

# Basal Ganglia Organization in Amphibians: Catecholaminergic Innervation of the Striatum and the Nucleus Accumbens

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## ABSTRACT

The aim of the present study was to determine the origin of the catecholaminergic inputs to the telencephalic basal ganglia of amphibians. For that purpose, retrograde tracing techniques were combined with tyrosine hydroxylase immunohistochemistry in the anurans *Xenopus laevis* and *Rana perezi* and the urodele *Pleurodeles waltl*. In all three species studied, a topographically organized dopaminergic projection was identified arising from the posterior tubercle/mesencephalic tegmentum and terminating in the striatum and the nucleus accumbens. Although essentially similar, the organization of the mesolimbic and mesostriatal connections in anurans seems to be more elaborate than in urodeles. The present study has also revealed the existence of a noradrenergic projection to the basal forebrain, which has its origin in the locus coeruleus. Additional catecholaminergic afferents to the striatum and the nucleus accumbens arise from the nucleus of the solitary tract, where catecholaminergic neurons appear to give rise to the bulk of the projections to the basal forebrain. In other regions, such as the olfactory bulb, the anterior preoptic area, the suprachiasmatic nucleus, and the thalamus, retrogradely labeled neurons (after basal forebrain tracer-applications) and catecholaminergic cells were intermingled, but none of these centers contained double-labeled cell bodies. It is concluded that the origin of the catecholaminergic innervation of the striatum and the nucleus accumbens in amphibians is largely comparable to that in amniotes. The present study, therefore, strongly supports the existence of a common pattern in the organization of the catecholaminergic inputs to the basal forebrain among tetrapod vertebrates. *J. Comp. Neurol.* 378:50-69, 1997. © 1997 Wiley-Liss, Inc.

**Indexing term:** ventral tegmental area; locus coeruleus; solitary tract nucleus; retrograde tracing; catecholamines

The striatum (caudate/putamen) and nucleus accumbens of amniotes are characterized by dense plexuses of catecholaminergic fibers. These plexuses consist of dopaminergic, noradrenergic and adrenergic fibers, and terminals. The cells from which the dopaminergic fibers arise lie in the ventral tegmental area, the substantia nigra, and the retrorubral area (for review, see Parent, 1986). In turn, noradrenergic and/or adrenergic fibers arise in the locus coeruleus and the solitary tract region (Wang et al., 1992; Reiner, 1994; Reiner et al., 1994; Smeets, 1994).

Of all catecholaminergic connections, the dopaminergic fibers originating in the mesencephalic tegmentum constitute the major part of the catecholaminergic input to the basal forebrain of amniotes. These midbrain cell groups

are well developed not only in mammals (Björklund and Lindvall, 1984; Hökfelt et al, 1984; Kitahama et al., 1994; Reiner, 1994), but also in birds (Bailhache and Balthazart, 1993; Reiner et al., 1994) and reptiles (Brauth et al., 1983; Smeets et al., 1986, 1987; Brauth, 1988; Smeets, 1994).

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Classically, these projections have been described as components of two different systems: 1) the mesolimbic system or ventral part of the mesostriatal system, whose fibers originate from cells of the ventral tegmental area and project to the nucleus accumbens, olfactory tubercle, bed nucleus of the stria terminalis, septum, and amygdala; and 2) the nigrostriatal system or dorsal part of the mesostriatal system, which is composed of cells in the substantia nigra that innervate the caudate/putamen of mammals and the corresponding structures in reptiles and birds (Björklund and Lindvall, 1984; Fuxe et al., 1985; Parent, 1986).

Immunohistochemical studies have also demonstrated that the basal forebrain of amphibians is characterized by dense plexuses of dopaminergic and noradrenergic fibers (González and Smeets, 1991, 1993, 1995; González et al., 1993). The distribution pattern of the catecholaminergic fibers made it possible to distinguish a putative striatum proper and nucleus accumbens in both anuran and urodele amphibians (González and Smeets, 1994). Results of previous studies by means of the horseradish peroxidase technique suggested that the source of the catecholaminergic innervation of the amphibian striatum and nucleus accumbens was located in the nucleus of the periventricular hypothalamic organ and the posterior tubercle (Dubé and Parent, 1982; Wilczynski and Northcutt, 1983a,b; Dubé et al., 1990). Moreover, the possibility of catecholaminergic projections arising from levels caudal to the midbrain was excluded experimentally (Tohyama et al., 1975, 1977).

As part of research attempting to answer the question of whether the organization of basal ganglia of amphibians is comparable to that of amniotes, we reinvestigated the afferent connections to the basal forebrain of anuran and urodele amphibians by means of recently developed, highly sensitive retrograde tracing techniques (Marín et al., 1996). The results of that study provided evidence that the distribution of cells projecting to the basal forebrain was more widespread than previously described (see, e.g., Wilczynski and Northcutt, 1983a; Neary, 1988; Wicht and Himstedt, 1988, 1990; Dubé et al., 1990). A comparison of brain regions that project to the basal forebrain with those that contain catecholaminergic cell bodies reveals that there are many candidates for contribution of catecholaminergic input to the basal forebrain of amphibians (see Table 1), including as well centers in the isthmic region and the hindbrain.

In the present study, we examine the actual sources of the catecholaminergic innervation of the striatum and the nucleus accumbens in anurans (*Rana perezi*, *Xenopus laevis*) and urodeles (*Pleurodeles waltl*). To investigate this, we used sensitive retrograde tracing techniques combined with immunohistochemistry for the rate limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (TH).

## MATERIALS AND METHODS

In the present study, a total of 16 adult green frogs (*Rana perezi*), 18 clawed toads (*Xenopus laevis*), and 12 Iberian ribbed newts (*Pleurodeles waltl*) were used. The animals were obtained from the laboratory stocks of the Department of Cell Biology, University Complutense of Madrid (*Rana perezi*, *Pleurodeles waltl*), and Department of Animal Physiology, University of Nijmegen (*Xenopus laevis*). Before surgical experiments all animals were deeply anesthetized by immersion in a 0.3% solution of tricaine methanesulfonate (MS222, Sandoz) in distilled water, pH 7.4.

The following tracers were used: FluoroGold (FG; Fluorochrome); 10 kD or 3 kD biotinylated dextran amines (BDA 10 kD, BDA 3 kD; Molecular Probes, Eugene, OR); and 10 kD or 3 kD Texas Red-conjugated dextran amines (TRDA 10 kD, TRDA 3 kD; Molecular Probes). Following a ventral approach through the roof of the mouth, the tracers were applied unilaterally to the striatum or the nucleus accumbens either iontophoretically (FG), or as crystals on the tip of sharp tungsten needles (BDA, TRDA). Following survival times of 7–14 days, the animals were anesthetized and perfused transcardially with 50 ml saline followed by 200 ml fixative (4% paraformaldehyde in 0.1 M phosphate buffer [PB], pH 7.4). The brains were removed, blocked in gelatin, and cut in the frontal or sagittal plane into 30–40 µm thin sections on a freezing microtome (see Marín et al., 1996, for details). Three different groups of experiments were carried out.

### Group A

In nine cases, FG tracing was combined with immunohistochemistry for TH. For this procedure, the sections were first mounted on glass slides and coverslipped with PB.

#### Abbreviations

A	anterior thalamic nucleus	oc	optic chiasm
Acc	nucleus accumbens	P	posterior thalamic nucleus
Ad	nucleus anterodorsalis tegmenti	Pb	parabrachial nucleus
AP	anterior parencephalon	POa	anterior preoptic area
Av	nucleus anteroventralis tegmenti	PP	posterior parencephalon
bs	basal synencephalic area	rm	retromammillary area
C	central thalamic nucleus	S	synencephalon
Cb	cerebellum	SPr	secondary prosencephalon
gl	glomerular layer of the olfactory bulb	Str	striatum
Ist	isthmic region	tect	tectum mesencephali
Lc	locus coeruleus	tp	tuberculum posterius area
Lp	lateral pallium	TP	tuberculum posterius
Lpv	lateral thalamic nucleus, posteroventral division	TPdm	dorsomedial part of the tuberculum posterius
Ls	lateral septum	TPvl	ventrolateral part of the tuberculum posterius
M	mesencephalon	VH	ventral hypothalamic nucleus
m	mesencephalic tegmental area	VM	ventromedial thalamic nucleus
mi	mitral cell layer of the olfactory bulb	vt	ventral thalamus
Mp	medial pallium	zpd	zona posterodorsalis
Ms	medial septum	III	nucleus nervi oculomotorii
nPT	nucleus pretectalis		

TABLE 1. Summary of Brain Centers That Project to the Amphibian Striatum and/or Nucleus Accumbens in Relation to Their Catecholaminergic Content<sup>1</sup>

Cell populations	Projecting to		
	Striatum	Accumbens	CA neurons
<b>Telencephalon</b>			
Olfactory bulb	+	+	+(DA)
Medial pallidum	+	+	-
Striato-Pallial transition area	+	+	-
Medial amygdala	+	+	-
Lateral amygdala	+	+	-
Anterior preoptic area	+	+	+(DA, NA)
Anterior entopeduncular nucleus (A) <sup>2</sup>	+	-	-
<b>Diencephalon</b>			
Suprachiasmatic nucleus	+	+	+(DA)
Ventral hypothalamus	+	+	-
Ventromedial thalamic nucleus (A)	+	+	+(DA)
Ventrolateral thalamic nucleus, ventral part (A)	+	-	-
Periventricular nucleus of the zona incerta (A)	-	+	+(DA)
Ventral thalamus (U) <sup>3</sup>	+	+	+(DA)
Nucleus of the periventricular organ	-	-	+(DA, NA)
Anterior thalamic nucleus (A)	-	+	-
Anteroventral thalamic zone (U)	-	+	-
Central thalamic nucleus (A)	+	-	-
Lateral thalamic nucleus, anterior division (A)	+	-	-
Lateral thalamic nucleus, posteroventral division (A)	+	-	-
Posterodorsal thalamic zone (U)	+	-	-
Posterior thalamic nucleus (A)	-	-	+(DA)
Nucleus pretectalis (U)	-	-	+(DA)
Posterior tubercle	+	+	+(DA)
<b>Mesencephalon</b>			
Mesencephalic tegmentum, medial part	+	+	+(DA)
Anteroventral tegmental nucleus	-	+	-
Anterodorsal tegmental nucleus	-	+	-
Superficial isthmal reticular nucleus (A)	+	-	-
Nucleus reticularis isthmi (U)	+	-	-
Torus semicircularis	+	-	-
<b>Isthmus</b>			
Raphe	+	+	-
Locus coeruleus	+	+	+(NA)
Parabrachial nucleus	+	-	-
Nucleus reticularis superior	+	+	-
<b>Rhombencephalon</b>			
Raphe	-	+	-
Nucleus reticularis medius	+	+	-
Nucleus reticularis inferior	+	+	-
Nucleus of the solitary tract	+	+	+(DA, NA, A)
Area postrema	-	-	+(DA, NA, A)
Midline CSF-contacting cells	-	-	+(DA)
<b>Spinal cord</b>			
Midline CSF-contacting cells	-	-	+(DA)

<sup>1</sup>A = adrenaline; DA = dopamine; NA = noradrenaline.

<sup>2</sup>Terminology exclusive for anurans.

<sup>3</sup>Terminology exclusive for urodeles.

Retrogradely labeled cells were photographed with a Zeiss microscope equipped with an epifluorescence set. Subsequently, the sections were gently dismantled and thoroughly rinsed in Tris buffer (TB), pH 7.6. The free-floating sections were then processed for TH immunohistochemistry (see González et al., 1993) with a mouse anti-TH antiserum (Incstar, Stillwater, MN), diluted 1:1,000 followed by the peroxidase anti-peroxidase technique (Sternberger, 1979). The sections were then mounted on glass slides and dried overnight. After ethanol dehydration and xylene cleaning, they were coverslipped with Entellan (Merck, Daemstadt, Germany) and photographed. Comparison of photographs from the same section allowed correlation of the distribution of FG retrogradely labeled cells and TH-immunoreactive (THir) neurons.

### Group B

In 18 cases, 10 kD or 3 kD BDA experiments were combined with indirect immunofluorescence for TH. Briefly,

brain sections were first incubated for 48 hours at 4°C with a mouse anti-TH antibody (Incstar), diluted 1:1,000. They were then incubated with a FITC-conjugated mouse-IgG complex (Incstar) diluted 1:150 for 90 minutes. BDA was visualized by incubation with a Texas Red-conjugated streptavidin complex (Vector Labs., Burlingame, CA, diluted 1:200) together with the secondary antibody. The sections were then mounted on glass slides and coverslipped with Vectashield (Vector Labs.). Alternating the appropriate filter combinations in a Zeiss fluorescence microscope allowed the identification of BDA retrogradely labeled cells and THir cells.

### Group C

In 19 cases, 10 kD and 3 kD TRDA experiments were combined with indirect immunofluorescence for TH. In this procedure, indirect immunofluorescence for TH was carried out as above (group B) and the combination of filters in the Zeiss fluorescent microscope allowed the observation of the red, retrogradely labeled cells and the green THir neurons.

For details about the specificity of the TH antibodies, the reader is referred to previous works (González and Smeets, 1991; González et al., 1993). In all cases, the distribution of retrogradely labeled, THir or double-labeled neurons in the brains of *Xenopus laevis*, *Rana perezi*, and *Pleurodeles waltl*, was charted in representative transverse sections by means of a computer-aided X-Y plotting system (Minnesota Datametrics, MD-2 digitizer and software). Finally, some sections that had been plotted were counterstained with cresyl violet to determine cytoarchitectonic boundaries.

## RESULTS

In the present study, several retrograde tracing techniques were used in combination with immunodetection of TH. Each method has advantages and disadvantages. Thus, FG tracing is very sensitive but is difficult to deliver iontophoretically. Hence, the injection sites are generally large and, therefore, not suited for topographical studies. Dextran amines, on the other hand, can be injected iontophoretically or applied as crystals on the tip of sharp needles. The latter procedure has the advantage that it can be done very quickly, keeping the animal only very briefly under anesthesia. Of the dextran amines, those with low molecular weight (3 kD) are very sensitive as retrograde tracers, and they have been shown to be transported faster than larger ones (see Fritzsche, 1993). However, the crystals of these 3 kD dextran amines dissolve quickly, which has a tendency to complicate the application procedure. Biotinylated dextran amines have the advantage that they result in Golgi-like staining of retrogradely labeled cells showing clearly the morphology of the soma and the dendrites. With Texas Red-conjugated dextran amines, on the other hand, the morphology of the labeled cells is less clearly observed, but the sensitivity of the tracer is very high. Another advantage of the latter tracer is that it is already fluorescent.

In the following description of the results, the different methods used will be referred to only when needed. In general, the pattern of retrogradely labeled cells obtained with each technique is similar (after identical injection sites), although the number of cells slightly varied depending on the tracer used.

### Catecholaminergic input to the basal forebrain

A comparison of the distribution of the cells that project to the basal forebrain with that of catecholaminergic cell groups reveals that there are several candidates for the catecholaminergic input to the striatum and nucleus accumbens of amphibians (see Table 1). However, in most of these centers, neurons that project to the basal forebrain intermingle with THir cells that do not project to the basal forebrain (Fig. 1A–F). These centers are, therefore, not considered as sources of the catecholaminergic input to the striatum and the nucleus accumbens. In some centers, however, a subpopulation of THir cells was also found to contain retrogradely transported tracer substances. In the following sections, we will first describe the brain centers that do provide the catecholaminergic inputs to the striatum and the nucleus accumbens of anuran and urodele amphibians. Subsequently, some comments are given on the centers that contain THir cell bodies, which do not provide catecholaminergic input to the basal forebrain.

As shown in Table 1, there are ten brain areas that contain both THir cells and cells that project to the basal forebrain. By means of double-labeling studies, cells that were both THir and also projected to the nucleus accumbens or striatum were found in only four of these centers. These centers are, from rostral to caudal: the posterior tubercle, the midbrain tegmentum, the locus coeruleus, and the nucleus of the solitary tract.

**Posterior tubercle/mesencephalic tegmentum.** The posterior tubercle harbors a large population of dopaminergic neurons in all amphibians. The distribution of these cells, however, differs between species. In *Xenopus*, a single group of cells is found at rostral levels of the posterior tubercle, consisting of small, round cells in its dorsal part and large, pearshaped neurons in its ventrolateral part. These two populations of cells can be recognized up to caudal diencephalic levels, where the large, ventrolaterally located cells disappear and the dorsal population shifts to a more ventral position. At actual mesencephalic levels, the posterior tubercular catecholaminergic cells are replaced by dopaminergic cells in the tegmentum, which form a single group in the midline and have dendrites that extend mainly dorsoventrally. In *Rana*, two separate populations of dopaminergic cells can be distinguished rostrally in the posterior tubercle, viz. a dorsomedial and a ventrolateral group. The morphology of the cells in these groups resembles that of the dorsally and ventrolaterally located cells in the single catecholaminergic cell group in the posterior tubercle of *Xenopus*. Another difference is that, in *Rana*, the midline dopaminergic cell group in the midbrain tegmentum is more elaborate in *Rana* than in *Xenopus*.

Afferent cells to the striatum of *Xenopus* were found ipsilaterally at all rostrocaudal levels of the posterior tubercle region (Fig. 2). Rostrally, they are organized in a compact cell group in the dorsomedial part of the posterior tubercle (Fig. 2A). More caudally, scattered cells are also located in the ventral aspect of the posterior tubercle, some of them contralateral to the application site (Fig. 2B,C). A few cells were found in the mesencephalic tegmentum (Fig. 2D). The combination of retrograde tracing and TH immunohistochemistry revealed that only a restricted number of the cells, projecting from the posterior tubercle/midbrain tegmentum to the striatum, are dopaminergic neurons. They lie at intermediate and caudal levels of the

posterior tubercle, mainly on the ipsilateral side (Fig. 2B,C,a–d). In the midbrain, only a few dopaminergic cells that project to the striatum are present at rostral levels (Fig. 2D).

Following injections of retrograde tracer in the nucleus accumbens, labeled cells were located in the region of the posterior tubercle and constitute a column extending from the rostral pole of the posterior tubercle (rostral to the most rostrally located striatal afferent cells) and continuing caudally into the midbrain tegmentum up to the caudal pole of the oculomotor nucleus (Fig. 3). Although the total number of cells projecting to the nucleus accumbens was similar to that projecting to the striatum, a notably higher density of cells was observed at caudal, compared to rostral, levels. A subpopulation of cells distributed throughout this column was found to be TH-immunoreactive (Fig. 3A–C,a–d). At rostral levels of the posterior tubercle, these cells occupy a dorsolateral position (Fig. 3A); more caudally, they extend ventromedially. Occasionally, double-labeled cells were present contralaterally (Fig. 3B). A majority of dopamine-containing cells was found in the midbrain tegmental group, where nearly 50% of the cells labeled after application of tracers to the nucleus accumbens are THir (Fig. 3C).

In *Rana*, striatal afferent cells were distributed along the whole rostro-caudal extent of the posterior tubercle region and extend into the midbrain tegmentum (Fig. 4A–D). The number of retrogradely labeled cells decreases gradually from rostral to caudal levels of the posterior tubercle, whereas only a few labeled cells were found in the midbrain tegmentum, close to the midline. The afferent neurons to the striatum intermingle to a large extent with THir cells in these regions, as seen in double-labeling experiments. At rostral levels, striatal afferent cells constitute a separate cell group ventrolateral to the dorsomedial portion of the nucleus of the posterior tubercle (Figs. 1D–E, 4A). At intermediate and caudal posterior tubercle levels, THir cells and retrograde labeled neurons were found to be largely intermingled, but double-labeled cells were restricted to the dorsomedial portion of the nucleus (Fig. 4B–C,a,b). In the midbrain, very few dopaminergic cells appear to project to the striatum (Fig. 4D).

The distribution of cells in the posterior tubercle of *Rana*, which project to the nucleus accumbens, resembles that observed in *Xenopus*. The whole rostrocaudal extent of the posterior tubercle contains cells that project to the nucleus accumbens, but the number of labeled cells is higher at caudal levels. In addition, numerous cells in the midbrain tegmentum project to the nucleus accumbens (Fig. 5). As observed with double-labeling techniques, dopaminergic cells that project to the nucleus accumbens form a column that extend throughout the posterior tubercle region and the midbrain tegmentum. The number of double-labeled cells increases from rostral to caudal and is remarkably high in the midbrain (Fig. 5A–D,a,b).

In the urodele, *Pleurodeles waltl*, dorsomedial and ventrolateral subdivisions of dopaminergic cells could be identified in the posterior tubercle. The cells in the dorsomedial group are small, whereas those in the ventrolateral portion are large, piriform cells with long processes directed dorsolaterally. At caudal diencephalic levels, the latter group occupies part of the dorsal infundibulum, whereas the dorsomedial group is continuous with the dopaminergic cells in the midbrain tegmentum. In contrast to the two anuran species studied, the midbrain

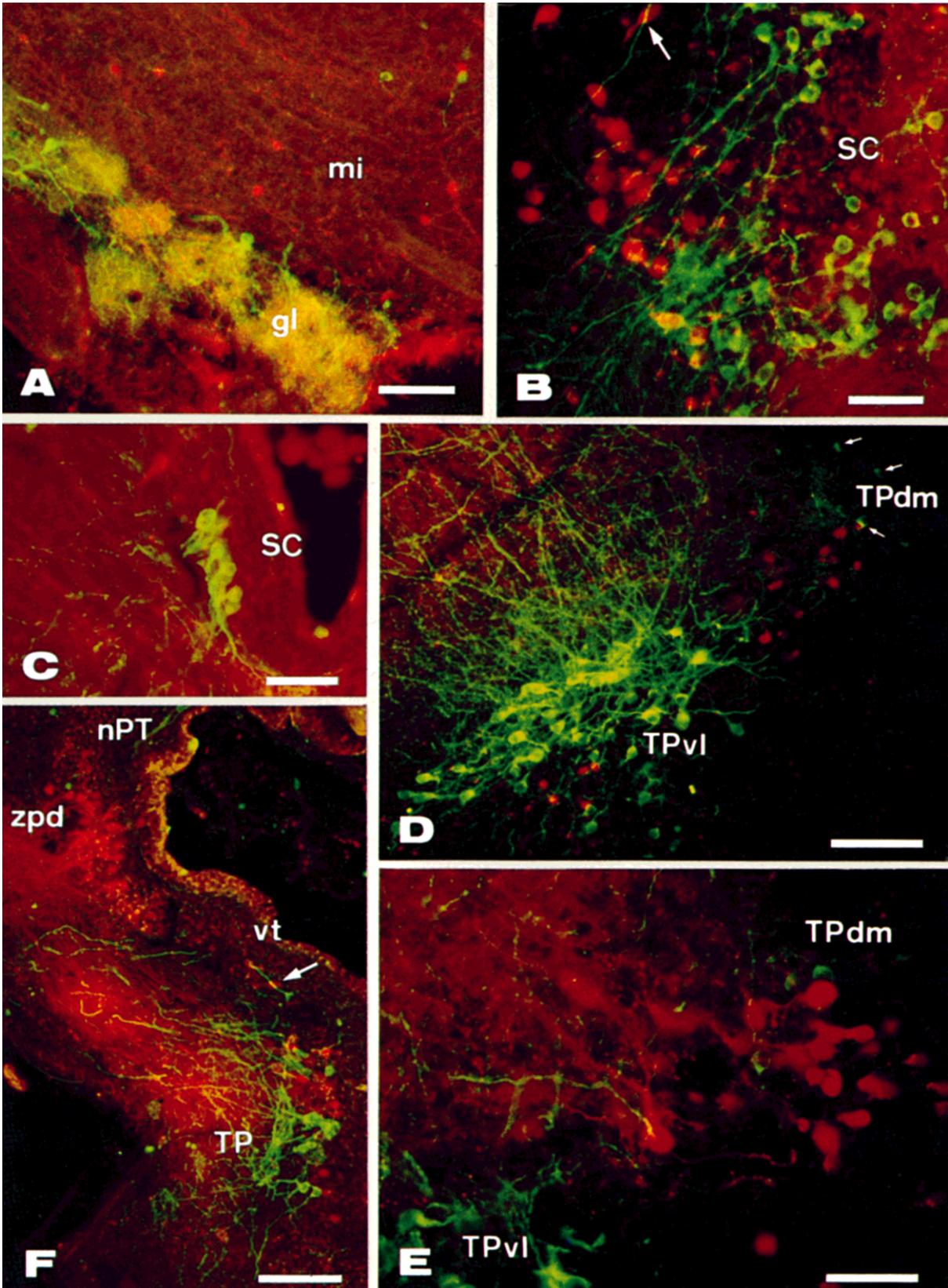


Fig. 1. Photomicrographs of transverse sections illustrating retrograde labeled cells in various biotinylated dextranamine (BDA) or Texas Red-conjugated dextran amines (TRDA) experiments (red fluorescence) in relation to THir neurons (green fluorescence) in the following brain regions. **A:** Olfactory bulb, after striatal BDA (10 kD) application in *Xenopus*. **B:** Suprachiasmatic nucleus, after striatal TRDA (10 kD) application in *Rana*. **C:** Suprachiasmatic nucleus, after striatal TRDA (3 kD) application in *Pleurodeles*. **D,E:** Posterior tubercle, after striatal TRDA (10 kD) application in *Rana*. Small

arrows in D point to small THir cells in the rostral pole of the dorsomedial group of the posterior tubercle, and this area is enlarged in E also showing the compact group of nondopaminergic retrogradely labeled cells; and F, caudal diencephalic level, after striatal TRDA (10 kD) application in *Pleurodeles*. The large arrows in B and F show examples of the yellowish appearance of overlapping fluorescences due to the section thickness (30–40  $\mu$ m). See list of abbreviations. Scale bars = 100  $\mu$ m in A,C,D, 50  $\mu$ m in B,E, 200  $\mu$ m in F.

*Xenopus laevis*

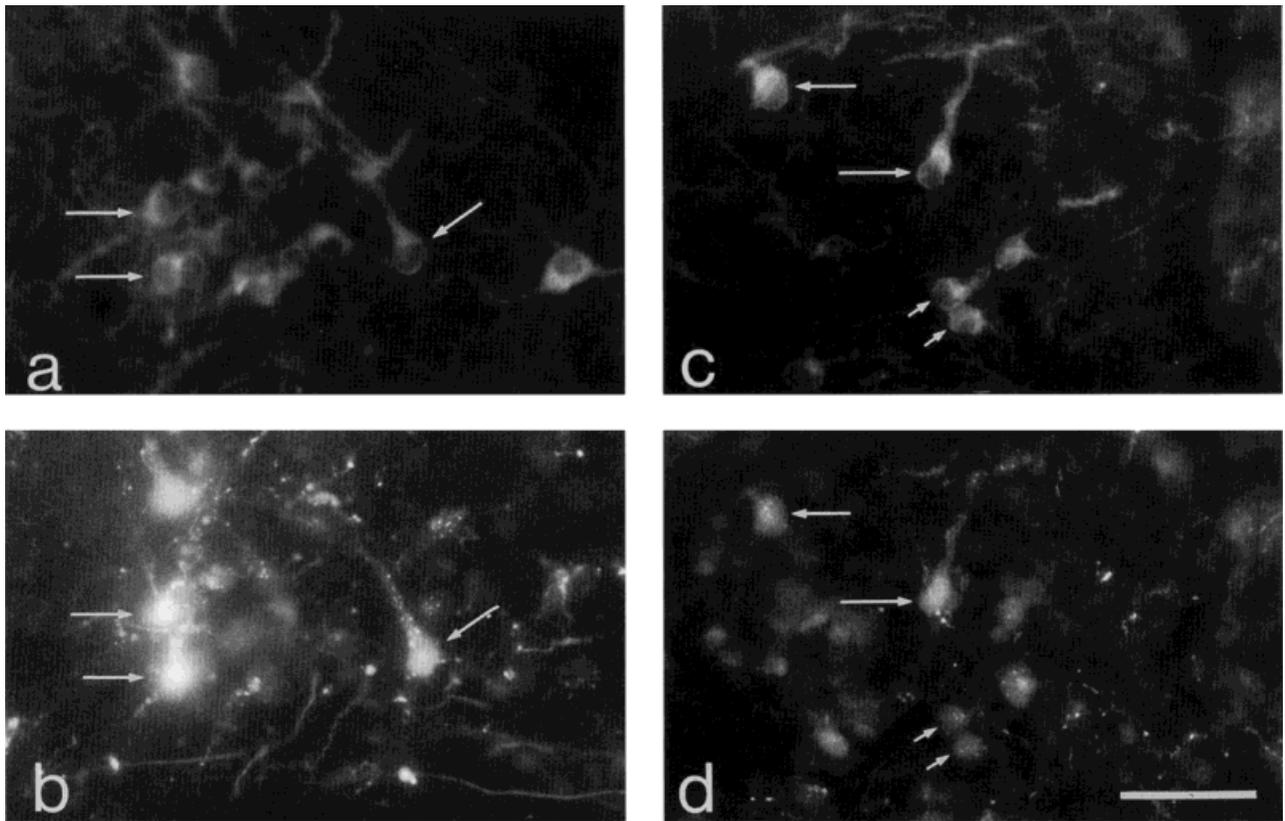
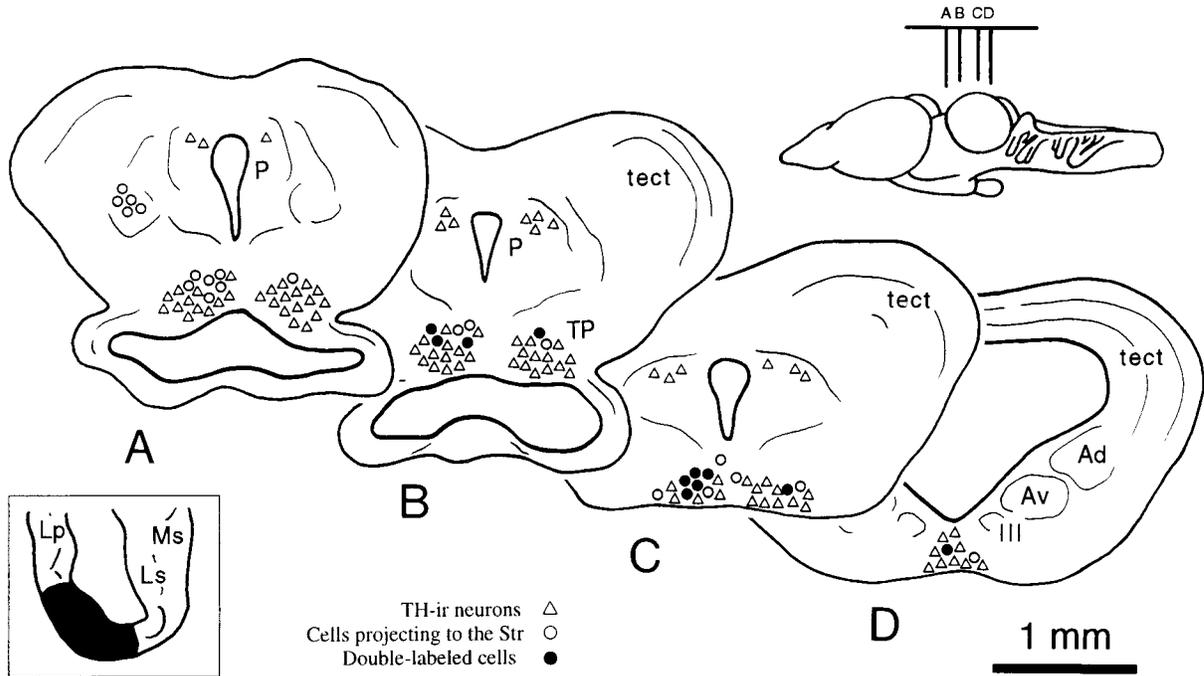


Fig. 2. **A-D:** Schematic drawings that illustrate the distribution of retrogradely labeled neurons in the posterior tubercle/mesencephalic tegmentum of *Xenopus* after tracer application to the striatum (**insert**). The localization of dopaminergic cells, as revealed by tyrosine hydroxylase (TH) immunohistochemistry, is indicated. Double-labeled cells are plotted in four selected transverse sections at levels indicated

in the upper right scheme. Photomicrographs in the lower part illustrate double-labeled cells (all arrows) at the level of the sections B (**a,b**) and C (**c,d**). In photomicrographs a and c, THir neurons are shown; in b and d, TRDA (3 kD)-labeled cells are seen. See list of abbreviations. Scale bar = 50  $\mu$ m.

# Xenopus laevis

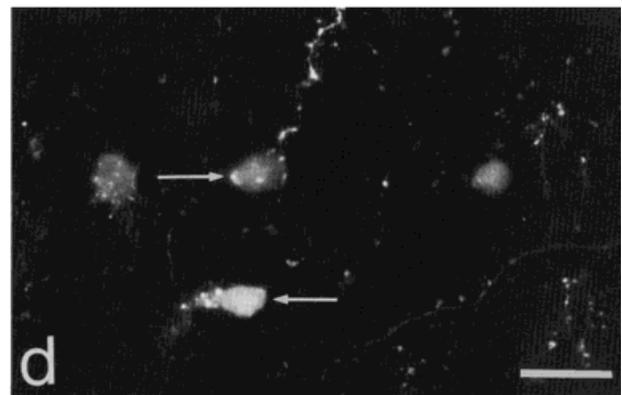
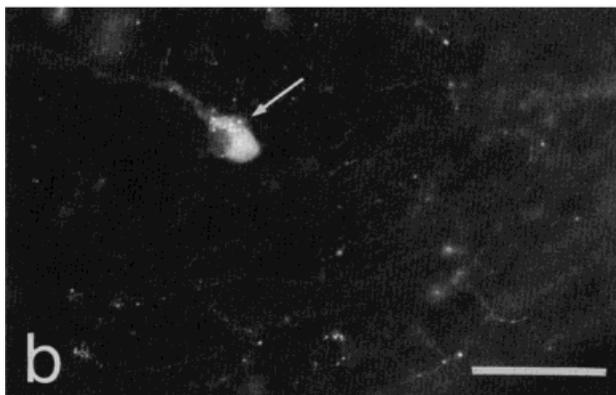
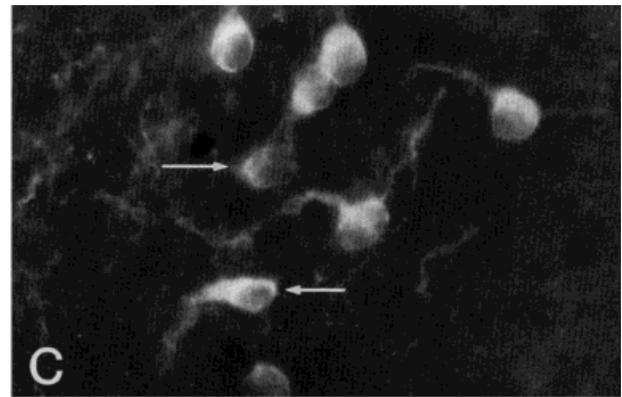
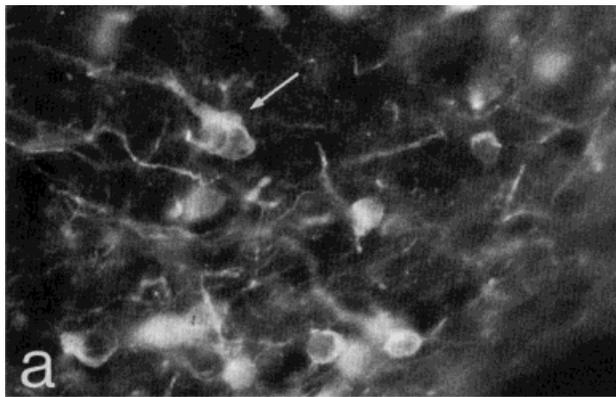
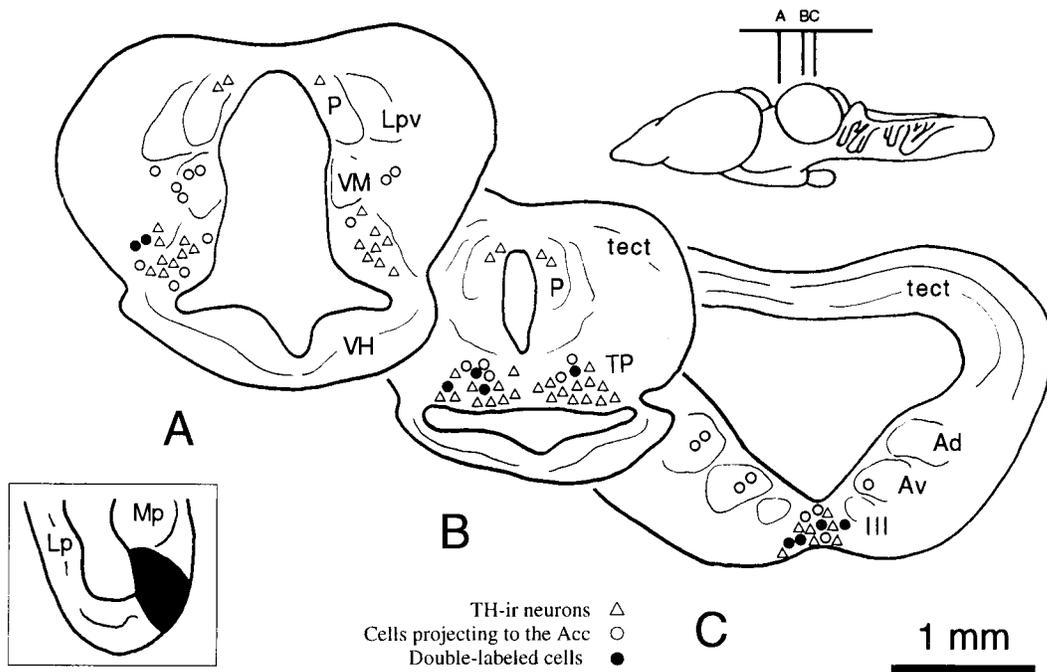


Fig. 3. A-C: Schematic drawings that illustrate the distribution of retrogradely labeled neurons in the posterior tubercle/mesencephalic tegmentum of *Xenopus* after tracer application to the nucleus accumbens (insert). The localization of dopaminergic cells, as revealed by TH immunohistochemistry, is indicated. Double-labeled cells are also plotted in three selected transverse sections at levels indicated in the

upper right scheme. Photomicrographs in the lower part illustrate double-labeled cells (arrows) at the level of the sections B (a,b) and C (c,d). In photomicrographs a and c, THir neurons are shown, whereas in b and d, TRDA (10 kD and 3 kD, respectively) labeled cells are seen. See list of abbreviations. Scale bars = 50  $\mu$ m in a,b, 25  $\mu$ m in c,d.

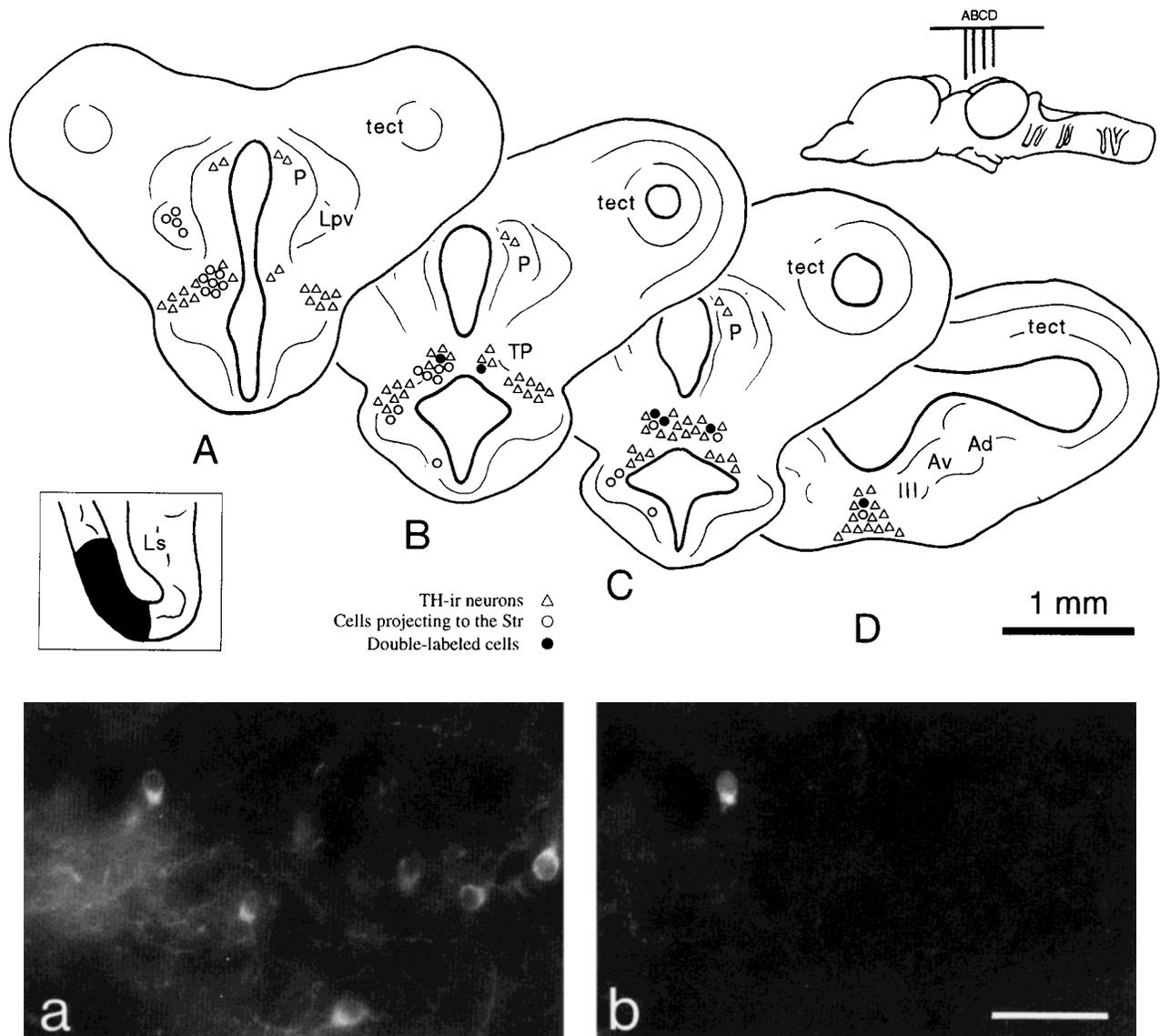
*Rana perezi*

Fig. 4. **A-D**: Schematic drawings that illustrate the distribution of retrogradely labeled neurons in the posterior tubercle/mesencephalic tegmentum of *Rana* after tracer application to the striatum (**insert**). The localization of dopaminergic cells, as revealed by TH immunohistochemistry, is indicated. Double-labeled cells are also plotted in four

selected transverse sections at levels indicated in the upper right scheme. Photomicrographs in the lower part illustrate a double-labeled cell at the level of the section C (**a,b**). In photomicrograph a, THir neurons are shown, whereas in b, a BDA (10 kD)-labeled cell is seen. See list of abbreviations. Scale bar = 50  $\mu$ m.

dopaminergic cells of *Pleurodeles* do not constitute a single cell group, but lie on both sides of the midline.

Afferent cells to the striatum of *Pleurodeles* were located mainly ipsilaterally in the posterior tubercle and the mesencephalic tegmentum. The majority of the striatal afferent cells were located in the rostral half of the posterior tubercle, exclusively confined to its dorsomedial portion (Fig. 6). Double-labeling revealed that THir cells projecting to the striatum occur only in the intermediocaudal portion of the dorsomedial posterior tubercle and, more frequently, in the midbrain tegmentum (Figs. 6A-C, 8a-c).

Afferent cells to the nucleus accumbens in *Pleurodeles* were found, as in the case of striatal afferent cells, along

the whole rostrocaudal extent of the posterior tubercle and in the midbrain tegmentum (Fig. 7A-C). However, the total number of cells as well as the number of contralaterally projecting cells was higher than after striatal applications. Dopaminergic cells projecting to the nucleus accumbens were found in small numbers from rostral levels of the posterior tubercle up to the caudal pole of the catecholaminergic cell group in the midbrain tegmentum (Figs. 7A-C, 9a-c), where they are more abundant in number.

**Locus coeruleus.** The amphibian locus coeruleus is formed by noradrenergic cells that lie in the isthmic region. In anurans, the THir cells of the locus coeruleus are scattered and vary in morphology from rostral to caudal

*Rana perezi*

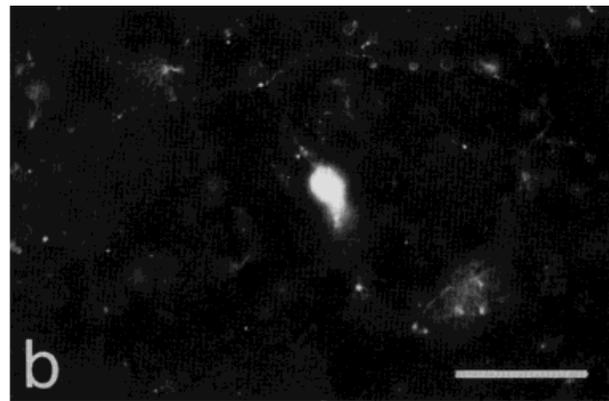
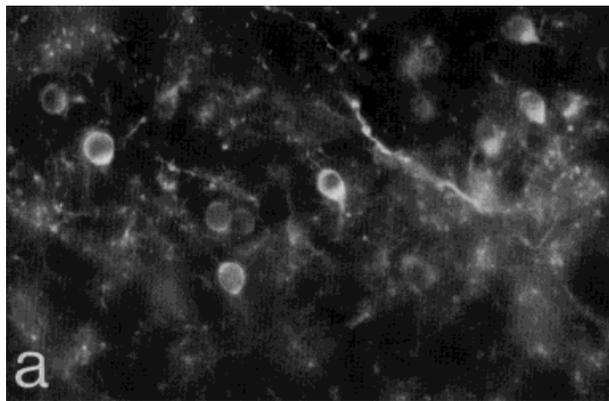
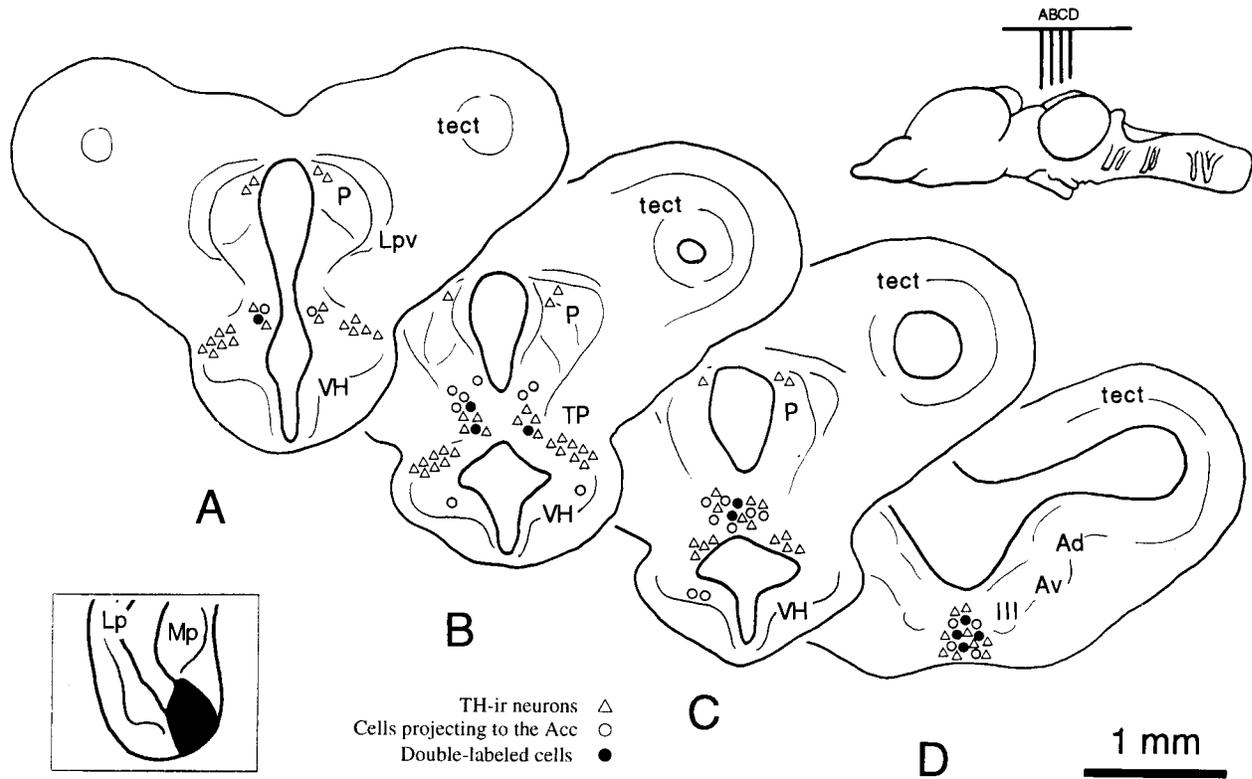


Fig. 5. **A-D:** Schematic drawings that illustrate the distribution of retrogradely labeled neurons in the posterior tubercle/mesencephalic tegmentum of *Rana* after tracer application to the nucleus accumbens (**insert**). The localization of dopaminergic cells, as revealed by TH immunohistochemistry, is indicated. Double-labeled cells are also plotted in four selected transverse sections at levels indicated in the

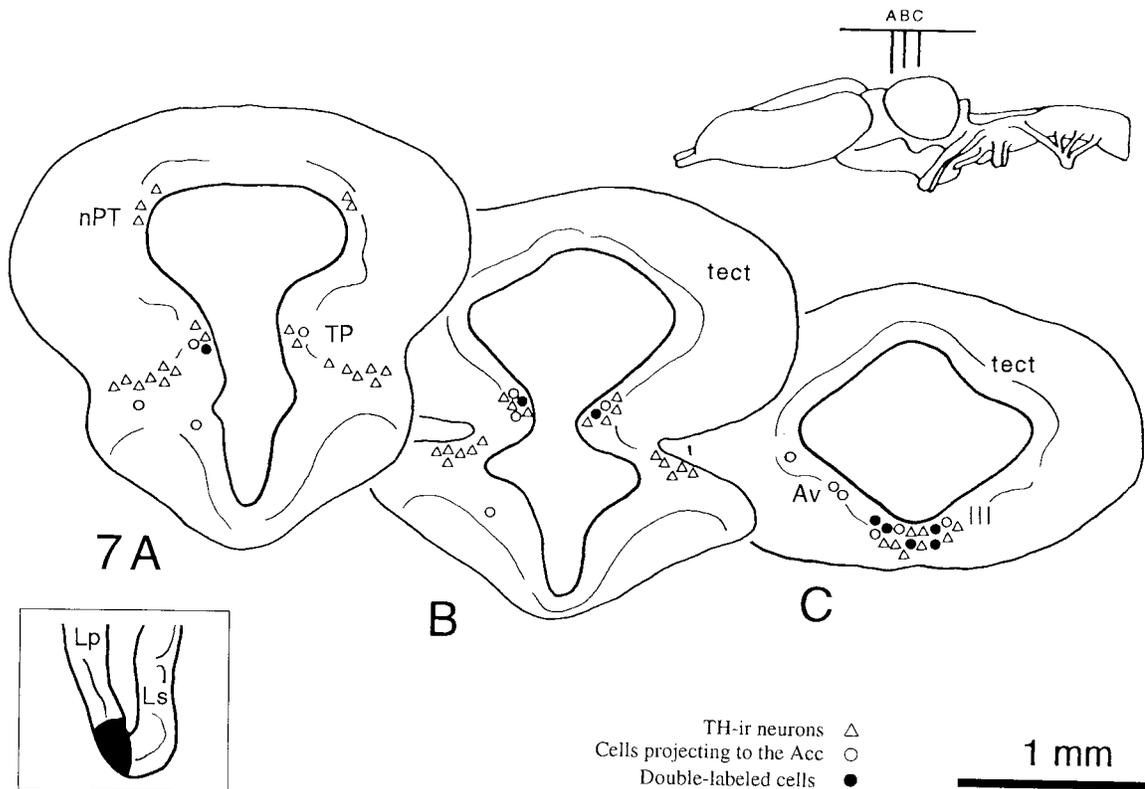
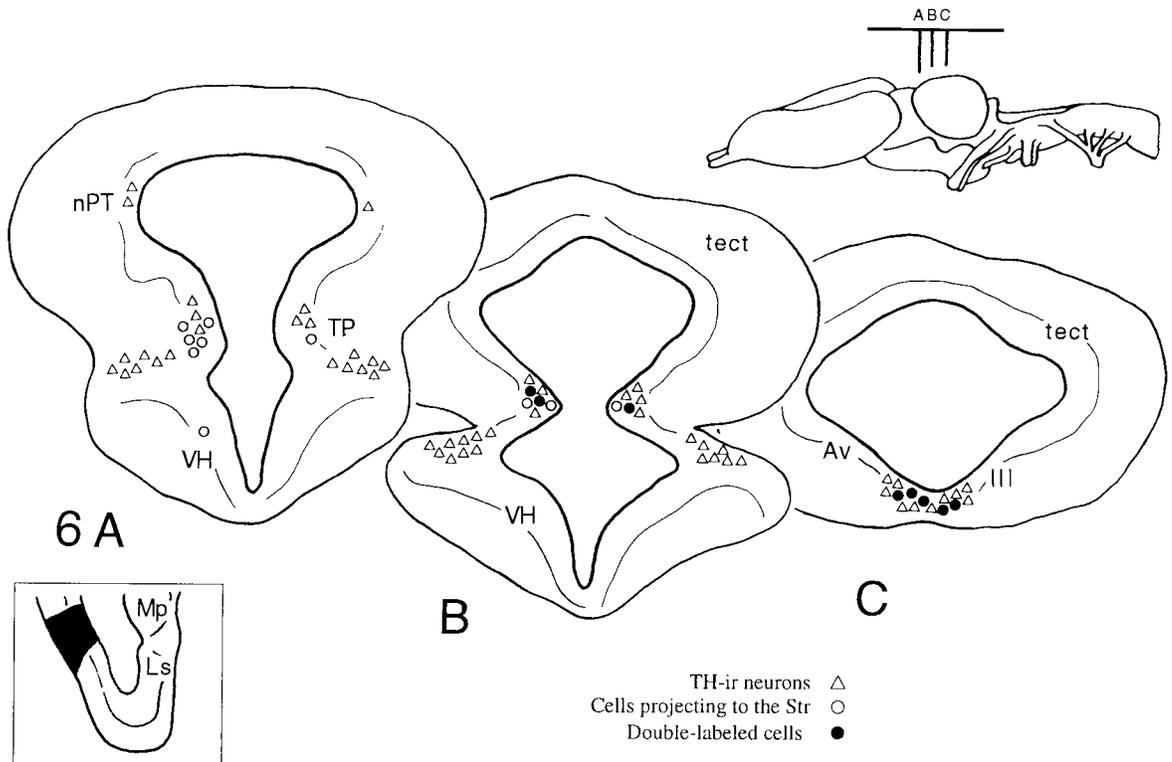
upper right scheme. Photomicrographs in the lower part illustrate a double-labeled cell at the level of the section C (**a,b**). In photomicrograph a, THir neurons are shown, whereas in b, a TRDA (10 kD)-labeled cell is observed. See list of abbreviations. Scale bar = 50  $\mu$ m.

levels. Rostrally, at the level of the isthmic nucleus, the cells of the locus coeruleus are medium-sized. More caudally, a group of large, multipolar noradrenergic cells occupies a more lateral position in the reticular formation from isthmic levels up to the caudal limit of the cerebellum. In addition, a population of small, fusiform noradrenergic cells extends medially to the isthmic nucleus and above the fourth ventricle. In *Pleurodeles*, on the other

hand, the cells of the locus coeruleus form a compact group at isthmic levels, immediately ventral to the cerebellum and ventromedial to the parabrachial nucleus.

In both anurans and urodeles, retrograde tracer applications to the striatum or the nucleus accumbens consistently labeled abundant cells in the isthmic region. The majority of the cells lie in the parabrachial area and in the reticular formation. However, combining TH immunohisto

*Pleurodeles waltl*



Figs. 6,7. Schematic drawings that illustrate the distribution of retrogradely labeled neurons in the posterior tubercle/mesencephalic tegmentum of *Pleurodeles* after TRDA (3 kD) application in the striatum (6) or the nucleus accumbens (7). The localization of dopamin-

ergic cells, as revealed by TH immunohistochemistry, is indicated. Double-labeled cells are also plotted in three selected transverse sections at levels indicated in the upper right scheme. See list of abbreviations.

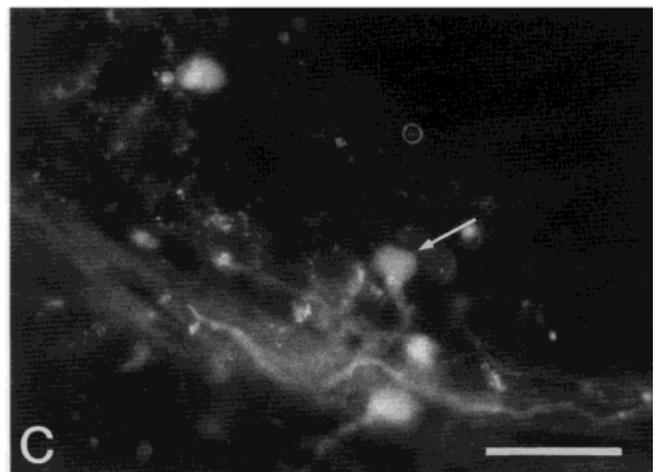
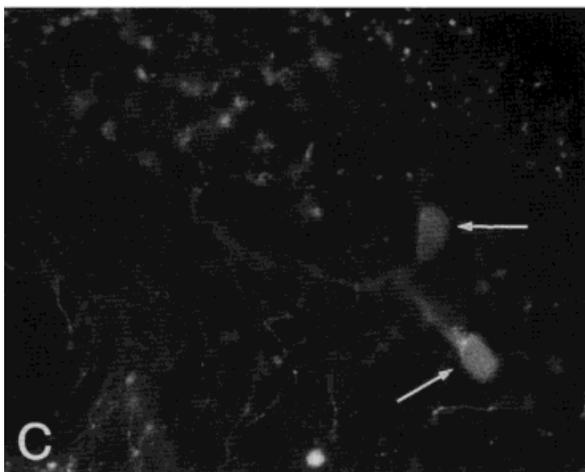
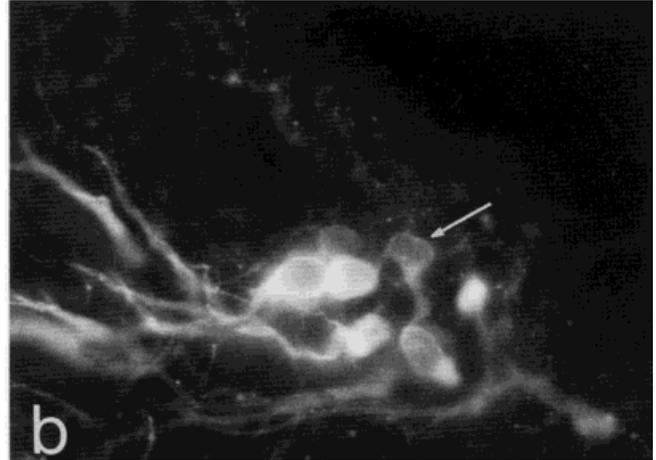
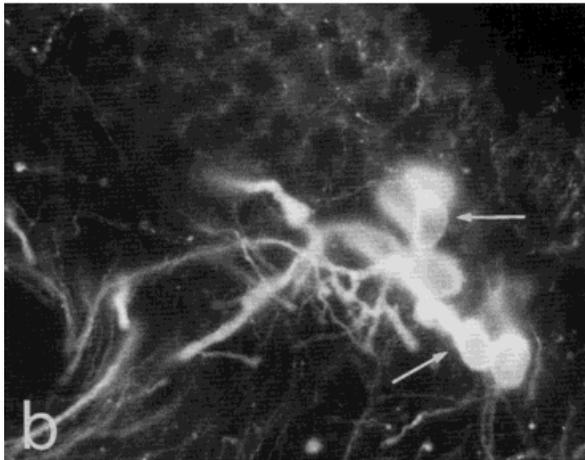
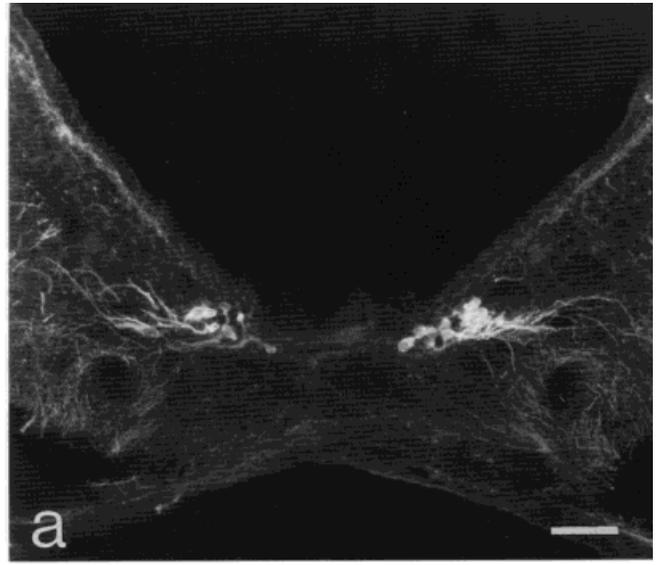
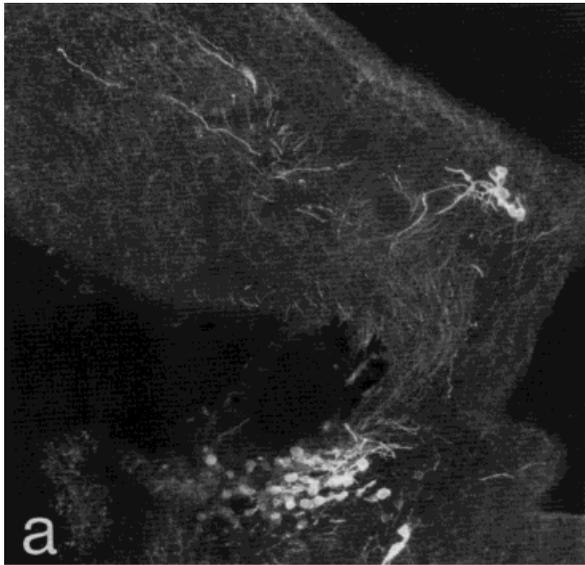


Fig. 8. Transverse sections through the brain of *Pleurodeles* that show the localization of THir cells (a,b) and retrogradely labeled cells (c). The panels illustrate the localization of THir cells in the two groups of the posterior tubercle (a) and, at higher magnification, the morphology of the cells in the dorsomedial posterior tubercular group (arrows; b) in the caudal diencephalon (level B in Fig. 6). Two cells of the latter group are double-labeled after TRDA (10 kD) application in the striatum (c, arrows). See list of abbreviations. Scale bars = 100  $\mu$ m in a, 50  $\mu$ m in b,c.

Fig. 9. Transverse sections through the brain of *Pleurodeles* that show the localization of THir cells (a,b) and retrogradely labeled cells (c). The panels illustrate the distribution of THir cells in the rostral mesencephalic tegmentum (a, level C in Fig. 7) and, at higher magnification, the morphology of the cells in the group on the left (b). One cell of the latter group is doubly labeled after TRDA (10 kD) application in the nucleus accumbens (c, arrow). See list of abbreviations. Scale bars = 100  $\mu$ m in a, 50  $\mu$ m in b, c.

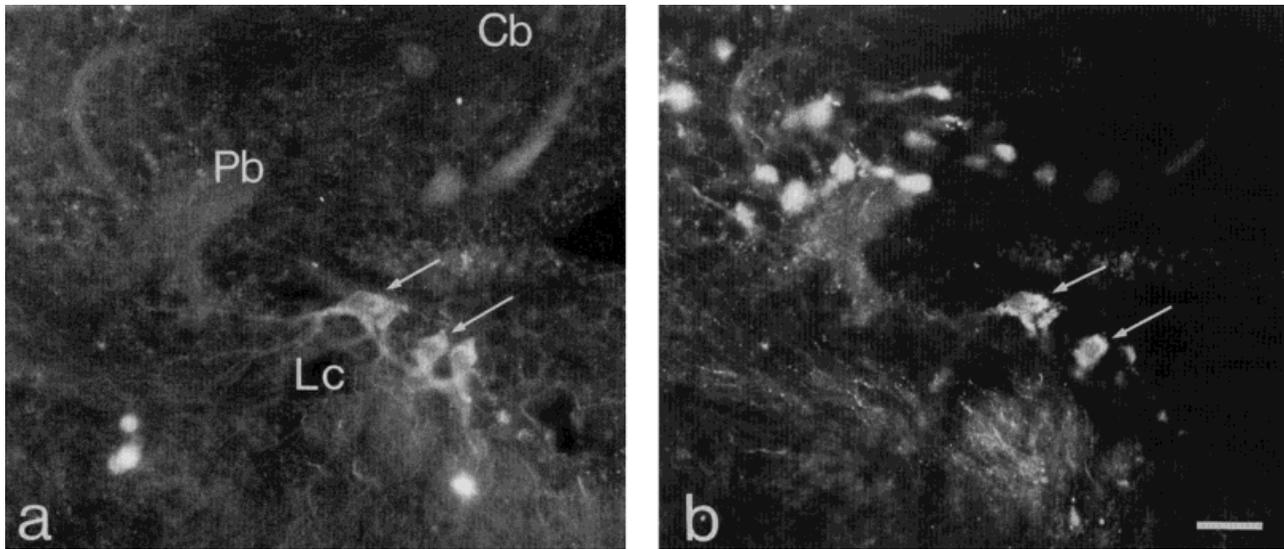


Fig. 10. Transverse section through the brain of *Pleurodeles* at the level of the locus coeruleus. The localization of the noradrenergic cells, as revealed by TH immunohistochemistry (a), was combined

with retrograde tracing of TRDA (3 kD) from the striatum (b). Double-labeled cells in the locus coeruleus are indicated by arrows. See list of abbreviations. Scale bar = 50  $\mu$ m.

chemistry with retrograde tracing techniques revealed a few double-labeled cells in the locus coeruleus. Thus, in anurans, independently from the tracer application site (striatum or nucleus accumbens), three to six double-labeled cells were observed within the population of large, multipolar, noradrenergic cells. Because of the small number of cells in the locus coeruleus that project to the basal ganglia, a topographical analysis of their distribution after different tracer applications failed to show any special arrangement between cells projecting to the striatum or the nucleus accumbens. It should be noted, however, that striatal afferent cells in the locus coeruleus of *Pleurodeles* (Fig. 10a,b) lie mainly rostral to those that project to the nucleus accumbens.

**Nucleus of the solitary tract.** In anurans and urodeles, a mixed population of dopaminergic, noradrenergic, and adrenergic cell bodies lie in the nucleus of the solitary tract, mainly medial and ventral to the tract. Following tracer applications to the striatum or the nucleus accumbens, retrogradely labeled cells were consistently observed in the ipsilateral nucleus of the solitary tract in the three amphibian species studied. The cells extend from the level of the caudal vagal motor nucleus to the obex. The number of cells labeled after striatal applications is always higher than after accumbal applications. Moreover, the cells projecting to the nucleus accumbens are restricted to the rostral half of the solitary tract nucleus. In general, the number of cells in the nucleus of the solitary tract that project to the basal ganglia is small. Double-labeling, however, showed that almost all cells that are retrogradely labeled, are also THir (Fig. 11).

### Other catecholaminergic cell groups

At many places in the brain of amphibians, catecholaminergic cell bodies intermingle with cells that project to the striatum and the nucleus accumbens. The following section considers the centers that do not provide a catecholaminergic input to the basal forebrain.

The most rostrally located catecholaminergic cells in the brain of amphibians lie in the olfactory bulbs around the glomeruli and in the mitral and internal granular layers. In experiments with retrograde tracer applications to either the striatum or the nucleus accumbens, labeled cells were always found in the mitral cell layer and, to a lesser extent, in the granular layer. The morphology of the cells projecting to the basal ganglia resembles that of catecholaminergic cells and a high degree of codistribution is found in several bulbar regions. However, double-labeled neurons were never found in the olfactory bulbs (Fig. 1A).

Previous studies in all three species have demonstrated a large population of catecholaminergic cells in the anterior preoptic area, surrounding the ventricle. The majority of these cells are in direct contact with the cerebrospinal fluid (CSF) by means of short processes with club-like endings. Only a few cells lie separated from the ventricular lining and differ in morphology from the CSF-contacting cells. Both in anurans and urodeles, cells in the anterior preoptic area project to the basal ganglia. However, although some of these cells lie close to the ependyma, they are not CSF-contacting neurons. The double-labeling experiments of this study did not reveal any double-labeled cell in this region.

Numerous THir cells were found in the suprachiasmatic nucleus of anurans and urodeles. Within the nucleus, two subpopulations of THir cells are recognized: a medial group of primarily CSF-contacting cells, and a lateral group with cells that lie further away from the ventricle. Following tracer application into the basal forebrain of anurans, a relatively large number of retrogradely labeled cells is found in the suprachiasmatic nucleus. From rostral to caudal, these cells occupy a lateral to lateroventral position within the nucleus. The cells that project to the striatum and nucleus accumbens in the rostral half of the suprachiasmatic nucleus lie lateral to the lateral group of THir cells, but, more caudally, projection cells are largely intermingled with the THir cells in the ventrolateral

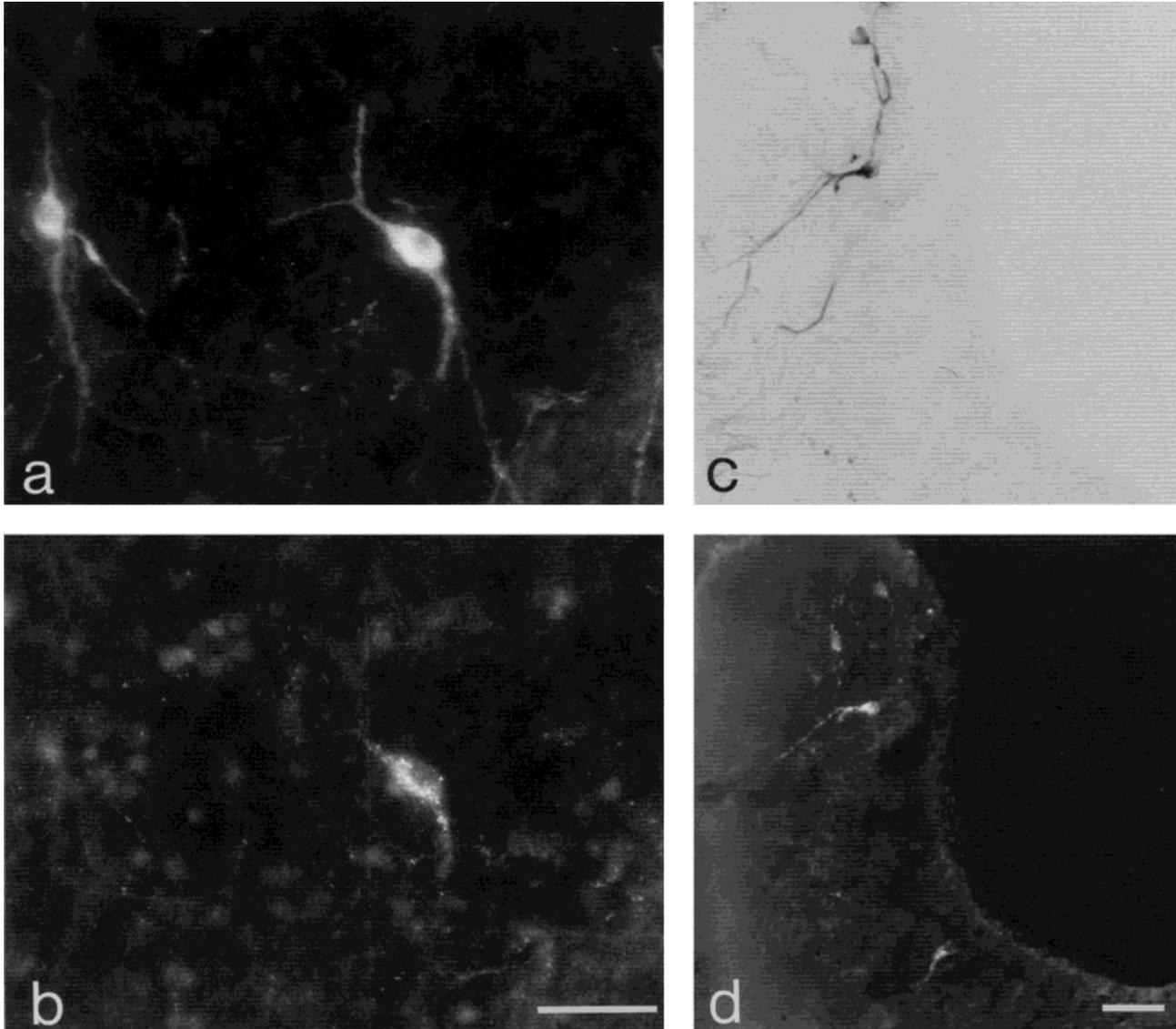


Fig. 11. Photomicrographs illustrating THir cells (a,c) and retrogradely labeled cells from the striatum (b,d) in *Xenopus* (a,b) and *Pleurodeles* (c,d). The catecholaminergic cell in b was retrogradely labeled after TRDA (3 kD) application, whereas those in d were found after a FluoroGold striatal injection. Scale bars = 50  $\mu$ m.

aspect of the nucleus (Fig. 1B). However, double-labeled cells were never observed in any region of the suprachiasmatic nucleus of anurans. In the suprachiasmatic nucleus of urodeles, a similar situation was observed. Only a few cells were found to project to the basal ganglia, and those are generally located dorsal to the lateral group of THir cells (Fig. 1C).

Both in anuran and urodeles, cells in the ventral thalamus project to the striatum and the nucleus accumbens, although the input to the latter seems to be stronger. CSF-contacting cells in the nucleus of the periventricular organ were never labeled after tracer applications into the basal forebrain. Scattered THir cells are present in the ventral thalamus of anurans and urodeles and are particularly numerous in an area lateral to the nucleus of the

periventricular organ (accompanying cells of the periventricular organ), but not in the nucleus of the periventricular organ itself. The latter structure is known to contain numerous CSF-contacting cells that are immunonegative for TH but immunopositive for antisera against dopamine and noradrenaline, suggesting accumulation of these catecholamines from the ventricle (for review, see González and Smeets, 1994). The combined immunohistochemical and tract-tracing techniques did not reveal double stained cells in these areas, although a high degree of codistribution of THir and projection neurons was observed in the area lateral to the periventricular organ.

The dorsal thalamus represents the main source of afferent fibers to the basal forebrain. The only group of catecholaminergic cells in the amphibian dorsal thalamus

is found at its caudal pole. However, these dopaminergic cells are clearly separated from the cell groups that project to the basal forebrain (Fig. 1F).

## DISCUSSION

By means of retrograde tracer techniques in combination with TH immunohistochemistry, the present study has revealed the sources of the catecholaminergic inputs to the striatum and the nucleus accumbens of amphibians. Catecholaminergic cell bodies in the posterior tubercle and the midbrain tegmentum are the major source of the dopaminergic input, whereas the locus coeruleus, most likely, provides the major noradrenergic input. Since it is well known that the nucleus of the solitary tract of amphibians contains dopaminergic, noradrenergic as well as adrenergic cell bodies (González and Smeets, 1994, 1995), its projection to the striatum and nucleus accumbens may also contain all three catecholamines.

### Comparison with previous studies in amphibians

Although the origin of the afferent connections to the basal forebrain of amphibians has been discussed in several studies (Tohyama et al., 1977; Vesselkin et al., 1980; Dubé and Parent, 1982; Wilczynski and Northcutt, 1983a; Dubé et al., 1990; Lázár and Kozicz, 1990), our knowledge is still limited for several reasons. Firstly, these studies were performed with horseradish peroxidase (HRP), a tracer that is less sensitive than the more recently developed tracers. Secondly, in the previous studies, large injections were made into the basal forebrain, primarily in the striatum, but sometimes including part of the nucleus accumbens. Another limitation was that those studies did not use double-labeling techniques. Nevertheless, by comparing the distribution of catecholaminergic cells with that of cells retrogradely labeled after basal forebrain injections, it was suggested that cells in the posterior tubercle were the major source of the dopaminergic innervation of the amphibian striatum (Wilczynski and Northcutt, 1983a; Parent, 1986). However, the present study has revealed the existence of two distinct cell populations within the posterior tubercle that project to the basal ganglia of amphibians. One population consists of nondopaminergic cells that form a compact group in the rostral part of the dorsomedial aspect of the posterior tubercle with additional cells sparsely distributed at more caudal levels, the other cell group consisting of dopaminergic cells that are scattered throughout the dorsomedial part of the posterior tubercle. The cells described by Wilczynski and Northcutt (1983a) as the putative source of the catecholaminergic innervation of the striatum in *Rana catesbeiana* most probably correspond to the noncatecholaminergic cells that occupy a similar position in the posterior tubercle of *Rana perezi* and *Xenopus laevis*. The use of highly sensitive tracers in the present study has revealed the existence of additional, noncatecholaminergic afferents from cells at intermediate and caudal levels of the posterior tubercle. Furthermore, these recently developed tracers enabled us to visualize the dopaminergic, striatal afferent neurons throughout the posterior tubercle, which cells are always less strongly labeled than the noncatecholaminergic cells. Similarly, the dopaminergic projections to the nucleus accumbens arising from the posterior tubercle

region seem to be a common feature in all amphibians studied (Marín et al., 1995; present study).

Another source for the catecholaminergic innervation of the amphibian basal ganglia, as suggested by previous studies, was the nucleus of the hypothalamic periventricular organ (Terlou and Ploemacher, 1973; Chacko et al., 1974; Dubé and Parent, 1982; Parent, 1986; Dubé et al., 1990). Ascending monoaminergic projections apparently arising from the area of the periventricular organ to the basal forebrain have been visualized in anurans (Terlou and Ploemacher, 1973; Chacko et al., 1974) and urodeles (Dubé and Parent, 1982). Lesion experiments in ranid frogs further corroborated the notion that the majority of monoaminergic afferent fibers to the telencephalon originated from the periventricular organ (Tohyama et al., 1977). Using the HRP technique, Dubé et al. (1990) suggested that the cells in the periventricular organ of the urodele, *Triturus cristatus*, project to the striatum, but this finding was not convincingly documented (see their Fig. 3A). In fact, all previous tracing studies failed to prove the existence of a basal forebrain projection arising from the hypothalamic periventricular organ in amphibians (see, e.g., Kicliter, 1979; Vesselkin et al., 1980; Wilczynski and Northcutt, 1983a). Even the use of very sensitive retrograde tracers in the present study did not reveal such a projection in any of the three amphibian species studied (Marín et al., 1996; present study). Therefore, it is concluded that the CSF-contacting cells of the nucleus of the periventricular organ do not contribute to the catecholaminergic innervation of the telencephalic basal ganglia of amphibians. In agreement with this conclusion is the observation that the striatum and the nucleus accumbens of anurans and urodeles contain distinct plexuses of THir fibers, whereas the cells in the periventricular organ are TH immunonegative (Smeets and González, 1990).

The existence of a telencephalic projection arising from the noradrenergic cells of the amphibian locus coeruleus has been frequently denied (Tohyama et al., 1975, 1977; Yamamoto et al., 1977). In fact, lesion experiments in *Rana catesbeiana* led to the conclusion that the catecholaminergic isthmus group in the latter species comprises neurons with short axons, which arborize only locally in the cerebellum (Tohyama et al., 1975). The present study, however, has provided strong evidence for a projection from the noradrenergic isthmal cell group to the basal forebrain in both anuran and urodele amphibians. Therefore, the conclusion that the amphibian locus coeruleus is poorly developed compared to the corresponding structure in amniotes (Tohyama et al., 1975; Schmidt, 1980; Dubé and Parent, 1982; Franzoni et al., 1986) must be reconsidered. Additional double-labeling studies are required to confirm whether other relevant efferent connections that characterize the locus coeruleus of amniotes, e.g., a projection to the spinal cord (Moore and Card, 1984), are also present in amphibians.

In contrast to an experimental lesion study in the frog, *Rana catesbeiana* (Tohyama et al., 1975), the present study has demonstrated a catecholaminergic projection from the nucleus of the solitary tract to the basal ganglia in both anuran and urodele amphibians. Furthermore, it was shown that the majority of the neurons within the nucleus of the solitary tract that project to the basal ganglia are catecholaminergic. Since dopaminergic and noradrenergic

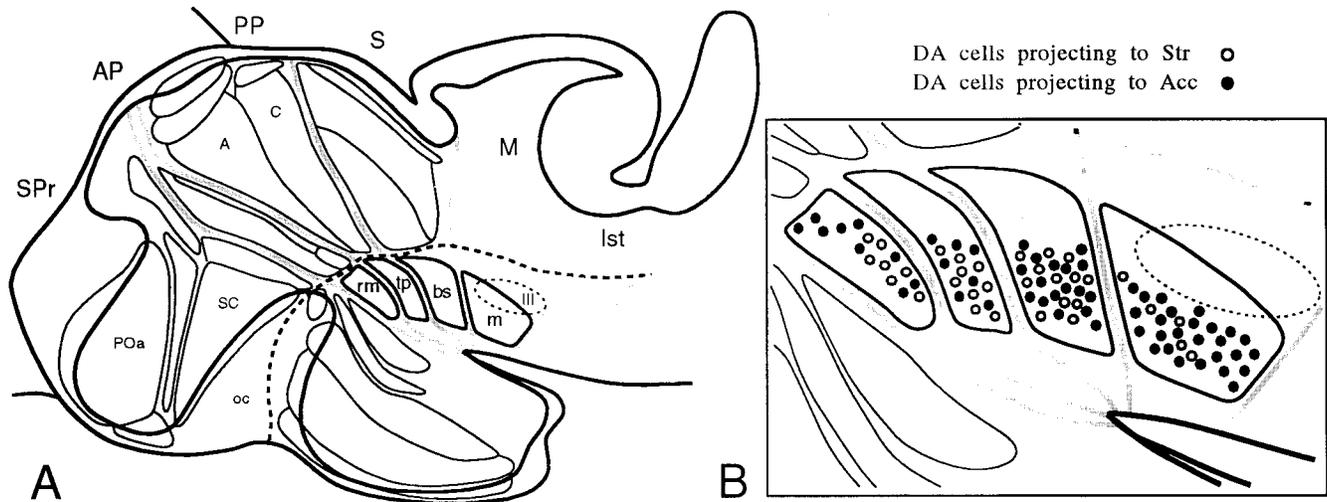


Fig. 12. Schematic diagram of a midsagittal section through the anuran brain illustrating, in projection, the mesencephalic and diencephalic centers with special reference to the organization of the basal plate structures (A, modified after Puelles et al., 1996). Intersegmental boundaries are represented as gray lines, whereas a striped line

marks the border between the alar and basal plates. B: Representation of the distribution of dopamine cells projecting to the anuran basal ganglia within the framework of the segmental organization of the diencephalic and mesencephalic basal plate. See list of abbreviations.

neurons are present in the latter nucleus (González and Smeets, 1991, 1993, 1995; González et al., 1993), it is possible that the two catecholaminergic systems project to the basal forebrain. Moreover, since adrenergic neurons constitute the major part of the catecholaminergic neurons within the nucleus of the solitary tract of *Pleurodeles* (González and Smeets, 1995) and adrenergic fibers were seen in the basal forebrain, this nucleus is the most likely source of adrenergic input to the telencephalic basal ganglia.

### The posterior tubercle/mesencephalic projection to the basal ganglia: Comparative considerations

The present study has demonstrated that the dopaminergic innervation of the striatum and the nucleus accumbens in amphibians arises primarily from the posterior tubercle and mesencephalic dopaminergic cell groups. Furthermore, this dopaminergic projection appears to be more elaborate in anurans than in urodeles. In *Rana*, where the dopaminergic innervation of the nucleus accumbens is particularly well developed (González and Smeets, 1991), the number of dopaminergic neurons projecting to the nucleus accumbens prevails over those projecting to the striatum. In *Xenopus*, the presence of almost equally dense plexuses of catecholaminergic fibers in the striatum and the nucleus accumbens (González et al., 1993) correlates well with the number of dopaminergic neurons projecting to each of the basal ganglia structures. For both anuran species, however, the organization of the dopaminergic input to the striatum and the nucleus accumbens is largely comparable. As can be concluded from the summarizing diagram (Fig. 12), a rostral-to-caudal topography exists in the organization of the cells of origin of the dopaminergic innervation of the striatum and the nucleus accumbens. In *Pleurodeles*, as in anurans, the more rostrally located dopaminergic neurons in the posterior tubercle only contribute to the mesolimbic pathway.

In all amniotes studied, the telencephalic basal ganglia receive a strong input from the A8–A10 dopaminergic cell groups (nomenclature after Dahlström and Fuxe, 1964; see, e.g., Parent, 1986; Smeets and Reiner, 1994). These projections are organized into distinct ventral and dorsal mesostriatal systems. In reptiles, the origin of the dorsal and ventral mesostriatal systems are largely segregated. In the lizard, *Gekko gekko*, a medial-to-lateral topography exists in the mesostriatal projections (González et al., 1990). The nucleus accumbens receives input from the ventral tegmental area (VTA), whereas the major input to the striatum originates in the substantia nigra (SN). In birds and mammals, however, the SN and the VTA contribute to both pathways. Thus, the dorsal mesostriatal system arise primarily from neurons in the SN, although cells in the VTA and the retrorubral area also contribute to this pathway. Moreover, a medio-to-lateral topography in the nigrostriatal projections was noted in rodents (Fallon and Moore, 1978; Nauta et al., 1978; Beckstead et al., 1979), but seems to be lacking in primates (Lynd-Balta and Haber, 1994b). Thus, even within the class of Mammalia, substantial variation is observed in the organization of the nigrostriatal projections.

The main source of the ventral mesostriatal system is the dopaminergic cells in the VTA, which, in mammals, are arranged into separate nuclei (see Oades and Holliday, 1987, for references). In rats, a small injection of retrograde tracer in the nucleus accumbens labels the whole anteroposterior extent of the VTA (Beckstead et al., 1979; Swanson, 1982; Albanese and Minciacchi, 1983; Phillipson and Griffiths, 1985) and, in addition, many cells in the supramammillary area (Phillipson and Griffiths, 1985). Similarly, small injections in the ventral striatum of primates result in labeling of neurons throughout the rostrocaudal extent of the ventral mesencephalon (Lynd-Balta and Haber, 1994a). Noteworthy, evidence for a medio-to-lateral topography in the arrangement of the ef-

ferent connections of the VTA to the nucleus accumbens has been found in rats (Fallon and Moore, 1978; Phillipson and Griffiths, 1985), but not in hamsters (Newman and Winans, 1980), cats (Groenewegen et al., 1980), and primates (Lynd-Balta and Haber, 1994a).

Traditionally, the cells of origin of the ventral mesostriatal system have been described as being confined to the boundaries of the VTA (A10 group). However, another compact group of dopaminergic neurons is found rostroventral to the A10 group within the supramammillary region of the hypothalamus of mammals (Kitahama et al., 1994; Tillet, 1994). This group of neurons extends in a rostrocaudal direction from the arcuate nucleus to the caudal pole of the mammillary nuclei, where they appear to be continuous with the dopaminergic cells of the inter fascicular nucleus (component of A10 group). It sends projections to several forebrain regions such as the prefrontal cortex, the lateral septum, and the nucleus accumbens (Carter and Fibiger, 1977; Newman and Winans, 1980; Swanson, 1982; Phillipson and Griffiths, 1985; Shepard et al., 1988). On the basis of cytological and hodological characteristics, the supramammillary dopaminergic neurons do not appear to be a rostral extension of the VTA cell group (Phillipson, 1979a,b; Swanson, 1982; Shepard et al., 1988). Although some neurons within the supramammillary region project to the nucleus accumbens (Phillipson and Griffiths, 1985), they mainly provide an input to the lateral septum (Swanson, 1982; Shepard et al., 1988). In addition to this dopaminergic projection, the supramammillary region contains a large population of nondopaminergic neurons that also project to limbic forebrain regions (Swanson, 1982; Shepard et al., 1988). The presence of both dopaminergic and nondopaminergic neurons within the supramammillary region resembles the situation found in the dorsal mesostriatal, ventral mesostriatal, and mesocortical systems (Guyenet and Aghajanian, 1978; Deniau et al., 1980; German et al., 1980).

The present study has revealed that the organization of the dopaminergic input to the telencephalic basal ganglia of amphibians shares many features with that of amniotes. For example, the rostrocaudal extent of the tegmental dopaminergic neurons is comparable to that of amniotes. Moreover, apart from those cells in the caudal posterior tubercle and the mesencephalic tegmentum, amphibians possess dopaminergic neurons in the rostral part of the posterior tubercle (retromammillary nucleus, after Puelles et al., 1996). The latter neurons project to forebrain structures such as the nucleus accumbens and resembles, therefore, the supramammillary dopaminergic neurons of mammals. Thus, subregions within the whole rostrocaudal extent of the paramedian dopaminergic cell group may have different morphological and hodological characteristics and idea previously suggested for mammals (Swanson, 1982).

### **The segmental concurrence of vertebrate A9–A10 cell groups**

Numerous studies have shown that the vertebrate brain develops in a segmental fashion (Puelles et al., 1987, 1991; Lumsden and Keynes, 1989; Bulfone et al., 1993; Figdor and Stern, 1993; Puelles and Rubenstein, 1993; Rubenstein et al., 1994). The results of these studies indicate that the segmental pattern of development leaves its imprint on the adult brain and appears to govern the location and topographic relationships of the various cell groups. Thus,

a segmental analysis of the organization of catecholaminergic cell groups provides a suitable approach to view evolutionary variation in brain organization among vertebrates (Smeets and Reiner, 1994). Despite the similarities found in the projections to the basal ganglia arising from the A9–A10 (SN-VTA) cell groups of amniotes and the posterior tubercle/mesencephalic groups of amphibians, the apparent differences in topographic location of these cell groups has traditionally raised questions about homology. Thus, whereas the A10 and the A9 cell groups are classically considered to be located in the mesencephalic tegmentum of amniotes (Björklund and Lindvall, 1984; Parent, 1986; Smeets et al., 1986; Kitahama et al., 1994; Reiner et al., 1994; Smeets, 1994), the posterior tubercle of amphibians is viewed as a diencephalic region (Neary and Northcutt, 1983; Puelles et al., 1996).

When a segmental approach is applied to localize the dopaminergic neurons of the VTA/SN of amniotes (Medina et al., 1994; Puelles and Medina, 1994; Medina and Reiner, 1995), it becomes obvious that the dopaminergic neurons of these groups are not restricted to the mesencephalic segment. Thus, the A10 group of amniotes comprises distinct retromammillary, posterior tubercular, basal synencephalic, mesencephalic, and isthmic portions (Puelles and Medina, 1994). Therefore, dopaminergic neurons extend rostrally in the diencephalon within the tegmental part of the three prosomeres (p3, retromammillary; p2, posterior tubercle; p1, basal synencephalic). As suggested by Puelles and Medina (1994), the paramedian proliferation zone of each of the segments (p1, p2, p3, mesencephalic, and isthmic) appears to produce its own part of the VTA. A similar, multisegmental origin may hold for the SN. Thus, the dopaminergic neurons of the pars compacta of the SN and the retrorubral area constitute a large plurisegmental complex in mammals (with laterally migrated cell groups in the p1, mesencephalic, and isthmic segments), but exist only as single segmental component in sauropsids (Puelles and Medina, 1994).

When the segmental analysis is applied to the diencephalon of amphibians (Puelles et al., 1996), corresponding basal diencephalic segments are found. Furthermore, our results reveal that the dopaminergic neurons in amphibians constitute a longitudinal complex that extends without interruption from the rostral posterior tubercular region (as defined by Neary and Northcutt, 1983) up to the caudal end of the oculomotor nucleus in the mesencephalic tegmentum (Fig. 12). In that perspective, the dopaminergic neurons of amphibians comprise distinct retromammillary, posterior tubercular, basal synencephalic, and mesencephalic segments. Thus, amphibians seem to lack the isthmic portion of the A10 complex and a laterally migrated SN and retrorubral area, but the dopaminergic cell field corresponding to the A10 complex is almost completely present. Furthermore, different portions of the dopaminergic tegmental complex of amphibians appear to have distinct hodological and morphological characteristics, as in amniotes.

### **Nondopaminergic basal ganglia projections from the posterior tubercle/mesencephalic tegmentum**

Numerous nondopaminergic neurons within the posterior tubercle and the mesencephalic tegmentum project to the striatum and the nucleus accumbens of amphibians. It is well known that nondopaminergic cells constitute part

of the dorsal and ventral mesostriatal systems of mammals (Thierry et al., 1980; Björklund and Lindvall, 1984; Gerfen et al., 1987a,b). However, the nondopaminergic contribution to the innervation of the striatum is considerably larger than to the nucleus accumbens, and arises specifically from the pars reticulata of the SN. In addition, numerous nondopaminergic neurons are located within the VTA, and also contribute to the innervation of the telencephalic basal ganglia (Thierry et al., 1980). Thus, the existence, in amphibians, of nondopaminergic neurons sparsely intermingled with the dopaminergic projecting cells in the posterior tubercle and the midbrain tegmentum resembles the situation in amniotes. This suggests that such nondopaminergic projections are a primitive character of the mesostriatal systems in tetrapod vertebrates. Although the general features seem to be common to the midbrain groups of amniote vertebrates, the search for an amphibian homologue of the amniote pars reticulata of the SN is a more troublesome subject. The mammalian pars reticulata is mainly confined to the mesencephalic tegmentum, ventral to the pars compacta. The pars reticulata sends minor projections to the telencephalon, but, on the contrary, it receives a massive input from the striatum. In addition, it projects to the tectum and to the thalamus, and it is reciprocally connected with the pedunculopontine nucleus (see Parent, 1986, for references).

In amphibians, the nondopaminergic cells within the posterior tubercular region are reciprocally connected with the striatum (Wilczynski and Northcutt, 1983a,b; present study) and project ipsilaterally to the optic tectum (Neary and Wilczynski, 1980). Furthermore, in *Rana perezi*, tracer injections into the subcerebellar region, where the presumptive homologue of the pedunculopontine tegmental nucleus has been suggested to be located (Muñoz et al., 1996), revealed a reciprocal connection with the posterior tubercle (unpublished observations), in the part that is reciprocally connected with the striatum and projects to the optic tectum. Nevertheless, the medial and rostral location of the posterior tubercular region in amphibians appears to be a topographical impediment in comparing this nondopaminergic group of amphibians with the pars reticulata of the amniote SN. However, it should be noted that in mammals, the nondopaminergic neurons of the pars reticulata extend further rostrally than the pars compacta, reaching as far into the diencephalon as the supramammillary region (Olszewski, 1952; Emmers and Akert, 1963; Berman, 1968; Winters et al., 1969; Berman and Jones, 1982; Paxinos and Watson, 1986). Only if one accepts that the amphibian equivalent of the SN-VTA groups represents an early stage in the evolution of these groups (which probably have developed during phylogeny by increasing the number of neurons and expanding caudally and laterally), can we hypothesize that a part of the posterior tubercle region represents the amphibian homologue of the pars reticulata of amniotes.

### Locus coeruleus input to the basal ganglia

The noradrenergic cells of the locus coeruleus may constitute the most conservative catecholaminergic cell group of the brain of vertebrates (Smeets and Reiner, 1994). The projection of the locus coeruleus of mammals and birds is extremely widespread innervating all major regions of the brain, including the basal ganglia (Jones and Moore, 1977; Fallon and Moore, 1978; Moore and Card, 1984; Aston-Jones, 1985; Kitt and Brauth, 1986; Siemen

and Künzle, 1994). All these brain areas are reached basically by the formation of extensive axon collateralization (Room et al., 1981; Dietrichs, 1985). This pattern, in which a relatively low number of noradrenergic neurons innervate almost all brain regions, most probably is constant throughout vertebrate evolution (Smeets and Reiner, 1994). The high degree of collateralization of locus coeruleus axons in the brain of amphibians and other nonmammalian vertebrates, however, awaits experimental investigation.

The present study has conclusively demonstrated the noradrenergic projection of the isthmus cell group to the basal ganglia of amphibians. This supports a homology of this cell group with the locus coeruleus of amniotes. Previously, it was hypothesized that the development of the locus coeruleus was correlated with the development of the neocortex (Yamamoto et al., 1977). It should be noted that the pallidum of amphibians receives noradrenergic inputs (González and Smeets, 1993, 1995) and, most likely, the noradrenergic neurons of the locus coeruleus are the source of this projection. This would be in accord with the idea that the extensive projections of the locus coeruleus represent a phylogenetically conserved component of the reticular formation projections (Moore and Card, 1984).

### Solitary tract nucleus input to the basal ganglia

Projections of the nucleus of the solitary tract to the basal forebrain have been reported in birds (Arends et al., 1988) and, primarily, in mammals (Smith and De Vito, 1984). However, the catecholaminergic nature of these projections was not corroborated with double-labeling techniques. In a comprehensive study dealing with the involvement of catecholamines and peptides in the efferent projections of the nucleus of the solitary tract in rats, Riche et al. (1990) revealed that the catecholaminergic cells constitute the bulk of those projections. Fibers were found to arise from all rostrocaudal levels of the nucleus and to project primarily ipsilaterally. In mammals and birds, the solitary tract nucleus has reciprocal projections with the parabrachial nuclei, hypothalamus, bed nucleus of the stria terminalis, substantia innominata, and amygdala (see Smith and De Vito, 1984; Kuenzel and Blähser, 1993, for references). Further, additional projections to the nucleus accumbens and the septum have been recently reported (Wang et al., 1992; Zagon et al., 1994). Following tracer injections in the nucleus accumbens, about one-third of the retrogradely labeled cells in the nucleus of the solitary tract were THir, and a small proportion of catecholaminergic cells were also labeled in the caudal ventrolateral medulla (Zagon et al., 1994). Therefore, the present finding, of the existence of catecholaminergic efferents from the nucleus of the solitary tract to the basal forebrain in amphibians, suggests that the processing of visceral information through these connections may be a primitive feature of the catecholaminergic systems in vertebrates.

### Functional considerations

The strong dopaminergic input to the striatum of amniotes plays a key role in the ability of the basal ganglia to mediate in movement control. In mammals, loss of dopaminergic input to the basal ganglia slows movement and impairs its initiation (see Reiner, 1994, for references), and similar results were observed in reptiles (Andersen et al., 1975). Despite the fact that amphibians lack laterally

migrated SN and retrorubral cell groups, the dopaminergic neurons within the posterior tubercle/mesencephalic groups may play a similar role in movement control as in amniotes (Barbeau et al., 1985).

In mammals, the nucleus accumbens is considered to be a sensorimotor integration area, which influences emotional and goal directed behaviors (see, e.g., Wise and Rompre, 1989; Everitt et al., 1990; Hooks and Kalivas, 1995; Melis and Argiolas, 1995). Moreover, the destruction of dopaminergic terminals in the nucleus accumbens has been associated with deficits in behavioral initiation, locomotion, and exploratory behavior (Taghzouti et al., 1985). Such data are not available for amphibians, but it would be of great interest to know whether the anatomically detected similarities in basal ganglia organization of amphibians are reflected in functional and behavioral aspects.

The locus coeruleus in mammals and birds has been shown to have widespread projections throughout the brain and spinal cord. The physiological effects of noradrenaline are complex and depend on the postsynaptic receptors present on the target cells (Moore and Bloom, 1979). In general, its actions are best described to be modulatory. Clearly, it is necessary to further characterize the amphibian locus coeruleus before forming hypotheses about its function.

In mammals and birds, the nucleus of the solitary tract is the major visceral sensory relay nucleus in the brain and, as such, it receives inputs from all the major organs of the body (Kalia et al., 1984; Onai et al., 1987; Arends et al., 1988; Loewy, 1990; Berk et al., 1993). Direct projections to the basal forebrain provide a link between viscerosensory regions of the medulla and limbic forebrain regions. Since the amphibian nucleus of the solitary tract also appears to receive visceral sensory inputs (Rubinson and Friedman, 1977; Matesz and Székely, 1978; Matsushima et al., 1987; Oka et al., 1987) and its ascending projections resemble those found in amniotes (Muñoz et al., 1995; Marín et al., 1996; the present study), this nucleus is likely to play a role in the processing of visceral information in amphibians.

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