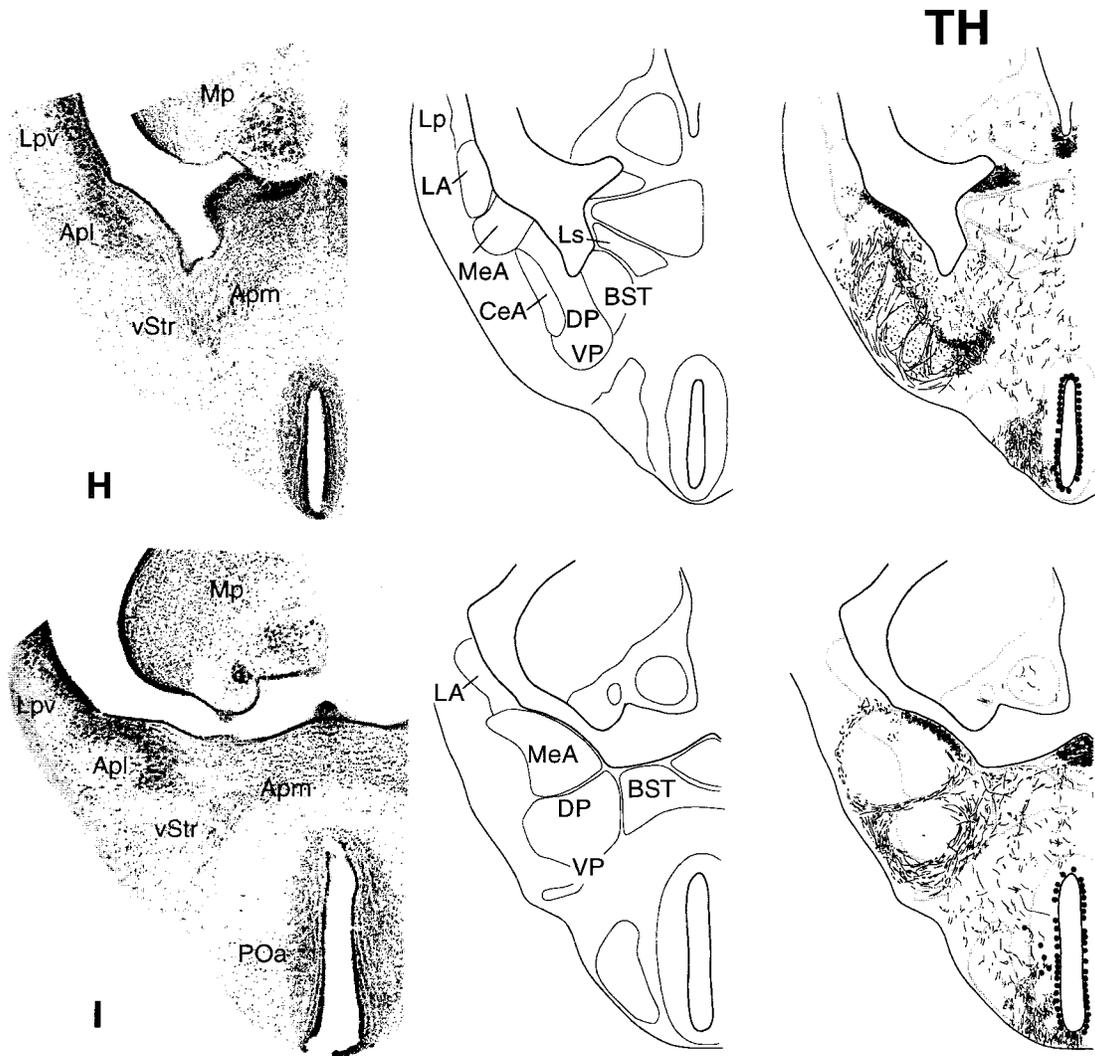


Figure 2 (Continued)

plate, the bowl-shaped main part of the nucleus, and its laterally located neuropil (Figs. 2J,K, 5A,D, 6B). In addition, numerous SPi fibers are found in a periventricular position, bringing together the MeA and the BST (Figs. 2G,H, 5E). Occasionally, some weakly stained SPi cells are found in the main part of the MeA. The region where the caudal pole of the MeA enters the medial preoptic area also contains a dense SPi neuropil, which is dorsomedially continuous with the caudal aspect of the olfactory amygdala (Fig. 2K). In contrast to other divisions of the amygdala, the central nucleus is almost completely devoid of SPi fibers (Fig. 5E).

Leu-ENK immunoreactivity. The Acc contains abundant ENKi fibers along its rostrocaudal extent (Fig. 2A–D) in sharp contrast with the rostral pole of the BST, which is almost completely devoid of ENKi fibers (Fig. 2E). The caudal part of the latter nucleus, however, contains relatively abundant ENKi fibers (Fig. 2G–J). The striatal neuropil possesses only weakly ENKi terminals along its rostrocaudal extent, whereas numerous coarse, ENKi fibers are present within the striatal cell plate (Fig. 2C–G).

Occasionally, weakly immunoreactive cell bodies are found in the Str. Strikingly, the enkephalergic innervation of the pallidum is remarkably high compared with other basal telencephalic regions. It is of note that the DP is innervated more prominently by ENKi fibers than the VP (Figs. 2G–J, 6C,D).

In the amygdaloid complex, the LA is almost completely devoid of ENKi fibers and contains only a few labeled, thin fibers and terminals within its caudal part (Fig. 6D). In contrast, the rostral, periventricular aspect of the AA contains a dense plexus of ENKi fibers, and scattered immunoreactive fibers are located laterally (Fig. 2B–E). In the MeA, sparse ENKi fibers and terminals are found around the cell plate and within its adjacent neuropil (Fig. 6D). In addition, a tightly packed ENKi cell group is observed in the rostral, plate-like division of the MeA (Figs. 2H–J, 6D). Finally, a neuropil that is very lightly labeled for ENK is found in the CeA.

NADPHd activity. The Acc is almost completely devoid of NADPHd fibers, which is in sharp contrast with neighboring structures (Figs. 2A–D, 5A,B, 7A). Thus,

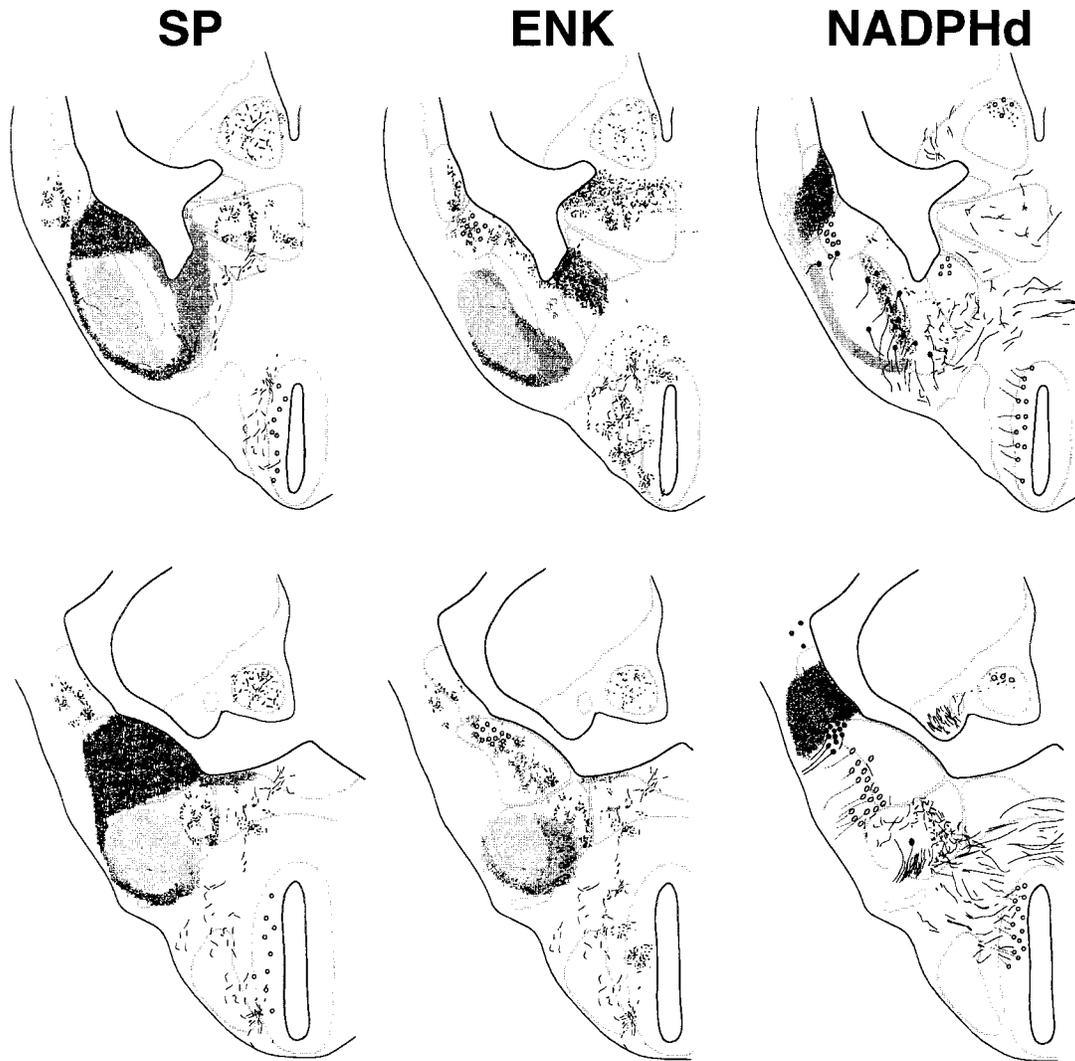


Figure 2 (Continued)

abundant NADPHd fibers are found in the lateral septum, strongly stained NADPHd cells and fibers are found in the DB, and numerous NADPHd neurons are embedded within a NADPHd⁺ neuropil in the striatal cell plate (Figs. 2B–H, 5B, 7A). The distribution of NADPHd activity serves to further differentiate between the Acc and BST, because the latter structure stains faintly for NADPHd activity and contains scattered, lightly stained cell bodies (Figs. 2E–I, 7B). Occasionally, some weakly stained neurons are found in the Acc.

The LA contains a strongly positive NADPHd neuropil along its rostrocaudal extent, although the densest NADPHd activity is found in its caudal pole (Figs. 2D–I, 5C,D, 7C). At intermediate-to-rostral telencephalic levels, the LA give rise to a diffuse bundle of NADPHd fibers that courses in a subpial position to terminate in an area just superficial to the ventromedial aspect of the Str, the ventral aspect of the Acc, the pallidum, and the ventrolateral part of the BST (Figs. 2D–I, 7B,C). On the other hand, the caudal pole of the LA contains abundant neurons that are heavily labeled for NADPHd (Figs. 2H,I, 5D) and that give rise to a compact bundle of faintly stained fibers that

courses dorsal and medially to the lateral forebrain bundle and reaches the hypothalamus.

Differing from the LA, the AA contains only moderate NADPHd activity (Figs. 2, 5C,D). The rostral part of the MeA contains numerous, weakly stained, NADPHd neurons located mainly in the superficial cell plate or scattered within the adjacent neuropil (Figs. 2H,I, 7C). In contrast, the caudal part of the MeA stains lightly for NADPHd activity and does not contain NADPHd neurons (Fig. 2J,K). NADPHd activity characteristically provides a guide for identification of the CeA, where strongly immunoreactive NADPHd neurons are found embedded in a profuse plexus of NADPHd fiber terminals (Figs. 2G,H, 7C). Remarkably, the NADPHd terminal plexus of the CeA extends rostrally through the Str, and, at rostral levels, it splits into two distinct fields: one above the Str and another that extends ventrally through the region of the VP and the lateral aspect of the BST, up to the caudal pole of the Acc (Figs. 2D–F, 7B).

CR immunoreactivity. The Acc is almost devoid of CRi fibers, although a few CRi neurons are located in a

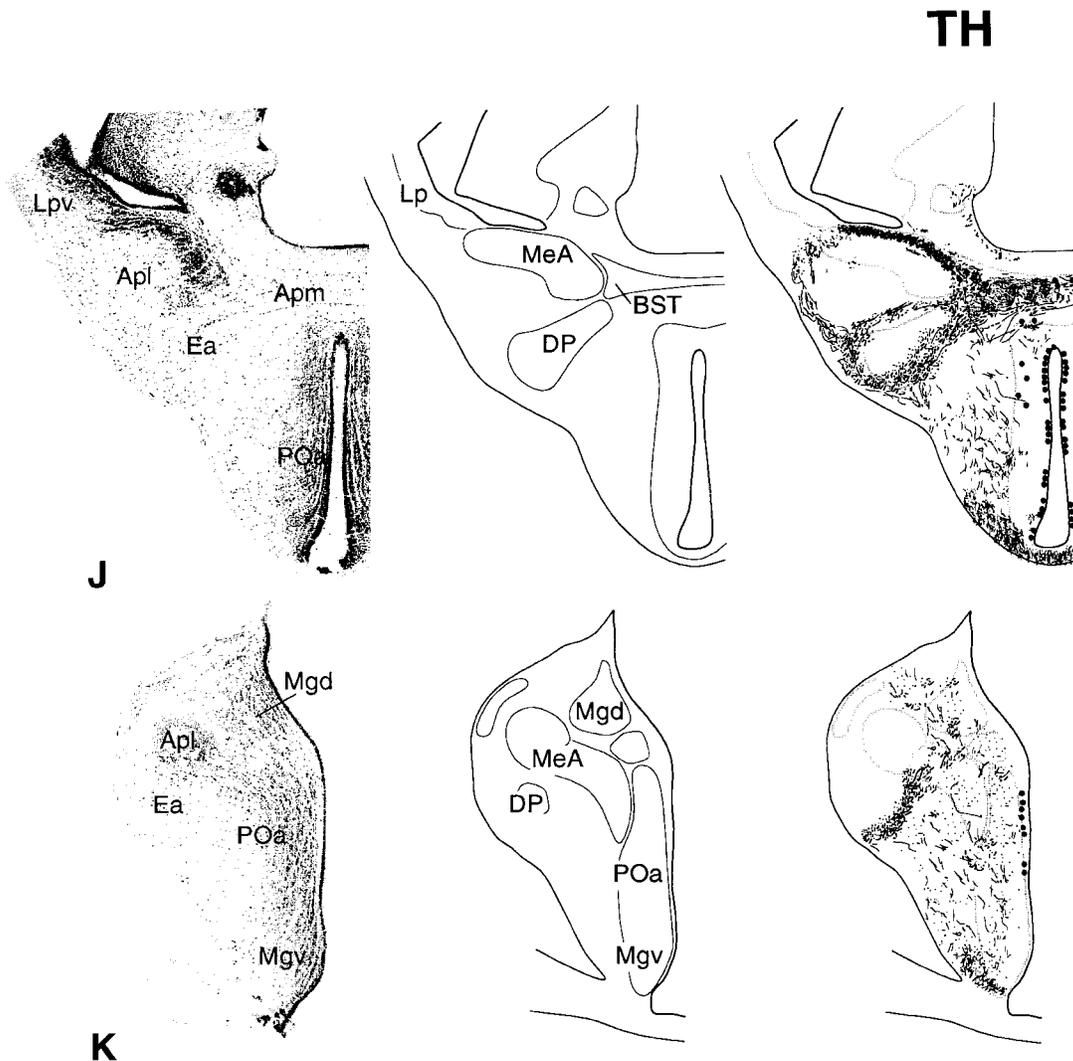


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periventricular position. The latter may be a caudal continuation of the cell population of the accessory olfactory bulb (Fig. 8A). Like with the NADPHd staining, CRi extends caudally from the accessory olfactory bulb in an arch-shaped trajectory within the rostral confines of the accessory olfactory tract, which also contains some CRi neurons (Figs. 5F, 8B). The Str does not contain CRi cells or fibers, but the lateral forebrain bundle is strongly stained for CR (Fig. 8C). CR labeling in the lateral forebrain bundle certainly corresponds to thalamic afferents to the ventrolateral telencephalic wall, because almost every neuron within the anterior division of the lateral thalamic nucleus and the central thalamic nucleus is CRi and projects mainly to the striatal neuropil (Marín et al., 1997a; present study). Scattered CRi cells are found specifically in the AA, whereas they are absent in other amygdaloid components and in the BST.

AVT and MST immunoreactivities. The ventral aspect of the Acc is characterized by the presence of a small population of AVTi neurons. These cells are embedded in a rather dense neuropil of AVTi varicose fibers and termi-

nals that also extends over the VP (Fig. 9A). In addition, the rostral parts of the Acc and the VP contain a moderate innervation of varicose MSTi fibers. The Str contains scattered AVTi and MSTi fibers. Some AVTi neurons and abundant AVTi fibers are found throughout the length of the AA.

Caudal to the Acc, the BST is innervated moderately by AVTi fibers, and some AVTi neurons are also found in the lateral part of the nucleus close to the lateral ventricle. The medial part of the BST, on the other hand, contains a population of MSTi neurons (Fig. 9B). The population of AVTi cells in the BST extends caudally and is located just ventral to the floor of the lateral ventricle, with some AVTi neurons observed in the periventricular fiber layer that links the MeA to the BST. The rostral part of the MeA contains a few scattered AVTi neurons and abundant fibers and terminals located in the periventricular zone.

DBH immunoreactivity. In contrast to the conspicuous THi innervation of the Acc and the Str, only a moderate number of varicose DBHi fibers are located in these regions, which accounts for the scarce noradrenergic innervation. The VP contains a prominent plexus of well-

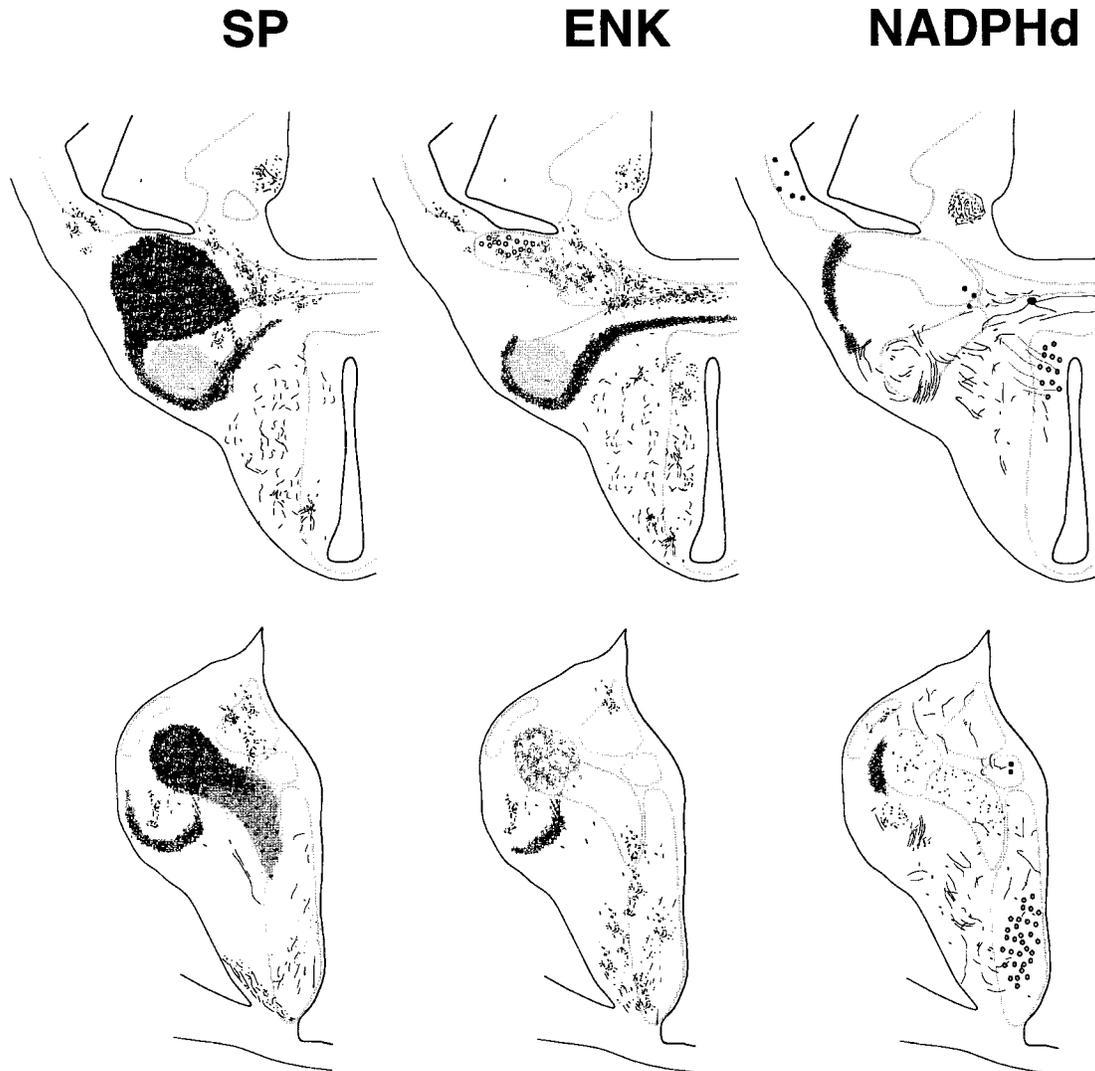


Figure 2 (Continued)

labeled, highly varicose fibers and terminals throughout its rostrocaudal extent (Fig. 10A,B). Caudally, the plexus of DBHi fibers extends through the floor of the lateral ventricle, reaching the ventral and medial parts of the BST (Fig. 10B). In the amygdaloid complex, the AA also contains sparse DBHi fibers that are restricted mainly to its laterally migrated part. In addition, the CeA contains numerous DBHi fibers, but the MeA is almost completely devoid of DBHi innervation (Fig. 10B).

ChAT immunoreactivity. The Str and the Acc contain only a few, scattered ChATi fibers. In contrast, the medially adjacent DB contains numerous ChATi fibers and neurons. Nevertheless, some weakly stained ChATi neurons are found throughout the rostrocaudal extent of the Str. Scattered ChATi fibers are found in the AA and the LA.

At caudal hemispheric levels, large numbers of scattered ChATi neurons are located in close relation to the lateral forebrain bundle, extending over several structures (Fig. 5G). ChATi neurons are found mainly in the DP and the VP, although some neurons are displaced dorsally and laterally by the lateral forebrain bundle. Slightly caudally,

ChATi cells are found around the lateral forebrain bundle and the anterior commissure as well as in the DP and the most lateral aspect of the BST. Occasionally, a few cells are found within the MeA.

SOM immunoreactivity. This marker labels characteristically striatal regions. In the Str, SOMi fibers constitute a rather dense neuropil adjacent to the striatal cell plate, which also contains a high density of coarse SOMi fibers (Fig. 11A,B). Some SOMi neurons are found occasionally in the ventral aspect of the Str. At rostral telencephalic levels, SOMi fibers extend medially to innervate the ventral part of the Acc (Fig. 11A). The dorsal part of the Acc lacks labeled fibers or terminals. A strongly SOMi innervation is found in the AA, but the dorsally located LA is completely devoid of immunoreactive fibers (Fig. 11B).

Caudally, the BST contains a moderately dense plexus of SOMi fibers, whereas numerous, strongly immunoreactive fibers are located in the CeA (Fig. 11C). In addition, a profuse terminal field is located in the main part of the MeA, in its adjacent neuropil, and in the extension of the MeA into the medial preoptic area (Fig. 11C,D). A few

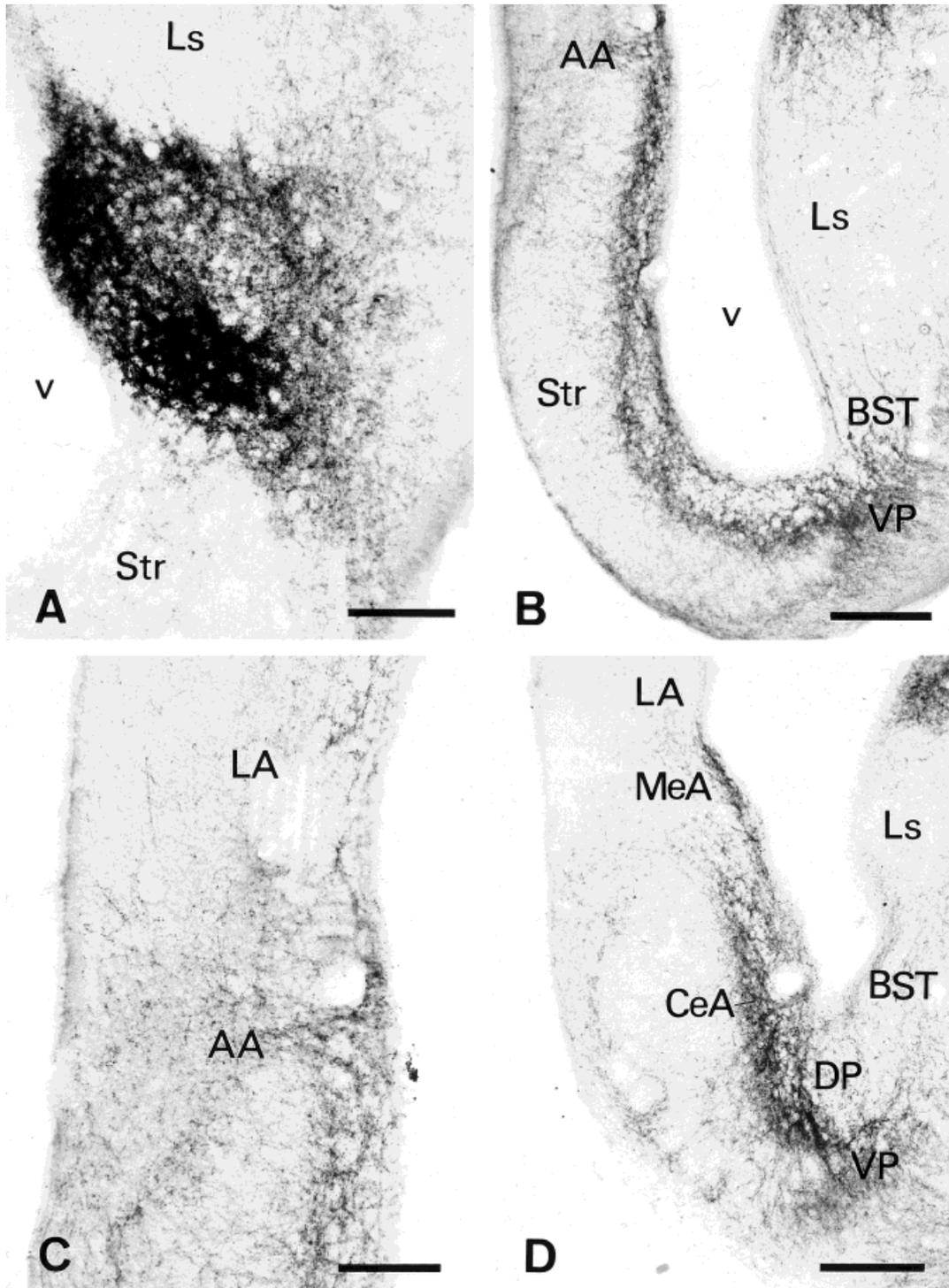


Fig. 3. Photomicrographs illustrating TH-immunoreactive (THi) fibers/terminals in the nucleus accumbens (A); in the striatum, ventral pallidum, and bed nucleus of the stria terminalis (B); in the lateral and anterior amygdaloid area (C); and in the medial and central amygdala and the pallidal structures (D). Note the less dense innerva-

tion of the dorsal part of the nucleus accumbens (Acc) compared with its ventral part. The levels of the photomicrographs are comparable to those of Figure 2C,E, and Figure 2E,G, respectively. For abbreviations, see list. Scale bars = 100 μ m in A,C, 200 μ m in B,D.

SOMi cell bodies are located in the caudal part of the MeA within the plexus of positive fibers.

NPY immunoreactivity. The Acc is moderately innervated by thin NPYi fibers, which are located mainly at

rostral levels. In contrast, the Str contains only a few scattered NPYi fibers, whereas the pallidum contains a weak immunoreactive neuropil. In addition, fibers labeled for NPY are sparse or largely absent from the rostral part

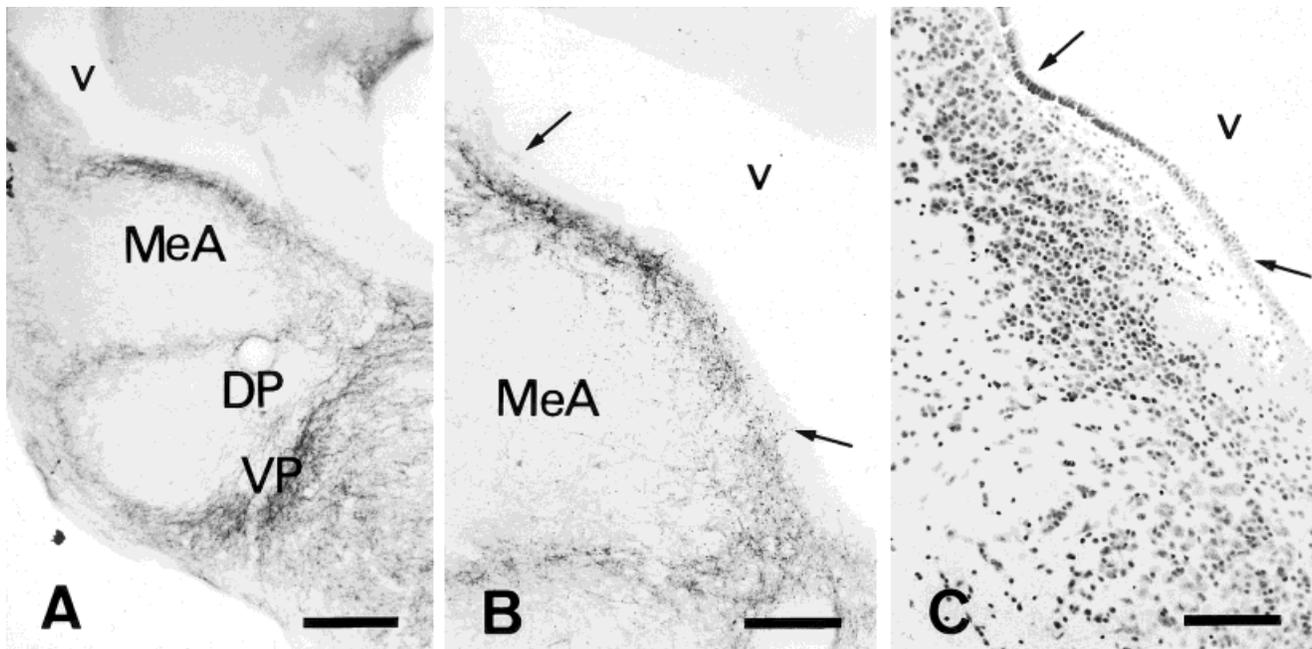


Fig. 4. A-C: Photomicrographs of transverse sections through caudal telencephalic levels illustrating THi fibers/terminals seen in the medial amygdala (MeA) and pallidal structures (A). The photomicrograph in B shows the dense innervation of the periventricular zone

of the MeA in a cell-free zone, as seen in a Nissl-stained section (C). Arrows indicate the lateral and medial boundaries of the periventricular zone of the MeA. For abbreviations, see list. Scale bars = 200 μ m in A, 100 μ m in B,C.

of the BST, which is in sharp contrast with the dense plexus of NPYi fibers located just dorsally in the ventral part of the lateral septum (Fig. 11E). It should be noted, however, that the density of NPYi fibers in the BST gradually increases in the caudal part of the nucleus.

Remarkably, immunohistochemistry for NPY characteristically labels the amygdaloid complex. Thus, moderate innervation is located throughout the AA, where some NPYi cell bodies are also found occasionally. In addition, numerous NPYi fibers occupy the rostral, plate-like part of the MeA and the periventricular fiber layer that connects the MeA and the BST (Fig. 11E). The caudal part of the MeA contains the densest NPYi innervation in the basal telencephalon. Thus, the bowl-shaped part of the MeA, its adjacent lateral neuropil, and the ventral extension of the MeA into the medial preoptic area strongly stain with the NPY antiserum (Fig. 11F). In contrast, the periventricular part of the MeA contains a moderately dense plexus of NPYi fibers and terminals, and the LA contains only a few varicose NPYi fibers, which are restricted to its caudal part. Finally, fibers labeled for NPY were sparse or were largely absent from the CeA.

NPFF immunoreactivity. At rostral telencephalic levels, the Acc contains a fine network of NPFFi fibers and terminals (Fig. 5H). In addition, numerous fibers course ventrally, close to the pial surface of the hemisphere. A few NPFFi fibers are located in the Str, the pallidum, and the rostral part of the BST. In contrast, the AA, the caudal part of the BST, and the CeA and MeA contain numerous fine and varicose NPFFi fibers. In addition, a pale, immunoreactive neuropil stains the entire extent of the MeA. It is of note that strongly NPFFi neurons are found in the medial part of the DB and, caudally, at the level of the lamina terminalis, in a medial position just dorsal to the preoptic recess. A few NPFFi cells are displaced occasionally within the medial part of the BST.

5-HT immunoreactivity. 5-HTi fibers and terminal fields are distributed widely throughout the basal telencephalon. However, the densest 5-HTi innervation is found throughout the neuropil adjacent to the Str and, remarkably, in the pallidum. In addition, numerous fine, varicose fibers are found in the LA, the AA, and the BST. In contrast, the MeA contains only a few 5-HTi fibers, which are restricted mainly to the periventricular cell plate.

DISCUSSION

In the present study, an attempt has been made to delineate histo- and immunohistochemically defined compartments that reflect structural and functional entities within the basal telencephalon of anuran amphibians. Previous studies on the chemoarchitecture of the basal forebrain of amphibians have noted the existence of an Acc and an Str (Inagaki et al., 1981a; Merchenthaler et al., 1989; González and Smeets, 1991, 1992a,b, 1993). More recently, data on the afferent and efferent connections of the basolateral hemisphere in frogs and newts obtained by means of sensitive anterograde- and retrograde-tracer techniques have led to the proposal for a further subdivision of the amphibian forebrain (Marín et al., 1997a-d). The present chemoarchitectonic analysis, together with previous studies on the connections of distinct zones of the basal forebrain, have laid the basis for a new compartmentalization of the basal forebrain, as proposed in the present account. A comparison between the new terminology and previous terminologies of basal forebrain areas is provided in Table 1.

Current concept of BG organization in amphibians

Str and Acc. The Str of amphibians, as classically defined, comprises almost the entire ventral part of the

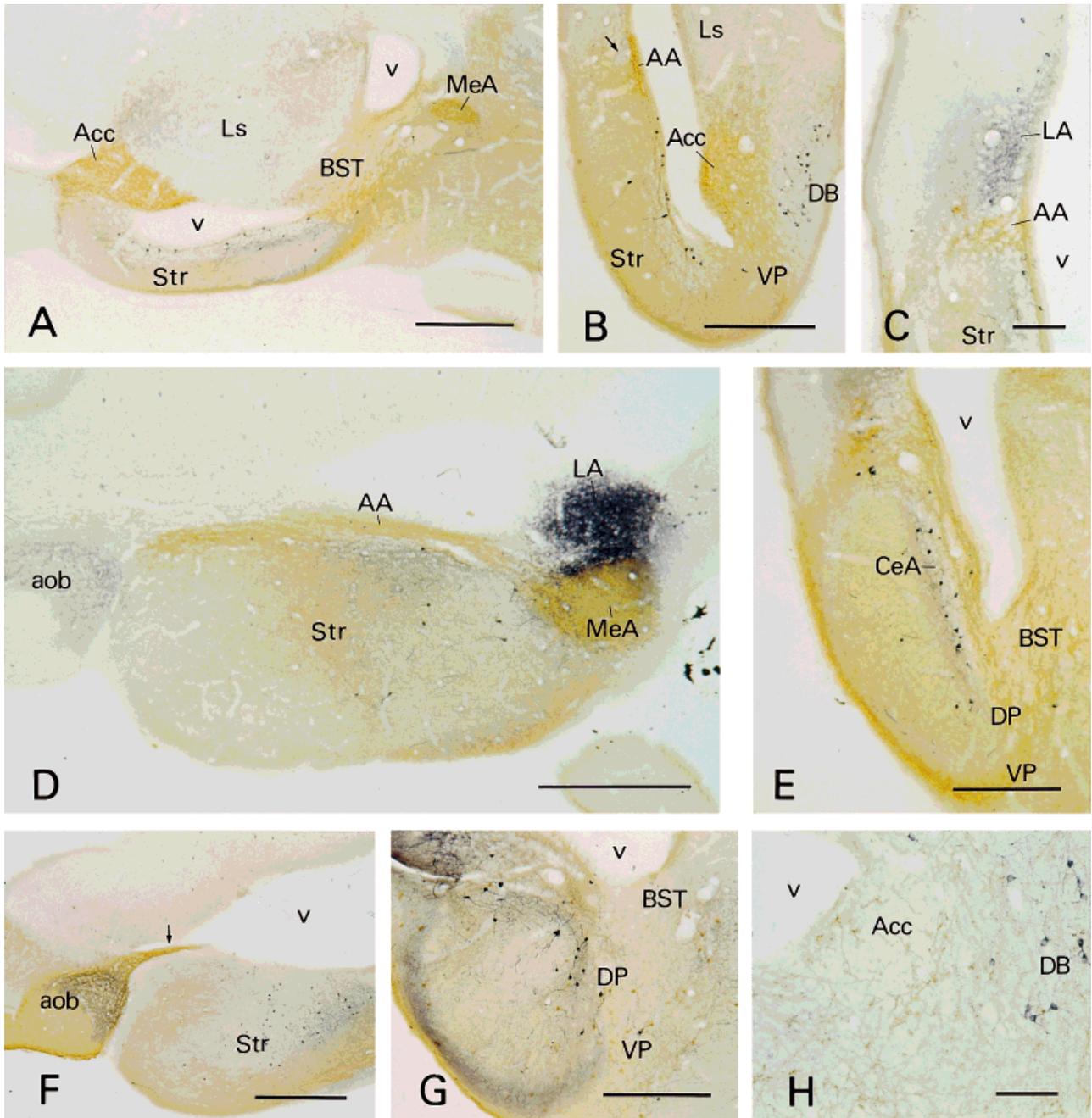


Fig. 5. Photomicrographs of brain sections through the telencephalon of *R. perezii*. Double-labeling techniques were used for SP (A–E), calretinin (CR; F), choline acetyltransferase (ChAT; G), and neuropeptide FF (NPFF; H, brown reaction) combined with NADPHd histochemistry (blue reaction). **A:** Sagittal section illustrating the position of the Acc and the bed nucleus of the stria terminalis (BST). **B:** Transverse section showing SP innervation of the Acc, ventral pallidum (VP), and AA as well as NADPHd cell bodies in the nucleus of the diagonal band of Broca (DB) and the striatum (Str). **C:** High-magnification photomicrograph of a transverse section through the lateral telencephalic wall showing NADPHd reaction in the lateral amygdala (LA) and SP immunoreactivity in the subjacent AA. **D:** Sagittal section illustrating the relative position of the LA and the medial amygdala (MeA) at caudal telencephalic levels (right side of the photomicrograph) and the

rostral position of the AA above the striatum up to the levels of the accessory olfactory bulb. **E:** Photomicrograph of a transverse section at caudal telencephalic levels showing the relative position of the central amygdala (CeA), the striatopallidal system, and the BST. **F:** Sagittal section at rostral telencephalic levels showing the relative distribution of NADPHd and CR in the accessory bulb and in its caudal prolongation above the striatum. **G:** Transverse section through caudal telencephalic levels showing the distribution of NADPHd and ChATⁱ cells. **H:** High-power photomicrograph showing NPFFⁱ fibers in the Acc and NADPHd activity in the surrounding regions. Arrows in B and F indicate the caudal extent of the accessory olfactory bulb. For abbreviations, see list. Scale bars = 500 μ m in A,D,F, 200 μ m in B,E,G, 100 μ m in C,H.

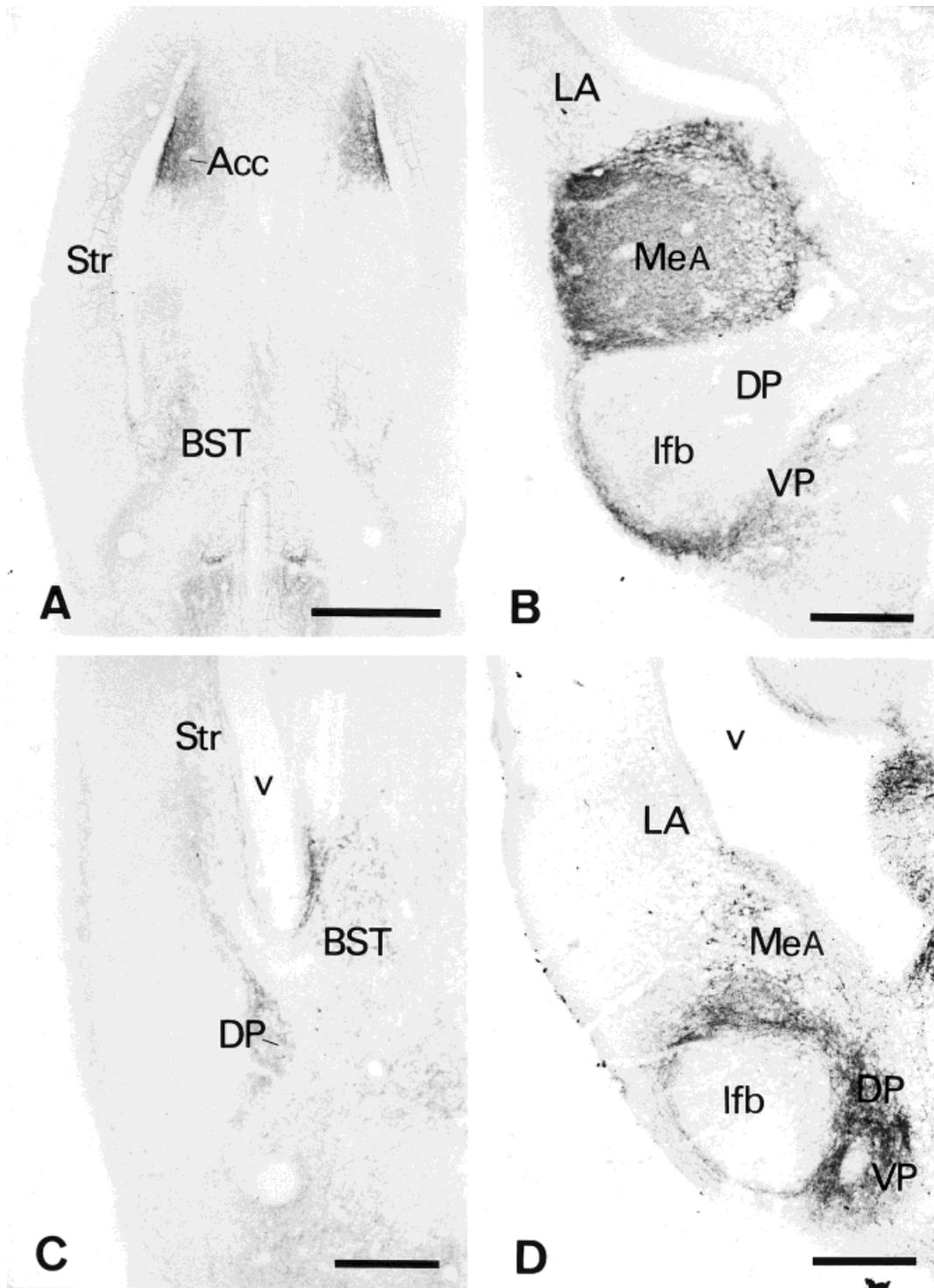


Fig. 6. A-D: Photomicrographs of horizontal (A,C) and transverse (B,D) sections through the telencephalon illustrating SPi fibers/terminals in the Acc and the BST (A) and in the MeA, BST, and pallidal regions (B). ENKi fibers/terminals are shown in the BST and the dorsal pallidum (DP; C,D). Note ENK⁺ cell bodies in the MeA. For abbreviations, see list. Scale bars = 500 μ m in A, 200 μ m in B-D.

lateral telencephalic wall (Herrick, 1910; Ramón y Cajal, 1946; Hoffman, 1963; Northcutt and Kicliter, 1980). However, recent studies on the connections of the basal telen-

cephalon of amphibians have suggested that the region occupied by the Str might contain several cell populations with distinct hodological features (Neary, 1995; Marin

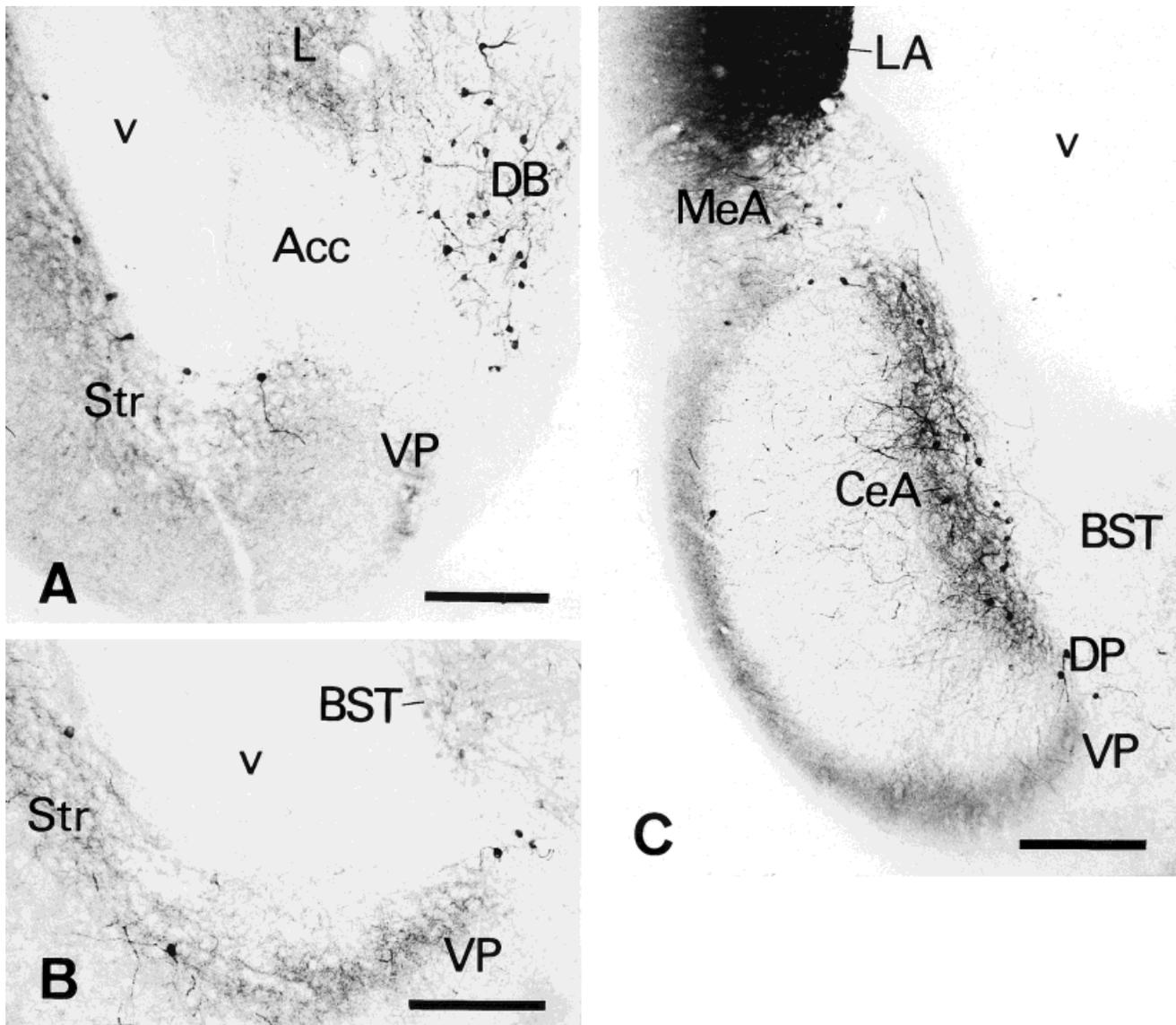


Fig. 7. Photomicrographs of transverse sections through the basal telencephalon illustrating NADPHd-reactive cell bodies and fibers at levels of the Acc (A), the ventral pallidum (B), and the CeA (C). Note the absence of NADPHd reactivity in the Acc. For abbreviations, see list. Scale bars = 200 μ m in A,C, 150 μ m in B.

et al., 1997a,b). In particular, rostral and dorsal striatal territories were demonstrated to project extensively to more caudal and ventral regions of the Str, suggesting the existence of a DP within the caudoventral aspect of the lateral telencephalic wall (Marín et al., 1997b). In addition, cells in the superficial aspect of the caudal Str were found to have a different pattern of connections compared with the rest of the Str (Marín et al., 1997a,b). On the basis of chemical data, the present results have allowed us to delineate the precise limits of the Str in anurans. It is now clear that the ventral part of the lateral telencephalic wall contains cell populations that differ in (immuno)histochemical and hodological characteristics from the other parts of the Str.

The precise extent and location of the Acc in anurans has also been a matter of discussion. The Acc, as previously

defined, extends from levels immediately caudal to the olfactory bulb up to the level of the lamina terminalis (Northcutt and Kicliter, 1980). However, recent hodological studies have suggested that the Acc is restricted to the rostral one-third of the medial telencephalic wall (Marín et al., 1997a,b), a notion that is supported by the results of the present report. In addition, the boundary between the Acc and the Str has now been defined clearly with several chemical markers, such as SP and TH, because the density of immunoreactive fibers is seen to be higher in the Acc than in the Str proper. Moreover, a careful analysis of the recent literature on the patterns of distribution of other neuropeptides and monoamines, such as galanin (GAL), pituitary adenylyl cyclase-activating polypeptide (PACAP), neuromedin U, and histamine (HA), reveals that all of these substances specifically label the Acc (Air-

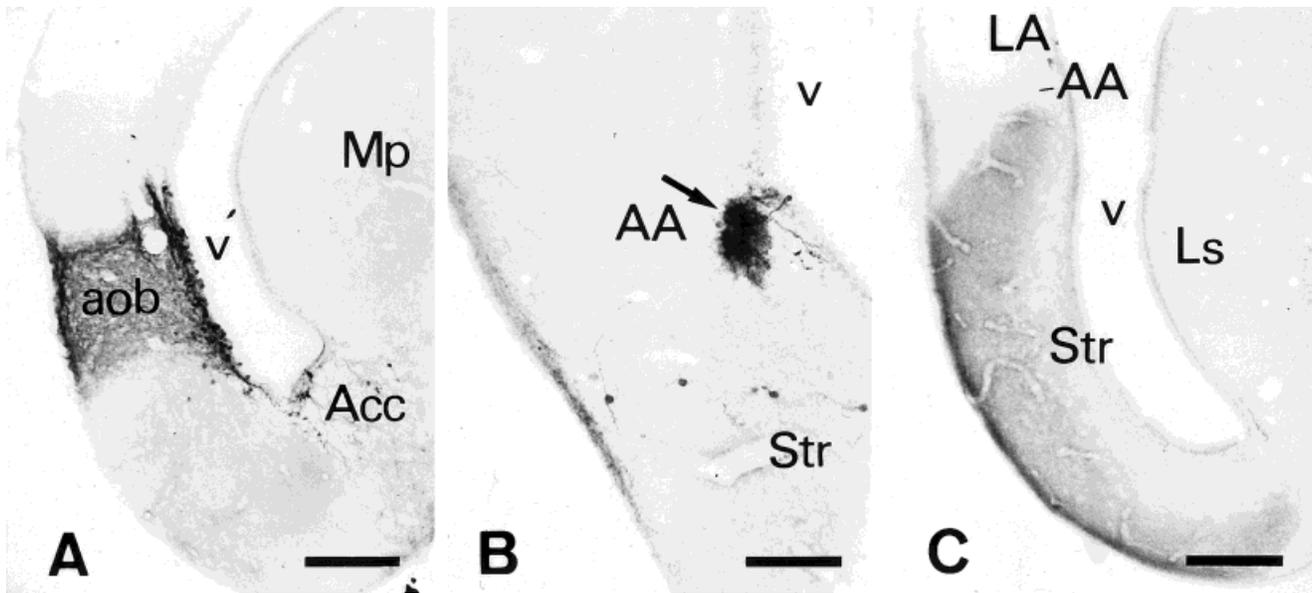


Fig. 8. Photomicrographs of transverse sections through the telencephalic hemisphere of *R. perezi* showing CRi cell bodies and fibers/terminals in the caudal part of the accessory bulb (A), in the AA just caudal to the accessory olfactory bulb (B), and in the AA and Str at

midtelencephalic levels (C). Note the concise CRi fiber tract and accompanying cells in the AA (B, arrow) and the diffuse labeling in the striatal neuropil in C. For abbreviations, see list. Scale bars = 200 μ m in A,C, 100 μ m in B.

aksinen and Panula, 1990; see Fig. 4c in Lázár et al., 1991; Yon et al., 1992; Maderdrut et al., 1996). In addition, dorsal and ventral subdivisions within the Acc can be recognized on the basis of innervation densities for most markers. Remarkably, the ventral part of the Acc differs from the dorsal part and appears to share some chemical features with the Str. For example, the Str and the ventral part of the Acc are innervated prominently by SOMi fibers, but the dorsal portion of the Acc is almost devoid of such fibers.

DP and VP. In the present study, TH, ENK, and SP immunoreactivity patterns were used to distinguish presumptive pallidal structures in the basal telencephalon of *R. perezi*. In addition, several other (immuno)histochemical markers have been used to corroborate the present delineation of the amphibian pallidum. The DP is located in the ventromedial part of what has been considered classically the ventral Str in anurans (Northcutt and Kicliter, 1980), extending from levels just rostral to the lamina terminalis up to the territory immediately caudal to the anterior commissure. In our view, the DP includes the anterior entopeduncular nucleus (see Neary and Northcutt, 1983) and forms a single entity on the basis of hodological and chemical features (Marín et al., 1997d,e; present study). In Nissl-stained sections, DP cells are larger and are packed less densely than the cells in adjacent structures. In addition, a ventral pallidal region has been identified in the ventromedial telencephalic wall. The amphibian pallidum, as shown in the present study, is characterized by dense ENKi and SPi fiber plexuses. Particularly, the DP contains a prominent ENK innervation and a less extensive SPi plexus, whereas the VP stains intensely for SP but moderately for ENK. In a previous immunohistochemical study, Merchenthaler et al. (1989) showed that the DP, as presently defined, contains a dense plexus of coarse ENKi fibers. In contrast, the dense SP

innervation that characterizes the VP was not noted in a previous study in the frog *Rana catesbiana* (Inagaki et al., 1981a). The VP also receives a prominent noradrenergic innervation (González and Smeets, 1993; present study). Remarkably, the region presently identified as the DP receives a prominent projection from the Str (Marín et al., 1997b), whereas injections of anterograde tracers into the rostral pole of the Acc give rise to descending projections that reach the VP and adjacent territories (Marín et al., 1997b). However, for a better characterization of these pallidal-like zones in amphibians, it will be necessary to determine whether ENKi and SPi fibers arise from striatal and accumbal neurons.

Amygdaloid complex and BST in amphibians

The proposed nomenclature clearly differs from previous studies of the amphibian telencephalon (Northcutt, 1974; Kicliter and Ebbesson, 1976; Northcutt and Kicliter, 1980; Wilczynski and Northcutt, 1983a,b; Neary, 1988, 1995; Bruce and Neary, 1995c). However, it seems unjustified and confusing, for example, to continue designating the main recipient of the accessory olfactory bulb projections as pars lateralis of the amygdala (see also Scalia et al., 1991). Our goal in presenting this account is to provide a more reliable terminology in light of the present findings and of recent immunohistochemical and hodological studies of the basal telencephalon in anurans. The current delineation of the basal telencephalon suggests that distinct pallial (cortical-like) and centromedial amygdaloid regions can be recognized within the amphibian amygdaloid complex. Moreover, as in mammals, an olfactory amygdala encompassing the nuclei related with the olfactory bulb and a vomeronasal amygdala, defined by the nuclei related to the accessory olfactory bulb, are recognized (Scalia et al., 1991). In addition, the presently defined BST comprises most of the territory occupied by

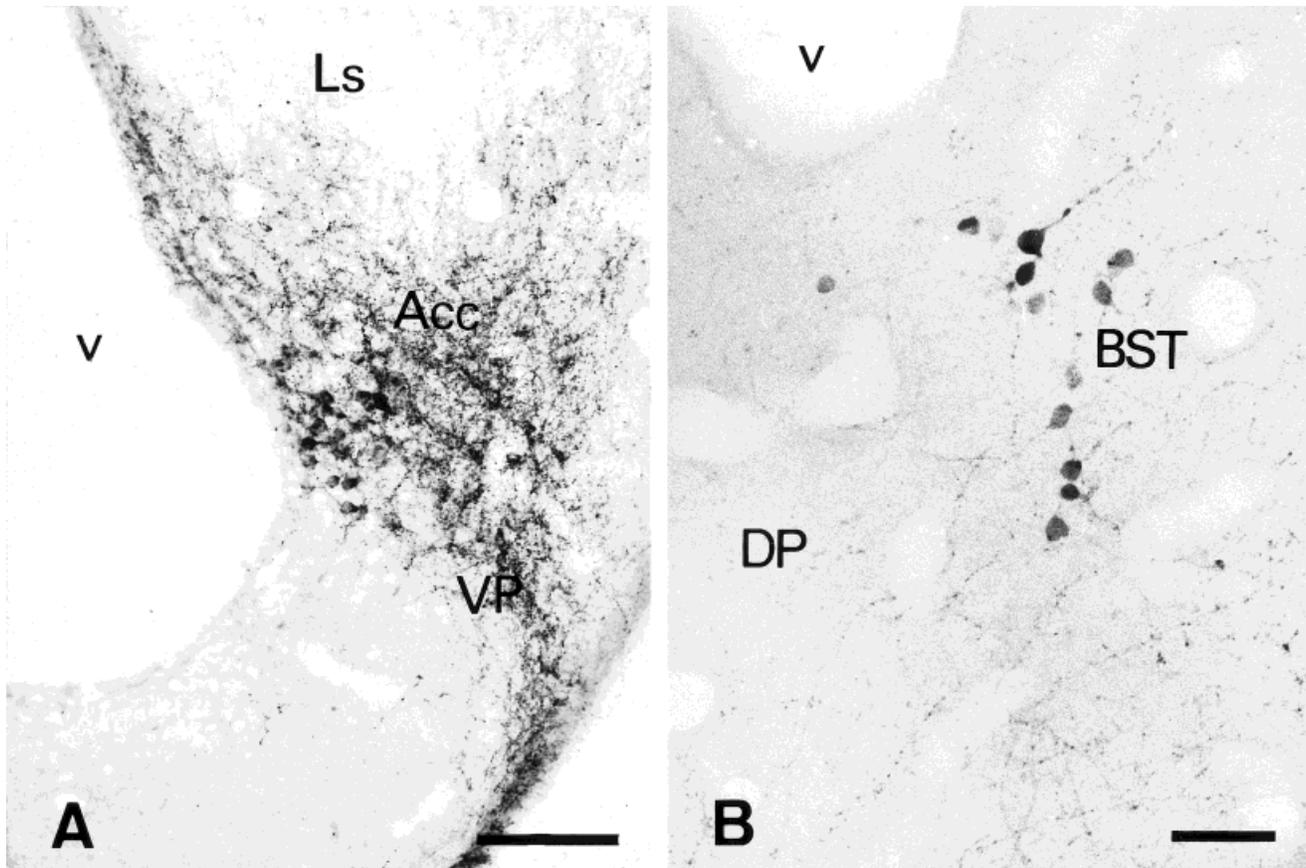


Fig. 9. Photomicrographs illustrating vasotocin (AVT)⁺ cell bodies and fibers in the Acc (A) and mesotocin (MST)⁺ neurons and fibers in the BST (B). For abbreviations, see list. Scale bars = 100 μ m in A, 50 μ m in B.

the pars medialis of the amygdala, as identified by Northcutt (1974).

LA. NADPHd histochemistry has been reported previously to differentiate between subdivisions of the lateral telencephalic wall in amphibians (González et al., 1996; Muñoz et al., 1996). In the present study, we have found that the NADPHd staining clearly delineates the LA, i.e., the ventral division of the lateral pallium, as defined in previous studies (Northcutt and Kicliter, 1980; Neary, 1990, 1995; Bruce and Neary, 1995c). The LA is characterized by a diffusely stained neuropil that extends throughout its rostrocaudal extent, clarifying the borders with surrounding structures. Hence, the LA is located between the lateral pallium proper and the olfactory amygdala throughout the length of the telencephalic hemisphere. Nevertheless, it seems probable that the territory presently defined as the LA is comprised of distinct cell populations. Thus, the pattern of NADPHd staining varies rostrocaudally and apparently defines several additional compartments, which might have different connections. For example, the most caudal portion of the LA projects to the hypothalamus (Neary, 1995), whereas cells located at intermediate-to-rostral levels in the LA project to the ventral part of the Str, the VP, and the Acc (Marín et al., 1997a,b). Little is known about the connections of the rostralmost part of the LA, but it apparently does not project to the hypothalamus or to the ventral part of the

medial telencephalic wall. Obviously, a detailed investigation of the hodological characteristics of this region is required to clarify the actual boundaries of LA subdivisions.

AA. The olfactory amygdala comprises an elongated territory that extends along the entire telencephalic hemisphere ventral to the LA and is continuous ventromedially with the striatal cell plate. In contrast to the LA, differences in cell-packing densities, which also reflect distinct chemical properties, make it possible to distinguish at least two parts within the olfactory amygdala, i.e., the AA and the MeA.

The AA is richly innervated by a great variety of neurotransmitters and neuropeptides. Among others, the AA contains fibers immunoreactive for dopamine, noradrenaline, SP, ENK, AVT, SOM, NPY, NPFF, and GAL (Inagaki et al., 1981a,b; Merchenthaler et al., 1989; Lázár et al., 1990, 1991; González and Smeets., 1991, 1992a,b, 1993, 1994; Tuinhof et al., 1994; present study). In addition, the AA contains CRi and AVTi cells (González and Smeets., 1992a,b; present study). The innervation of this region is particularly dense in its periventricular part rather than its superficial zones, suggesting the existence of compartments. The AA is located in close relation with the accessory olfactory tract and at least its rostral part receives projections from the main olfactory bulb (North-

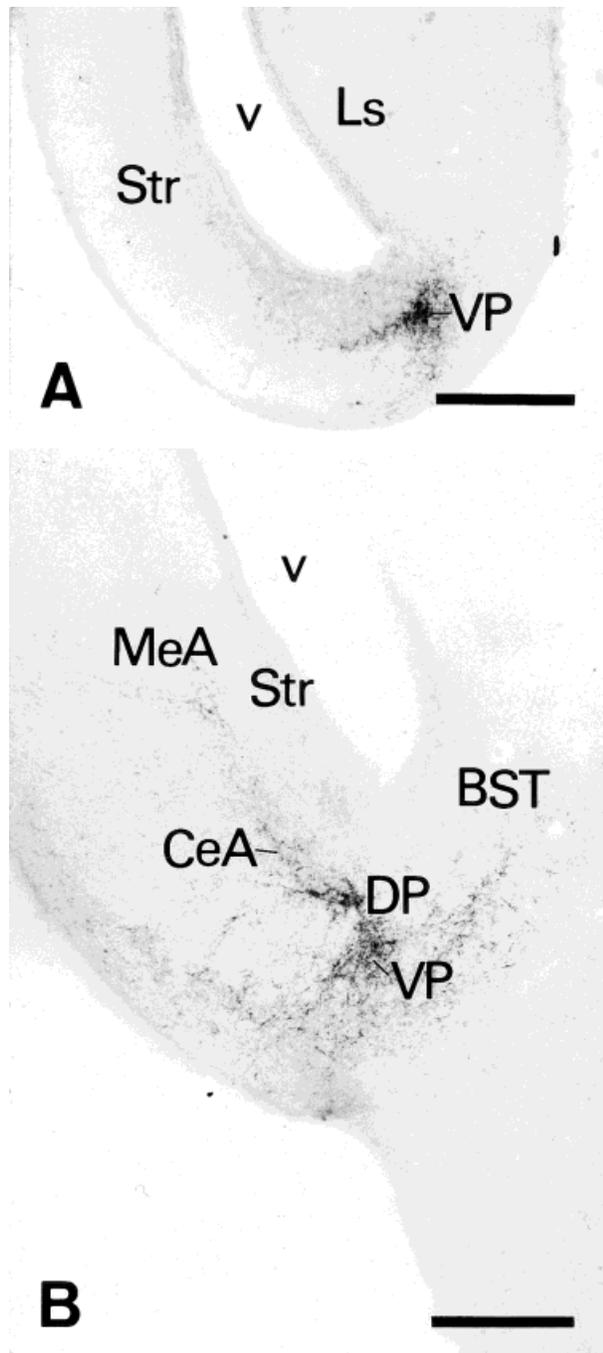


Fig. 10. Photomicrographs of transverse sections through the rostral (A) and caudal (B) telencephalic levels showing dopamine β -hydroxylase (DBH) fibers/terminals in the VP, DP, and CeA. For abbreviations, see list. Scale bars = 100 μ m.

cutt and Royce, 1975; Scalia et al., 1991; Marin et al., 1997a).

MeA. The MeA constitutes a prominent, bowl-shaped structure located in the caudal part of the amphibian amygdaloid complex. Characteristically, the MeA is clearly delineated by a prominent peptidergic staining, which includes SPi, NPYi, SOMi, GALi, and PACAPi fibers (Inagaki et al., 1981a; Lázár et al., 1991, 1993; labeled as

the nucleus entopeduncularis on Figs. 3 and 8B in Yon et al., 1992; present study). In addition, ENKi, AVTi, and calcitonin gene-related peptide (CGRP)-immunoreactive cells are located in the MeA (Merchenthaler et al., 1989; González and Smeets, 1992a,b; Petkó and Sánta, 1992; present study). The MeA, as presently defined, includes the medial amygdaloid nucleus and the cortical amygdaloid nucleus, as defined by Scalia et al. (1991) in their thorough study of the projections of the main and accessory olfactory bulbs in the frog *Rana pipiens*. Although, cytoarchitecturally, the rostral part of the MeA resembles the cortical amygdaloid nucleus, we have not identified this nucleus separately, because the precise boundary between both groups is indistinguishable in the frog *R. perezi*, and the distribution patterns of most peptidergic systems in the MeA do not identify distinct rostrocaudal compartments. Nevertheless, ENKi cell bodies appear to be restricted to the rostradorsal, plate-like portion of the MeA in *R. perezi*. In addition, the periventricular part of the MeA exhibits immunohistochemical features that distinguish it from the rest of the nucleus. For example, the periventricular part of the MeA receives a prominent catecholaminergic innervation, which is in sharp contrast with the rest of the MeA.

The MeA is the main recipient of the projections from the accessory olfactory bulb, although its rostral part also receives a projection from the main olfactory bulb (Northcutt and Royce, 1975; Scalia et al., 1991). In addition, the MeA is connected reciprocally with the hypothalamus (Scalia, 1976; Neary and Wilczynski, 1977; Allison and Wilczynski, 1991, 1994; Neary, 1995) and receives projections from the medial pallium (Northcutt and Ronan, 1992).

CeA. The CeA consists of a superficial cell population located in the caudoventral part of the lateral telencephalic wall, adjacent to the Str and the pallidum. Characteristically, the CeA contains large NADPHd⁺ cells that give rise to a compact bundle of descending fibers. In addition to the cells, the CeA contains a dense field of NADPHd fiber terminals and a prominent dopaminergic, noradrenergic, and somatostatinergic innervation. Dopaminergic fibers arise from the posterior tubercle region and the mesencephalic tegmentum, whereas the locus coeruleus and the nucleus of the solitary tract account for the noradrenergic innervation of the CeA (Marin et al., 1997d). Misinterpretation of the caudal boundaries of the Str led some to attribute the long, descending projections to the brainstem and the upper spinal cord from the caudoventral part of the lateral telencephalic wall to the Str rather than to a part of the amygdaloid complex (ten Donkelaar et al., 1981; Tóth et al., 1985; Wetzel et al., 1985). However, recent hodological studies have demonstrated that the CeA, rather than the Str, is widely interconnected with areas in the caudal brainstem, which include the parabrachial region, the rhombencephalic lateral reticular zone, and the nucleus of the solitary tract (Marin et al., 1997a,b). Similarly, the projections to the infundibular hypothalamus ascribed to the Str most likely arise from the CeA (Neary, 1995; Marin et al., 1997b).

BST. The BST occupies a ventral, periventricular position within the medial telencephalic wall from midtelencephalic levels up to the level of the anterior commissure. Immunohistochemically, the BST shares many features with the CeA and MeA regions. Thus, the BST receives a prominent peptidergic innervation, which includes SPi,

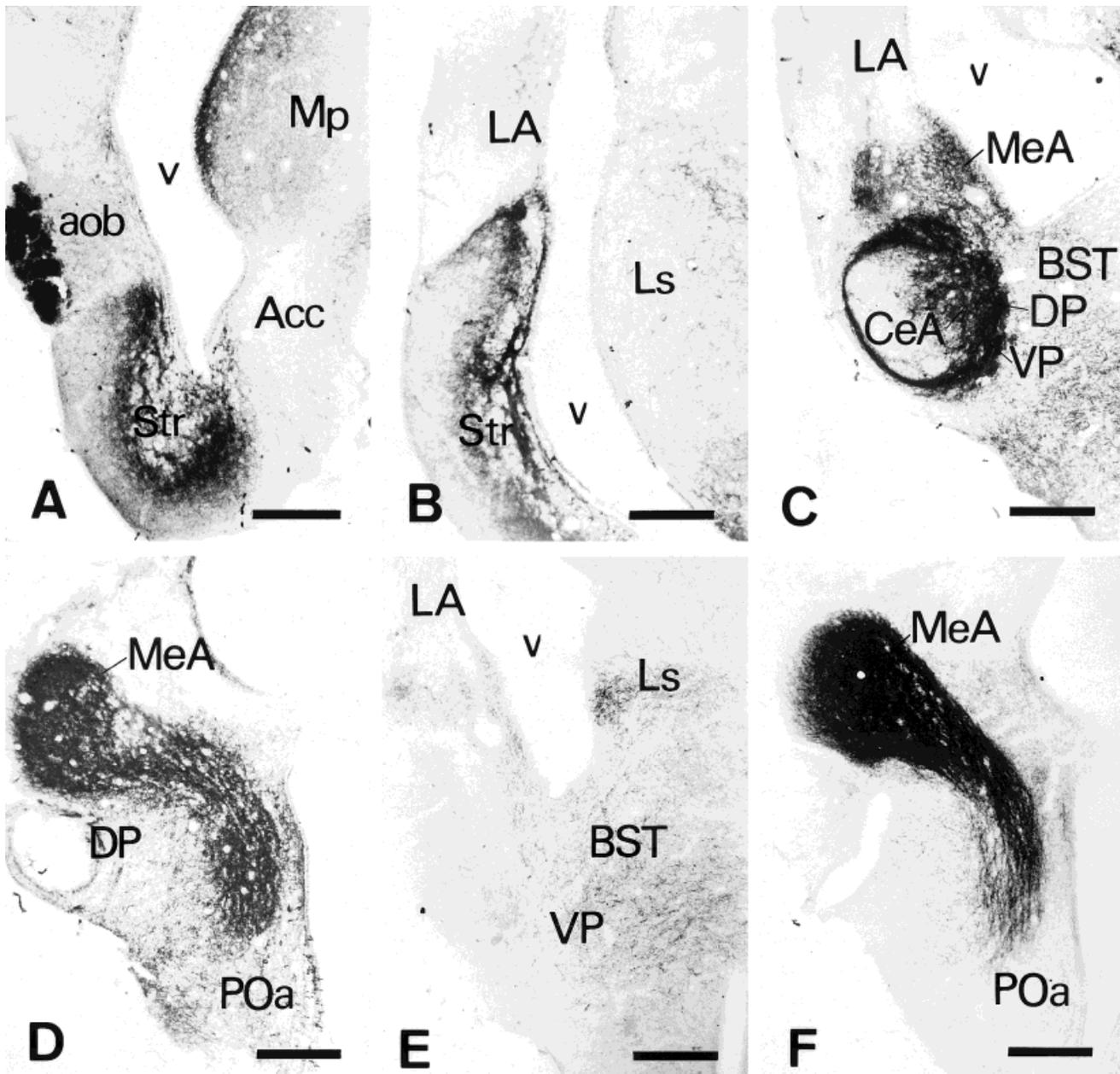


Fig. 11. A-F: Photomicrographs of transverse sections through the telencephalon illustrating somatostatin immunoreactive fibers/terminals at rostral Acc and rostral Str levels (A), at midstriatal levels (B), at levels of the CeA (C), and through the caudal pole of the MeA

(D). E and F show neuropeptide Y (NPY) staining in the transition area between the AA and the MeA and in the caudal pole of the MeA, respectively. For abbreviations, see list. Scale bars = 500 μ m.

ENKi, GALi, AVTi, HAI, and SOMi fibers (Merchenthaler et al., 1989; Airaksinen and Panula, 1990; Lázár et al., 1991; González and Smeets, 1992a,b; present study). Abundant catecholaminergic fibers are also distributed widely in the BST (González and Smeets, 1991, 1993), and the rostral part of the nucleus contains weakly NADPH-stained neurons as well as cells immunoreactive for AVT, MST, and neurotensin (González and Smeets, 1992a,b; Bello et al., 1994; present study). In contrast, the caudal, commissural pole of the BST contains GALi and ENKi neurons (Merchenthaler et al., 1989; Lázár et al., 1991). In

addition, some AVT and MST cells as well as a few dopaminergic neurons are found around the anterior commissure (González and Smeets, 1991, 1992a,b, 1994; present study). Remarkably, the distribution of several markers within the BST shows clear medial-to-lateral differences, suggesting the existence of a further subdivision within the amphibian BST.

Hodologically, the BST also appears to share many features with the CeA and the MeA. Thus, the BST projects to the hypothalamus, from which, in turn, receives a prominent projection (Neary and Wilczynski, 1977; Alli-

TABLE 1. Comparative Nomenclature of the Anuran Basal Telencephalon¹

Present study	Northcutt and Kicliter, 1980	Bruce and Neary, 1995c	Marín et al., 1997a,b
Striatopallidal system			
Nucleus accumbens	Rostral part of the Acc	Rostral part of the Acc	Nucleus accumbens
Striatum	Dorsal Str + Part of the ventral Str	Dorsal Str + part of the ventral Str	Rostral and dorsal divisions of the Str
Dorsal pallidum	Part of the ventral Str + Ea	Part of the ventral Str + Ea	Part of the ventral Str + Ea
Ventral pallidum	Part of the ventral Str + ventral part of the Acc	Part of the ventral Str + ventral part of the Acc	Part of the caudal half of the medial telencephalic wall
Amygdaloid complex			
Lateral amygdala	Dorsal part of the ventral division of the Lp	Dorsal part of the ventral division of the Lp	Ventral division of the Lp
Anterior amygdaloid area	Ventral part of the ventral division of the Lp	Ventral part of the ventral division of the Lp	Striatopallial transition area
Central amygdala	Part of the ventral Str	Caudal division of the Str	Part of the caudal division of the Str
Medial amygdala	Amygdala, pars lateralis	Amygdala, pars lateralis	Amygdala, pars lateralis
Bed nucleus of the stria terminalis	Amygdala, pars medialis + caudal part of the Acc	Amygdala, pars medialis + caudal part of the Acc	Amygdala, pars medialis

¹For abbreviations, see list.

son and Wilczynski, 1991; Neary, 1995) and is reciprocally connected with the medial pallidum (Northcutt and Ronan, 1992). However, additional studies are needed to further characterize hodological features of the amphibian BST as it is described in the present study.

Comparison with the basal forebrain organization in amniotes

In the present study, the distribution patterns for several markers have been used to delineate the striatopallidal system, the amygdaloid complex, and the BST in amphibians. Similar histo- and immunohistochemical investigations have been made in amniotes to achieve the same goal, i.e., to characterize structural and functional structures in the basal forebrain. It seems appropriate, therefore, to compare the presently found staining patterns with those of amniotes, because they most probably identify structures that share major characteristics.

Striatopallidal system. The present results strengthen the notion that, in all tetrapods, the BG consist of dorsal (Str and DP) and ventral (Acc and VP) striatopallidal systems (Medina and Reiner, 1995, 1997; Heimer et al., 1995, 1997). The division of the amphibian striatal complex into an Acc and an Str originally evolved from immunohistochemical considerations (Dubé and Parent, 1982; Parent, 1979; González and Smeets, 1991), but it has been reinforced subsequently by immunohistochemical, connective, and developmental evidence (Marín et al., 1995, 1997a,b,d,f). On the other hand, the pattern of ENK and SP immunoreactivity in the amphibian DP and VP is essentially similar to that reported for the DP and VP in sauropsids (Reiner et al., 1983, 1984a,b; Reiner, 1987; Russchen et al., 1987; Anderson and Reiner, 1990) and for the external segment of the globus pallidus and the VP in mammals (Haber and Elde, 1981; Haber and Nauta, 1983; Groenewegen and Russchen, 1984). Moreover, the connections of both pallidal structures, as presently defined, basically resemble those of the DP and the VP in amniotes (Reiner et al., 1980; Medina and Smeets, 1991; Heimer et al., 1995; Medina and Reiner, 1995, 1997; Marín et al., 1997a,b). It is suggested that numerous connections previously attributed to part of the formerly considered Str or Acc correspond to pallidal connections (Marín et al., 1997a–d; present study). For example, noradrenergic inputs to the ventral part of the medial telencephalic wall arising from the locus coeruleus and the nucleus of the solitary tract primarily reach the VP rather than the Acc (Marín et al., 1997d; present study). Moreover, the efferent projections of the caudoventral part of the lateral telencephalic wall to the pretectum and the mesencephalic and

isthmus tegmentum may be now interpreted alternatively as true dorsal pallidal projections (Marín et al., 1997b). In that view, most of the direct striatotectal connections described in amphibians most probably account for true pallidotectal projections (Finkenstädt et al., 1983; Rettig, 1988; Marín et al., 1997b,c), as demonstrated in reptiles (Welker et al., 1983) and, more recently, in mammals (Takada et al., 1994). Another interesting point is the interpretation of the striking intrinsic connections of the amphibian Str. These projections, which arise from caudal "striatal" regions and terminate at intermediate-to-rostral levels of the Str (Marín et al., 1997a,b), may now be explained as pallidostriatal pathways. In mammals, the pallidostriatal connections constitute extensive and topographically organized feedback pathways that may reciprocate the striatopallidal input (Staines et al., 1981; Staines and Fibiger, 1984; Spooren et al., 1996). Finally, another major feature that the amphibian pallidum shares with its presumptive counterpart in amniotes is the topographical relation with the distribution of the cholinergic neurons in the basal forebrain complex. Thus, the cholinergic neurons of the basal telencephalon in amphibians are distributed over several territories, including the DP and the VP, as it also occurs in amniotes (Medina et al., 1993; Medina and Reiner, 1994; Heimer et al., 1995; Marín et al., 1997e; present study).

Amygdala and BST. Although it is generally accepted that the amygdaloid complex and the BST occupy a prominent place in the telencephalon of amniotes due to their participation in multiple functions, little is known about the organization of the amygdala in the ancestors of amniotes. In the present study, a new delineation of the amphibian amygdala is proposed to provide a comprehensive framework for further comparative anatomical studies.

Although it seems clear that the LA and the AA constitute two separate cell populations on the basis of chemoarchitectonic characteristics, assigning strict homologies to each component seems difficult. The amphibian LA, as presently defined, appears to resemble the basolateral complex of the mammalian amygdala. Thus, the intermediate part of the LA gives rise to projections that reach the Str, Acc, ventral pallidal areas, DB, and hypothalamus, whereas it does not contribute to the innervation of caudal brainstem areas (Neary, 1995; Marín et al., 1997a,b). In turn, it receives a projection from the ventral forebrain and the parabrachial nucleus (Marín et al., 1997a,b). On the other hand, the caudal portion of the amphibian LA is connected reciprocally with the hypothalamus, whereas it does not project to the ventral forebrain; neither receives

projections from the parabrachial nucleus (Marín et al., 1997a,b). In that view, both the intermediate and the caudal parts of the amphibian LA appear to resemble the basolateral and lateral amygdaloid nuclei of mammals, respectively (de Olmos et al., 1985; Amaral et al., 1992).

The AA shares many features with structures of the mammalian olfactory amygdala, which includes several nuclei characterized by receiving direct inputs from the olfactory bulb, piriform cortex, diagonal band, locus coeruleus, and raphe nuclei, whereas efferents from this region reach the ventral Str and the hypothalamus (de Olmos et al., 1985; Amaral et al., 1992). Among the components of the olfactory amygdala, the existence of structures that are homologous to the mammalian bed nucleus of the accessory olfactory tract and nucleus of the lateral olfactory tract has been suggested in reptiles (Martínez-García et al., 1993; Bruce and Neary, 1995a,b). Such nuclei, if they exist in amphibians, might be located within the boundaries of the AA.

Comparison of the available data on the connections of the LA and the AA in amphibians leads to the conclusion that both structures have a similar pattern of connections, making it difficult to establish precise homologies between these groups. A similar problem has been described in reptiles, where several nuclei, including the ventral anterior amygdala, the centromedial dorsal ventricular ridge, and the lateral amygdalar nucleus, were compared with the basolateral and basomedial amygdala of mammals, whereas the external amygdala and the ventral anterior amygdala were related to the olfactory amygdala (Bruce and Neary, 1995a,b,c). In addition, it also seems likely that the amphibian LA and AA might include a field that is homologous to at least part of the mammalian piriform cortex and claustrum, as it has been proposed in amniotes (Díaz et al., 1990; Striedter, 1997). Obviously, a more detailed study of the connections of the LA and the AA is required to gain more insight into the organization of this part of the amygdala.

In amphibians, reptiles, and mammals, the MeA is the main recipient of the projections from the accessory olfactory bulb (Northcutt and Royce, 1975; Scalia and Winans, 1975; de Olmos et al., 1985; Martínez-García et al., 1991; Scalia et al., 1991; Lohman and Smeets, 1993). In some reptiles, an additional target of the accessory bulb is the nucleus sphericus (Martínez-García et al., 1991; Lohman and Smeets, 1993), which does not have an evident equivalent in amphibians or mammals. Interestingly, in addition to the accessory bulb projection, the rostral part of the amphibian MeA receives an input from the main olfactory bulb (Scalia et al., 1991), which was demonstrated previously in reptiles and mammals (Scalia and Winans, 1975; de Olmos et al., 1985; Martínez-García et al., 1991; Lohman and Smeets, 1993). Moreover, the projections from the amphibian MeA to the hypothalamus reinforce the comparison to its counterpart in amniotes (de Olmos et al., 1985; Bruce and Neary, 1995a,c). In addition, several immunohistochemical features of the amphibian MeA deserve some comments. For example, the MeA in amphibians contains a population of AVTi cells, similar to the functionally equivalent vasopressinergic cells present among medial amygdaloid neurons in mammals (Caffé and van Leeuwen, 1983; Caffé et al., 1989; Dubois-Dauphin et al., 1989; Urban et al., 1990; González and Smeets, 1992a,b; present study). Moreover, it is worth mentioning that the catecholaminergic innervation of the MeA is relatively sparse in all

tetrapods studied (Hökfelt et al., 1984; de Olmos et al., 1985; Amaral et al., 1992; González and Smeets, 1994; Reiner et al., 1994; Smeets, 1994; present study).

The amphibian CeA shares numerous features with the CeA of mammals, occupying similar positions within the lateral subpallium and having correspondent immunohistochemical features, e.g., a prominent catecholaminergic and peptidergic innervation (Hökfelt et al., 1984; de Olmos et al., 1985; Amaral et al., 1992; present study). In addition, the amphibian CeA receives abundant projections from the nucleus of the solitary tract and the parabrachial nucleus (Marín et al., 1997a,d) and, in turn, projects to the hypothalamus, midbrain and isthmus tegmentum, lateral nucleus of the torus semicircularis, parabrachial nucleus, and caudal rhombencephalon (ten Donckelaar et al., 1981; Wetzel et al., 1985; Neary, 1995; Marín et al., 1997b). A similar pattern of connections has been reported for the mammalian CeA (Saper and Loewy, 1980; van der Kooy et al., 1984; de Olmos et al., 1985; Holstege, 1991; Bernard et al., 1993; Alden et al., 1994). The striatoamygdaloid transition area in reptiles and a portion of the avian archistriatum also resemble the main features of the mammalian CeA (Zeier and Karten, 1971; Nottebohm et al., 1976; Russchen and Jonker, 1988; Bruce and Neary, 1995c).

In reptiles, birds, and mammals, the BST occupies a similar position on the floor of the lateral ventricle. The chemoarchitecture and hodological characteristics of the mammalian BST have been studied extensively (de Olmos et al., 1985; Alheid et al., 1995), but data on sauropsides are more sparse. Nevertheless, the available data suggest that the BST in reptiles and birds shares numerous features with its presumptive mammalian homologue. For example, there are afferent connections to the BST from the Acc, hypothalamus, and caudal brainstem areas (Swanson and Cowan, 1979; de Olmos et al., 1985; Russchen and Jonker, 1988; González et al., 1990; Wild et al., 1990; Smeets and Medina, 1995), whereas abundant projections from the BST reach the hypothalamus, mesencephalon, and rhombencephalon (de Olmos et al., 1985; Russchen and Jonker, 1988; Bruce and Neary, 1995a,b,c). A similar pattern of connections has been suggested for the region occupied by the BST in amphibians (Marín et al., 1997a,d). In addition, the caudal part of the BST in reptiles and mammals receives a significant input from the accessory bulb (de Olmos et al., 1985; Martínez-García et al., 1991; Lohman and Smeets, 1993), which may occur in amphibians (see Figs. 8, 9 in Scalia et al., 1991).

Extended amygdala and the fundamental plan of forebrain organization in vertebrates

The term extended amygdala concerns the notion that portions of the amygdaloid complex, i.e., the CeA and MeA, extend rostrally through the basal forebrain and include the BST. The extended amygdala consists of two subdivisions, a medial division, which is related to the medial amygdaloid nucleus and the medial BST, and a central division, which is related to the central amygdaloid nucleus and the lateral nucleus of the stria terminalis (Alheid and Heimer, 1988; Alheid et al., 1995). In addition, the two divisions form segregated corridors through the caudal substantia innominata, bridging the gap between the centromedial amygdaloid complex and the BST. Therefore, the concept of the extended amygdala provides a systematic framework for understanding the function of a broad

rostrocaudal territory of the basal forebrain in preference to analysis the amygdala as an area that is restricted to the complex of nuclei within the caudal part of the telencephalon (Alheid and Heimer, 1988; Alheid et al., 1995; Heimer et al., 1997).

The identification of the extended amygdala is supported by histological, histochemical, and hodological evidence (for extensive reviews, see Alheid et al., 1995; Heimer et al., 1997), although, remarkably, the concept of the extended amygdala originally evolved from developmental and comparative anatomical studies (Johnston, 1923). Thus, there are several markers that serve to distinguish it from nearby components of the BG or to discern between both divisions of this structure. For example, catecholamine-immunoreactive terminals in rat and monkey appear to label the central division of the extended amygdala in preference to the medial division (Hökfelt et al., 1984; Heimer et al., 1997). In addition, the central division of the extended amygdala has prominent connections with autonomic and somatomotor centers in the brainstem. For instance, cells located throughout the central division of the extended amygdala are retrogradely labeled by a single tracer injection in the parabrachial area, and, reciprocally, the central extended amygdala is the main telencephalic target of the parabrachial region (de Olmos, 1972; Saper and Loewy, 1980; Bernard et al., 1993; Alden et al., 1994).

The present results, together with data from recent studies in amphibians (Marín et al., 1997a,b,d,e), provide substantial comparative support to the concept of the extended amygdala as an early established, fundamental organizational plan of the basal telencephalon in vertebrates. The centromedial amygdala and the BST, as discussed above, share many chemical and hodological features with their respective counterparts in amniotes. Furthermore, the amphibian centromedial amygdala is rostrally continuous through the floor of the lateral ventricle with lateral and medial portions of the BST, thus sustaining the notion that these structures may be considered as a part of a large forebrain continuum. It should be noted that ventral pallidal and extended amygdaloid elements intermingle to some extent in the medial part of the VP through the floor of the lateral ventricle. A similar situation, however, is also found in mammals (Heimer et al., 1997). Interestingly, NADPHd activity can be of help in identifying the central division of the amygdala and its rostral extension in amphibians, because it appears to specifically label parabrachial projections to the basal telencephalon (Marín et al., 1997b; present study). In addition, the medial shell portion of the Acc in mammals is directly continuous with the central division of the extended amygdala, and these share many important neurochemical and connectional similarities. For example, the shell part of the Acc projects to the hypothalamus and caudal brainstem regions, a feature that is more common to the extended amygdala than to the Str (Zahm and Heimer, 1993) and that is also shared by the amphibian Acc (Marín et al., 1997b).

In summary, the fundamental organization plan of the basal telencephalon recently proposed by Heimer et al. (1995, 1997) for the mammalian brain, which proposes that the main subcortical structures of the basal telencephalon in mammals, i.e., the striatopallidal system, extended amygdala, and septal-diagonal band system, are organized such that they constitute large, rostrocaudal,

adjacent corridors (Heimer et al., 1995, 1997), might be a common feature of the organization of the basal telencephalon in all tetrapods, because most of its characteristics appear to be present in extant amphibians. Accordingly, further support is provided by the expression of homeobox genes during the early development of the vertebrate forebrain, which defines compartments that correspond with the main subcortical systems present in the adult brain (Bulfone et al., 1993; Papalopulu and Kintner, 1993; Puelles and Rubenstein, 1993; Rubenstein et al., 1994).

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