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Nucleus Accumbens in the Lizard *Psammodromus algirus*: Chemoarchitecture and Cortical Afferent Connections

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ABSTRACT

To better understand the organization and evolution of the basal ganglia of vertebrates, in the present study we have analyzed the chemoarchitecture and the cortical input to the nucleus accumbens in the lacertid lizard *Psammodromus algirus*. The nucleus accumbens contains many γ-aminobutyric acid (GABA)-positive neurons and calbindin-positive neurons, the majority of which may be spiny projection neurons, and a few dispersed neuropeptide Y–positive neurons that likely represent aspiny interneurons. The nucleus accumbens contains two chemoarchitectonically different fields: a rostromedial field that stains heavily for substance P, dopamine, $GABA_A$ receptor, and a caudolateral field that stains only lightly to moderately for them, appearing more similar to the adjacent striatum. Injections of biotinylated dextran amine were placed in either the medial, dorsomedial, or dorsal cortices of *Psammodromus*. The medial and the dorsal cortices project heavily to the rostromedial field of the accumbens, whereas they project lightly to moderately to the caudolateral field. Cortical terminals make asymmetric, presumably excitatory, synaptic contacts with distal dendrites and the head of spines. Our results indicate that the hippocampal-like projection to the nucleus accumbens is similar between mammals and reptiles in that cortical terminals make mainly excitatory synapses on spiny, putatively projection neurons. However, our results and results from previous investigations indicate that important differences exist between the nucleus accumbens of mammals and reptiles regarding local modulatory interactions between cortical, dopaminergic, and cholinergic elements, which suggest that the reptilian nucleus accumbens may be as a whole comparable to the shell of the mammalian nucleus accumbens. J. Comp. Neurol. 405:15–31, 1999.

Indexing terms: basal ganglia; ventral striatum; reptile; hippocampus; ultrastructure

The reptilian nucleus accumbens seems very similar to that of mammals in terms of location, histo- and immunohistochemical features, as well as connections (Russchen et al., 1987; Russchen and Jonker, 1988; González et al., 1990; Smeets and Medina, 1995; Pérez-Santana et al., 1997). Similarly to mammals, the nucleus accumbens of reptiles is located in the basal telencephalon ventromedially to the dorsal striatum and is rich in acetylcholinesterase activity and in fibers and terminals containing substance P (SP), enkephalin (ENK), and dopamine (DA) (Russchen et al., 1987; Smeets et al., 1986, 1987; Smeets, 1988; Zahm and Brog, 1992; Jongen-Rêlo et al., 1993). In addition, in both mammals and reptiles the nucleus accumbens contains the same basic cell types, i.e., spiny projection neurons that contain the neurotransmitter γ-aminobutyric acid (GABA) as well as the neuropeptides SP, ENK, or both (Reiner et al., 1984; Reiner, 1987; Anderson and Reiner, 1990; Reiner and Anderson, 1990), and aspiny

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interneurons containing either choline acetyltransferase (CHAT, a marker for acetylcholine) or both neuropeptide Y (NPY) and somatostatin (SS) (Reiner and Oliver, 1987; Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1992, 1993; Powers and Reiner, 1993). Finally, the nucleus accumbens of mammals and reptiles receive an input from the limbic and limbic-related cortex, the amygdala, the dorsomedial thalamus, the A8–A10 tegmental dopaminergic neurons, and from the noradrenergic neurons of the locus coeruleus, and project to the ventral pallidum, bed nucleus of the stria terminalis, hypothalamus, A8–A10 dopaminergic cell field, and raphe nuclei (Russchen and Jonker, 1988; Hoogland and Vermeulen-VanderZee, 1989; González et al., 1990; Berendse et al., 1992a,b; Zahm and Heimer, 1993; Bruce and Neary, 1995; Smeets and Medina, 1995).

In mammals, the nucleus accumbens has been shown to be a heterogeneous structure, and on the basis of both chemoarchitecture and connections can be divided into a shell and a core (Jongen-Rêlo et al., 1994; Meredith et al., 1996). The nucleus accumbens in reptiles also shows nonhomogeneous staining patterns for SP, ENK, and DA (Russchen et al., 1987). However, no evidence has been obtained for a subdivision of the nucleus into regions comparable to the shell and core of the mammalian accumbens.

Despite the high similarity of mammalian and reptilian nucleus accumbens with respect to cell types and connections (references above), and with respect to the presence of the same basic types of dopaminergic and cholinergic receptors (Richfield et al., 1987; Schelegel and Kriegstein, 1987), pharmacologic studies have shown that dopamine or specific agonists of the dopamine receptor subtype D2 do not produce the same effect on acetylcholine release in the striatum/nucleus accumbens of mammals and reptiles (Stoof et al., 1987; Henselmans and Stoof, 1991; Henselmans et al., 1991). Consistent with this finding, a recent electron microscopic study has shown that, in contrast to mammals, in the nucleus accumbens of reptiles dopaminergic terminals do not establish any contact with perikarya, dendritic shafts or terminals of cholinergic interneurons (Henselmans and Wouterlood, 1994). To further investigate the organization of the reptilian nucleus accumbens and to better understand the function and evolution of the basal ganglia of vertebrates, the aim of the present study has been to analyze the chemoarchitectonic features and the cortical input at both light and electron microscopic levels of the nucleus accumbens of a reptile, the lizard *Psammodromus algirus*. To chemoarchitectonically define the nucleus accumbens of *Psammodromus*, telencephalic sections were immunohistochemically stained for either SP, DA, GABA, $GABA_A$ receptor subtype, or NPY. In addition, for this study brain sections of *Psammodromus* were also stained for the calcium-binding protein calbindin D-28k (CaBP), because this protein shows a distinctive pattern of distribution in the nucleus accumbens of rats and primates that makes it possible to distinguish the two functionally different regions of the nucleus, i.e., the core and the shell (Jongen-Rêlo et al., 1994; Meredith et al., 1996).

MATERIAL AND METHODS

Adult lizards of the species *P. algirus* (Lacertidae) were used in the present work (body length 65–80 mm). Throughout the experimental work, animals were treated following the European Union guidelines on treatment of experimental animals.

Immunohistochemistry

Animals were deeply anesthetized with urethane, perfused transcardially, and their brains were processed as described previously (Dávila et al., 1993; Andreu et al., 1994; Guirado and Dávila, 1994). Brain sections were immunostained following protocols and antisera described elsewhere for GABA and NPY (Dávila et al., 1991, 1993), $GABA_A$ receptor subtype (Guirado and Dávila, 1994), DA (Andreu et al., 1994), and CaBP (Dávila et al., 1999). The immunostaining for SP was performed on lizards that were perfused transcardially with phosphate buffer saline 0.1 M, pH 7.4 (PBS) under deep anesthesia with urethane, followed by 4% paraformaldehyde in PBS for 30 minutes at room temperature. Brains were removed from the skulls and stored overnight in the same fixative at 4°C; then they were embedded in 4% agar and cut into 50-mm-thick sections on a Vibratome. The sections were washed extensively in PBS and processed for immunocytochemistry following the peroxidase-antiperoxidase (PAP) method. Free floating sections were incubated in 2% normal goat serum and 0.3% Triton X-100 in PBS for 60–90 minutes at room temperature to block nonspecific binding (the same solution was used as a solvent for the primary and link antibodies). Sections were transferred to a rabbit anti-SP antibody (Amersham, Buckinghamshire, United Kingdom) diluted 1:500 for 18 hours, and then washed three times in PBS for 45 minutes, followed by incubation in a goat anti-rabbit IgG diluted 1:35 for 1 hour, another wash in PBS for 45 minutes, and finally an incubation in the rabbit PAP complex diluted 1:100 for 1 hour. All steps were done at room temperature under constant agitation. The immunolabeling was revealed with 0.05% diaminobenzidine (DAB) and 0.03% H_2O_2 in PBS. After a thorough wash in PBS, the sections were mounted on gelatinized slides, air-dried, dehydrated in ethanol, cleared in xylene, and cover-slipped with Eukitt.

Biotinylated dextran amine injections

Under ether anesthesia, animals were injected with of biotinylated dextran amine 10,000 molecular weight (BDA 10K, Molecular Probes, Eugene, OR) in the medial (n = 3), dorsomedial (n = 2), or dorsal (n = 6) telencephalic cortices. Tracer injections were made iontophoretically by applying a 5 µA positive pulsed current (7 seconds on/7 seconds off for 15–20 minutes) to a 5% solution of BDA 10K in 0.01 M phosphate buffer (PB, pH 7.4), by using glass micropipettes (inner diameter, 5–10 µm). After a survival time of 7–10 days, lizards were deeply anesthetized with urethane, transcardially perfused with 0.1 M phosphate buffered saline (PBS, pH 7.4) and subsequently with PB containing 4% paraformaldehyde, 0.075 M lysine, and 0.01 M sodium periodate at room temperature for 30 minutes. The perfused brains were removed from the skulls and stored at 4°C overnight in the same fixative; then they were embedded in 4% agar, and 50-µm-thick frontal sections were obtained with a Vibratome.

Sections were incubated in avidin-biotin complex (Vectastain ABC standard kit, Vector Laboratories, Burlingame, CA) at room temperature for 3 hours. Peroxidase activity was revealed with 0.05% DAB and 0.03% hydrogen peroxide (H_2O_2) in PBS. Sections were then washed

with PBS, mounted on gelatinized slides, air dried, dehydrated in ethanol, cleared in xylene, and coverslipped with Eukitt.

Electron microscopy

After a light microscopy examination, selected BDA-labeled sections were processed for electron microscopy. Sections were washed in PBS, treated with 1% OsO_4 in PBS for 1 hour, dehydrated in acetone, and flat embedded in Araldite between two aluminum sheets. To increase contrast for electron microscopy, these sections were stained with 1% uranyl acetate in 70% acetone during dehydration. Flat-embedded sections were glued onto prepolymerized resin blocks and cut at 70–80 nm on a Reichert Ultra-Cut E ultramicrotome. Ultrathin sections were collected on copper grids and examined with a Philips CM100 electron microscope.

To ascertain the existence of putative spiny projection neurons in the nucleus accumbens of *Psammodromus* (which are known targets of cortical terminals in the mammalian nucleus accumbens), we analyzed Golgi-impregnated sections from previous investigations of our laboratory (Guirado et al., 1984, 1987; Andreu et al., 1996).

RESULTS

Chemoarchitecture of the nucleus accumbens in *Psammodromus*

As in other reptiles, the nucleus accumbens in *Psammodromus* is a cell field located within the basal telencephalon, ventromedially to the dorsal striatum, extending from rostral until mid-telencephalic levels (Fig. 1). Our results on immunohistochemistry clearly showed that the nucleus presented nonhomogeneous patterns of staining for SP, DA, $rGABA_A$, and CaBP (Figs. 2, 3). These staining patterns are shown at three comparable rostrocaudal levels of frontal sections through the nucleus accumbens of *Psammodromus* (SP, DA, $rGABA_A$, and CaBP; Figs. 2, 3), as well as at three mediolateral levels of sagittal sections through the nucleus accumbens of this lizard (CaBP; Fig. 4). On the basis of these patterns, the nucleus could be divided into a heavily stained rostromedial region and a less intensely stained caudolateral region that appeared similar to the adjacent striatum.

As observed in frontal sections, the rostral pole of the nucleus was characterized by the presence of distinctive, extremely rich plexuses of fibers and terminals immunopositive for DA (DA+) and SP (SP+) (Fig. 2A,D). At intermediate and caudal levels, the densest immunoreactive area for DA and SP appeared progressively restricted to the most medial part of the nucleus, just below the septum, until it finally disappeared at its most caudal level (Fig. 2). As the densest staining area shrank and finally disappeared caudalward, a less intensely stained field for DA or SP became progressively enlarged laterally (Fig. 2B,C,E), to occupy the whole area of the nucleus at its most caudal level (Fig. 2F).

A similar staining pattern was shown by $rGABA_A$ in frontal sections: the strongest staining was found at the rostral pole of the nucleus, and it became progressively restricted to the most medial part at intermediate and caudal levels of the nucleus (Fig. 3A,B). The densest staining area was no longer present at the most caudal pole of the nucleus (Fig. 3C). As the densest staining area of the nucleus progressively disappeared caudalward, the lateral and caudal region of the nucleus showing a moderate to light immunostaining for $rGABA_A$ became progressively enlarged and occupied the whole area of the nucleus at its most caudal pole (Fig. 3B,C).

The CaBP staining pattern also revealed a similar division of the nucleus accumbens into a rostromedial field and a caudolateral field as observed in both frontal and sagittal sections. The rostromedial field contained numerous densely packed CaBP-positive (CaBP+) cell bodies and a very dense CaBP+ neuropil (Fig. 3D,E). The caudolateral field contained many loosely packed CaBP+ cell bodies and a moderately stained neuropil (Fig. 3E,F). These two compartments of the nucleus were better observed in sagittal sections, which showed the existence of a sharp boundary between a heavily stained rostral pole and a moderately stained caudal pole in the nucleus accumbens (Fig. 4). These rostral and caudal compartments observed with CaBP in sagittal sections appeared to represent the rostromedial field or the caudolateral field, respectively, observed with DA, SP, $rGABA_A$, and CaBP stainings in frontal sections of the nucleus.

Finally, the nucleus accumbens in *Psammodromus* contained numerous GABA-immunopositive (GABA+) cell bodies (Fig. 5A), as well as a few SP+ cell bodies. These GABA+ cell bodies were uniformly distributed throughout the nucleus, resembling in both number and distribution the CaBP+ neurons. A few dispersed NPY+ cell bodies were also observed in the nucleus accumbens of *Psammodromus* (Fig. 5B,C). These cells were preferentially located at the lateral aspect of the nucleus. Observation of Golgi-impregnated sections indicated the presence in the nucleus accumbens of a variety of medium-sized spiny neurons, which typically showed many long dendritic spines of different morphologies (Fig. 5D,E).

Cortical projections to the nucleus accumbens in *Psammodromus*

Injections of BDA were placed into either the reptilian medial, dorsomedial, or dorsal cortices. The medial and dorsomedial cortices as well as both the medial and intermediate parts of the dorsal cortex of reptiles are considered to be comparable to the mammalian hippocampal cortex on the basis of their similar cyto- and chemoarchitecture, ontogenesis, and connections with the hypothalamus (Lohman and Van Woerden-Verkley, 1976; Molowny and López-García, 1978; Hoogland and Vermeulen-VanderZee, 1988; López-García and Martínez-Guijarro, 1988; López-García et al., 1988; Martínez-García and Olucha, 1988; Dávila et al., 1995, 1997).

Light microscopy

After BDA injections in either the intermediate or the lateral part of the dorsal cortex of the lizard *Psammodromus*, a moderate to high number of anterogradely labeled fibers and terminals were observed in the nucleus accumbens (Fig. 6A–D). Extremely few retrogradely labeled neurons were also present in the nucleus accumbens after BDA injections in the dorsal cortex. The anterogradely labeled fibers and terminals of dorsal cortical origin were not homogeneously distributed in the nucleus accumbens. Injections in either the intermediate or lateral part of the dorsal cortex produced a heavy anterograde labeling of fibers and terminals in the rostral pole as well as in the medial part of the nucleus at intermediate and caudal

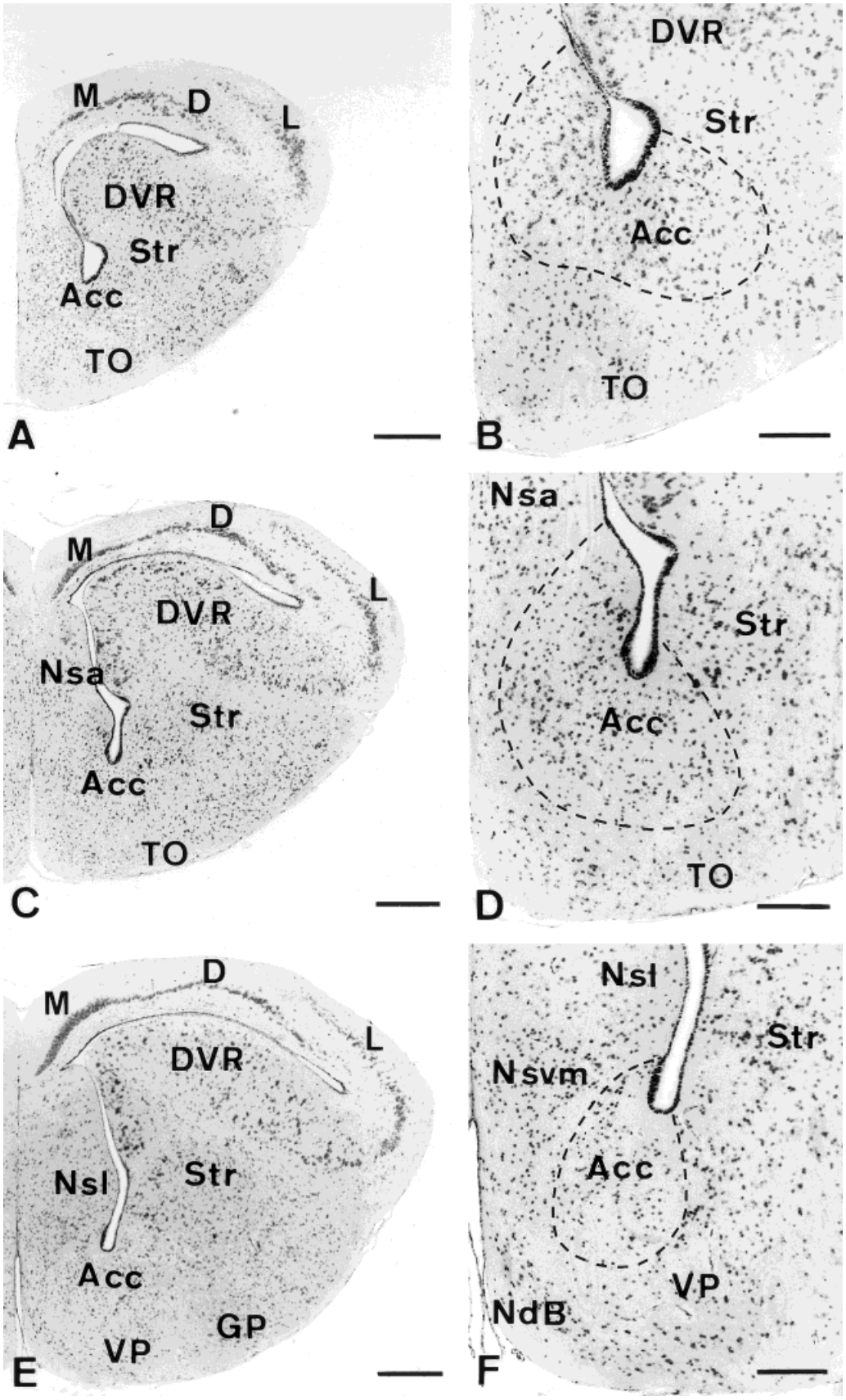


Fig. 1. Nissl-stained frontal sections at three rostrocaudal levels of the nucleus accumbens (Acc) of *Psammodromus*. **A,C,E:** Low-magnification photomicrographs of the telencephalon. **B,D,F:** Details of the accumbal region at the three rostrocaudal levels. The cytoarchitectonic boundaries of the Acc are marked by dashed lines. From hereon, medial is to the left and dorsal to the top in frontal sections. M, D, and L: medial, dorsal, and lateral cortices, respectively. DVR, dorsal ventricular ridge; GP, globus pallidus; NdB, nucleus of the diagonal band of Broca; Nsa, nucleus septalis anterior; Nsl, nucleus septalis lateralis; Nsvm, nucleus septalis ventromedialis; Str, striatum; TO, tuberculum olfactorium; VP, ventral pallidum. Scale bars = 250 µm in A,C,E, 125 µm in B,D,F.

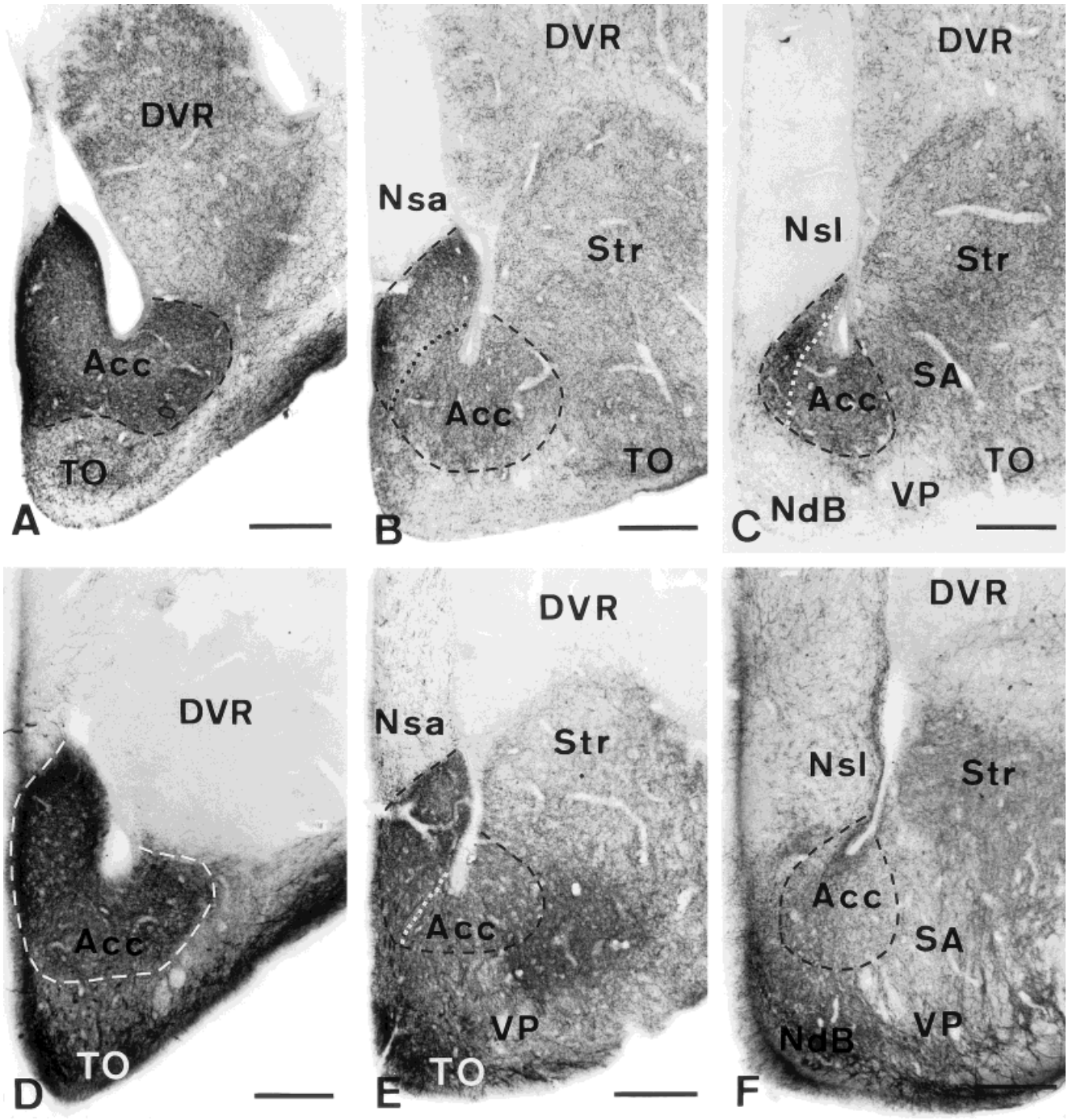


Fig. 2. Chemoarchitecture of the nucleus accumbens (Acc). **A–C:** Immunostainings for dopamine (DA) at a rostral, an intermediate, and a caudal level of the nucleus, respectively. **D–F:** immunostainings for substance P (SP) at three rostrocaudal accumbal levels. Dashed lines mark the limits of the nucleus accumbens. Dotted lines separate the two accumbal chemoarchitectonic fields. Note that the rostral pole of the nucleus is heavily stained for DA and SP. DVR, dorsal ventricular ridge; NdB, nucleus of the diagonal band of Broca; Nsa, nucleus septalis anterior; Nsl, nucleus septalis lateralis; SA, striato-amygdaloid transition area; Str, striatum; TO, tuberculum olfactorium; VP, ventral pallidum. Scale bars = 200 μm in A–F.

levels (Fig. 6C). In contrast, the anterograde labeling in the lateral and caudal part of the nucleus accumbens was moderate and resembled that observed in the adjacent striatum. In transverse sections, the projection appeared as fine, short segments of varicose axons as well as small punctiform profiles that presumably correspond to axon terminals (Fig. 6D). The dorsal cortical projection to the nucleus accumbens was predominantly ipsilateral, but a few labeled terminals were also found in the medial part of the contralateral nucleus.

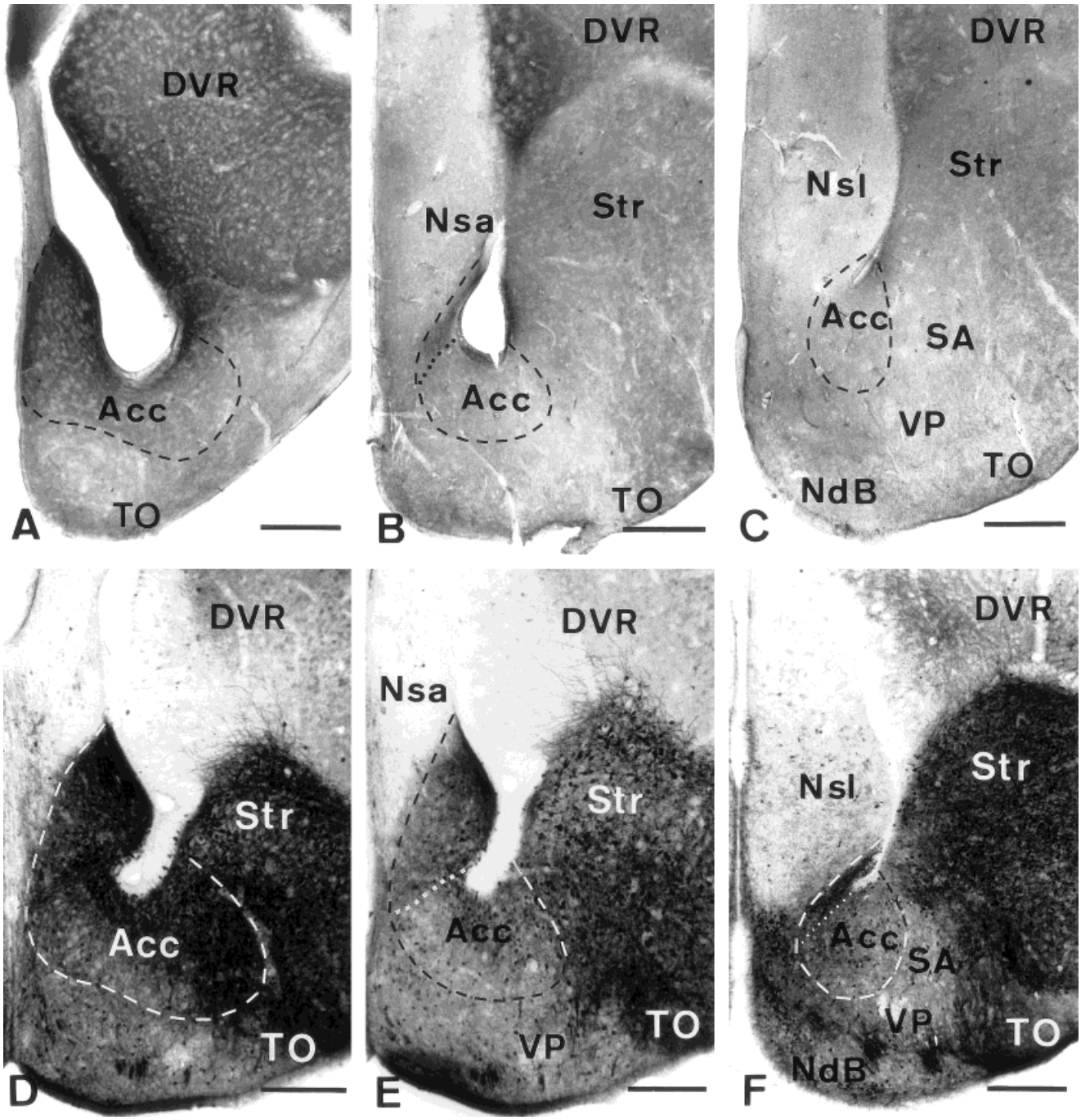


Fig. 3. Chemoarchitecture of the nucleus accumbens (Acc). As in Figure 2, dashed lines mark the limits of the nucleus accumbens, whereas dotted lines separate the two accumbal chemoarchitectonic fields. **A–C:** Immunostainings for γ-aminobutyric acid receptor subtype ($rGABA_A$) at a rostral, an intermediate, and a caudal level of the nucleus, respectively. The rostromedial region is moderate to heavily stained for $rGABA_A$, whereas the caudolateral region is less intensely stained. **D–F:** Immunostainings for CaBP at three rostrocaudal accumbal levels. Note that the CaBP-positive neuropil is less intense at the caudolateral edge of the nucleus, close to the striatum. Abbreviations as in Figure 2. Scale bars = 200 μm in A–F.

After BDA injections in the medial cortex, anterograde labeling of fibers and terminals was also found in the nucleus accumbens (Fig. 6E–G). The labeling was heavy in the rostral and medial portions of the nucleus, whereas the lateral and caudal portion received only a minor input from the medial cortex resembling the scarce input from this cortex to the adjacent striatum (Fig. 6G). In comparison to the input from the dorsal cortex, the medial cortex projection to the lateral part of the nucleus was much less prominent. The medial cortex projection to the nucleus accumbens was mostly ipsilateral and only extremely few BDA-labeled varicose fibers were found in the contralat-

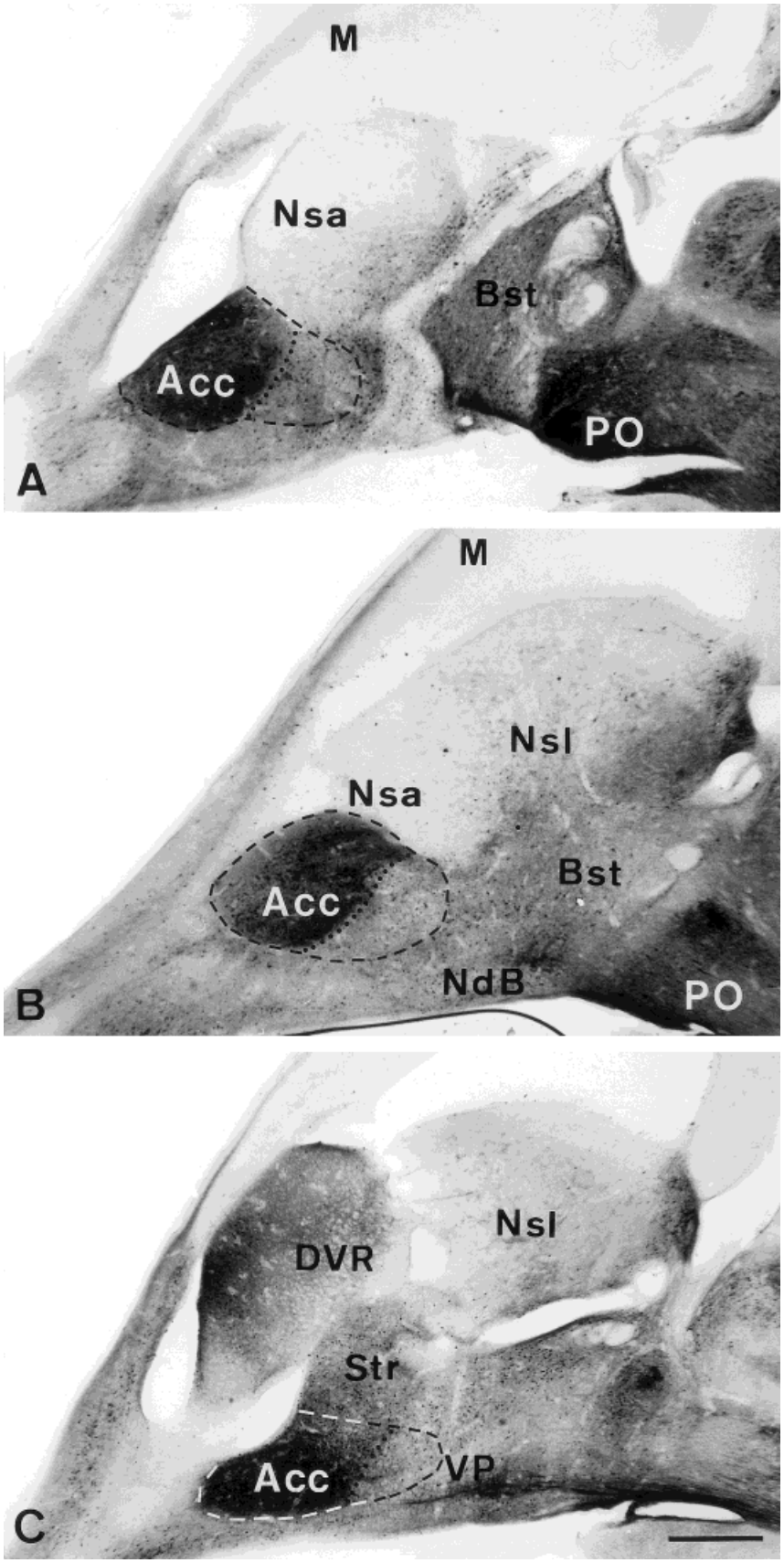


Fig. 4. Sagittal sections of the brain of *Psammodromus* immunostained for the calcium-binding protein calbindin D-28k at a medial (**A**), an intermediate (**B**), and a lateral (**C**) aspects of the nucleus accumbens (Acc). Dashed lines mark the boundaries of the nucleus, and dotted lines separate two fields within the nucleus. Rostral is to the left, and dorsal is to the top. Bst, bed nucleus of the stria terminalis; DVR, dorsal ventricular ridge; NdB, nucleus of the diagonal band of Broca; Nsa, nucleus septalis anterior; Nsl, nucleus septalis lateralis; M, medial cortex; Str, striatum; VP, ventral pallidum; PO, preoptic region. Scale bar = 400 µm in C (applies to A–C).

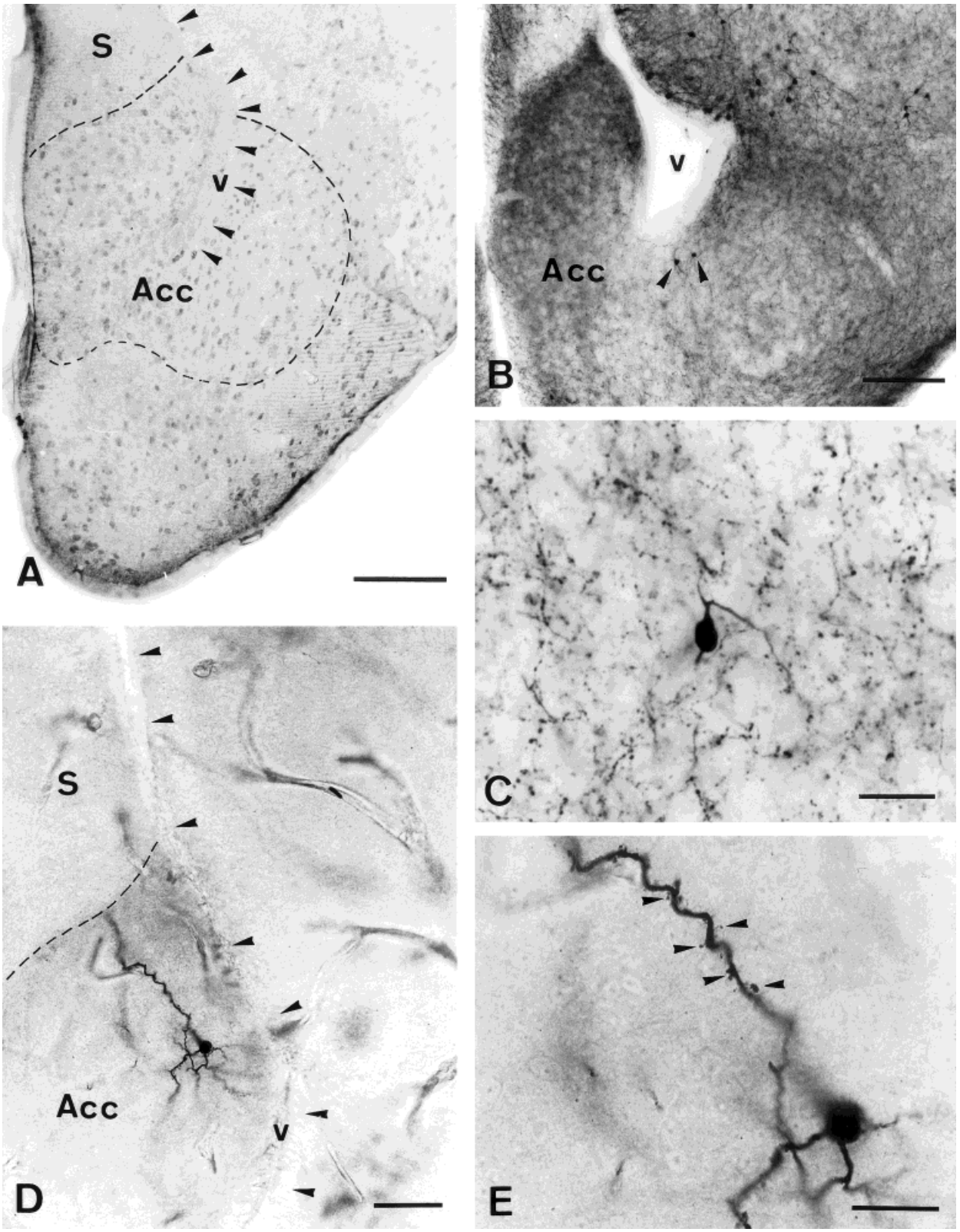


Fig. 5. Neurons in the nucleus accumbens. **A:** Frontal section through the nucleus accumbens immunostained for γ-aminobutyric acid. Numerous immunoreactive cell bodies can be observed throughout the nucleus accumbens (Acc), which appears delimited by dashed lines. Arrowheads indicate the position of the ventricle (v). S, septum. **B:** Frontal section through the nucleus accumbens immunostained for neuropeptide Y (NPY). Two NPY-immunoreactive (NPY-ir) neurons (arrowheads) can be observed in the lateral part of the nucleus accumbens (Acc), near the ventricle (v). Note the dense plexus of NPY-ir fibers in the medial region of the nucleus. **C:** High magnification of a NPY-ir neuron in the accumbens. **D:** Frontal section through the nucleus accumbens showing a Golgi-impregnated neuron that extends its dendrites within the limits of the nucleus accumbens (Acc). The border between this nucleus and the overlying septum (S) is indicated by a dashed line. Arrowheads indicate the position of the ventricle (v). **E:** High magnification of the neuron in D, showing a dendrite with many heterogeneous dendritic spines (arrowheads). Scale bars = 150 μm in A,B, 25 μm in C, 50 μm in D, 20 μm in E.

eral nucleus. In contrast to the injections in the medial or dorsal cortices, no labeling was found in the nucleus accumbens after injections of the tracer in the dorsomedial cortex.

Electron microscopy

No differences were observed between the medial and lateral parts of the nucleus accumbens regarding the morphology of labeled terminals and the type of synaptic contacts that they established. Labeled boutons were large, had plenty of rounded synaptic vesicles, and contained a few mitochondrial profiles (Fig. 7). Smaller boutons with rounded synaptic vesicles were also found. Labeled terminals were usually observed contacting small dendritic profiles or dendritic spines (Fig. 7). When observed, the synaptic specialization was always of the asymmetric type (Fig. 7). No perisomatic synaptic contacts were observed, although labeled boutons were occasionally found in close apposition to a cell body.

In a number of cases, the dendritic profile on which the labeled bouton made synapse was smaller than the bouton proper. In these cases, the labeled terminal appeared to enwrap the dendritic profile (Fig. 7F). When the dendritic spine was evident, the labeled bouton made the synapse on the head of the spine (Fig. 7D). Some of the dendritic profiles of the nucleus accumbens contacted by labeled terminals were also observed to be contacted by one or two unlabeled terminals, which were sometimes observed to make symmetric synapses (Fig. 7B,C,E). Labeled terminals were sometimes observed in apposition with unlabeled terminals, although no synaptic specialization could be identified in such cases (Fig. 8). Finally, a few BDA-labeled myelinated fibers were also observed in the nucleus accumbens.

DISCUSSION

Our results indicate that in the lizard *Psammodromus* the nucleus accumbens show two different chemoarchitectonic regions on the basis of their distinctive staining patterns for SP, DA, $GABA_A$ receptor, and CaBP. These two regions also show different patterns of connections with respect to the input from the cerebral cortex, suggesting that they represent functionally different fields. Our results indicate that cortical projections to the nucleus accumbens end mainly on distal dendrites and the head of spines of spiny, putatively projection neurons and that cortical terminals make asymmetric, presumably excitatory, contacts with them. As discussed below, these results indicate that corticoaccumbal projections are very similar in reptiles and mammals in that they have a direct excitatory influence on projection neurons of the nucleus accumbens, although some important differences exist between reptiles and mammals regarding other local modulatory contacts of cortical terminals.

Chemoarchitecture of the nucleus accumbens in reptiles

Our results indicate that the nucleus accumbens of *Psammodromus* shows immunohistochemical features that are similar to those of the nucleus accumbens of other reptiles (Reiner et al., 1984; Smeets et al., 1986, 1987; Reiner, 1987; Reiner and Oliver, 1987; Russchen et al., 1987; Smeets, 1988; Bennis et al., 1991, 1994; Medina et al., 1992; Henselmans and Wouterlood, 1994). In reptiles, the nucleus accumbens (as well as the dorsal striatum) contains numerous GABAergic neurons as well as SP+ and ENK+ neurons (present results in *Psammodromus*; Reiner et al., 1984; Russchen et al., 1987; Reiner, 1987; Bennis et al., 1991, 1994). Studies in turtles have indicated that in the dorsal striatum the vast majority of GABAergic neurons represent two separate populations of spiny projection neurons: neurons co-containing GABA and SP, and neurons co-containing GABA and ENK (Reiner, 1987; Anderson and Reiner, 1990; Reiner and Anderson, 1990). Projection neurons of the nucleus accumbens of reptiles are also thought to be spiny GABAergic neurons co-containing ENK, SP, or both, although more double-label studies are needed to establish the degree of colocalization of these substances in this nucleus (Reiner et al., 1984; Reiner, 1987; Anderson and Reiner, 1990; Reiner and Anderson, 1990). Our observations of Golgi-impregnated cells in the nucleus accumbens of *Psammodromus* agree with the presence of many spiny neurons, which probably represent GABAergic projection neurons. In addition, the nucleus accumbens of *Psammodromus* contains a large number of CaBP+ cells, whose number and distribution closely resemble those of GABA+ cells, suggesting that they may mostly represent the same cell population.

The nucleus accumbens of reptiles is also characterized by its high AChE+ activity (Russchen et al., 1987), and a number of studies have shown that this is related to the presence in the nucleus accumbens of a few, dispersed cholinergic neurons and their processes that represent intrinsic aspiny neurons (Hoogland and Vermeulen-VanderZee, 1990; Medina et al., 1993; Powers and Reiner, 1993; Henselmans and Wouterlood, 1994). Considering that cholinergic interneurons are present in the nucleus accumbens of different species of lizards and in turtles, it is likely that this interneuron type also exists in the nucleus accumbens of *Psammodromus*. Finally, dispersed NPY+ or SS+ intrinsic aspiny neurons and their processes are present in the nucleus accumbens of reptiles (present results in *Psammodromus*; Reiner and Oliver, 1987; Medina et al., 1992). Studies in turtles have shown that NPY and SS are colocalized in the same cells in the striatum and nucleus accumbens (Reiner and Oliver, 1987), and this is probably true also in other reptiles. In conclusion, the nucleus accumbens seems to possess the same basic projection neurons and the same basic interneurons in all or most reptiles studied thus far, with the projection neurons being spiny GABAergic cells co-containing either SP, ENK, or both, and the interneurons being aspiny cells containing either NPY/SS or acetylcholine.

In addition, the nucleus accumbens in all reptiles studied thus far contains rich plexuses of SP+ or ENK+ fibers and terminals that represent collaterals of the projection neurons (present results in *Psammodromus*; references above). The nucleus accumbens in reptiles is also densely innervated by TH+ or DA+ fibers and terminals (present results in *Psammodromus*; Smeets et al., 1986, 1987; Smeets, 1988) and moderately innervated by NA+ fibers (Smeets and Steinbusch, 1989; Smeets, 1994). Double-labeling studies in a snake indicate that most TH+ fibers arise in the dopaminergic tegmental field A8–A10, whereas a part of them represent noradrenergic fibers arising in the locus coeruleus (Pérez-Santana et al., 1997).

A remarkable finding of the present study is the observation that the nucleus accumbens can be divided into two separate chemoarchitectonic fields: a rostromedial field

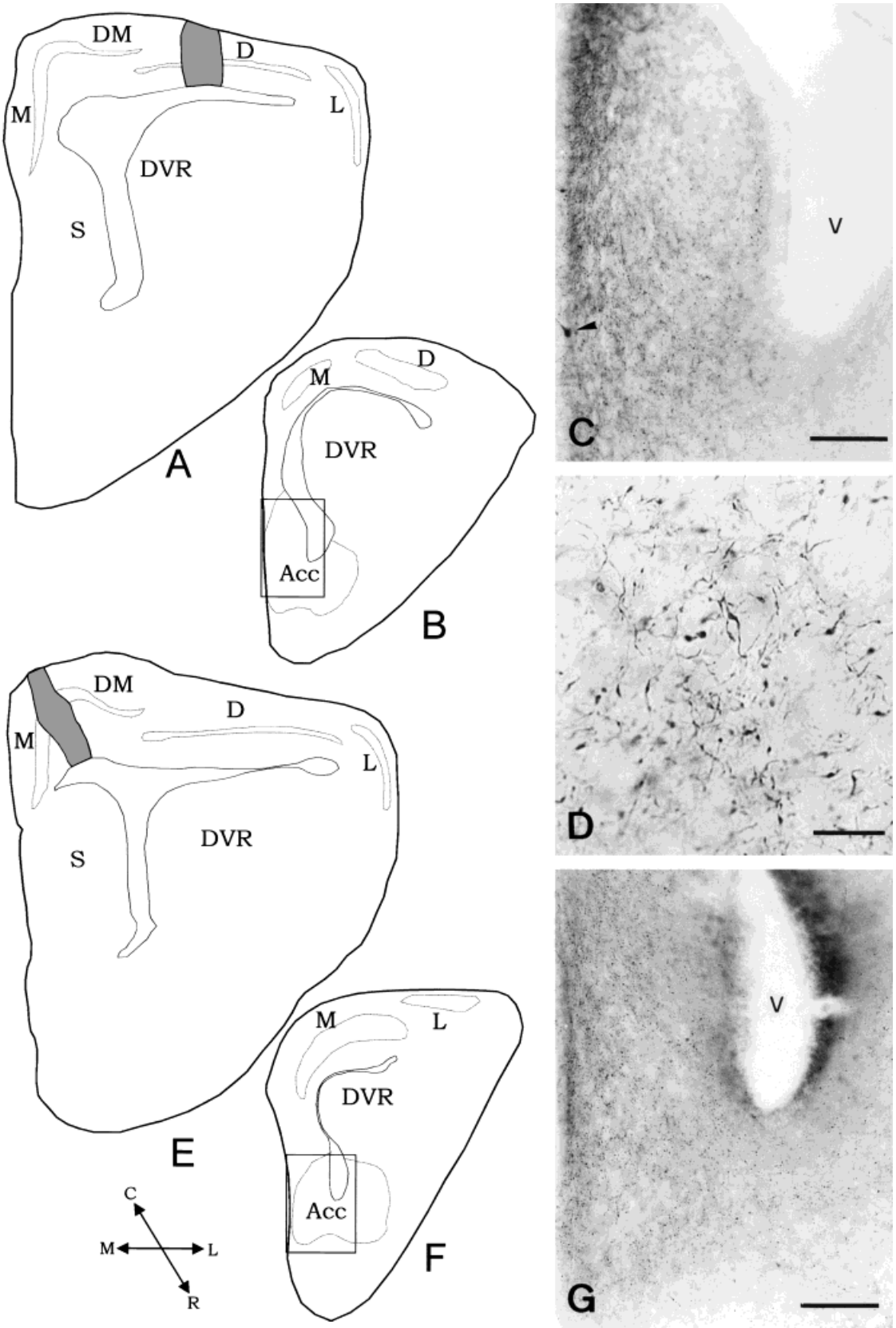


Figure 6

that is heavily stained for SP, DA, $GABA_A$ receptor, and CaBP; and a caudolateral field that stains only lightly to moderately for these markers and appears to be similar to the adjacent striatum. Previous studies in reptiles have also found that the nucleus accumbens shows heterogeneous staining patterns for AChE and NADPH-diaphorase as well as for ENK, SP, DA, and zinc (Russchen et al., 1987; Pérez-Clausell and Fredens, 1988; Smeets et al., 1997). However, no attempt was made to further describe these patterns or to correlate them to the accumbal connectivity. As discussed further below, our results provide evidence that the two separate chemoarchitectonic fields of the nucleus accumbens show also a difference with respect to their cortical input (present results) as well as their efferent connections (Smeets and Medina, 1995).

Corticoaccumbal projection in reptiles

Our results have indicated that in the lizard *Psammodromus* the dorsal cortex projects to the nucleus accumbens, thus corroborating previous results in other reptiles (Hoogland and Vermeulen-VanderZee, 1989; González et al., 1990; Pérez-Santana et al., 1997). In the *Gekko*, evidence has been provided for the existence of a topographic organization in the projection from the dorsal cortex to the nucleus accumbens (González et al., 1990). Thus, a rostral-to-caudal gradient in the dorsal cortex corresponds to a ventromedial-to-dorsolateral gradient in the nucleus accumbens. Our injections in the lacertid lizard *Psammodromus* did not allow us to find a topographic organization in the dorsal cortical input to the accumbens, because they were performed at a single rostrocaudal level, although at different mediolateral portions of the cortex. Our results indicate that both the intermediate and lateral parts of the dorsal cortex project heavily to the rostral and medial portions of the accumbens and moderately to the lateral part of the nucleus. This finding also seems to be true for the lizard *Gekko* (see Fig. 8 in González et al., 1990), suggesting that this pattern may be a general feature in lizards.

Our results indicate that the medial cortex also projects to the nucleus accumbens. This projection ends mainly in the rostral and medial part of the nucleus, whereas the lateral and caudal part is only lightly innervated. Previous studies using an anterograde tracer in *Gekko* have not described a similar projection (Hoogland and Vermeulen-VanderZee, 1993). Studies in snakes reported retrogradely labeled neurons in the medial cortex after injections in the nucleus accumbens, but they were considered to be likely due to fibers of passage (Pérez-Santana et al., 1997). Although we cannot completely rule out the possibility that our labeling in the accumbens after injections in the medial cortex of *Psammodromus* was due to uptake of the tracer by descending dorsal cortical fibers, this possibility seems very unlikely because deep injections in the dorsomedial cortex produced no anterograde labeling in the accumbens.

As noted above, there is a correlation between the two chemoarchitectonic fields of the nucleus and the density of the cortical input, suggesting that these two fields may be functionally different. Studies on the efferent projections from the nucleus accumbens in *Gekko* have also found differences in the projections from the medial or lateral parts of the nucleus (Smeets and Medina, 1995) (see further discussion in comparison with mammals).

Targets of cortical terminals and intrinsic circuits in the nucleus accumbens of reptiles

Electron microscopic (EM) observations in *Psammodromus* indicated that cortical terminals contacted mainly distal dendrites and dendritic spines of accumbal neurons. In addition, our EM results showed that, when a synaptic specialization was observed, cortical terminals were seen to make asymmetric, presumably excitatory synapses. This finding suggests that cortical fibers provide a direct excitatory input to spiny projection neurons of the nucleus accumbens in *Psammodromus*. Considering that the chemoarchitecture, neuron populations, and connections of the nucleus accumbens seem very similar among reptiles (see discussion above), this finding in *Psammodromus* may also be true for most reptiles. Our results also provide evidence that cortical terminals do not seem to make synaptic contacts with other terminals or with perikarya, although they are sometimes observed in apposition with such structures. In this respect, a previous study in the lizard *Gekko* reported that in the nucleus accumbens cholinergic neurons were rarely contacted by terminals making asymmetric contacts and such rare asymmetric contacts occurred with perikarya or proximal dendrites of cholinergic neurons (Henselmans and Wouterlood, 1994). Thus, ultrastructural observations indicate that in the nucleus accumbens of reptiles cholinergic interneurons are not contacted by cortical terminals. However, in the nucleus accumbens of *Gekko*, glutamate was observed to have a weak, although significant, effect on the release of acetylcholine (Henselmans et al., 1991). Although we cannot rule out the possibility that cholinergic interneurons in the nucleus accumbens of reptiles may receive a small direct input from the cortex until double labeling studies are performed, it is also possible that the effect of glutamate on acetylcholine release is indirect. Previous studies in *Gekko* also indicated that DA+ terminals make no synaptic contacts with any part of cholinergic interneurons of the nucleus accumbens, although both cholinergic terminals and DA+ terminals do often converge on the same spiny projection neuron, and form primarily symmetrical synapses (Henselmans and Wouterlood, 1994). Our results in *Psammodromus* have also indicated that cortical terminals do sometimes converge on the same

Fig. 6. Cortical afferent fibers to the nucleus accumbens. A–D: Biotinylated dextran amine (BDA) injection in the dorsal cortex. **A:** Camera lucida drawing indicating the injection site. The shadowed area corresponds to the highest density of the tracer. M, DM, D, and L: medial, dorsomedial, dorsal, and lateral cortices, respectively. DVR, dorsal ventricular ridge; S, septum. **B:** Drawing of a frontal, rostral telencephalic section at the level of the nucleus accumbens (Acc); areas with BDA labeling are delimited by dotted lines. **C:** Photomicrograph of the nucleus accumbens showing BDA labeling of dorsal cortical origin in an area similar to that area in the square in B. Arrowhead indicates a retrogradely labeled neuron. V, ventricle. **D:** High magnification of afferent cortical fibers and varicosities in the nucleus accumbens. E–G: BDA injection in the medial cortex. **E:** Camera lucida drawing indicating the injection site. The shadowed area corresponds to the highest density of the tracer. **F:** Drawing of a frontal, rostral telencephalic section at the level of the nucleus accumbens (Acc); areas with BDA labeling are delimited by dotted lines. **G:** Photomicrograph of the nucleus accumbens showing BDA labeling of medial cortical origin in an area similar to that area in the square in F. V, ventricle. Crossed arrows at bottom-left corner indicate caudorostral (C-R) and mediolateral (M-L) directions for each pair of drawn sections (A–B and E–F). Scale bars = 75 μm in C, 15 μm in D, 75 μm in G.

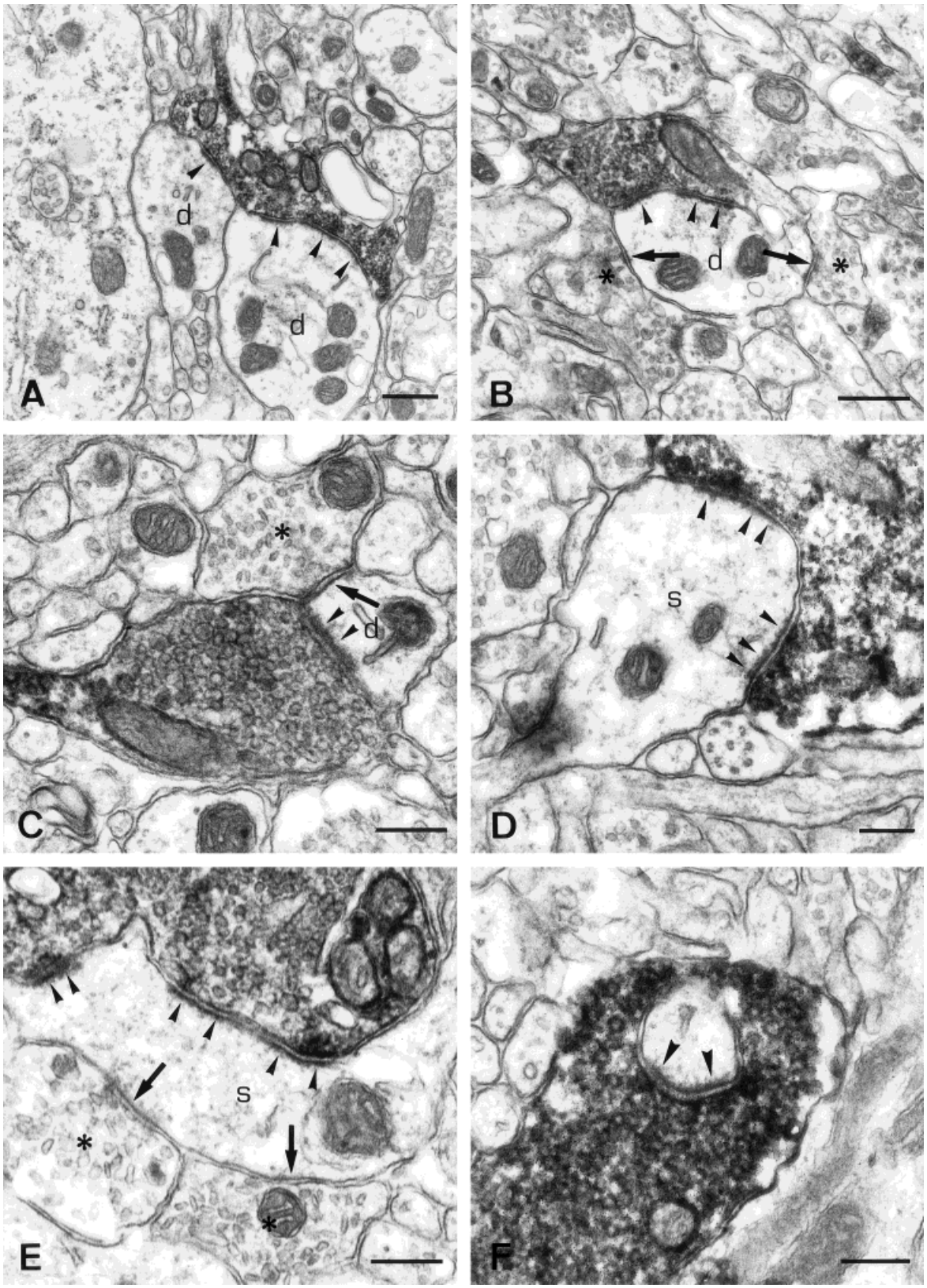
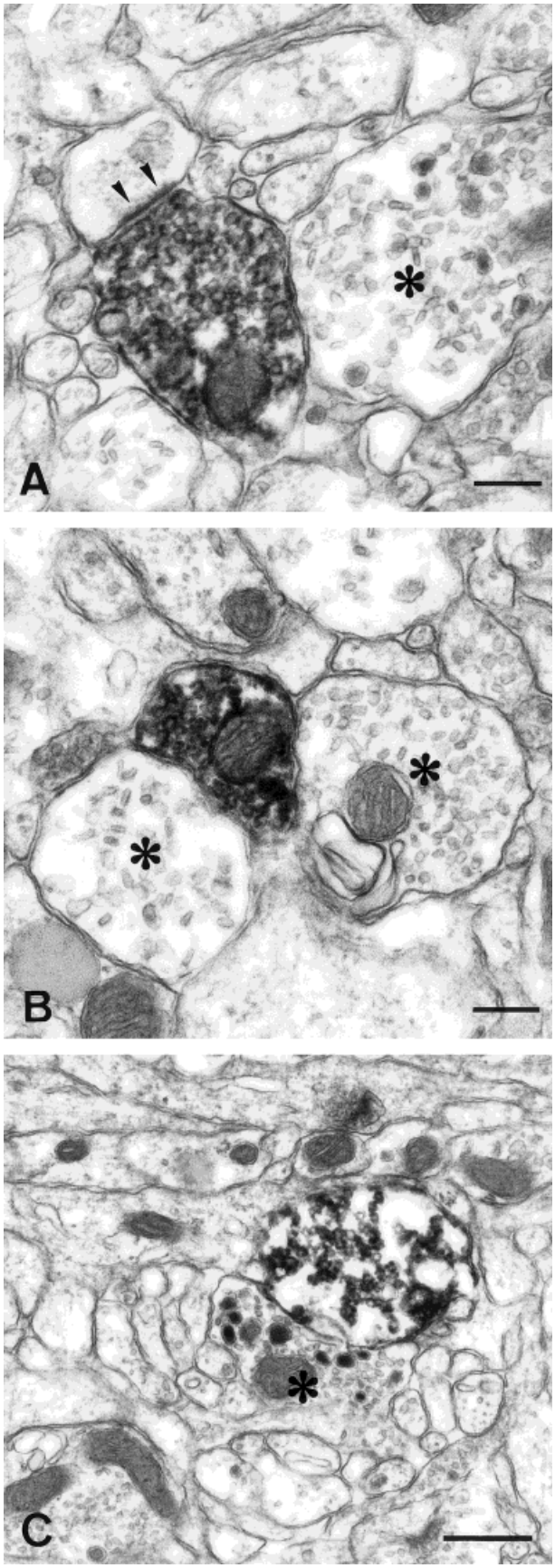


Fig. 7. Electron photomicrographs of biotinylated dextran amine (BDA)–labeled boutons of cortical origin in the nucleus accumbens. **A:** A large labeled bouton makes asymmetrical synapses (arrowheads) with two adjacent dendritic profiles (d). **B:** A BDA-positive bouton makes an asymmetrical synapse (arrowheads) with a dendrite (d), on which two other nonlabeled boutons (asterisks) make symmetrical synapses (arrows). **C:** A small dendritic profile (d) receives convergent synapses of two apposed boutons: one is a BDA-labeled terminal that makes an asymmetrical contact (arrowheads) with it, whereas the other is a nonlabeled terminal (asterisk) with flattened vesicles, and makes a symmetrical contact (arrow) with the dendrite. No synaptic specialization is evident between the apposed terminals. **D:** Asymmetrical synapse (arrowheads) onto the head of a dendritic spine (s) made by a BDA-labeled bouton. **E:** A dendritic spine (s) is contacted by a labeled bouton and two other unlabeled terminals (asterisks). Asymmetrical (arrowheads) as well as symmetrical (arrows) synaptic specializations can be observed between the three terminals and the dendrite. **F:** A labeled bouton, plenty of rounded synaptic vesicles, enwrap a small dendritic profile or perhaps the head of a spine, making a prominent asymmetrical contact (arrowheads). Scale bars = 500 nm in A,B, 250 nm in C–F.



dendrite with one or two nonlabeled terminals making symmetrical synapses. This finding together with the results in *Gekko* suggest that cortical terminals may sometimes converge on the same accumbal neurons with cholinergic terminals, dopaminergic terminals, or both.

All of these results together indicate that in the nucleus accumbens of reptiles the major extrastriatal inputs (from the cortex or the A8–A10 DA+ cell field) and the axons of the cholinergic interneurons seem to exert a direct influence on the spiny projection neurons. These results also indicate that in the nucleus accumbens of reptiles cholinergic interneurons receive little or no input from cortical or DA+ terminals, and only extremely few axoaxonic contacts are present between cortical, DA+, and/or cholinergic terminals, suggesting that only a minor local modulation of their input to the spiny projection neurons takes place.

Comparison of the chemoarchitecture of the nucleus accumbens between mammals and reptiles

The nucleus accumbens of reptiles appears to be very similar to that of mammals in terms of location, cell populations, as well as connections (Russchen et al., 1987; Smeets and Medina, 1995; Medina and Reiner, 1995; Pérez-Santana et al., 1997). In both mammals and reptiles, the nucleus accumbens is located ventromedially to the dorsal striatum (also called striatum proper in reptiles and neostriatum in mammals) and is rich in AChE+ activity as well as in fibers and terminals SP+, ENK+, and DA+ (Graybiel et al., 1981; Reiner et al., 1984; Voorn et al., 1986, 1989, 1994; Reiner, 1987; Russchen et al., 1987; Zahm and Brog, 1992; Jongen-Rêlo et al., 1993). In addition, the nucleus accumbens of mammals and reptiles contains spiny projection neurons containing GABA, ENK and/or SP, and aspiny interneurons containing either acetylcholine or NPY and SS (Reiner et al., 1984; Reiner, 1987; Russchen et al., 1987; Sugimoto and Mizuno, 1987; Meredith et al., 1989; Hoogland and Vermeulen-Vander-Zee, 1990; Reiner and Anderson, 1990; Medina et al., 1992, 1993; Kalivas et al., 1993; Powers and Reiner, 1993). Finally, in both reptiles and mammals, the nucleus accumbens projects to the ventral pallidum, the bed nucleus of the stria terminalis, the preoptic region and lateral hypothalamus, the A8–A10 dopaminergic cell field, parabrachial region and raphe nuclei, and receives input from the limbic/perilimbic cortex, the amygdala, the ventral pallidum, the dorsomedial thalamus, the A8–A10 DA+ cell field, and the locus coeruleus (Kelley and Domesick, 1982; Groenewegen et al., 1987; Berendse et al., 1988, 1992a,b; McGeorge and Faull, 1989; Berendse and Groenewegen, 1990; González et al., 1990; Haber et al., 1990; Heimer et al., 1991; Spooren et al., 1991; Martínez-García et al.,

Fig. 8. Electron photomicrographs of biotinylated dextran amine–labeled boutons of cortical origin in the nucleus accumbens. **A:** Labeled bouton making an asymmetrical synapsis (arrowheads) with a small spinous-like profile is in close apposition with an unlabeled bouton (asterisk) showing numerous flattened synaptic vesicles and some dense-cored ones. Synaptic specializations are not observed between both boutons. **B:** Labeled bouton in close apposition with two unlabeled boutons (asterisks). **C:** A nonlabeled bouton with some large dense-cored vesicles, likely peptidergic (asterisk), is in apposition to a labeled bouton. No synaptic specializations are evident between the apposed terminals in B and C. Scale bars = 250 nm in A–C.

1993; Zahm and Heimer, 1993; Kunishio and Haber, 1994; Lynd-Balta and Haber, 1994; Bruce and Neary, 1995; Giménez-Amaya et al., 1995; Smeets and Medina, 1995; Pérez-Santana et al., 1997).

In mammals, many studies have shown that the nucleus accumbens is nonhomogeneous with respect to its cyto- and chemoarchitecture, its connections as well as its function, and on this basis the nucleus accumbens of mammals can be divided into a core and a shell (Herkenham et al., 1984; Zàborsky et al., 1985; Zahm and Heimer, 1988; Berendse et al., 1988, 1992a,b; Groenewegen et al., 1989; Meredith et al., 1989, 1996; Henselmans and Stoof, 1991; Deutch and Cameron, 1992; Pennartz et al., 1992; Zahm and Brog, 1992; Jongen-Rêlo et al., 1993, 1994; Rogard et al., 1993; Voorn et al., 1994). The core is the dorsal and laterally located region in the nucleus and seems more similar to the dorsal striatum in its sensorimotor-related connections and functions, whereas the shell is located ventral and medial in the nucleus, peripheral to the core, and by its connections seems more related to the limbic system and extended amygdala (Berendse et al., 1988, 1992a,b; Zahm and Brog, 1992). In both rats and primates, the division between the shell and the core can best be visualized by using CaBP as a marker (Jongen-Rêlo et al., 1994; Meredith et al., 1996). By using this marker, the core is characterized by being generally CaBP-rich, whereas the shell stains only low to moderately for CaBP (Jongen-Rêlo et al., 1994; Meredith et al., 1996). Both shell and core regions are present along all the rostrocaudal extent of the nucleus accumbens, with the shell being largest and the core being smallest at the rostral pole of the nucleus (Jongen-Rêlo et al., 1994). In rats, the core and the shell can be further subdivided into ENK-rich and ENK-poor compartments that present distinctive connections with the cortex, the medial thalamus, and the A8–A10 dopaminergic cell field (Berendse et al., 1988, 1992a,b; Berendse and Groenewegen, 1990; Zahm and Brog, 1992; Jongen-Rêlo et al., 1993).

As noted above, the nucleus accumbens of reptiles can also be divided into rostromedial and caudolateral parts, which in some respects can be comparable to the shell and core, respectively, of mammals. For example, the rostromedial part of the nucleus accumbens of reptiles projects to the bed nucleus of the stria terminalis, the preoptic region and lateral hypothalamus, the A8–A10 dopaminergic cell field, and the raphe nuclei, resembling in part the pattern of projections of the shell of mammals (Zahm and Heimer, 1993; Smeets and Medina, 1995). On the other hand, the caudolateral part of the reptilian nucleus accumbens is connected mainly with more caudal parts of the tegmental dopaminergic cell field (A8–A9), and is connected neither with the bed nucleus of the stria terminalis nor with the raphe nuclei, partly resembling the projections of the core (Zahm and Heimer, 1993; Smeets and Medina, 1995). In addition, the two divisions of the reptilian nucleus accumbens can be distinguished by their different CaBP staining pattern, also resembling the shell and core of mammals. However, unlike the shell, the rostromedial division of the reptilian accumbens is rich in CaBP. On the other hand, unlike the core, the caudolateral division of the reptilian accumbens shows only a moderate CaBP staining. Therefore, regarding their CaBP staining pattern, the rostromedial and caudolateral divisions of the nucleus accumbens of reptiles do not appear comparable to the shell and core, respectively, of the mammalian nucleus accumbens. As discussed below in further detail, the nucleus accumbens of mammals and reptiles also show some differences with respect to the input from the cortex (see below).

Comparison of the corticoaccumbal projection and the intrinsic circuit in the nucleus accumbens between mammals and reptiles

As noted above, the specific pattern of connections of the shell and the core indicates that these two regions of the accumbens are related either to the limbic and extended-amygdala system or to the neostriatum, respectively (Zahm and Brog, 1992; Zahm and Heimer, 1993). With respect to the input from the cortex, in rats and primates only the shell receives input from the hippocampus (Russchen et al., 1985; Groenewegen et al., 1987, 1991; Brog et al., 1993), whereas the prefrontal cortex projects to both shell and core (Berendse et al., 1992b; Haber et al., 1995). In *Psammodromus*, the whole nucleus accumbens receives a limbic cortical input, although the rostromedial part of the nucleus is more heavily innervated.

Ultrastructural studies of the input from the hippocampus to the nucleus accumbens in rats have shown that, similarly to our finding in the lizard *Psammodromus*, cortical terminals form mainly asymmetric synapses on distal dendrites and spines of accumbal neurons (Sesack and Pickel, 1990). In the rat, hippocampal terminals synapse on dendrites receiving convergent input from TH+ terminals (Sesack and Pickel, 1990). As noted above, this may be true also in lizards because in *Psammodromus*, we sometimes observed cortical terminals converging on the same dendrite with non-labeled terminals making symmetrical synapses that in part could be DA+.

Axoaxonic appositions, although without apparent synaptic specialization, were sometimes observed between hippocampal and TH+ terminals in the rat nucleus accumbens (Sesack and Pickel, 1990). This type of nonsynaptic axoaxonic apposition has been suggested to play an important role on the dopamine-mediated modulation of the cortical input to cholinergic and/or to projection neurons in both the striatum and the nucleus accumbens in mammals (Di Chiara and Morelli, 1993; Wu et al., 1993; Di Chiara et al., 1994; Kalivas and Duffy, 1997). This modulatory effect appears mediated by dopamine receptor subtype D2 (Di Chiara and Morelli, 1993; Wu et al., 1993; Di Chiara et al., 1994; Kalivas and Duffy, 1997). However, the nucleus accumbens responds differently to dopamine depletion or to dopamine receptor subtype D2, depending on what part of the nucleus is affected, i.e., the core or the shell (Henselmans and Stoof, 1991; Meredith et al., 1995). In this respect, the core of the nucleus accumbens also seems more similar to the neostriatum than the shell (Henselmans and Stoof, 1991). In the whole neostriatum and in the accumbens core, specific agonists of D2 dopamine receptors produce 70% or 50% inhibition, respectively, on the acetylcholine release, whereas no effect is observed in the shell (Henselmans and Stoof, 1991). As noted above, in reptiles the cholinergic interneurons of the nucleus accumbens and dorsal striatum do not appear to receive any input from DA+ terminals (Henselmans and Wouterlood, 1994), and they seem to receive little or no input from the cortex. As in the shell of rat accumbens, in the striatum and nucleus accumbens of reptiles, agonists of D2 dopamine receptors do not produce any effect on acetylcholine release (Henselmans et al., 1991). This finding may be

related to the general lack of D2-mediated modulatory intrinsic circuits and/or the lack of axoaxonic interactions between dopaminergic, cortical, and cholinergic terminals or neurons in both the accumbens/striatum of reptiles and the accumbal shell of mammals. More ultrastructural studies on the synaptic contacts in the different regions of the accumbens, and studies on the cellular distribution of dopamine receptor subtypes in mammals and reptiles may help to resolve the differences observed in pharmacologic studies.

CONCLUSION

The nucleus accumbens in mammals and lacertid lizards appear to be highly similar in terms of location, cell populations, and general connections. However, the nucleus accumbens of mammals shows a clear division into a core and a shell, which are chemically different and possess distinctive patterns of connections that make the core more similar to the neostriatum and the shell more related to the extended amygdala and limbic system. The nucleus accumbens of reptiles is also chemically heterogeneous and shows slightly different connections between its rostromedial or caudolateral parts, which partly resemble the shell and core of the mammalian accumbens, respectively. With respect to the cortical input to the nucleus accumbens, this is very similar between mammals and reptiles in that cortical (hippocampal) terminals contact mainly spines and distal dendrites of putative projection neurons, forming primarily asymmetric, excitatory synapses. However, some important differences seem to exist between the accumbens in mammals and reptiles regarding the existence of axoaxonic contacts and in the interaction between dopamine, glutamate, and acetylcholine, which suggest that the lacertilian nucleus accumbens is, as a whole, similar to the accumbal shell of mammals.

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