Anuran Dorsal Column Nucleus: Organization, Immunohistochemical Characterization, and Fiber Connections in Rana perezi and Xenopus laevis

A. MUÑOZ, M. MUÑOZ, A. GONZÁLEZ, AND H.J. TEN DONKELAAR
Department of Cell Biology, Universidad Complutense de Madrid, 28040 Madrid, Spain (A.M., M.M., A.G.), and Department of Anatomy and Embryology, University of Nijmegen, 6500 HB Nijmegen, The Netherlands (A.M., H.J.T.D.)

ABSTRACT

As part of a research program on the evolution of somatosensory systems in vertebrates, the dorsal column nucleus (DCN) was studied with (immuno)histochemical and tract-tracing techniques in anurans (the large green frog, Rana perezi, and the clawed toad, Xenopus laevis). The anuran DCN contains some nicotinamide adenine dinucleotide phosphate diaphorase-positive neurons, very little calbindin D-28k, and a distinct parvalbumin-positive cell population. The anuran DCN is innervated by primary and non-primary spinal afferents, by primary afferents from cranial nerves V, VII, IX, and X, by serotonin-immunoreactive fibers, and by peptidergic fibers. Non-primary DCN afferents from the spinal cord appear to arise throughout the spinal cord, but particularly from the ipsilateral dorsal gray. The present study focused on the efferent connections of the DCN, in particular the targets of the medial lemniscus. The medial lemniscus could be traced throughout the brainstem and into the diencephalon. Along its course, the medial lemniscus gives off collaterals to various parts of the reticular formation, to the octavolateral area, and to the granular layer of the cerebellum. At mesencephalic levels, the medial lemniscus innervates the lateral part of the torus semicircularis as well as various tegmental nuclei. A striking difference between the two species studied is that while in R. perezi medial lemniscal fibers do not reach the tectum mesencephali, in X. laevis, intermediate and deep tectal layers are innervated. Beyond the midbrain, both dorsal and ventral thalamic areas are innervated by the medial lemniscus. The present study shows that the anuran "lemniscal pathway" is basically similar to that of amniotes. © 1995 Wiley-Liss, Inc.

Indexing terms: amphibians, somatosensory system, medial lemniscus, thalamus, torus semicircularis

In terrestrial vertebrates, two basic systems of ascending spinal projections are found (Willis and Coggeshall, 1991): 1) a primary afferent ascending spinal projection via the dorsal funiculus to the dorsal column nucleus, giving rise to the mediallemniscal pathway to the thalamus; and 2) a secondary afferent projection via the lateral funiculus (i.e., the spinal lemniscus) to the reticular formation, mesencephalon, and thalamus.

In anurans, anterograde degeneration studies (e.g., Ebbesson, 1969, 1976; Hayle, 1973a,b) did not demonstrate a spinotectal tract, and the existence of a dorsal column-medial lemniscal system remained a much debated question until the early 1980s. A recent anterograde tracer study showed a distinct direct spinotectal projection in anurans (A. Muñoz et al., 1994). The anuran dorsal column nucleus (DCN) is somatotopically arranged in such a fashion that its medial ("gracile") compartment is innervated by dorsal root fibers from lumbar and thoracic segments, whereas those of the cervical enlargement project to the lateral ("cuneate") compartment (Antal et al., 1980; Nikundiwe et al., 1982; Jhaveri and Frank, 1983). In Xenopus laevis a non-primary afferent projection to the DCN or postsynaptic dorsal column system was also demonstrated (ten Donkelaar and de Boer-van Huizen, 1991). In ranid frogs, Vesselkin and co-workers (Vesselkin et al., 1971; Vesselkin and Kovacević, 1973), Silvey et al. (1974), and Neery and Wilczynski (1977) described a contralateral projection of the DCN (or perisolitary band) to thalamic nuclei. More recent cobalt labeling studies in Rana escu-

Accepted May 25, 1995.

Address reprint requests to Dr. H.J. ten Donkelaar, Department of Anatomy and Embryology, Faculty of Medical Sciences, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.
The anuran medial lemniscus also innervates the lateral part of the torus semicircularis (Comer and Grobstein, 1981; Wilczynski, 1981; Neary and Wilczynski, 1986).

The present study is part of a research program on the evolution of somatosensory systems in vertebrates. The development, chemical neuroanatomy, and circuitry of somatosensory systems is being studied in amphibians, urodèles as well as anurans. In the present study, the connectivity of the dorsal column nucleus in two anuran amphibians, the Spanish green frog, *R. perezi* (formerly *R. ridibunda*) and the clawed toad, *X. laevis*, was analyzed, using mainly HRP and biotinylated dextran amine (BDA) tracing techniques. Little is known about the chemical neuroanatomy of the anuran DCN. The mammalian cuneate and gracile nuclei are characterized by the presence of γ-aminobutyric acid (GABA)ergic (inter)neurons, and are innervated by substance P-positive and many other peptidergic fibers (see Rustioni and Weinberg, 1989, for review). Recent studies (e.g., Celio, 1990; Rausell and Jones, 1991a,b; Rassell et al., 1992; Menétry et al., 1992a,b; Maslany et al., 1992; Ren and Ruda, 1994) also showed a certain preferential distribution of calcium-binding proteins like calbindin D-28k and parvalbumin for somatosensory structures including the dorsal column nuclei. Nitric oxide synthase (NOS) possibly marks a population of local circuit neurons within the DCN (Valtschanoff et al., 1993). No such data are available for anurans. Therefore, the existence of different cell populations within the anuran dorsal column nucleus was studied using nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase (d) histochemistry (NADPHd being a marker for NOS), calbindin D-28k, parvalbumin, GABA, and glycine immunohistochemistry. Additionally, data on the serotonergic and peptidergic innervation of the DCN will be discussed. It will be shown that the anuran dorsal column-medial lemniscal system is basically similar to that of amniotes.

**MATERIALS AND METHODS**

The animals (60 adult specimens of *R. perezi* and 45 young adult *X. laevis*) were obtained from laboratory stock of the Department of Cell Biology, University Complutense of Madrid (*R. perezi*) and the Department of Animal Physiology, University of Nijmegen (*X. laevis*). For a cytoarchitectonic analysis of the obex region, Nissl (creollysche) stained sections of both anurans were available, cut either transversally, horizontally, or sagittally at a thickness of 20 μm. Adjacent sections were stained with silver proteinate, according to either Bodian’s (1936) or Klüver and Barrera’s (1953) technique. The histochemical, immunohistochemical, and tract-tracing techniques used in this study are discussed below. The nomenclature used is based on studies by Opdam et al. (1976) and Nikundiwe and Nieuwenhuys (1983) on the brainstem, and by Neary and Northcutt (1983) on the anuran diencephalon.

**NADPH-diaphorase histochemistry**

Four adult frogs (*R. perezi*) were anesthetized in a 0.3% solution of tricaine methanesulphonate (MS222, Sandoz) and subsequently perfused transcardially with a 0.9% saline solution followed by a fixative containing 4% parafor-
maldehyde and 15% saturated picric acid in 0.1 M phosphate buffer (pH 7.4). The brain and spinal cord were taken out and further fixed in the same fixative for 6–8 hours at room temperature. They were subsequently immersed in a 30% sucrose phosphate buffer solution at 4°C, embedded in a 15% gelatin and 30% sucrose solution, and stored for 5 hours in a 4% formaldehyde solution at room temperature. On a freezing microtome, 30 or 40 μm frontal sections were cut and collected in phosphate buffer. Free-floating sections were incubated in a medium containing 1 μM β-NAPDH, 0.8 μM nitroblue tetrazolium, and 0.06% Triton X-100 in 0.1 M phosphate buffer (pH 7.6) at 37°C for 1–2 hours. After incubation, the sections were thoroughly rinsed in phosphate buffer, mounted on gelatin-coated glass slides, and, after drying overnight, coverslipped. Selected sections were counterstained with 1% cresyl violet. In two cases, the histochemistry after rinsing.

**Immunohistochemical procedures**

For the immunohistochemical procedures used, animals were anesthetized with an overdose of MS222 and transcardially perfused with saline followed by a mixture of 4% paraformaldehyde, 0.05% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4). The brain and spinal cord were removed and postfixed for 4–7 hours in the same fixative, embedded in 15% gelatin with 30% sucrose (R. perezi) or in polyacrylamide (X. laevis). Brains were cut frontally on a freezing microtome or on a Vibratome at 40 μm, and the sections were collected in a Tris-saline (TBS) buffer (0.05 M, pH 7.6). All antibodies were diluted in 0.1% phosphate buffer (pH 7.4). The sections were preincubated for 1–2 hours in TBS containing 3% normal serum and 0.1% Triton X-100 and subsequently incubated in the primary antibody-containing solution for 24–36 hours at 4°C. Controls for the immunohistochemistry experiments included: 1) staining some selected sections with pre-immune mouse serum (1:1,000 overnight; 2) swine anti-rabbit (Nordic), diluted 1:50, for 2 hours; and 3) rabbit PAP complex (Dakopatts), diluted 1:600, for 2 hours.

**Calbindin D-28k and parvalbumin immunohistochemistry**

1) Mouse anti-calbindin D-28k (Sigma) and mouse anti-parvalbumin (Sigma), diluted 1:1,000, for 24–72 hours; 2) goat anti-mouse (Nordic), diluted 1:100, for 3–5 hours; and 3) PAP, diluted 1:500, for 2 hours.

**GABA immunohistochemistry**

After colchicine (Sigma) injections (3 μl, containing 20 μg/ml) into the fourth ventricle of deeply anesthetized animals, and perfusion after survival times of 5–12 hours, the following procedure was used: 1) rabbit anti-GABA (Sigma), diluted 1:1,000, overnight; 2) swine anti-rabbit (Nordic), diluted 1:50, for 2 hours; and 3) PAP-rabbit (Dakopatts), diluted 1:600, for 2 hours.

**Tyrosine hydroxylase (TH) immunohistochemistry**

GABA immunohistochemistry (eight cases). After colchicine (Sigma) injections (3 μl, containing 20 μg/ml) into the fourth ventricle of deeply anesthetized animals, and perfusion after survival times of 5–12 hours, the following procedure was used: 1) rabbit anti-GABA (Sigma), diluted 1:1,000, overnight; 2) swine anti-rabbit (Nordic), diluted 1:50, for 2 hours; and 3) PAP-rabbit (Dakopatts), diluted 1:600, for 2 hours.

**Neuropeptide Y (NPY) immunohistochemistry**

1) Rabbit anti-parvalbumin (Sigma), diluted 1:1,000, overnight; 2) swine anti-rabbit (Nordic), diluted 1:50, for 2 hours; and 3) rabbit PAP complex (Dakopatts), diluted 1:600, for 2 hours.

**Tract-tracing experiments**

In vivo technique. All experiments were carried out under surgical anesthesia with MS222. The following tracers were used as retrograde and anterograde tracers: HRP (Boehringer), BDA, 10 kD (Molecular Probes D-1956), and rhodamine dextran amine (RDA; Molecular Probes D-1817). HRP was applied iontophoretically (a 15% HRP solution in distilled water onto fine tungsten needles and applied to the DCN (five cases), to the thalamus (three cases), or to the most lateral part of the torus semicircularis (two cases), or to the thalamus (three cases). BDA was recrystallized from distilled water onto fine tungsten needles and applied to the proximal stumps of cut trigeminal nerves (four cases) or third spinal dorsal roots (three cases). Previous experiments (Nikundiwe et al., 1982, X. laevis; M. Muñoz et al., 1991, R. perezi) in which HRP was applied to thoracic and lumbar dorsal roots were used for the analysis of DCN projections of the more caudal dorsal roots. Additionally, material in which HRP or BDA was used to the proximal stumps of the facial, glosopharyngeal, and vagal nerves could be analyzed. Alternatively, BDA was injected ionto-
etry as a 10% solution in phosphate buffer, into the DCN (three cases), cervical dorsal horn (three cases), cerebellum (three cases), and thalamus (four cases). Survival times varied from 5 to 10 days. Subsequently, the animals were re-anesthetized and perfused through the heart with isotonic saline followed by a fixative containing 4% paraformaldehyde for the BDA experiments and 1.5% paraformaldehyde and 2% glutaraldehyde for the HRP cases. The brain and spinal cord were removed, postfixed for 2–4 hours, and embedded in gelatin or polyacrylamide. Sections were cut transversally or horizontally at 40 μm on a freezing microtome. Histochemistry for HRP followed the heavy metal intensification of the DAB-based HRP reaction product (Adams, 1981). For visualizing BDA, an avidine biotin complex (Vectastain ABC Elite Kit, Vector Laboratories) was used. Some BDA-reacted sections were rinsed and further processed for calbindin D-28K or parvalbumin immunohistochemistry as described above. The black color of the BDA labeling contrasts with the calbindin protein labeling stained brown by using the DAB reaction without heavy metal intensification. RDA, recrystallized from distilled water onto sharp tungsten needles, was applied to the thalamus and the torus semicircularis. Applications of 2–4 days, animals were re-anesthetized with an overdose of MS222 and perfused with 0.1 M phosphate buffer (pH 7.4) followed by a fixative containing 4% paraformaldehyde in phosphate buffer. The brain and (rostral) spinal cord were taken out, embedded in polyacrylamide, left overnight in 15% saccharose in 0.1 M phosphate buffer, and cut transversally on a freezing microtome at 40 μm. They were mounted in glycerin-gelatin and viewed with a Zeiss fluorescence microscope with appropriate filter combinations.

In vitro technique. In ten young adult X. laevis, an in vitro approach was used, based on Cochran et al. (1987). The animals were deeply anesthetized with a 0.2% solution of MS222 and perfused with iced Ringer’s solution (78 mM NaCl, 25 mM NaHCO₃, 2 mM CaCl₂, 2 mM KCl, 0.5 mM MgCl₂, 11 mM glucose; pH 7.4). The brains were removed, submerged in the same iced Ringer’s solution, and cut at middiencephalic or midmesencephalic levels. Applications of 3 kD BDA (Molecular Probes, D-7135), recrystallized at the tip of sharp tungsten needles, were made at the ventral thalamus (five cases) or the torus semicircularis (five cases). The brains were kept for 5–18 hours at room temperature in continuously oxygenated Ringer’s solution (pH 7.4) with carbogen, and subsequently processed as described for the in vivo BDA experiments.

RESULTS

Delineation and (immuno)histochemical characterization of the anuran dorsal column nucleus

Cytoarchitecture. No distinct DCN or nucleus funiculi dorsalis could be distinguished in most cytoarchitectonic studies of the anuran brain stem (e.g., Ariëns Kappers and Hammer, 1918; Zeehandelaar, 1921; Opdam et al., 1976). Therefore, since Woodburne’s (1939) Marchi studies, the anuran DCN is defined as the site of termination of dorsal funicular fibers in the caudal brainstem, rather than as a cytoarchitectonic entity. Nevertheless, Nissl-stained sections of the brainstem at obex levels allow the delineation of a DCN (Fig. 1), although labeling of spinal primary afferent projections to the obex level by cobalt staining (Antal et al., 1980), HRP (Nikundiwe et al., 1982) or BDA (see Fig. 6) much more clearly delineates the DCN.

In X. laevis, medial (“gracile”) and lateral (“cuneate”) compartments of the DCN can be distinguished above and lateral to the distinct solitary tract (Nikundiwe et al., 1982; Nikundiwe and Nieuwenhuys, 1983). A dorsal indentation suggests such a subdivision. Medially, the DCN is difficult to distinguish from the nucleus of the solitary tract, and laterally it is very poorly segregated from the nucleus of the descending tract of the trigeminal nerve. Both compartments of the DCN consist of small (8–10 μm) and medium-sized (15 μm) multipolar elements. The medial, gracile part begins at the level of the second spinal nerve and extends into the brainstem, where it is situated dorsal and dorsolateral to the solitary tract (Fig. 1A,B). The lateral, cuneate part extends further rostrally than the gracile part, and borders on the nucleus of the descending trigeminal tract. In R. perezi, as in R. esculenta (Antal et al., 1980), the segregation of the DCN from the surrounding cell structures such as the nucleus of the descending trigeminal tract and the nucleus of the solitary tract is also rather poor. In R. perezi, the cell area dorsal and lateral to the solitary tract only occasionally shows an indentation allowing the distinction of medial and lateral compartments in the DCN (Fig. 1C–F). These gracile and cuneate subdivisions rostrally extend to the level of the glossopharyngeal nucleus where they are slightly more laterally located since in that position the most medial part of the rhombencephalic alar plate is occupied by the caudal pole of the vestibular nuclear complex and related descending vestibular root fibres (Fig. 1C,D). Lateral to the DCN cells, the cells in the dorsolateral position of the alar gray are mingled with afferent fibers of cranial nerves V, VII, IX, and X, forming the descending tract of the trigeminal nerve. These dorsolateral alar gray cells can be regarded as a component of the nucleus of the descending trigeminal tract.

In X. laevis and to a lesser extent also in R. perezi, the cell area above and lateral to the solitary tract at the obex level is composed of two DCN compartments, the lateral of which fades into the nucleus of the descending trigeminal tract.

Chemical neuroanatomy

NADPH-diaphorase histochemistry. In the caudal part of the rhombencephalic alar plate in R. perezi, NADPHd-positive neurons were observed in the DCN, in the adjacent descending trigeminal nucleus, and in the nucleus of the solitary tract (Fig. 2A,B). In X. laevis, the caudal lateral line nucleus was labeled as well. In the DCN, a cluster of NADPHd-positive neurons was found, the dendrites of which are directed dorsally toward the dorsal funiculus. It should be noted, however, that NADPHd-positive neuron populations are more distinct in the nucleus of the solitary tract, mainly in its medial and ventromedial parts below the solitary tract, and in the nucleus of the descending trigeminal tract (Fig. 2A,C). By combining NADPHd staining with immunohistochemistry against TH, the NADPHd-positive part of the nucleus of the solitary tract was shown to be intermingled with the catecholaminergic cells (González and Smeets, 1991) situated ventral to the solitary tract. In the caudal part of the rhombencephalic alar plate of both anuran species studied, NADPHd-positive fibers were found predominantly in two bundles, i.e., the descending trigeminal tract and the solitary tract. In the solitary tract, NADPHd-positive fibers were observed in its most dorsal
observed (Fig. 1A-D’), although caudal to the level of the dorsomedial (DM), and lateral to the ventral anterior-tegmental area (VTA), a similar pattern of Calb immunoreactivity was observed to the level of the hypothalamus (Fig. 1D-D’). The second group was located in the nucleus accumbens (NAc) and the ventral tegmental area (VTA). A characteristic feature of the VTA is the presence of Calb-immunoreactive cells, which are scattered throughout the area. These cells appear to be more numerous in the ventral part of the VTA, where they are distributed in clusters. The distribution of Calb-immunoreactive neurons is consistent with the idea that the VTA plays a role in the regulation of reward and reinforcement. It is possible that the presence of Calb-immunoreactive cells in the VTA is related to the processing of reward-related information, which is important for the regulation of motivation and behavior. The ventral tegmental area is a key structure in the reward system, receiving inputs from the ventral pallidum and the nucleus accumbens, and projecting to the dorsomedial nucleus of the thalamus, the amygdala, and the basal ganglia. The presence of Calb-immunoreactive cells in the VTA may suggest a role in the processing of reward-related information, which is important for the regulation of motivation and behavior.
number of Calb-positive neurons in the nucleus of the
descending trigeminal tract was much higher. In both
anuran species, hardly any Calb-positive neurons were
found in the DCN. In the dorsal horn of the spinal cord,
however, an abundant Calb-positive cell population was
observed.

In striking contrast to the lack of Calb-positive neurons
in the DCN, in both anuran species a distinct Parv-positive
DCN cell population was observed, particularly in X. laevis
(Fig. 4A–C). Apart from the DCN, Parv-positive neurons
were observed in the reticular formation, octavolateral
area, rostral part of the nucleus of the solitary tract,
trigeminal nuclear complex, and dorsal and ventral horn of
the spinal cord. In R. perezi, relatively few Parv-positive
DCN neurons were observed, spread throughout the
nucleus. The dendrites of these rather large cells are mainly
directed dorsally or laterally into the adjacent white matter.
In some sections more dorsally located and smaller Parv-
positive cells were observed as well. In young adult speci-
mens of X. laevis, many more Parv-positive neurons were
observed in the DCN. Even a clear segregation into a medial
and a lateral component could be observed (Fig. 4A–C).
More ventrally located neurons probably form part of the
nucleus of the solitary tract. In experiments in which the
immunostaining against Parv was combined with a BDA
application to the third dorsal root, BDA-labeled endings
close to the somata of Parv-positive neurons were observed,
suggesting that they receive primary spinal afferents.

The data presented suggest that Parv can be used as a
marker for the DCN in anurans, and that Calb almost
certainly cannot. However, since Calb is abundantly pre-
sent in the nucleus of the solitary tract as well as in the
nucleus of the descending trigeminal tract, it can help to
delineate the lateral and medial borders of the DCN.

In the caudal part of the medulla oblongata, scattered
GABAergic neurons were observed in the alar plate. They
are more densely grouped in two different locations: one in
the nucleus of the solitary tract, the other in the DCN and
the adjacent nucleus of the descending trigeminal tract. In
the DCN, small, round or oval-shaped, neurons were found
(Fig. 5A, B). Figure 5C shows a control experiment. In the
DCN of the anuran species studied no glycine-immunoreac-
tive neurons were observed. In R. perezi, however, some
large glycinergic neurons were observed just ventrolateral
of the DCN (Fig. 5A, D). These bipolar neurons are oriented
dorsoventrally with processes directed into dorsal and
ventral directions.
Tract-tracing experiments

In order to characterize the afferent and efferent connections of the DCN in *R. perezi* and *X. laevis*, HRP histochemistry, and BDA immunodetection were used. Additionally, the fluorescent tracer RDA was used as a retrograde tracer. Even though the iontophoretic injections of the tracers were rather small, particularly the BDA injections, it was nearly impossible to restrict the application site to the DCN. Medially located injections often partially involved the nucleus of the solitary tract, whereas more lateral injections included part of the nucleus of the descending trigeminal tract. It is therefore not possible to discriminate fully the connectivity of the DCN using only this type of tracer application. Subsequent retrograde tracing experiments (HRP, BDA, and RDA) were used to confirm the projections from the DCN, whereas anterograde tracing experiments were done to corroborate and describe the terminal fields of the efferent connections of the DCN. Since the results in both species are largely comparable, in the following section the general pattern of the connections...
Fig. 4. **A:** Distribution of calcium-binding proteins in the caudal part of the rhombencephalon and most rostral part of the spinal cord of *X. laevis.* on the left the distribution of parvalbumin-immunoreactive neurons; on the right the distribution of calbindin D-28k-immunoreactive neurons. **B–E:** Photomicrographs of examples of parvalbumin (B,C) and calbindin (D,E) labeling. Scale bars = 100 μm.
In this area largely resemble the organization of the thalamus, with the input of the thalamic nuclei. The thalamus nuclei with rostral, external was observed, but its caudal and, therefore, a similar the entrance of the visual nuclei. In Y, Y, a similar area found from the level of the spinal nerve up to the dorsolateral transition. In the alpha region the thalamus are subcortical, particularly anterior. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha- dorso-lateral transition are somatotopically organized. y-Alpha-
Fig. 6. A: Schematic drawing of a series of transverse sections through the brainstem and cervical spinal cord showing the distribution of hot-sensitive (left) and dorsal root (right) afferent fibers. B: Photomicrographs showing unilateral trigeminal (bulb and dorsal root) termination pattern of BDA-labeled trigeminal afferents to the DCN of R. perzi. C: Photomicrographs showing the third dorsal root at the retinal DCN of R. perzi. Scale bars = 100 μm.

In addition to this peptidergic primary afferent projection to the DCN, the DCN is innervated by Leu-Enk, NPY, and 5-HT-immunoreactive fibers. Leu-Enk-immunoreactive terminal boutons of the DCN are distributed throughout the dorsal gray at levels of the spinal cord and a thin rim around the DCN. NPY-immunoreactive fibers enter the DCN from the lateral funiculus and form varicose fibers that strongly innervate the DCN and adjacent structures such as the nucleus of the solitary tract and the nucleus of the descending trigeminal tract (Fig. 7B).
The nucleus of the solitary tract (NTS) and the paraganglionic area are major sources of afferent projections to the dorsal column nuclei (DCN). These projections are mediated by the cranial nerves V, IX, and X and appear to be organized in a topographic manner. The DCN receives inputs from the thalamus, the cerebellum, and the spinal cord, among other areas. The DCN plays a critical role in processing somatosensory information, including pain, temperature, and touch. The DCN has been shown to be involved in the modulation of autonomic functions, such as cardiovascular regulation.

**References:**


**Figure Legend:**

A. Diagram illustrating the projections from the cranial nerves to the DCN. B. Image showing the anatomical structure of the DCN with various neural pathways indicated in different colors.
Fig. 8. Labeling observed after a BDA injection into the DCN of *R. perezi* (for injection site see also Fig. 10A). In a series of transverse sections through the diencephalon (*A*-*D*), brainstem (*E*-*L*), and spinal cord (*M*-*O*), the pattern of anterogradely labeled fibers and retrogradely labeled cells (black dots) is shown.
Fig. 9. Labeling observed after a BDA injection into the DCN of X. laevis. In a series of transverse sections through the diencephalon (A–D), brainstem (E–M), and spinal cord (N–P), the pattern of anterogradely labeled fibers and retrogradely labeled cells (black dots) is shown.
eral to the isthmic nucleus. At caudal mesencephalic levels, the fibers turn dorsally along the lateral aspect of the midbrain, and most of them bend medially, where they terminate in the torus semicircularis (Figs. 8F,G, 9F,G, 10C,D). The principal, magnocellular, and commissural nuclei receive only a sparse DCN projection, but the laminar nucleus is densely innervated, mainly in its lateral portion. A few fibers pass to the contralateral commissural and principal nuclei of the torus semicircularis. In R. perezi medial lemniscal fibers do not reach the mesencephalic tectum, while in X. laevis the intermediate and deep tectal layers are innervated. These rather thick medial lemniscal fibers innervating the tectum mesencephalic often give off thin collaterals that terminate in the laminar nucleus of the torus semicircularis (Figs. 9E–G, 10C). In both species, at more rostral mesencephalic levels, the anterodorsal and anteroventral segmental nuclei as well as the red nucleus and the interstitial nucleus of the fasciculus longitudinalis medialis are innervated by medial lemniscal fibers (Figs. 8E,F, 9E,F).

At rostral mesencephalic levels, scattered labeled fibers distribute to the pretorial gray, also in X. laevis, also to the pretectal gray (Figs. 8E, 9E). Beyond the midbrain, both the dorsal and ventral thalamic areas are innervated by medial lemniscal fibers (Figs. 8A–D, 9A–D). A few thin, varicose fibers innervate the ventral parts of the posterior and central dorsal thalamic nuclei, whereas the ventromedial thalamic nucleus and the posterior tubercle are far more densely innervated. The fibers reaching the ventromedial nucleus pass through the dorsal and ventral parts of the ventrolateral thalamic nucleus and varicocities are also found among its cells. Apart from a few fibers reaching the anterior nucleus of the dorsal thalamus (in two cases in R. perezi), no labeling was found more rostrally in the anterior diencephalon or in the telencephalon in any of the cases.

2. Extralemniscal ascending projections. Apart from the medial lemniscus, the DCN gives rise to a distinct ipsilateral ascending projection (Figs. 8I–K, 9I–L). Due to their proximity, the ascending primary afferent spinal fibers bypassing the injection site were most likely to be involved. Such fibers are known to project to the octavolateral area and the cerebellum (Antal et al., 1980; Nikuniwe et al., 1982). Additionally, adjacent cell groups such as the nucleus of the descending trigeminal nucleus and the nucleus of the solitary tract might have incorporated the tracer from the injection sites. Therefore, ipsilateral projections from the DCN are difficult to demonstrate in anterograde experiments.

Retrograde tracer experiments. To verify whether these ascending projections really arise in the DCN, in both anuran species injections of HRP, BDA, or RDA were placed into the thalamus, torus semicircularis, and cerebellar region.

1. Thalamic applications. In this group of experiments, a retrograde tracer was applied to the thalamus in such a way that both the dorsal and ventral thalamic nuclei were implicated. Retrogradely labeled cells in the region of the DCN formed a mixed population of irregular, large cells, and round, small cells (Fig. 11A,D). Although the majority of the cells were located contralaterally, a minor component of ipsilateral cells was also present. The dendrites of these cells are long and directed both dorsally and ventrally, reaching the dorsal and the dorsolateral funiculi respectively. Their axons were followed into the contralateral medial lemniscus. In addition, a few cells were labeled bilaterally in the dorsolateral descending trigeminal tract. In in vitro BDA experiments in young adult X. laevis a similar pattern of labeling was observed (Fig. 12A,C).

2. Toral applications. When the injection sites were limited to the torus semicircularis, neurons were retrogradely labeled within the DCN. They were particularly found on the contralateral side, although an ipsilateral component was present as well. Two distinct cell groups were observed in R. perezi (Fig. 11B,E). The first one is made up of large cells with a minor component of small cells located in the dorsalmost gray. They possess several processes extending into the dorsal fiber layer. Their axons course ventromedially, cross the midline, and form part of the medial lemniscus. The second group of labeled cells is located in the lateral marginal zone of the dorsal gray from the level of the obex to the second spinal segment. These are large bipolar and irregular cells with long processes directed mainly to the dorsal part of the lateral funiculus and into the dorsal funiculus, while their axons participate in the medial lemniscus. In addition, retrogradely labeled cells were always found in the ipsilateral descending nucleus of the trigeminal nerve following toral injections. After similar toral injections in X. laevis, retrogradely labeled cells in the DCN form a band positioned from dorsomedial to ventrolateral above the solitary tract. The most dorsally located cells possess dendrites extending into the dorsal funiculus, whereas more ventrolateral cells have dendrites reaching the dorsal aspect of the lateral funiculus. Some neurons were observed with dendrites reaching both the dorsal and dorsolateral funiculi (Fig. 12D). In in vitro experiments, BDA was applied to the torus semicircularis of X. laevis. The pattern of labeling in the DCN is shown in Figure 12B,D,E.

3. Cerebellar applications. In experiments with tracer applications into the lateral portion of the cerebellar plate, the underlying cerebellar nucleus and the adjacent gray were mostly implicated as well. At the obex region, these applications resulted in the labeling of three cell groups. Most labeled cells were found at the ventromedial margin of the caudal extent of the fasciculus solitarius, on both sides of the medulla, probably due to the uptake of the tracer by fibers projecting from the nucleus of the solitary tract to the nucleus visceralis secundarius (parabrachial region). In the nucleus of the descending trigeminal tract labeled cells were found as well, mainly ipsilateral to the application site. The third group of retrogradely labeled cells was found, bilaterally, in the DCN (Fig. 11C,F). These cells were mainly found ipsilaterally. Their axons seem to run together with the ascending primary afferent fibers from the spinal dorsal roots.

Efferent projections: descending. In experiments with BDA or HRP applications into the DCN region anterogradely labeled axons could be followed from the injection site caudalwards into the spinal cord. These fibers course via the ipsilateral dorsal funiculus and form fine arborizations of thin varicose fibers terminating among the cells in the dorsal horn throughout the cord, but particularly at cervical levels. A sparse bilateral innervation of the intermediate and ventral zones was also observed. These fibers could, however, represent fibers by-passing the injection site. Therefore, spinal injections with retrograde tracers were studied. A small population of cells, scattered in the area of the ipsilateral DCN, was always labeled after injection of the various spinal segments (Fig. 10G).
Fig. 10. Photomicrographs illustrating the labeling observed after retrograde HRP injection into the BNST. A: BDA injection site in the contralateral BNST. B: Retrograde labeling in the contralateral BNST following a BDA application into the BNST. Scalebars = 100 μm.

The anuran dorsal column nucleus is shown in A. Arrow points to a cell group giving rise to the lumbar nuclei of the t...
DISCUSSION

In the present study the organization, immunohistochemical characterization, and particularly the fiber connections of the anuran DCN were investigated. Although it is obvious that the anuran DCN remains a rather ill-defined area in the caudal part of the rhombencephalic alar plate, and no selective markers for the DCN other than its labeling by primary afferents from the spinal cord are available, NADPHd staining and immunohistochemical staining of calcium-binding proteins and various neurotransmitters certainly help in delineating and characterizing the DCN. Since no clear cytoarchitectonic separation of the DCN into a medial, ‘gracile’ nucleus and a lateral, ‘cuneate’ nucleus is obvious, the term dorsal column nucleus is preferred.

The NADPHd histochemical technique, known to stain specific neurons (Thomas and Pearse, 1964), can selectively stain particular populations of neurons in a Golgi-like manner (Scherer-Singler et al., 1983). Throughout the brain, NADPHd and NOS localizations are identical (Bredt and Snyder, 1992). Therefore, NADPHd can be used as a marker for NOS. Nitric oxide probably plays a major role as a neuronal messenger (Bredt and Snyder, 1992; Meller and Gebhart, 1993; Schuman and Madison, 1994). The presence of NADPHd-positive cells and fibers in the mammalian spinal cord (Valtschanoff et al., 1992) suggests that nitric oxide may be involved in spinal sensory processing. In the rat DCN, Valtschanoff et al. (1993) found that most NOS-positive neurons are also immunoreactive for GABA, but not for the excitatory transmitters glutamate and aspartate. Moreover, since NOS-positive neurons could not be labeled retrogradely from the thalamus or spinal cord, they are probably local circuit neurons (Valtschanoff et al., 1993). In the anuran species studied, NADPHd-positive neurons were found in the DCN, but especially in the adjacent nucleus of the solitary tract and the descending nucleus of the trigeminal nerve, in keeping with data in mammals (e.g., Leight et al., 1990; Vincent and Kimura, 1992; Dohrn et al., 1994; Takemura et al., 1994). Since no double-labeling studies for GABA or excitatory transmitters were carried out, it remains to be analyzed whether these NADPHd-positive neurons are local circuit neurons or give rise to efferent projections such as the medial lemniscus.

Calcium-binding proteins such as Calb, calretinin, and Parv are found in certain subpopulations of neurons in the central and peripheral nervous system (Baimbridge et al., 1982; Garcia-Segura et al., 1984; Braun, 1990; Celio, 1990; Ren and Ruda, 1994). They even label entire pathways, and sometimes whole functional systems (Celio, 1990; Andressen et al., 1998). In mammals, calcium-binding proteins like calbindin and Parv show a preferential distribution in somatosensory structures, including the DCN (e.g., Celio, 1990; Rausell and Jones, 1991a,b; Rausell et al., 1992; Menetrey et al., 1992a,b; Maslany et al., 1992; Ren and Ruda, 1994). Parv appears to be abundant in the pathway for epicritic sensibility, i.e., the dorsal column-medial lemniscal system, and Calb occurs in the whole taste pathway of rats (Celio, 1990). In rats, Calb-positive neurons are found in certain laminae of the dorsal horn (Antal et al., 1990; Ren and Ruda, 1994) including the cells of origin of ascending spinal projections (Menetrey et al., 1992b), in the sensory trigeminal nuclei, and in the gracile and cuneate nuclei (Celio, 1990). In rats, Menetrey et al. (1992a) showed that Calb-positive neurons form a major part of the solitary and trigeminal projection systems. In the trigeminal system of monkeys, both proteins are differentially expressed in the ascending trigeminothalamic projections to the ventral posteromedial (VPM) nucleus (Rausell and Jones, 1991a,b). Antisera to parvalbumin and calbindin mark VPM rods and matrix, which receive principal and spinal trigeminal input, respectively. A similar segregation has been demonstrated for the ascending somatosensory projections from the spinal cord (Rausell et al., 1992): A non-nociceptive Parv-positive dorsal column-medial lemniscal projection terminates in cytochrome oxidase (CO)-rich domains of the ventral posterolateral thalamic nucleus (VPL) where Parv-positive neurons are found. Nociceptive Calb-positive spinothalamic fibers terminate in CO-poor domains of the VPL where Calb-positive cells are present (Rausell et al., 1992).

Against this background, the presence of Calb and Parv in the anuran DCN was studied. It appeared that in the alar plate of the caudal rhombencephalon, Calb-positive neurons were found particularly in the nucleus of the solitary tract and in the descending nucleus of the trigeminal nerve, continuing into the dorsal horn of the spinal cord. In both anuran species studied, hardly any Calb-positive neurons were found in the DCN itself. This pattern of distribution of Calb-positive neurons suggests that in anuran amphibians, as in mammals, Calb could be restricted to the nociceptive part of the somatosensory system including neurons in the dorsal horn of the spinal cord, and the descending nucleus of the trigeminal nerve. In striking contrast, in both anuran species a distinct Parv-positive DCN population was observed, particularly in X. laevis. Parv-positive neurons were found throughout the DCN, and their dendrites were mainly directed dorsally or laterally into the adjacent dorsal funiculus. Immunostaining of Parv can therefore be used to delineate the anuran DCN. It should be noted, however, that the pattern of distribution of calcium-binding proteins in the rat DCN is quite different. Maslany et al. (1992) found both Calb- and Parv-positive neurons in the cuneate and gracile nuclei, although Parv-positive DCN cells were more numerous. The distribution of Parv cells appeared to be similar to the known distribution of thalamic projection neurons.

In mammals, the presence of small GABAergic interneurons within the DCN has been extensively described (for reviews see Mugnaini and Oertel, 1985; Rustioni and Weinberg, 1989). Also glycinergic inhibitory effects within the DCN were observed. Does the anuran DCN contain GABAergic interneurons? In the present study small, round or oval-shaped GABA-immunoreactive neurons were observed. The pattern of labeling in other parts of the brain stem is comparable to that described by Franzoni and Morino (1989, R. esculenta), who, unfortunately, did not include the most caudal part of the brainstem in their analysis. Double-labeling studies, i.e., combinations with tract tracing or NADPHd, are needed to demonstrate whether these GABA-immunoreactive neurons in the anuran DCN are actually interneurons. In this respect, it should be noted that Pritz and Stritzel (1989a) suggested that the reptilian (Caiman crocodilids) DCN lacks glutamic acid decarboxylase (GAD)-immunoreactive neurons, indicating that the reptilian DCN—like the dorsal thalamus (see Pritz and Stritzel, 1988)—lacks local circuit neurons. A few glycinergic neurons were found at the ventrolateral border of the DCN. In the lamprey, such glycinergic neurons are known to inhibit reticulospinal neurons (Dubuc et al.,...
Fig. 11  A–C: Schematic drawings illustrating the distribution of retrogradely labeled neurons in the caudal part of the rhombencephalic alar plate of *R. perezi* following BDA applications to the thalamus (A), torus semicircularis (B), and cerebellum (C). D–F: Photomicrographs showing examples of labeling for each experiment. In D and E the contralateral DCN is labeled after dorsal thalamic and toral injections, respectively. In F labeling in the ipsilateral DCN after a cerebellar injection is shown. Scale bars = 100 μm.
Fig. 12. A, B: Two in vitro experiments in X. laevis. BDA was applied to the ventral thalamus (A) and torus semicircularis (B), respectively. In the photomicrographs C–E examples of labeling are shown. C: DCN neurons projecting to the contralateral ventral thalamus. D, E: DCN neurons projecting to the contralateral torus semicircularis. In D, one contralaterally projecting toral projection neuron at the ventrolateral aspect of the caudal DCN area with dorsally and ventrally oriented dendrites is shown; the arrow marks two axons crossing the midline to join the medial lemniscus. Scale bars = 100 µm.
THE ANURAN DORSAL COLUMN NUCLEUS

1983b). In mammals, glycinenergic cells of different sizes have been observed in the gracile and cuneate nuclei (Poruchko et al., 1992).

Even though cytoarchitectonic studies do not clearly define the anuran DCN, this nucleus is characterized by its somatotopic organization of primary afferent projections from the spinal cord (Antal et al., 1980; R. esculenta; Nikundiwe et al., 1982, *X. laevis*). Data in *R. pererei* (M. Muñoz et al., 1991) indicate a similar pattern of arrangement, whereby primary afferents from lumbar and thoracic dorsal root ganglia innervate the medial, “gracile” compartment of the DCN, whereas those from cervical ganglia innervate its lateral, “cuneate” compartment as well as the spinal or descending trigeminal nucleus. The dorsal funicular projection continues rostrally to innervate the vestibular nuclear complex and, rather abundantly, the granular layer of the cerebellum (Antal et al., 1980; Székely et al., 1980). Fibers terminating in the vestibular nuclei and in the cerebellum arise from limb-innervating spinal ganglia (Antal et al., 1980; González et al., 1984). The non-primary spinal afferents or postsynaptic dorsal column system (PDCS) also appears to be arranged somatotopically. The presence of such a PDCS has now been demonstrated throughout terrestrial vertebrates (e.g., Rustioni, 1973; Angaut-Petit, 1975b; Rustioni and Kaufman, 1977; Bennett et al., 1984; Giesler et al., 1984; Funke, 1988; ten Donkelaar and de Boer-van Huizen, 1991; Fritz and Stritzel, 1994). In mammals, the cells of origin of these non-primary afferent projections to the DCN, or postsynaptic dorsal column neurons, have been shown to transmit nociceptive information (Uddenberg, 1968; Angaut-Petit, 1975b; Bennett et al., 1984; Kamogawa and Bennett, 1986), at least in cats.

The lateral part of the anuran DCN is innervated by fibers from the descending tract of the trigeminal nerve, arising from the descending part of the trigeminal, facial, glossopharyngeal, and vagal nerves (Fig. 12; see also Rabinson and Friedman, 1977; Mateu and Székely, 1978; Fuller, 1979; Lowe and Russell, 1982; Altman and Dawes, 1983; Stuesse et al., 1984; Oka et al., 1987; González et al., 1993; M. Muñoz et al., 1994). In contrast, lateral line nerve projections, present in permanently aquatic species such as *X. laevis*, strictly avoid the DCN (Lowe and Russell, 1982; Altman and Dawes, 1983; Fritzsch et al., 1988; Will et al., 1985a).

The most lateral part of the anuran DCN is also innervated by substance P- and CGRP-immunoreactive fibers passing via the tract of Lissauer (see also Rosenthal and Croom, 1985; Adli et al., 1988; Petkó and Sánta, 1992). In addition to this peptidergic primary afferent projection to the DCN, the anuran DCN is innervated by Leu-Enk, NPY, and 5-HT-immunoreactive fibers in line with data by Ueda et al. (1984), Merchencheter et al. (1989), and Lázár et al. (1990). This serotonergic and peptidergic innervation of the DCN is in line with immunohistochemical data in mammals (e.g., Steinbusch, 1981; Westman et al., 1984; Hulliday et al., 1988; Ihikü et al., 1989; Tamatsani et al., 1989; Conti et al., 1990; Fabri and Conti, 1990; Blomqvist and Broman, 1993). Since after tracer applications to the DCN retrogradely labeled neurons were observed in the serotonergic—see Ueda et al., 1984—raphe nucleus, it seems likely that this nucleus is the source of the serotonergic innervation of the DCN. In rats, Wilcockson et al. (1987) observed serotonergic terminals in apposition to neurons of the DCN that project to the thalamus, whereas in cats and monkeys, Blomqvist and Broman (1993) observed serotonergic input to DCN neurons projecting to various brainstem areas including pretectum, superior colliculus, and pontine nuclei, related to motor processing.

Descending control of the DCN, so prominent in mammals (see Willis and Coggshall, 1991 for review), seems to be rather restricted in anurans. After injections of tracers into the DCN, labeled cells were found bilaterally in the cerebellar nucleus, in the ventral nucleus of nerve VIII, and in the reticular formation at levels between motor nuclei VII and IX including the inferior raphe nucleus. In mammals, the transmission of sensory information through the dorsal column-medial lemniscus pathway is controlled by pathways from the cerebral cortex (e.g., Kuypers, 1958; Kuypers and Tuerck, 1964), red nucleus (Edwards, 1972; Weinberg and Rustioni, 1989), vestibular nuclei (Weinberg and Rustioni, 1989), cerebellum (Sotgiu and Cesa-Bianchi, 1972), and reticular formation (Wilcockson et al., 1987; Weinberg and Rustioni, 1989). Therefore, with the possible exception of the red nucleus, a comparable brainstem “control” of the DCN is found in anurans.

A major part of the present study focused on the efferent connections of the DCN, particularly on the targets of the medial lemniscus. The existence of a dorsal column-medial lemniscal system in amphibians remained a much debated question until the early 1980s. Subsequently, Vesselkin and co-workers (Vesselkin et al., 1971; Vesselkin and Kovačević, 1973), Silvey et al. (1974), and Neary and Wilczynski (1977) described a contralateral projection of the DCN or “perisoli¬tary band” (Neary and Wilczynski, 1977) to thalamic nuclei. With electrophysiological techniques, Urbán and Székely (1982) noted slow negative potentials from the posteroventral nucleus of the thalamus in response to stimulation of the second dorsal root, the dorsal column, and the DCN. In the present study the course and site of termination of the medial lemniscus was shown by anterograde tracing (Figs. 8, 9) and its cells of origin by retrograde labeling of the DCN from its main targets, i.e., the ventral thalamus, the lateral part of the torus semicircularis, and the cerebellar cortex (Figs. 11, 12). The use of a new and powerful anterograde tracer like BDA made it possible to identify even fine terminal fields and the scattered fibers in the thalamus. The data obtained are summarized in Figure 13. Since it was hardly possible to restrict tracer applications to the DCN, in such injections the adjacent nucleus of the solitary tract and the descending nucleus of the trigeminal nerve as well as fibers of passage (e.g., spinal primary afferents to the cerebellum) might be involved. By retrograde labeling the origin of the medial lemniscal projections was verified. It should be emphasized that the ventrolateral part of the DCN found to project to the thalamus and particularly to the torus extends caudally as far as the second spinal segment. The dendrites of these cells are mainly directed to the dorsolateral funiculus, whereas their axons join the contralateral medial lemniscus. A certain similarity to the mammalian lateral cervical nucleus, known to receive somatosensory information via the spinocervical tract and projecting contralaterally via the medial lemniscus (Willis and Coggshall, 1991), seems likely.

The medial lemniscus could be traced throughout the brainstem and into the diencephalon. Along its course, the medial lemniscus gives off collaterals to various parts of the reticular formation, to the octavolateral area, and to the granular layer of the cerebellum. At mesencephalic levels, the medial lemniscus primarily innervates the lateral part of the torus semicircularis (also noted by Comer and Grobstein, 1981; Wilczynski, 1981; Neary and Wilczynski,
and the anterodorsal and anteroventral tegmental nuclei as well as the red nucleus and the interstitial nucleus of the fasciculus longitudinalis medialis. Whereas in *R. perezi* medial lemniscal fibers do not reach the tectum mesencephali, in *X. laevis* intermediate and deep tectal layers are innervated in agreement with retrograde tracer data (Wilczynski and Northcutt, 1977; Zittlau et al., 1988; Hofmann et al., 1990; Masino and Grobstein, 1990). Beyond the midbrain, both dorsal and ventral thalamic areas are innervated by the medial lemniscus. The ventral parts of the posterior and central nuclei of the dorsal thalamus are reached by a few thin, varicose, fibers, but the ventromedial thalamic nucleus and the nucleus of the posterior tubercle are far more densely innervated. In two cases in *R. perezi*, a few fibers also reached the anterior nucleus of the dorsal thalamus. No projections beyond the diencephalon were observed. Extralemniscal projections were found to the ipsilateral cerebellar cortex, confirming retrograde tracer data (González et al., 1984), and bilaterally to the spinal cord. The ipsilateral spinal projection from the DCN was previously observed in *X. laevis* (ten Donkelaar et al., 1981).

Hence, the present study not only further substantiated the presence of a rather well-developed dorsal column-medial lemniscus system in anurans, but also showed that its mesencephalic and diencephalic targets are much more extensive and diverse than suggested in previous studies (Vesselkin et al., 1971; Silvey et al., 1974; Neary and Wilczynski, 1977; Comer and Grobstein, 1981; Wilczynski, 1981; Forehand and Farel, 1982; Urbán and Székely, 1982; Neary, 1988). The anuran "lemniscal pathway" appears to be basically similar to that of amniotes (reptiles: Ebbesson, 1978; Siemen and Künzle, 1994a; birds: Wild, 1989; mammals: e.g., Hazlett et al., 1972; Hand and van Winkle, 1977; Feldman and Kruger, 1980; Berkley et al., 1986; see also Willis and Coggeshall, 1991 for a summary of mammalian studies), although in mammals the widespread thalamic projections should be particularly emphasized. In the red-eared turtle, *Pseudemys scripta elegans*, Siemen and Künzle (1994a, b) noted a direct ascending projection from the most medial part of the DCN area, by-passing the thalamus, to the basal part of the telencephalon.

At first sight, the mesencephalic target of the anuran medial lemniscus seems to be quite different from the amniote mesencephalic target. For the opossum, RoBards et al. (1976) introduced the term *intercollicular terminal zone* for the common target of projections from the dorsal column nuclei, spinal cord, and sensorimotor cortex in the central midbrain. In reptiles, Ebbesson (1967, 1969) introduced the term *intercollicular nucleus* for the mesencephalic target of ascending spinal projections. In this nucleus, a projection from the dorsal column nucleus terminates as well (Ebbesson, 1978; see also Belekhova et al., 1985; Pritz and Stritzel, 1989b). It seems likely that this intercollicular zone, nucleus, or "midbrain somatosensory area" (Pritz and Stritzel, 1989b), characterized by at least an input from the spinal cord and DCN, is a major integrative center of the somatosensory system. Pritz and Stritzel (1990) showed that the medial complex in the dorsal thalamus is the thalamic target of the midbrain somatosensory intercollicular area. In anurans, the main midbrain target of the medial lemniscus is formed by the lateral part of the torus semicircularis (Comer and Grobstein, 1981; Wilczynski, 1981; Neary and Wilczynski, 1986; Neary, 1988; the present study). The anuran torus semicircularis is a major integrating center for a number of sensory and non-sensory affer-

---

**Fig. 13.** Diagram summarizing the fiber connections of the anuran dorsal column nucleus shown in a dorsal view of the brain of *R. perezi.*
ents in addition to its auditory input and may well serve a role similar to the one the tectum mesencephali serves for the visual system (Wilczynski and Capranica, 1984). It includes a laminar nucleus, a principal nucleus, a magnocellular nucleus, and two smaller nuclei (Potter, 1965), each of which receives a particular set of afferents (e.g., Wilczynski, 1988; Feng and Lin, 1991). Physiological studies (Comer and Grobstein, 1981) in R. pipiens suggest a certain overlap of tactile and auditory information: the very dorsolateral torus is almost exclusively concerned with tactile information; auditory activity is most often found to be localized in central parts of the torus, but in between the two, multimodal (tactile and auditory) activity is found. Torial afferents also arrive from the vestibular (Wilczynski, 1981), and, in X. laevis, from the lateral line system (Will et al., 1985b; Love, 1986; Zittlau et al., 1986). The laminar toral nucleus not only receives DCN efferents but also spinal (Ebbesson, 1976; A. Muñoz et al., in preparation) and trigeminal afferents (Comer and Grobstein, 1981; M. Muñoz et al., 1994), and so—at least partly—represents a midbrain somatosensory area. The multimodal laminar nucleus as well as the mainly auditory magnocellular nucleus extensively innervate the central and posterior nuclei (Frontera’s nucleus posteroentralis; see Frontera, 1952) of the dorsal thalamus, whereas the ascending projections of the principal nuclei are restricted to the caudal part of the posterior thalamic nucleus (Hall and Feng, 1987; Feng and Lin, 1991). The laminar nucleus also innervates the ventromedial thalamic nucleus (Feng and Lin, 1991; A. Muñoz and ten Donkelaar, unpublished observations), i.e., the main diencephalic target of the medial lemniscus (present study) as well as of the spinothalamic tract (A. Muñoz et al., 1994). The central thalamic nucleus extensively projects to the ipsilateral striatum (Vesselkin et al., 1980; Wilczynski and Northcutt, 1983a; Neary, 1988). Hence, this DCN-torus-central thalamic nucleus–striatal pathway is one way by which somatosensory information may reach the telencephalon.

Although the anuran dorsal column-medial lemniscus system is basically similar to that of amniotes, large differences are found with regard to the telencephalic targets of this pathway. Therefore, a few remarks on the telencephalic structures receiving somatosensory information in anurans seem appropriate. Physiological studies revealed somatosensory activity within the medial pallium (e.g., Supin and Gusevnikov, 1964; Karamian et al., 1966; Northcutt, 1979; Vesselkin and Kovačević, 1973), possibly relayed in the dorsal thalamus. Since the anterior thalamic nucleus is the only thalamic nucleus innervating the medial pallium (Scalia and Colman, 1975; Vesselkin and Ermanova, 1978; Kicliter, 1979; Neary, 1984; Northcutt and Ronan, 1992), somatosensory information to this pronounced telencephalic structure, also known as the archipallium (Ariëns Kappers et al., 1936; Clairambault and Derer, 1968) or the primordium hippocampi (Herrick, 1910; Hoffman, 1963), must relay in the anterior nucleus. However, since spinal afferents to the anterior thalamic nucleus—either via the spinothalamic tract (A. Muñoz et al., 1994) or via the dorsal column-medial lemniscal pathway (Neary and Wilczynski, 1977; present study)—are rather limited, alternative routes must be available, possibly via the posterior thalamic nucleus, known to project to the anterior thalamic nucleus (Neary and Wilczynski, 1979; see also Neary, 1990; Northcutt and Ronan, 1992). It should also be noted that the dendrites of cells in the anterior thalamic nucleus penetrate the central nucleus (Neary, 1990). Therefore, somatosensory information could reach the medial pallium via multisynaptic routes.

Another telencephalic structure in which somatosensory activity was recorded is the striatum (Vesselkin et al., 1971; Vesselkin and Kovačević, 1973). The anuran striatum receives a major thalamotelencephalic input from nuclei relaying sensory information from the midbrain roof, from the torus semicircularis, and from ventral diencephalic structures, receiving spinal and DCN-medial lemniscal afferents (Scalia and Colman, 1975; Vesselkin et al., 1980; Wilczynski and Northcutt, 1983a; Lázár and Kozicz, 1990). The striatum in amphibians appears to be able to influence the midbrain roof via the pretectum and various midbrain and isthmal nuclei (Wilczynski and Northcutt, 1983b). The anuran striatum plays a crucial role in processing sensory information as well as in coordinating all telencephalic output to lower brainstem motor centers. Both visual (Gruberg and Ambros, 1974) and auditory (Mudry and Capranica, 1980) activity was recorded from the striatum. Further, electrical stimulation of the sciatic nerve evoked potentials in the striatum (Vesselkin et al., 1971; Vesselkin and Kovačević, 1973). The sensory input to the striatum is relayed in the anterior division of the lateral thalamic nucleus (visual information: Lázár, 1969; Scalia, 1978), in the central thalamic nucleus (auditory and also somatosensory information: Hall and Feng, 1987; Feng and Lin, 1991; present study), and in the ventromedial thalamic nucleus (somatosensory information: Neary and Wilczynski, 1977; present study). In R. perezi, tracer applications to the striatum showed a direct striatal projection arising from cells in the lateral aspect of the ventromedial thalamic nucleus (A. Muñoz, unpublished observations), in line with observations by Vesselkin et al. (1980) as well as by Lázár and Kozicz (1990). Vesselkin et al. (1980) also noted a direct striatal projection from the torus semicircularis, whereas Lázár and Kozicz (1990) found a few faintly labeled small cells in the nucleus of the posterior tubercle projecting to the lateral wall of the telencephalon including the striatum (see also Wilczynski and Northcutt, 1983a). Somatosensory information to the striatum may thus be relayed via the dorsal thalamus (the central thalamic nucleus), the ventral thalamus (the ventromedial thalamic nucleus), and the nucleus of the posterior tubercle, a separate diencephalic region (Neary and Northcutt, 1983). Which thalamic nuclei really relay somatosensory information to the striatum (and medial pallium) is now being studied in a series of double-labeling experiments.

**ACKNOWLEDGMENTS**

This research was supported by an ENP short-term fellowship of the European Science Foundation to A. Muñoz, a NATO Collaborative Research Grant (930542) to H.J. ten Donkelaar, and by a grant from the Spanish Government (DGICYT PB 93-0083) to A. González. The authors thank Mrs. Roelie de Boer-van Huizen and Mrs. Ine Bergervoet-Vernooy for excellent technical assistance, Mrs. Marilú Ackermans for making the final versions of the drawings, and Ms. Inge Eijkhout for secretarial assistance. The valuable comments of Dr. William L.R. Cruce are gratefully acknowledged.

**LITERATURE CITED**

lin, and serotonin in the spinal cord of the Northern leopard frog, Rana 

projections from the lateral line nerves, associated cutaneous nerves, and the 

Andressen, C., I. Blümcke, and M.R. Cello (1993) Calcium-binding proteins: 

ascending postynaptic fibres in the cat’s fasciculus gracilis. Exp. Brain 

Anguat-Petit, D. (1975b) The dorsal column system. II. Functional proper-
eties and bulbar relay of the postynaptic fibres of the cat’s fasciculus 

fibres in the spinal cord and brain stem of the frog. Neuroscience 
5:1311–1322.

Antal, M., T.F. Freund, and E. Polgar (1990) Calcium-binding proteins, 
parvalbumin- and calbindin-D28k-immunoreactive neurons in the rat 
spinal cord and dorsal root ganglia: A light and electron microscopic 

Ariëns Kappers, C.U., and E. Hammer (1918) Das Zentralnervensystem des 
fibres in the spinal cord and brain stem of the frog. Neuroscience 
5:1311–1322.

Antal, M., T.F. Freund, and E. Polgar (1990) Calcium-binding proteins, 
parvalbumin- and calbindin-D28k-immunoreactive neurons in the rat 
spinal cord and dorsal root ganglia: A light and electron microscopic 

Ariëns Kappers, C.U., and E. Hammer (1918) Das Zentralnervensystem des 
fibres in the spinal cord and brain stem of the frog. Neuroscience 
5:1311–1322.

Antal, M., T.F. Freund, and E. Polgar (1990) Calcium-binding proteins, 
parvalbumin- and calbindin-D28k-immunoreactive neurons in the rat 
spinal cord and dorsal root ganglia: A light and electron microscopic 

Ariëns Kappers, C.U., and E. Hammer (1918) Das Zentralnervensystem des 
fibres in the spinal cord and brain stem of the frog. Neuroscience 
5:1311–1322.

Antal, M., T.F. Freund, and E. Polgar (1990) Calcium-binding proteins, 
parvalbumin- and calbindin-D28k-immunoreactive neurons in the rat 
spinal cord and dorsal root ganglia: A light and electron microscopic 

Ariëns Kappers, C.U., and E. Hammer (1918) Das Zentralnervensystem des 
fibres in the spinal cord and brain stem of the frog. Neuroscience 
5:1311–1322.

Antal, M., T.F. Freund, and E. Polgar (1990) Calcium-binding proteins, 
parvalbumin- and calbindin-D28k-immunoreactive neurons in the rat 
spinal cord and dorsal root ganglia: A light and electron microscopic 


