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The Dopaminergic Innervation of the Ventral Striatum in the Rat: A Light- and Electron-Microscopical Study With Antibodies Against Dopamine

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ABSTRACT

Dopamine (DA) in the dorsal and ventral striatum is associated with different aspects of locomotor activity control. The ventral striatum may form an interface between the limbic system and the extrapyramidal motor system. The distribution of dopaminergic fibers in this interface position was studied in detail with a method applying antibodies against DA. Furthermore, the ultrastructural morphology of the DA fibers was examined by means of immuno-electron microscopy. The results show that DA immunoreactivity is distributed over the ventral striatum in a highly compartmentalized fashion. In the dorsal striatum few compartments were found. The DA fibers in the ventral striatum establish mainly symmetric synaptic contacts, preferably with dendritic shafts and spines. The results are discussed in relation to previous data concerning the light and electron-microscopic identification of catecholaminergic fibers in the ventral and dorsal striatum.

Key words: dopamine-compartments, nucleus accumbens, olfactory tubercle, immuno-electron microscopy

The dopaminergic neurotransmission in the striatum plays an essential role in the control of extrapyramidal motor functions. Dopamine (DA) is released by fibers originating from the dopaminergic cell groups in the substantia nigra and ventral tegmental area, which reach the striatum through the mesostriatal pathways (see Björklund and Lindvall, '84, for review). Injections of DA or DA agonists in the caudate-putamen or the nucleus accumbens produce stereotypy or locomotor hyperactivity, respectively (Pijnenburg and van Rossum, '73; Jackson et al., '75; Costall and Naylor, '75; Costall et al., '77). The same differentiation between these two aspects of DA-stimulated behavior in the striatum can be noted when the DA innervation of the caudate-putamen or the nucleus accumbens is selectively lesioned by injections of 6-hydroxydopamine, and the animals are subsequently challenged with systemically applied DA agonists (Kelly et al., '75). The involvement of the nucleus accumbens in locomotor activity control is also suggested on the basis of experiments on turning behavior. This behavior can be induced by administering DA agonists to animals with unilateral lesions of the nigrostriatal DA system (for review see Pycock, '80). But besides this striatal DA imbalance, a functional DA neurotransmission in the nucleus accumbens seems to be required to provide a "locomotor component" for execution of the circling activity (Kelly and Moore, '77; Pycock and Marsden, '78). Control over locomotor activity can be exerted by the efferent projections from the nucleus accumbens to the ventral pallidum, the substantia nigra pars compacta, and directly or indirectly via the ventral pallidum to the mesencephalic locomotor region (Swanson and Cowan, '75; Nauta et al., '78; Mogenson et al., '83, '85; Moon Edley and Graybiel, '83; Swanson et al., '83; Groenewegen and Russchen, '84; Brudzyński and Mogenson, '85).

The nucleus accumbens, together with the parvicellular core of the olfactory tubercle, constitutes the ventral striatum, which is characterized by its limbic input from the amygdala and allocortical regions (Heimer and Wilson, '75; Krayniak et al., '81; Groenewegen et al., '80, '81; Sørensen

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and Witter,'83;Kelleyand Domesick,'82).Stimulation of the hippocampus evokes excitatory responses in the nucleus accumbens, which can be modulated by DA (Groenewegen et al., '81; Lopes da Silva et al., '84; Yang and Mogenson, '84). Behavioral studies indicate that after hippocampal lesions enhancement of locomotor activity may occur, which is associated with dopaminergic neurotransmission in the nucleus accumbens (Isaacson, '84). On the basis of these findings it has been suggested that the nucleus accumbens functions as an "interface" between the limbic system and the extrapyramidal motor system (Mogenson and Yim, '81; Heimer et al., '82). In view of the importance of the dopaminergic neurotransmission at this "interface" position, it was considered necessary to gain insight into the anatomical distribution of the DA fibers at the light- and electron-microscopic level. Although many authors have reported on the distributional patterns of dopaminergic fibers or mesencephalic afferents to the telencephalon (see Björklund and Lindvall, '84, for review), there is little detailed information on the dopaminergic innervation of the ventral striatum (Hökfelt et al., '77; Lindvall and Stenevi, '78; Fallon and Moore, '78; Moore, '78; Fallon et al., '83). Likewise, there is only little knowledge of the ultrastructural features of the dopaminergic nerve endings in the ventral striatum (Arluison et al., '84; Bouyer et al., '84a). Therefore, we sought to determine the distribution of DA-containing fibers in the ventral striatum and related areas and to establish the ultrastructural characteristics of these very fibers. Direct labeling of DA was achieved by employing recently developed specific antibodies against DA (Geffard et al., '84).

MATERIALS AND METHODS

Fourteen male, adult Wistar rats were anesthetized with Nembutal (0.1 ml/100 g) and perfused transcardially with Ringer, followed by 750 ml of a solution of 5% glutaraldehyde buffered with 0.01 M sodium-cacodylate + 1% $Na_2S_2O_5$; pH ranges 4–7. Subsequently, 4-mm transverse slices of the rostral part of the brain, including the nucleus accumbens, were taken for immersion fixation in the same fixative during 30–60 minutes. Sections (50 μm) were cut on a Vibratome (Oxford) and incubated overnight in antisera against DA (A1 or Jannes) diluted 1:3,000 or 1:6,000. Further immunostaining was according to the PAP technique or with horseradish peroxidase (Boehringer, Mannheim) conjugated with antirabbit IgG (raised in swine, IgG 7S fraction, Nordic, The Netherlands), kindly provided by Dr. D.M. Boorsma (Boorsma and Van der Raay-Helmers, '85). Production and specificity of the dopamine antisera have been described elsewhere (Geffard et al., '84; Buijs et al., '84). Sectioning and incubation in the first antiserum was in Tris-buffered saline (pH=7.1–7.6) + 1% $Na_2S_2O_5$ and 0.5% Triton X-100. In all other incubations the reductor was omitted. For immuno-electron microscopy no detergent was used. After DAB incubation sections were postfixed for 30 minutes in 1% OsO_4 buffered with 0.1 M sodium cacodylate, dehydrated, and flat embedded in Epon. Regions from the nucleus accumbens and olfactory tubercle were excised, mounted on top of an Epon block, and sectioned for electron microscopy. Ultrathin sections were lightly contrasted with Reynold's lead citrate and examined in a Philips EM 301.

The results are presented with reference to the atlas of König and Klippel ('63).

RESULTS

Light microscopy

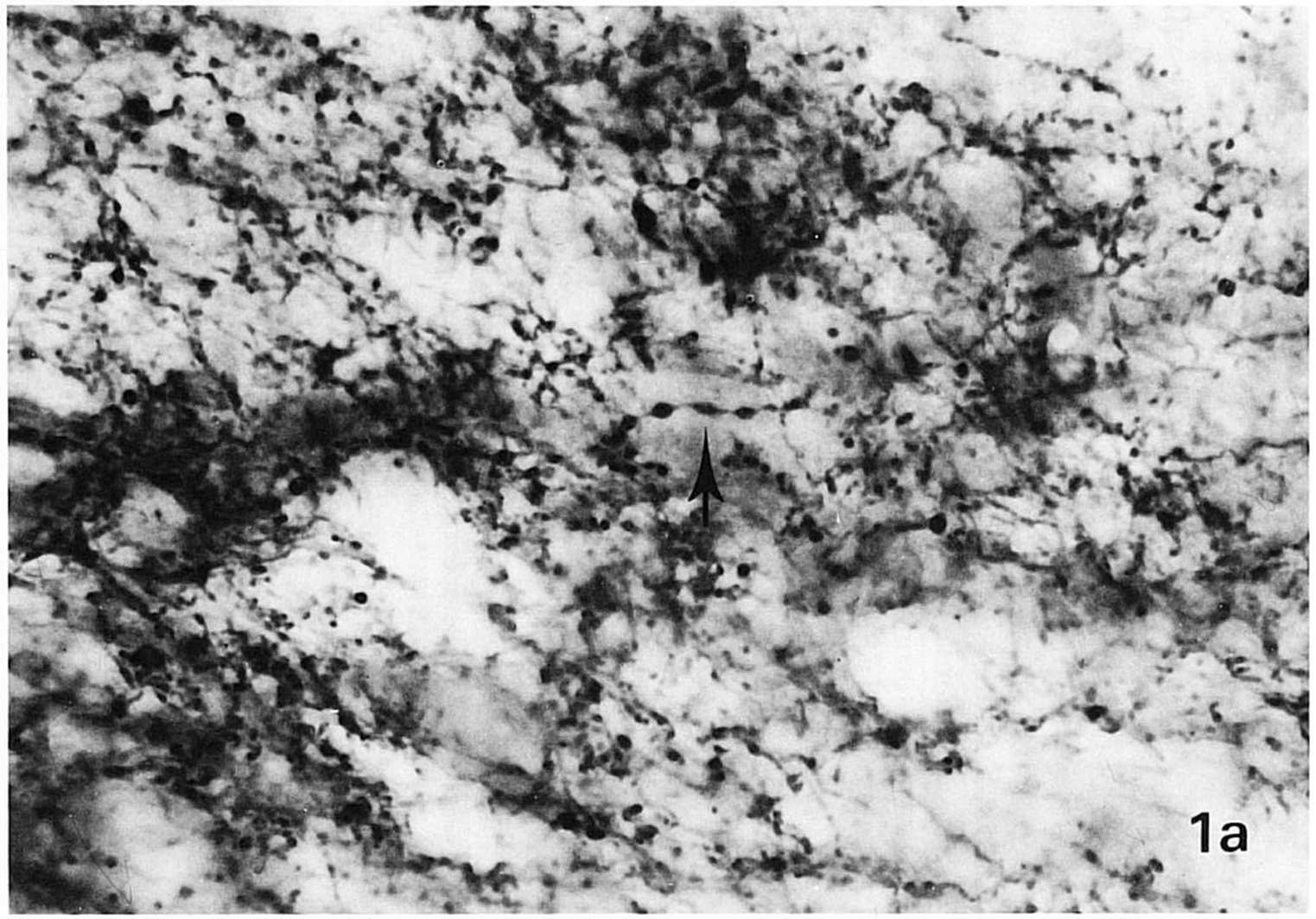
Ventral and dorsal striatal regions are characterized by the presence of a dense dopamine (DA) plexus consisting of delicate, thin fibers with many varicosities. Comparison of the morphology of these fibers in the two regions shows that the fibers in the ventral striatum (Fig. 1a) display more and larger varicosities than those in the dorsal striatum (Fig. 1b). This difference is most conspicuous in the patches of high DA immunoreactivity (see below). DA projection fibers coursing along the rostroventral aspect of the nucleus accumbens are composed of varicose and nonvaricose axons that can be seen entering the striatum or proceeding to prefrontal cortical regions (Fig. 2). In contiguous regions, such as the anterior olfactory nucleus, prepiriform cortex, and prefrontal cortex, DA fibers are elongate with large, regularly spaced varicosities and few ramifications (Fig. 3).

The most rostral region that evinces the characteristic dense striatal DA plexus is the olfactory tubercle, as shown in Figures 4–6. The DA innervation is present over its entire rostrocaudal extent and covers all three layers. DA immunoreactivity (DA-IR) is strongest, however, in the plexiform layer (Figs. 5, 6). Between the olfactory tubercle and the equally dense DA-innervated anterior part of the nucleus accumbens, the posterior part of the anterior olfactory nucleus is situated, where a more loosely arranged network of DA fibers is found (Figs. 3–5). This network is intermingled with beaded fibers running in a dorsomedial direction toward the pregenual part of the prefrontal cortex. Apparently, these fibers arise from the medial forebrain bundle and course along the ventral and dorsal aspects of the nucleus accumbens and the anterior commissure (Fig. 5).

Two structures are marked off from the ventral striatum by a different pattern of DA innervation: the ventral pallidum and the islands of Calleja. The rostral extension of the ventral pallidum, as defined by Heimer and Wilson ('75) and Haber and Nauta ('83), is relatively sparsely innervated by DA fibers (Fig. 2). In the islands of Calleja a moderately dense DA innervation is found in the granule cell compartment. This pattern continues into the so-called cores of the islands of Calleja, which, according to Fallon and co-workers ('83), connect to the striatal cell bridges or

Abbreviations

AC	anterior commissure
AON	anterior olfactory nucleus
BST	bed nucleus of the stria terminalis
c	core of the islands of Calleja
CI	capsula interna
DA	dopamine
DA-IR	dopamine immunoreactivity
g	granule cell compartment of the islands of Calleja
IC	islands of Calleja
LV	lateral ventricle
LS	lateral septum
NA	nucleus accumbens
OT	olfactory tubercle
P	pallidum, composing ventral pallidum and globus pallidus
pl	plexiform layer of olfactory tubercle
po	polymorph cell layer of olfactory tubercle
py	pyramidal cell layer of olfactory tubercle
r	outer rim of islands of Calleja
VP	ventral pallidum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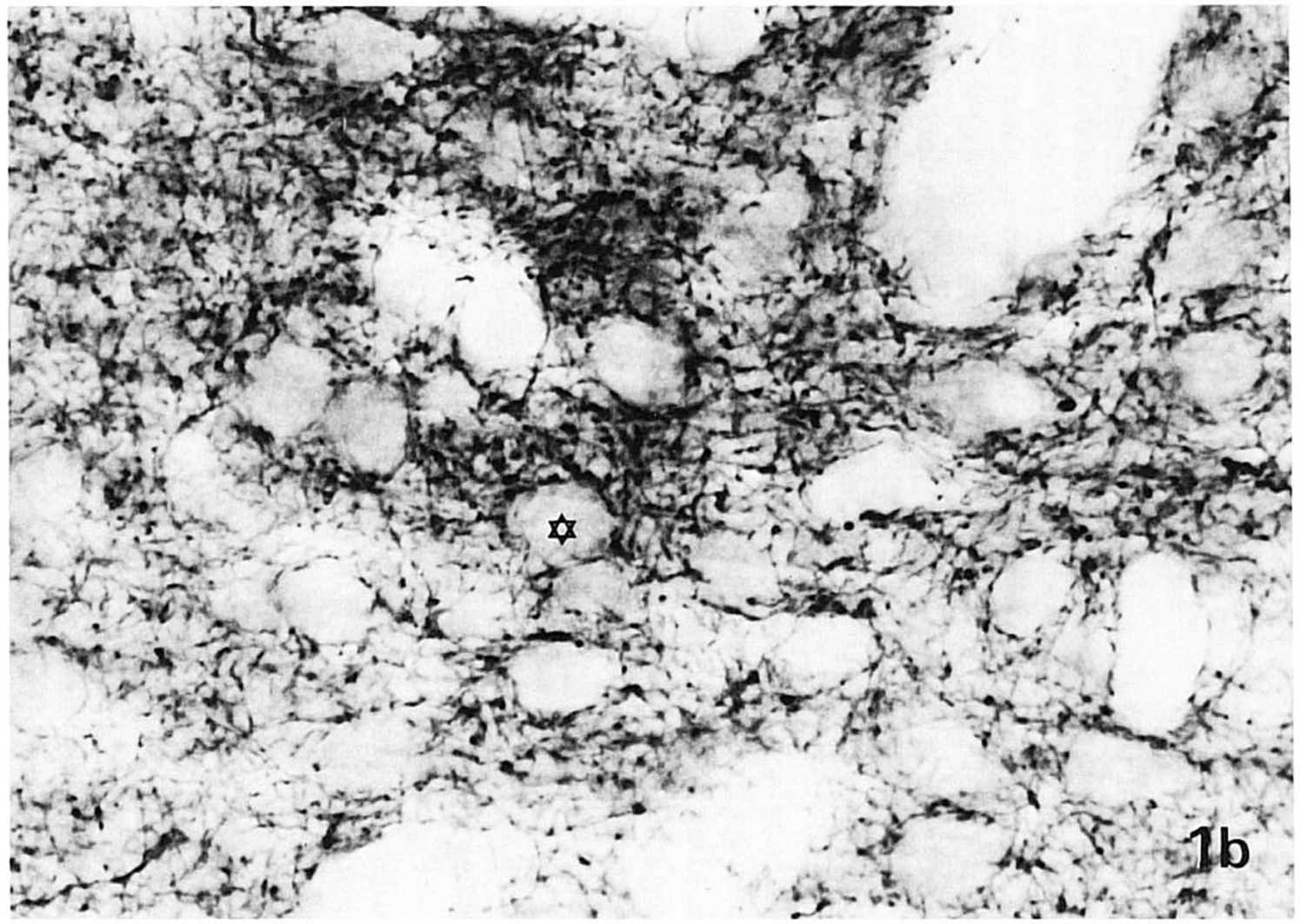
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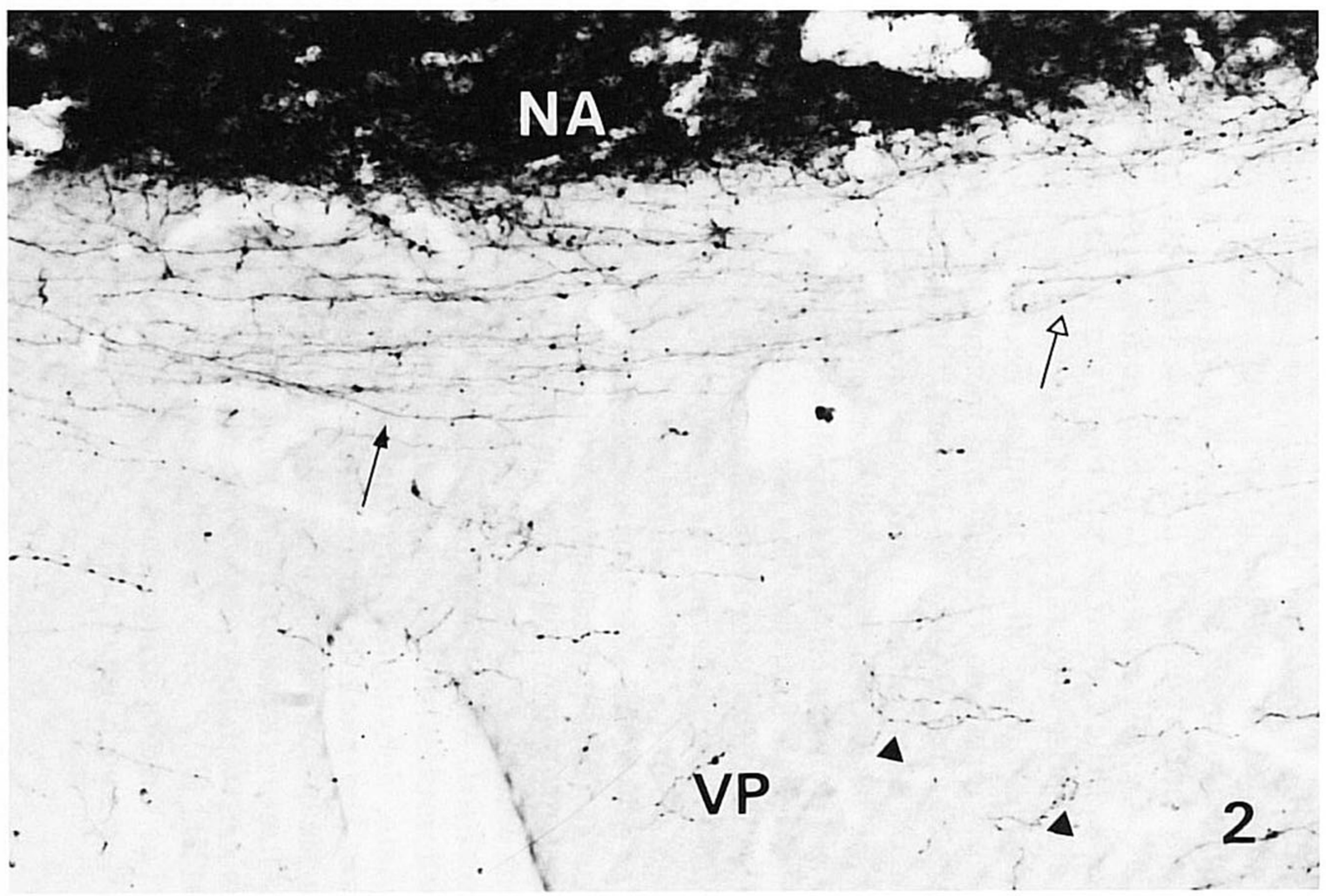
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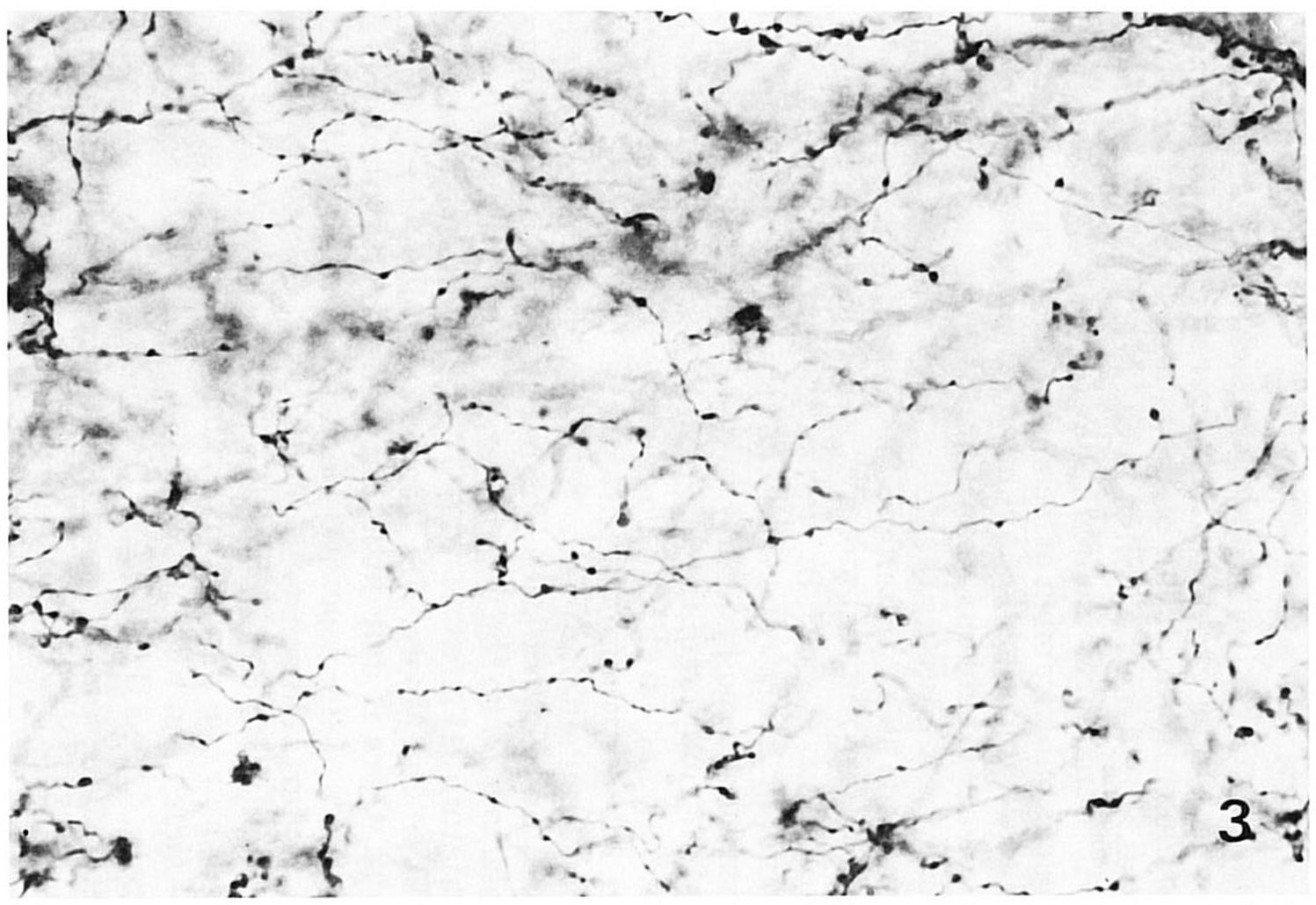
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to the pallidal extensions (Figs. 6, 7). The outer rim of the islands is heavily labeled. Usually three small patches of extremely high DA-IR are located dorsal and ventral to the insula Calleja magna and in the dorsomedial tip of the olfactory tubercle (Figs. 7, 11–13). These patches seem to be associated with the islands of Calleja. As illustrated in Figures 12 and 13, the patches can be localized outside the boundaries of the nucleus accumbens, in a group of cells that resemble the striatal cells of the nucleus accumbens.

At rostral levels a small number of DA-IR cells (size 4–6 μm) are located along the dorsomedial edge of the olfactory tubercle and also in the nucleus accumbens (Fig. 8).

More caudally, at the level of Figure 9, the DA plexuses in the nucleus accumbens and the olfactory tubercle become interconnected via the DA innervation of the striatal cell bridges (Fig. 6), only to be interrupted by the ventral pallidum and the ventrally located fiber bundles. At this level DA-IR is not uniformly distributed over the ventral striatum. Instead, the pattern of DA fibers resembles a patchwork with areas of strong and weak immunoreactivity. Similar areas with strong DA-IR are also seen dorsally in the caudate-putamen (Fig. 10). The differential aspect of DA-IR in the ventral striatum seems to be caused by the presence of regions with lower and higher staining intensity, which is in contrast with the more uniform, heavy labelling of DA-IR medially in the caudate-putamen (Figs. 13, 14). Continuing in a caudal direction this differential staining pattern in the ventral striatum becomes increasingly clear (Figs. 11, 13, 14). Although there is much individual variation in the pattern, the arrangement of a cone-shaped patch of strong DA-IR, capped by a weakly immunoreactive area in the "septal pole" of the nucleus accumbens, features in all examined animals. Also, the top of this pole is occupied by a dark patch. This particular pattern extends into the most caudal part of the nucleus accumbens (Figs. 11–14). From the examination of serial sections processed for either DA immunostaining or cellular Nissl staining it became clear that the heterogeneity of the DA-IR pattern can be matched quite well with cytoarchitectonical features. The patch of high DA-IR in the septal pole of the nucleus accumbens tends to avoid areas with dense cell populations and favours more sparsely populated sites (cf. Fig 12a,b). The same pattern is visible ventromedially in the nucleus accumbens (Fig. 7). However, the region in the top of the septal pole with strong DA-IR seems to be in register with a cell-dense area (Fig. 12).

Caudoventrally the nucleus accumbens recedes to make way for the subcommissural part of the ventral pallidum which is dispersed with bundles of fibers apparently heading for the nucleus accumbens (Fig. 15). The fact that some of these fibers branch in the ventral pallidum and exhibit varicosities suggests that also the ventral pallidum is a target area for the DA fibers (Fig. 16). Ventrally in this region a dense plexus of thin, varicose DA fibers is present (Fig. 15). The ventral pallidum continues caudally underneath the posterior part of the anterior commissure where DA fibers course in a ventrodorsal direction (Fig. 17). At this level heavy labeling is seen in the bed nucleus of the stria terminalis and in the septal area (Fig. 18). In the latter area a characteristic DA innervation pattern is found consisting of thin, highly varicose fibers surrounding unlabeled cell bodies and processes (Fig. 19). These perineuronal configurations are already encountered in rostral areas at the level shown in Figure 9.

TABLE 1. Quantitative Estimate of the Synaptic Relations of DA-Immunoreactive Neurons in the Ventral Striatum

	n. accumbens		Olfactory tubercle	
Type of synaptic contact	No.	% Total	No.	% Total
Symmetric axodendritic[1]	158	25	146	30
Asymmetric axodendritic	7	1	—	—
Axosomatic	18	3	6	1
Postsynaptic dendrite receives other unlabeled input	37	6	19	4
DA profiles without synaptic specializations	405		317	
Total number of examined DA profiles (n)	625		488	

[1]The symmetric axodendritic contacts are divided in 41% axospinous and 59% axonal-dendritic shaft contacts in the nucleus accumbens (n = 170), in the olfactory tubercle the division is 38% and 62%, respectively (n = 198).

Electron microscopy

Tissue samples were taken from different areas in the ventral striatum: the ventromedial and dorsomedial nucleus accumbens and the two inner layers of the olfactory tubercle, where a cellular continuum is found with the striatal cell bridges (Hedreen, '81; Millhouse and Heimer, '85). From the "septal pole" of the nucleus accumbens, regions with high or low immunoreactivity were studied. No obvious differences were observed in the ultrastructural morphology or the synaptic relations of the DA profiles in these different areas (Table 1).

DAB reaction product, indicating the presence of DA-IR, is found in unmyelinated axons and axon varicosities. The reaction product is located in the plasmalemma and around mitochondria, microtubuli, and vesicles. Only in heavily labeled profiles can DAB precipitate be seen inside vesicles, apparently caused by diffusion of the reaction product (Figs. 20, 24). Boutons and varicosities are stacked with round or flattened vesicles varying between 40 and 70 nm in diameter (Fig. 24). Smaller vesiclelike structures of approximately 10 nm probably represent microtubules. Some of the DA nerve endings contain large vesicles (80–140 nm), some of which are cored. These vesicles do not contain any reaction product (Fig. 21).

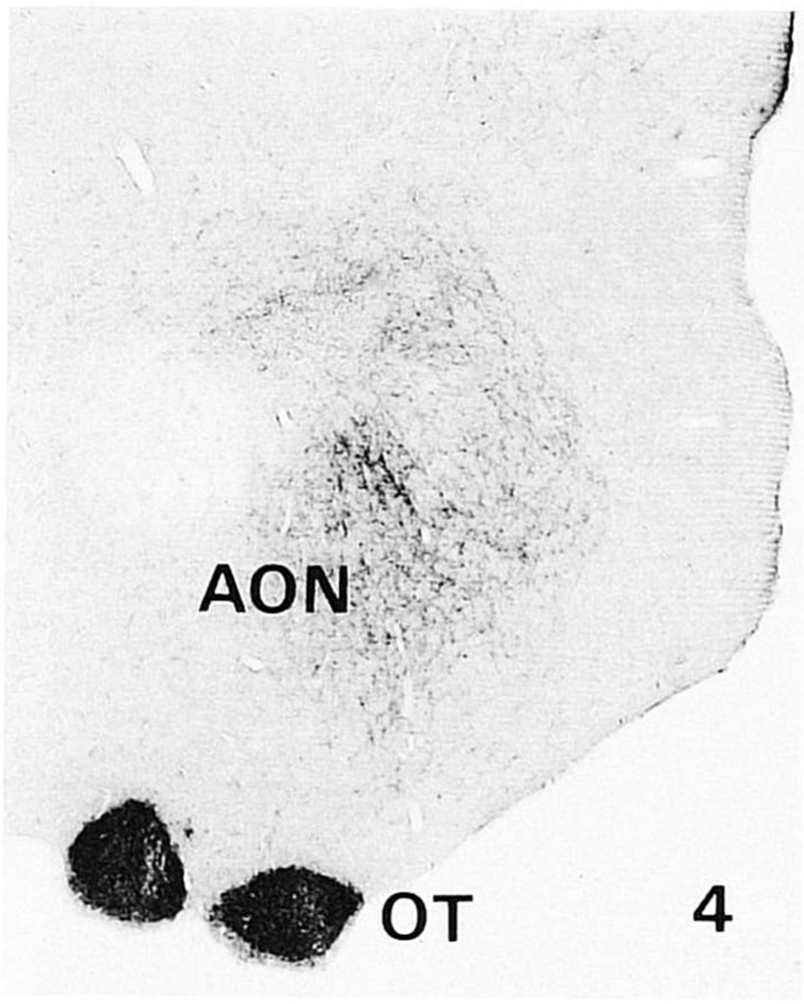
The location of lines of DAB precipitate in the plasmalemma of axon profiles and varicosities apposing possible postsynaptic targets makes it difficult to establish unequivocally the presence of synaptic contacts, since the reaction product either obscures or suggests the presence of presynaptic membrane specializations (Fig. 20). By studying labeled profiles in a series of sequential sections, it could be

Figs. 1–3. Examples of the different DA fiber configurations in the striatum.

Fig. 1. The fibers of the DA plexus in the nucleus accumbens (1a) have more and larger varicosities (arrow) than those of the DA plexus in the caudate nucleus (1b). Star marks position of unlabeled striatal cells. ×560.

Fig. 2. Along the ventromedial aspect of the nucleus accumbens (NA) course smooth (arrow) and varicose (open arrow) DA fibers. Ramifying varicose axons are present in ventral pallidal regions (arrowheads). ×220.

Fig. 3. The DA plexus in the prepiriform cortex is constituted by thick, elongate, varicose axons. A similar fiber morphology is found in the anterior olfactory nucleus and the prefrontal cortex. ×560.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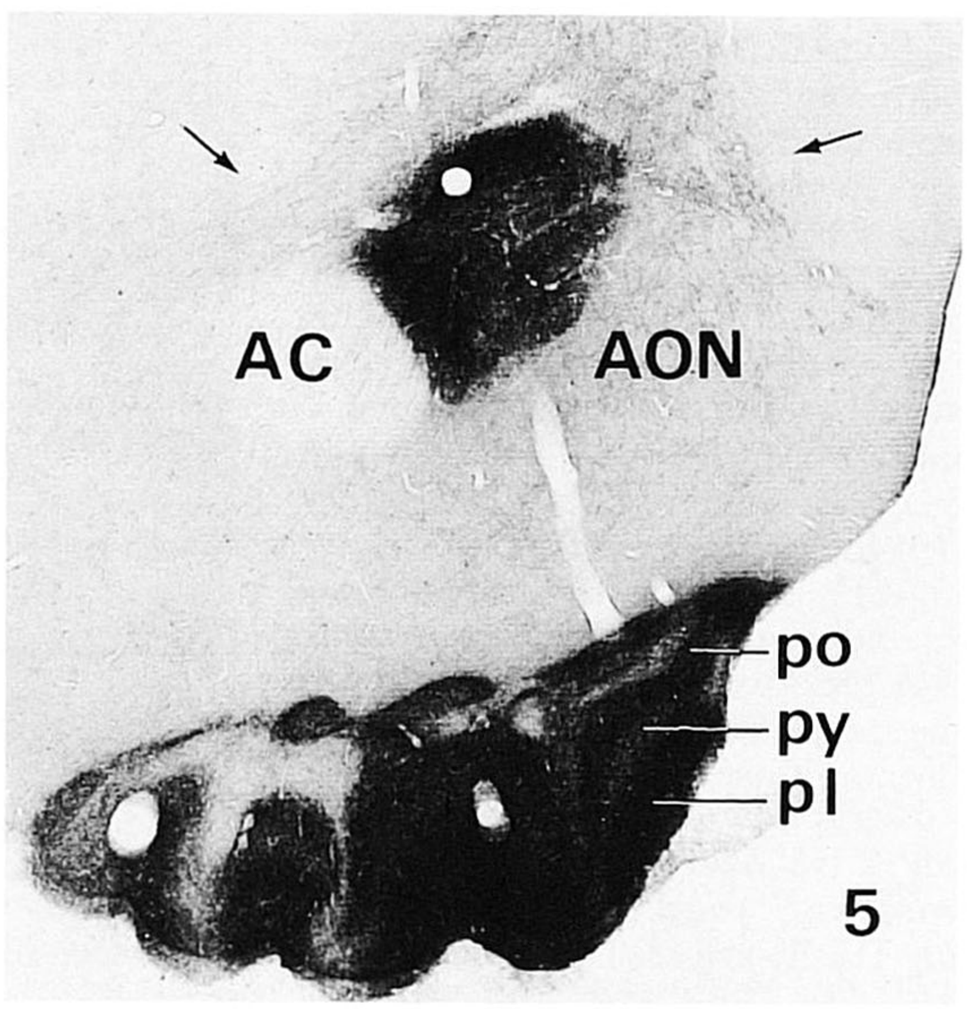
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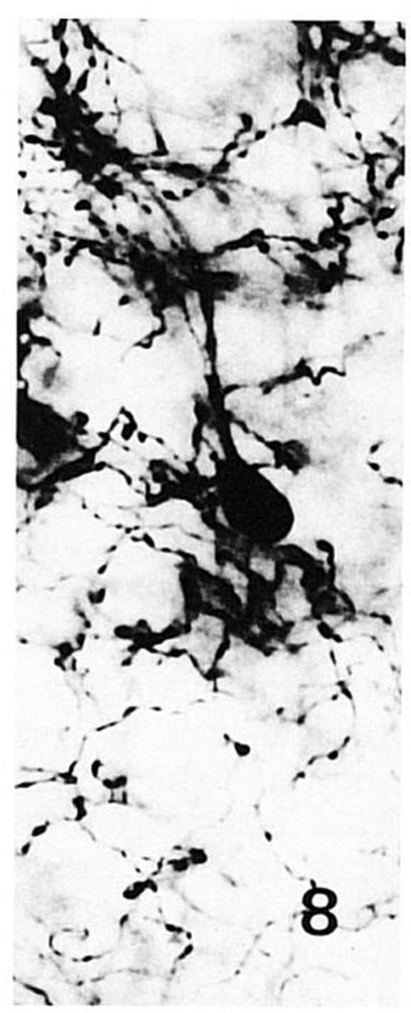
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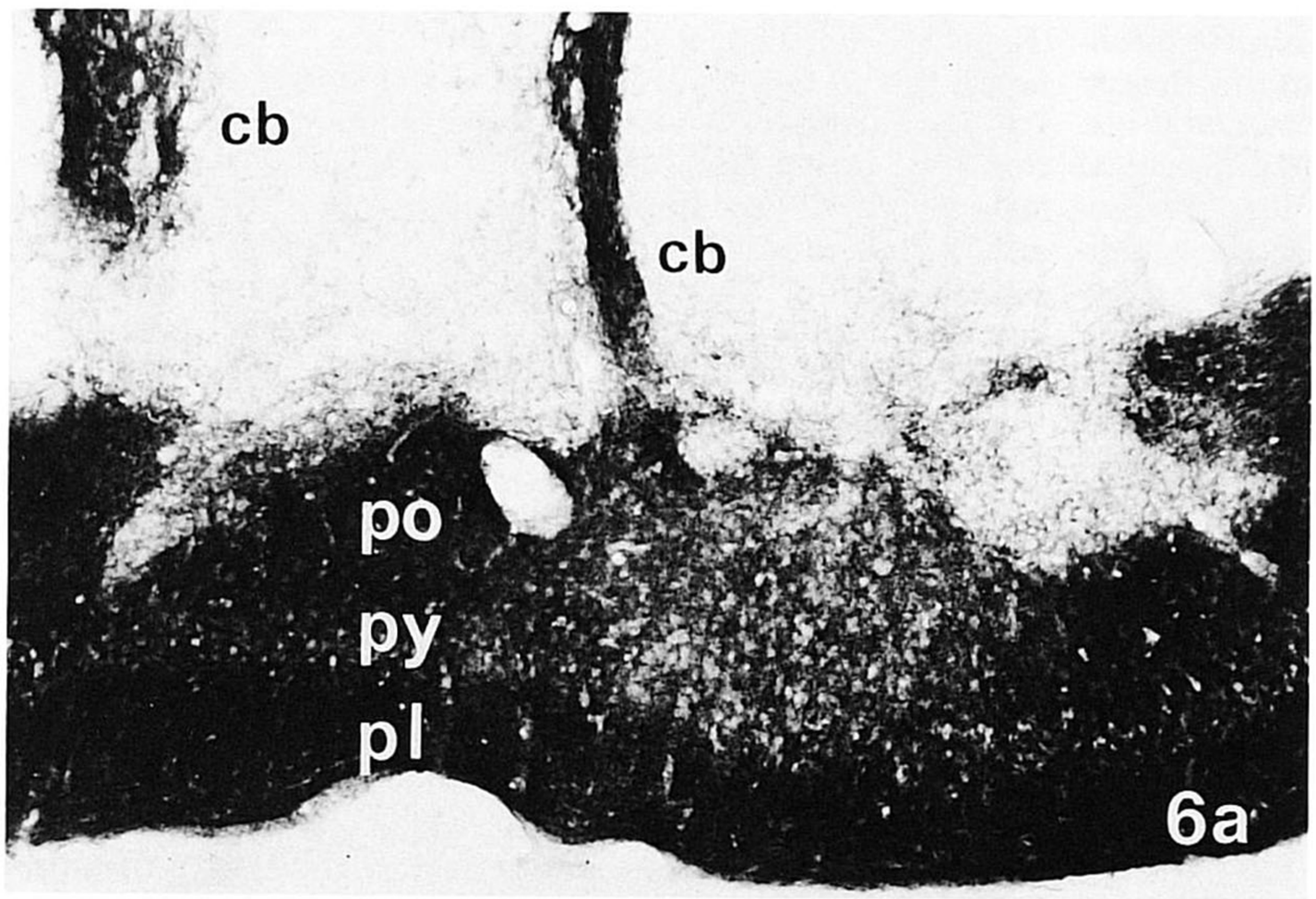
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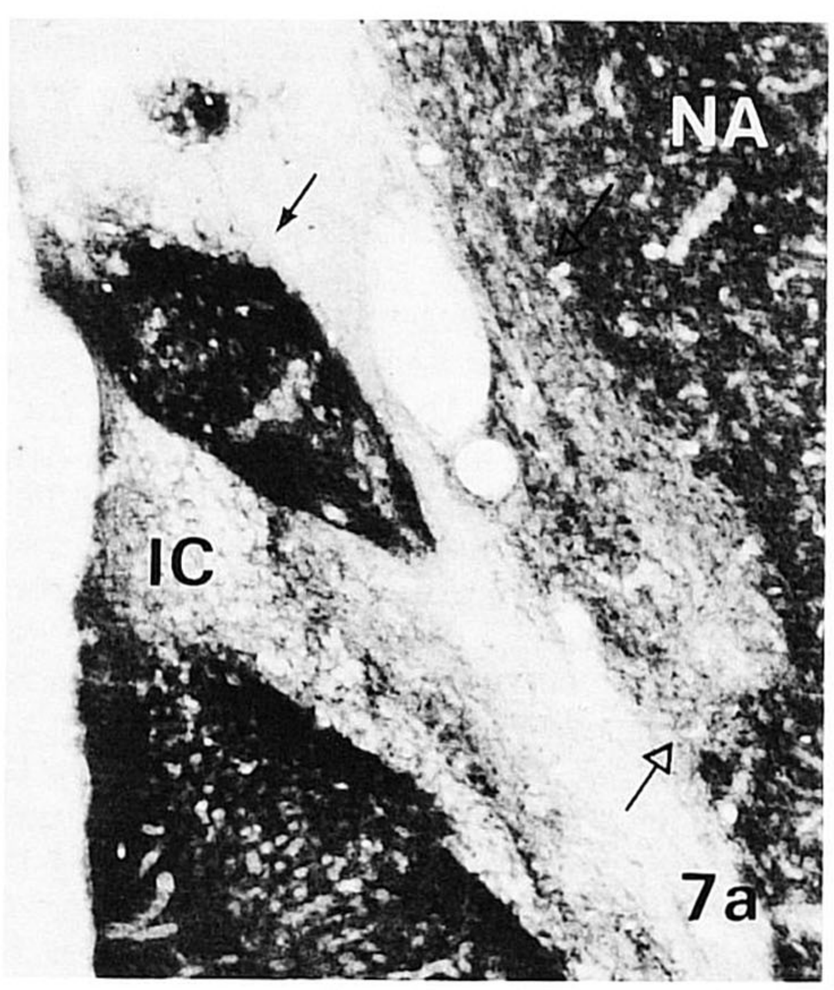
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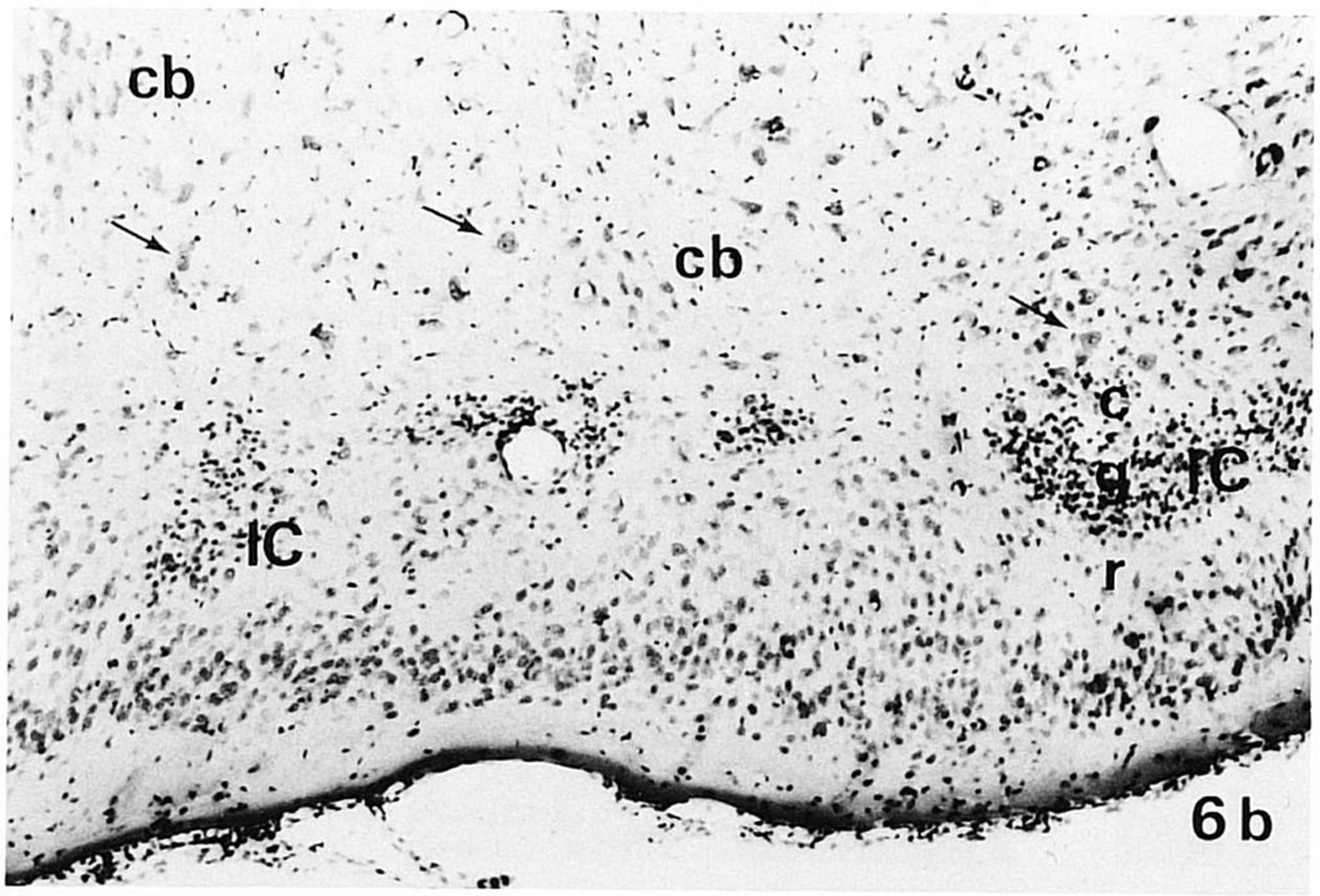
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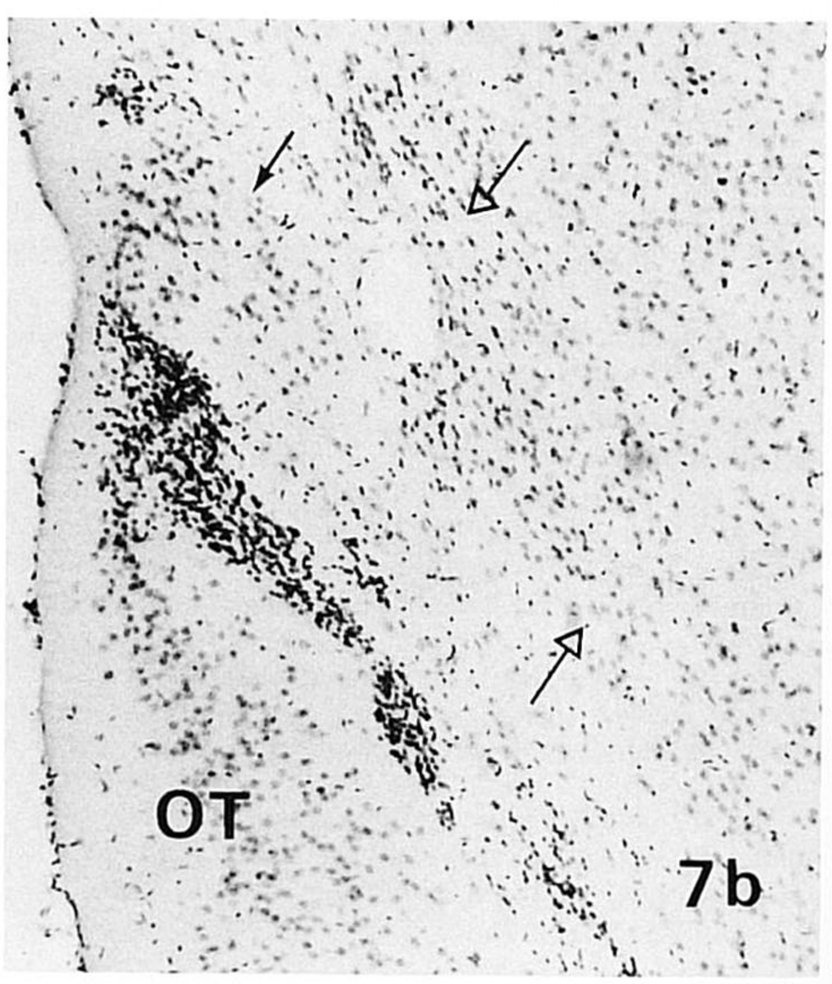
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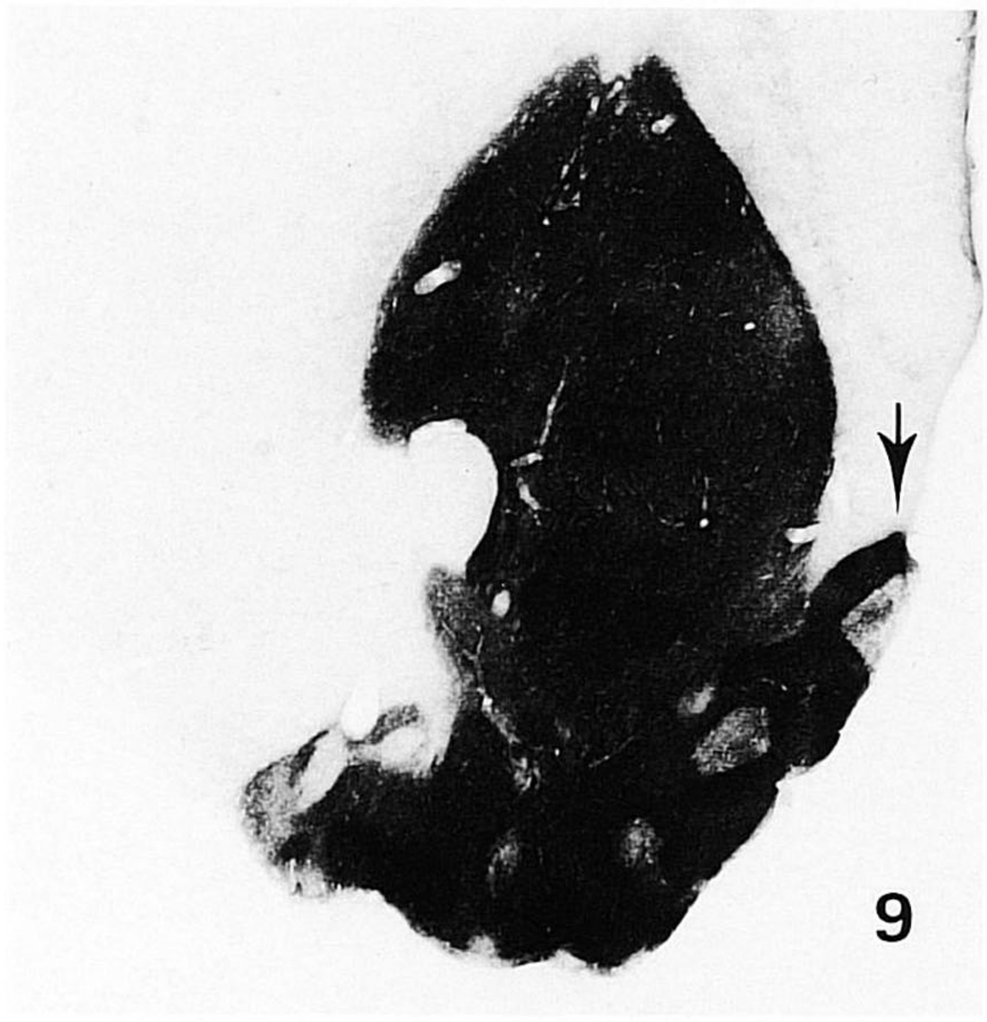
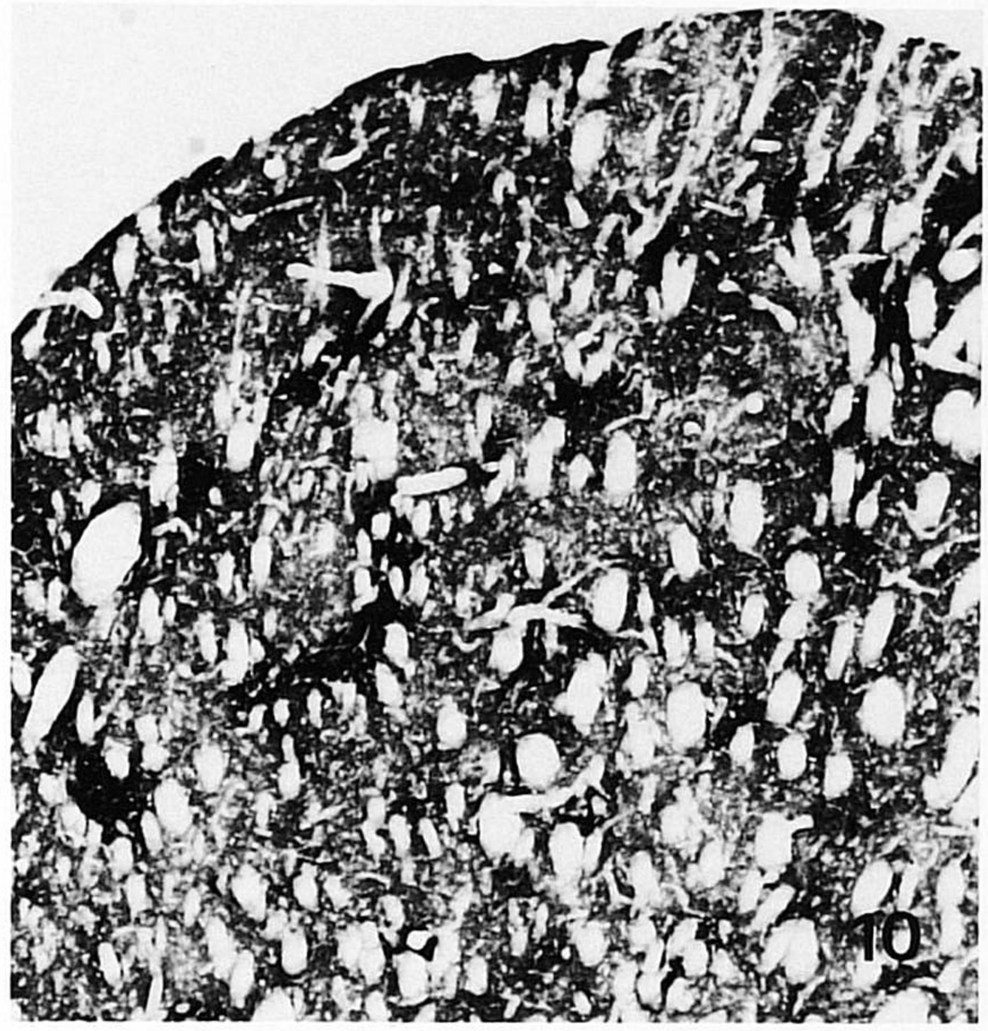




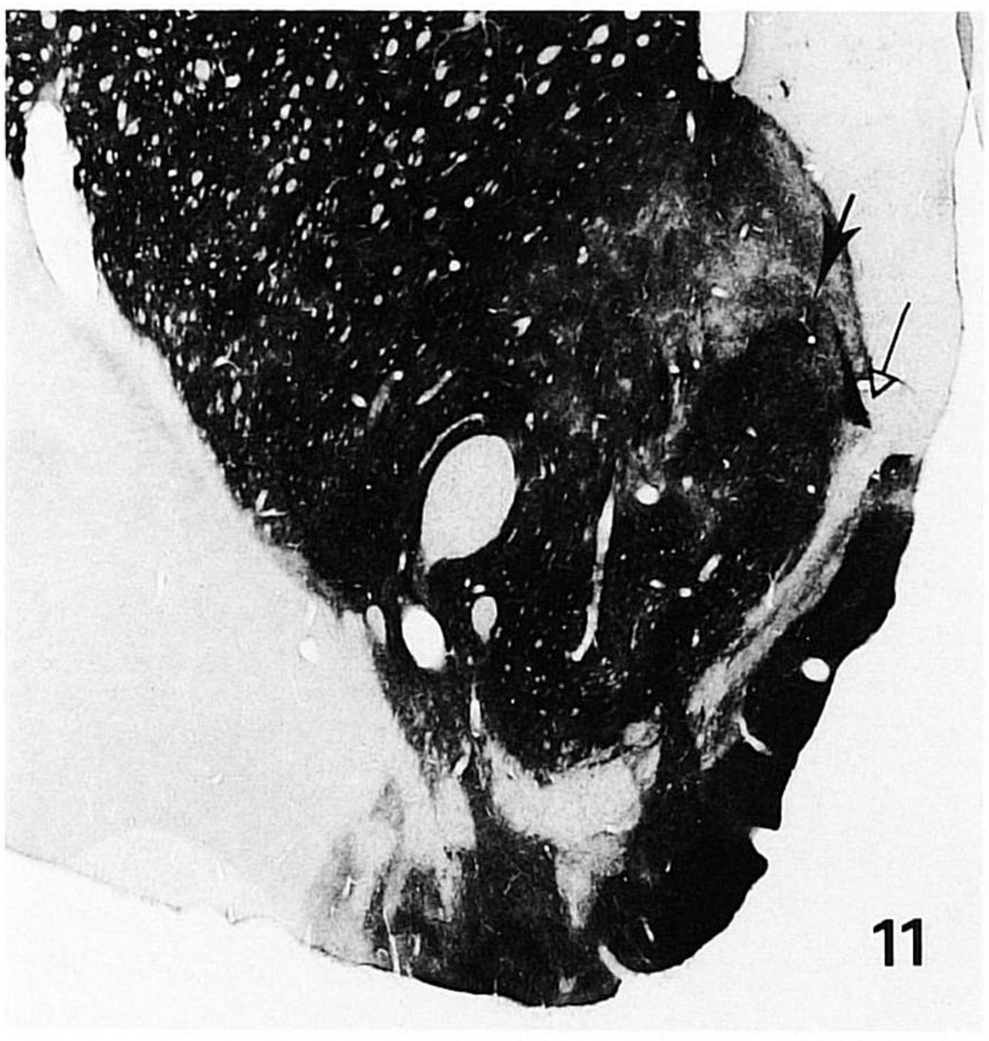


Fig. 9. The DA innervation in the nucleus accumbens and the olfactory tubercle is continuous and has a patch-like appearance (approx. A 9820). Note that the area with strong DA-IR dorsomedial in the tip of the olfactory tubercle is already present at this rostral level (arrow). ×28.

Fig. 10. Areas with strong DA-IR dorsally in the caudate-putamen (approx. A 8620). The micrograph has been slightly underexposed for better visualization of these areas. ×30.

Fig. 11. Patches of strong and weak DA-IR in the nucleus accumbens (A 9410). The rostral extension of the "septal pole" patch of strong DA-IR is located medially in the nucleus accumbens (arrow). Note the presence of areas with extremely strong DA-IR (open arrow). ×21.

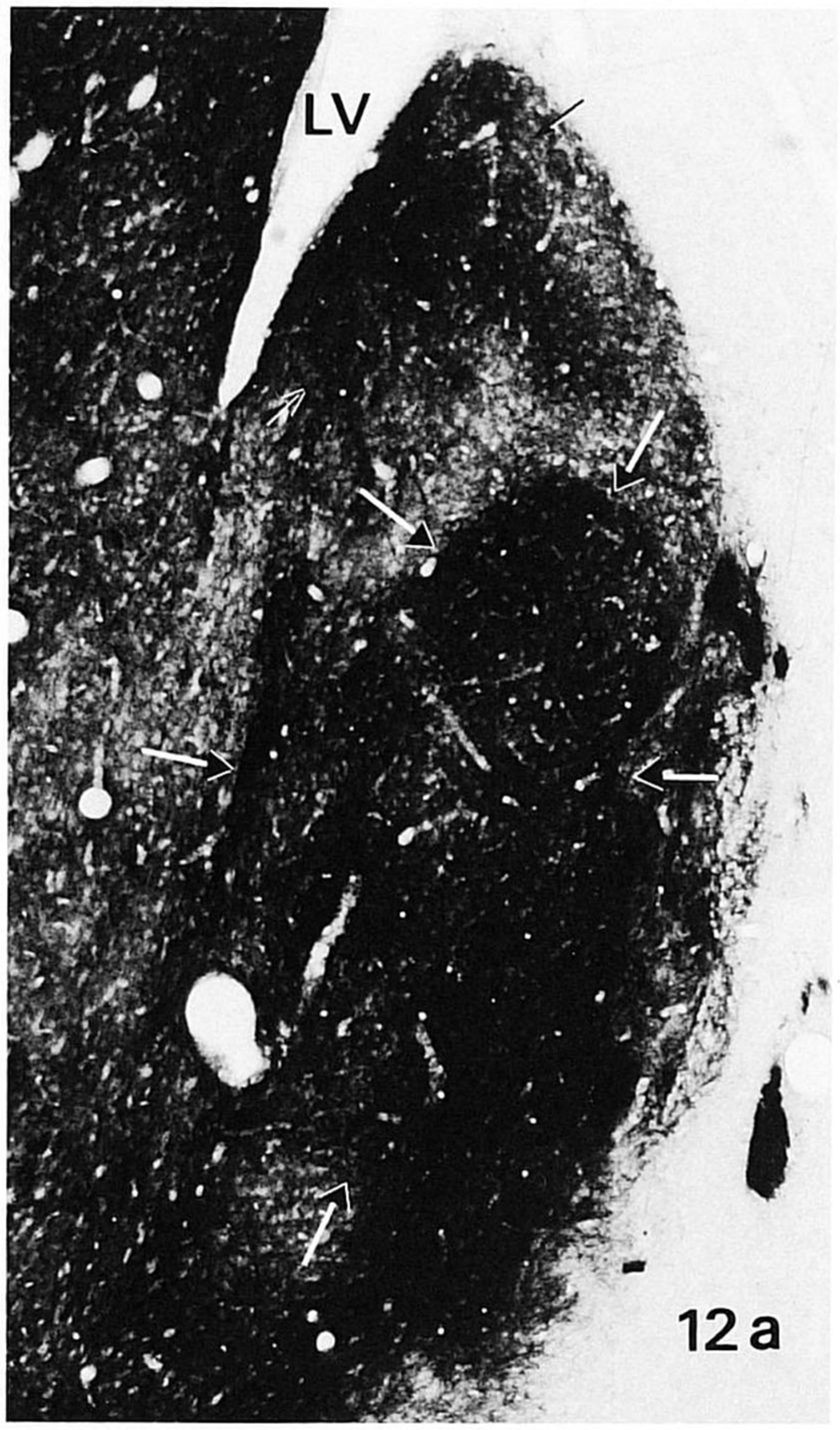
Fig. 4. The olfactory tubercle (OT) is the most rostral region in the telencephalon where a dense DA plexus is found (level A 10500). A less dense network is present in the anterior olfactory nucleus (AON). ×30.

Fig. 5. At level A 10050 a dense DA network is present rostrally in the nucleus accumbens and in the olfactory tubercle, where the DA innervation extends over the plexiform (pl), the pyramidal (py), and the polymorph (po) cell layers. Along the dorsolateral aspect of the anterior commissure (AC) and the nucleus accumbens, and through the posterior part of the anterior olfactory nucleus (AON) fibers are coursing toward prefrontal cortical regions (arrows). ×38.

Fig. 6. Two consecutive sections through the olfactory tubercle taken at level A 8920 are stained for DA (a) and according to the Nissl procedure (b). The olfactory tubercle and the nucleus accumbens are interconnected by striatal cell bridges (cb) that are innervated by DA fibers (cb in a). Although all three layers of the olfactory tubercle receive DA fibers, the densest plexus is found in the plexiform layer (pl). Also the outer rim (r) of the islands of Calleja (IC) is heavily labeled, whereas the granule cell compartment (g) and the core (c) of the islands display a moderately dense innervation. Overlying the olfactory tubercle and the islands of Calleja is the ventral pallidum, which presence is indicated by the large pallidal neurons (arrows in b). × 56.

Fig. 7. Two consecutive sections through the dorsomedial tip of the olfactory tubercle (OT) immunostained for DA (a) and Nissl stained (b). The island of Calleja (IC) shows the same innervation pattern as described in Figure 6. Dorsal to the island of Calleja a patch of strong DA-IR (a, arrow) is overlying an area with striatal cells (b, arrow). In the nucleus accumbens (NA) an area with a moderately dense DA innervation coincides with a cell-dense region (open arrows). Left side of the figure represents medial aspect of the brain. ×56.

Fig. 8. Unipolar DA cell in the ventrolateral nucleus accumbens, at the level of Figure 5. ×560.



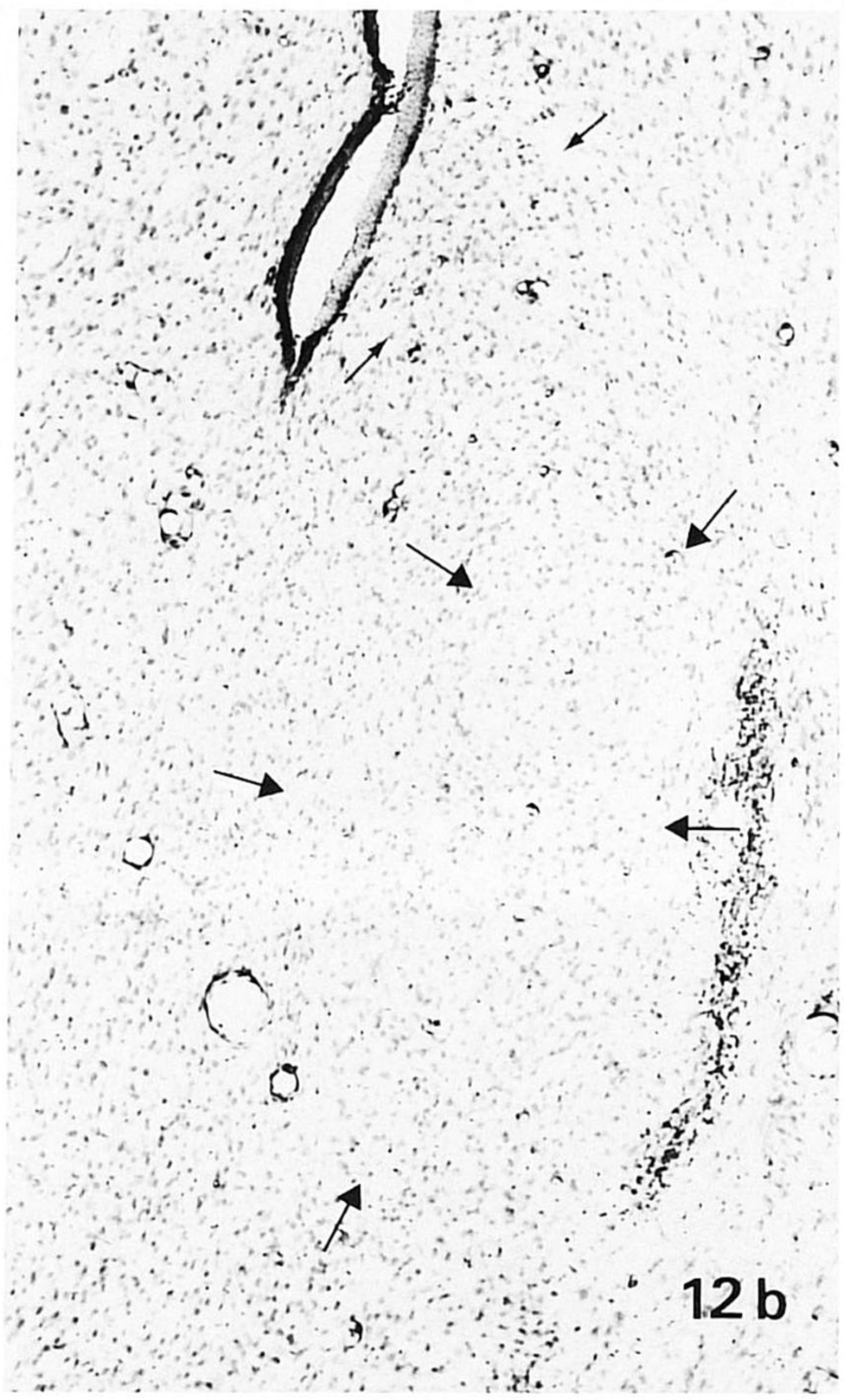


Fig. 12. Two consecutive sections through the dorsomedial tip—the "septal pole" — of the nucleus accumbens immunostained for DA (a) and Nissl stained (b). The "septal pole" patch of strong DA-IR (arrows in a) coincides with an area having a moderately dense cell population, as outlined by the arrows in b (arrows in a and b are at identical positions). In contrast, in the top of the "septal pole" another area with strong DA-IR seems to be in register with a cell dense area (small arrows in a, b). a, ×64; b, ×56.

demonstrated that, although many profiles exhibit this membrane staining, this does not necessarily indicate the presence of a synapse (Fig. 22). Thus, only appositions showing obvious synaptic clefts and postsynaptic membrane specializations were considered synapses.

About 38% of the examined DA-containing profiles establish synaptic contacts, preferably with dendritic spines and shafts (Figs. 23–25, 27, 28, see Table 1). Synapses were observed in small fibers (Fig. 23) as well as in varicosities. With a few exceptions (asymmetric axodendritic contacts, see Fig. 26), the synapses are of the symmetric type (Gray type II). It was frequently observed that spines contacted by DA axon terminals also receive input from unlabeled axons, which establish asymmetric or symmetric (not illustrated) contacts (Figs. 27, 28, Table 1).

Close appositions between DA profiles and cell bodies, resembling symmetric synapses, are also observed (Figs. 25, 29). However, membrane specializations are seldom as prominent as in the case of axodendritic synapses. In the present material it was not possible to establish unequivocally the identity of the neurons approached by the DA fibers.

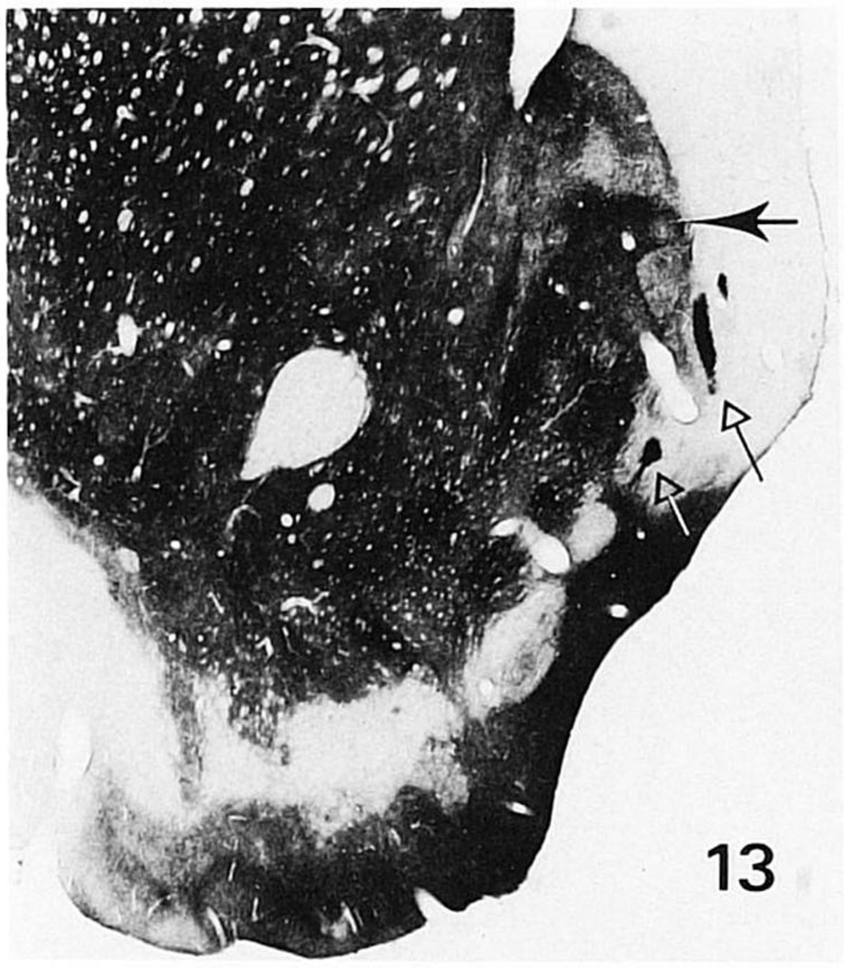
Figs. 13–15. The differential aspect of the distribution of DA-IR in the nucleus accumbens at levels A 8920 (13), A 8620 (14), and A 7890 (15). A region with strong DA-IR in the "septal pole" can be appreciated in all three levels (arrows). Patches of strong DA-IR are also present just outside the nucleus accumbens (open arrows in Fig. 13). In Figure 15 some DA fibers can be seen in the ventral pallidum (VP), whose ventral border is occupied by a dense DA plexus (small arrow). ×21.

Fig. 16. Bundles of DA fibers traverse the ventral pallidum on their way to the striatum; they give off branches in the ventral pallidum (large arrow) that exhibit varicosities (small arrows). ×220.

Fig. 17. Immediately dorsal to the anterior commissure thick DA fibers in the caudate nucleus are oriented in a ventrodorsal direction (arrows). ×220.

Fig. 18. The bed nucleus of the stria terminalis (BST) and the lateral septum (LS) display a dense DA plexus, whereas the ventral pallidum and the globus pallidus (continuous at this level, P) are relatively sparsely innervated by thick DA fibers (A7020). ×21.

Fig. 19. DA fibers surround a cell body and its processes, thereby forming a perineuronal configuration. The cell is located along the ventromedial border of the rostral nucleus accumbens, approximately at level A 9820. ×220.



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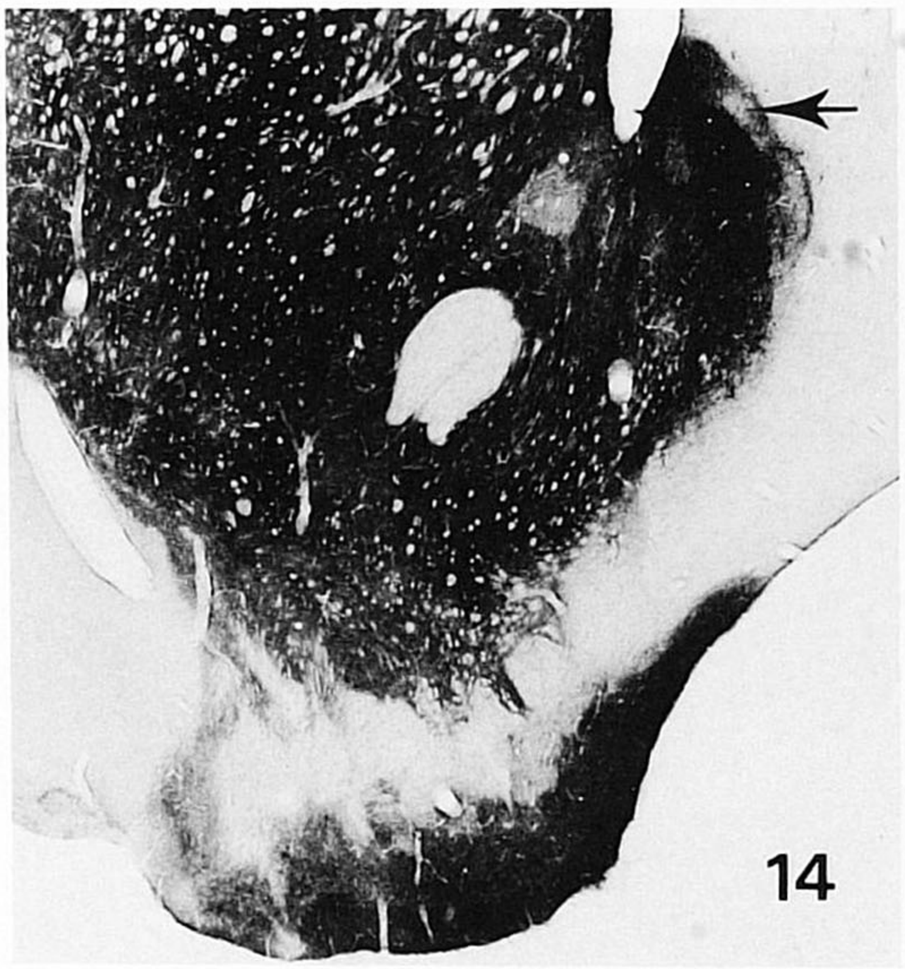

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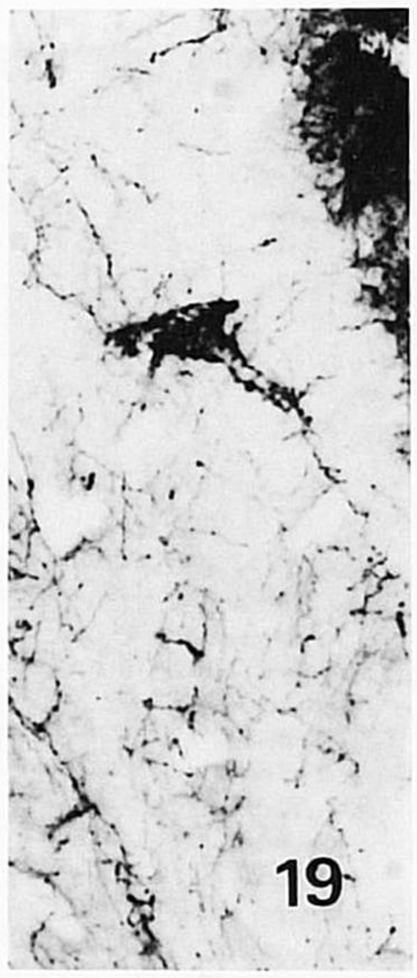

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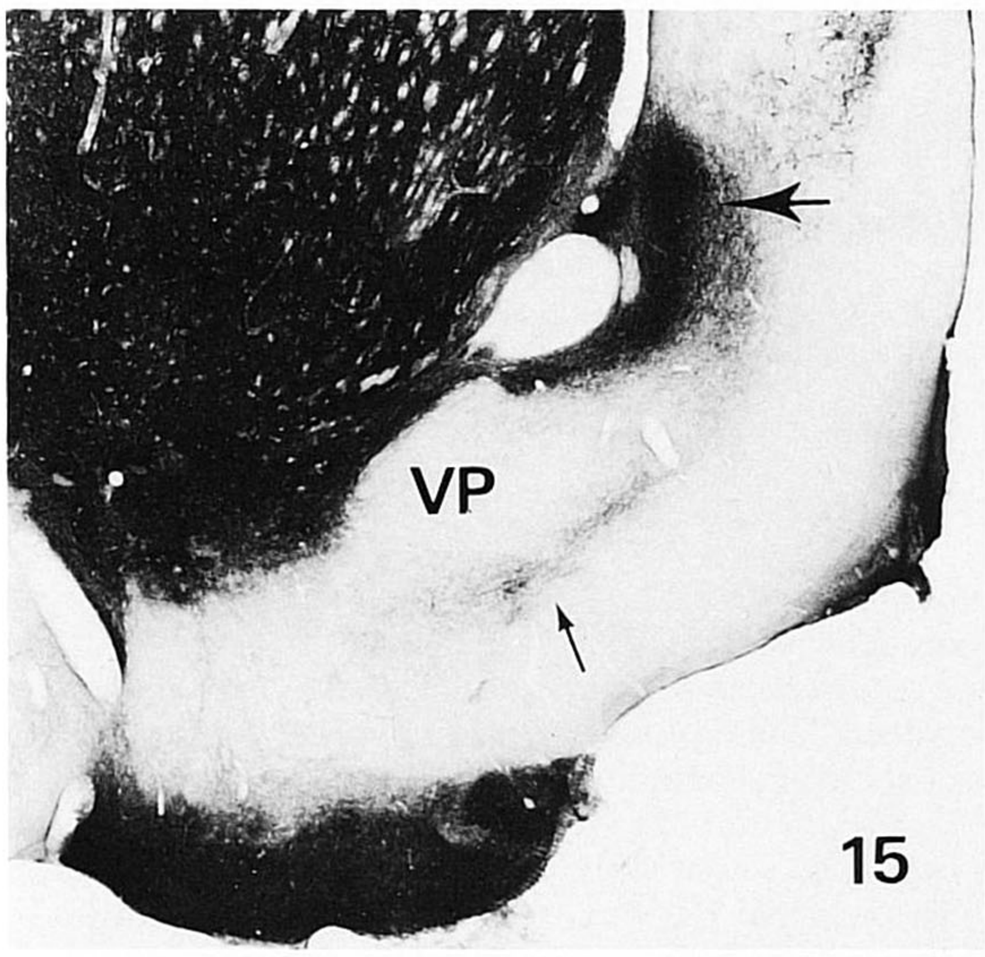

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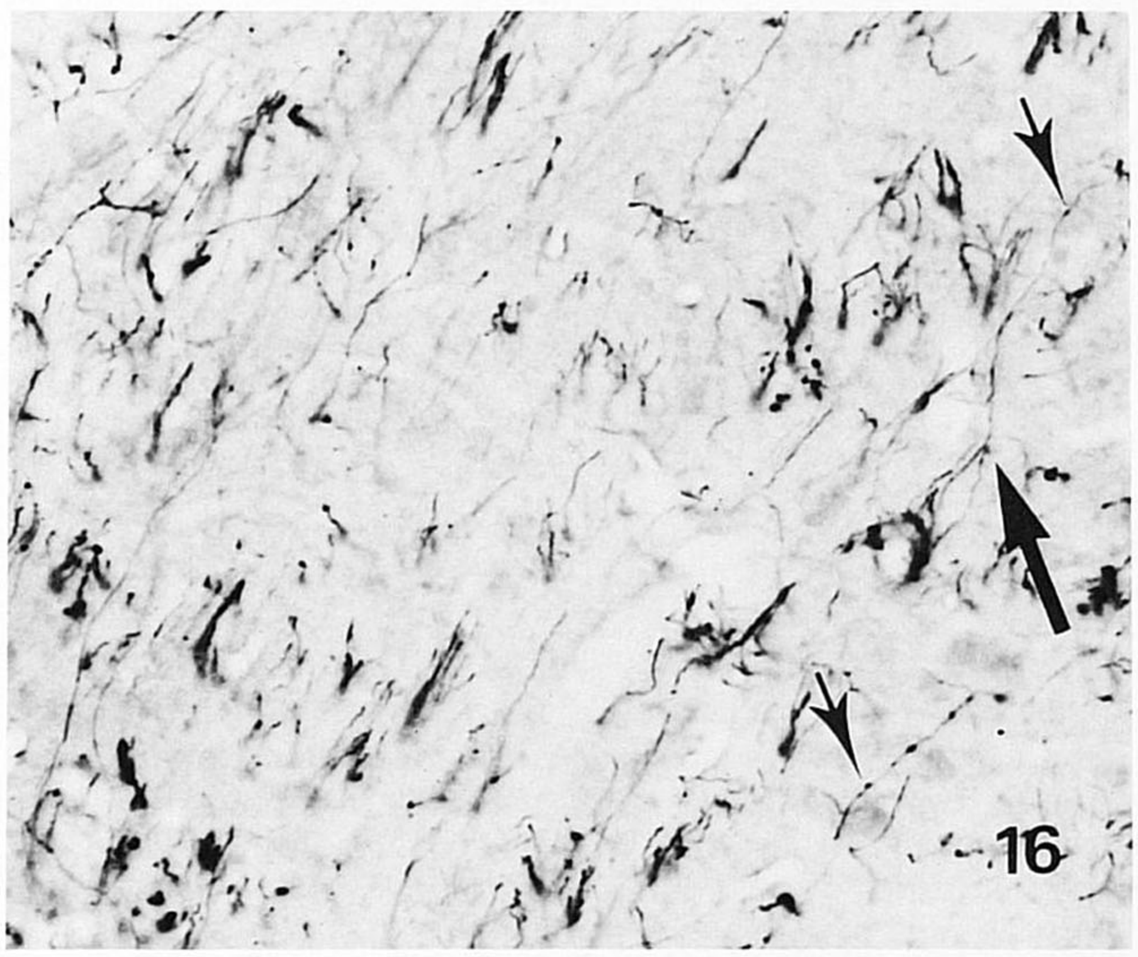

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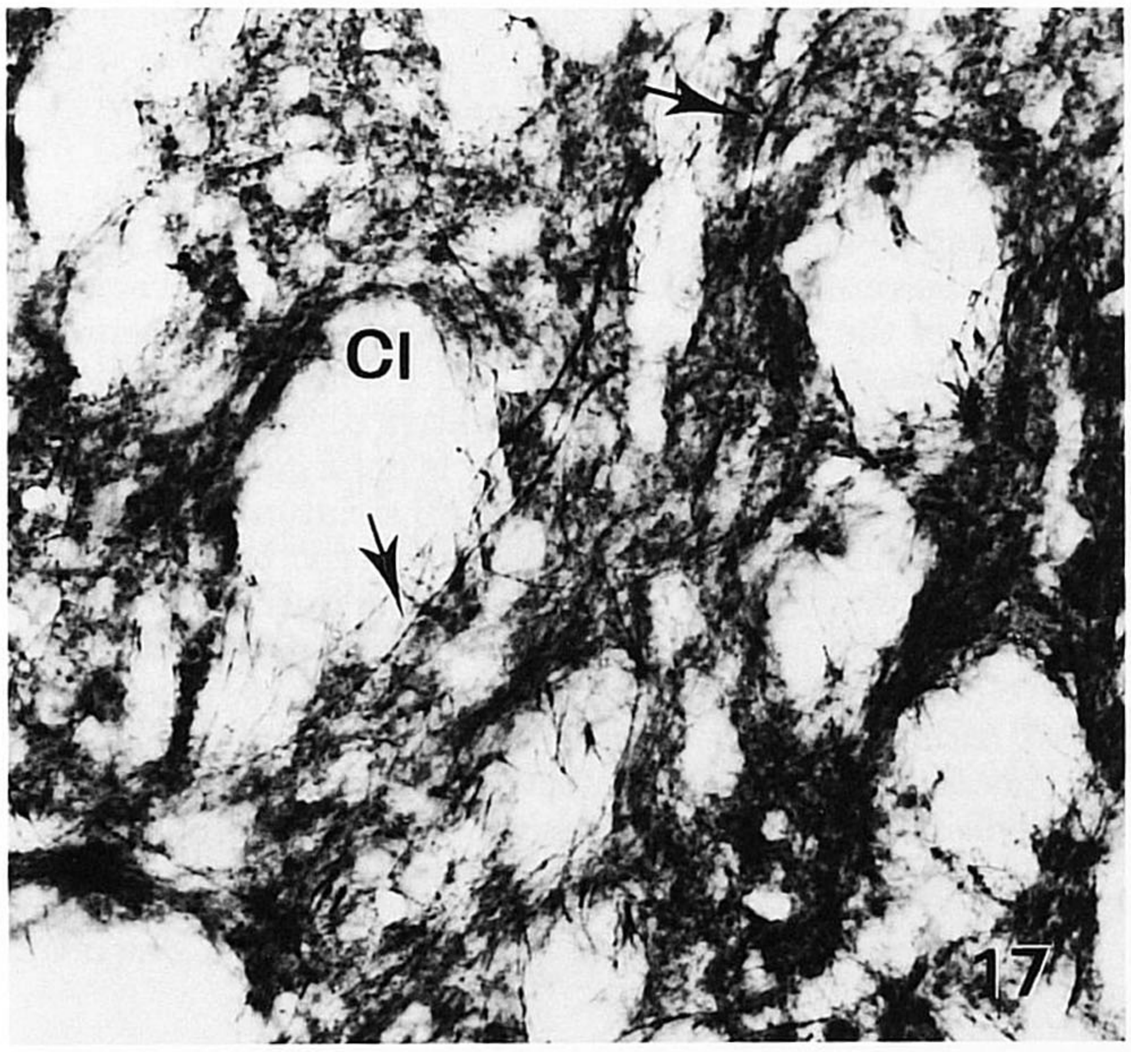

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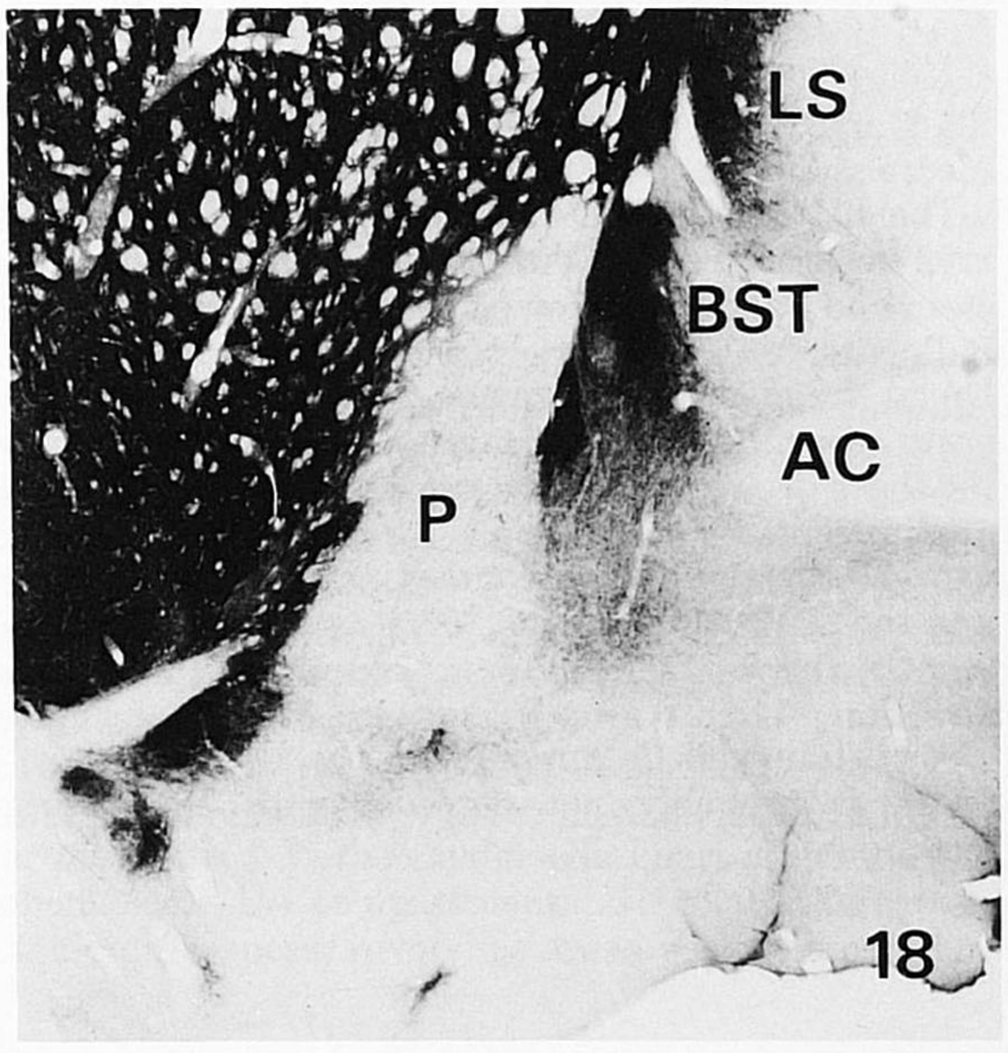

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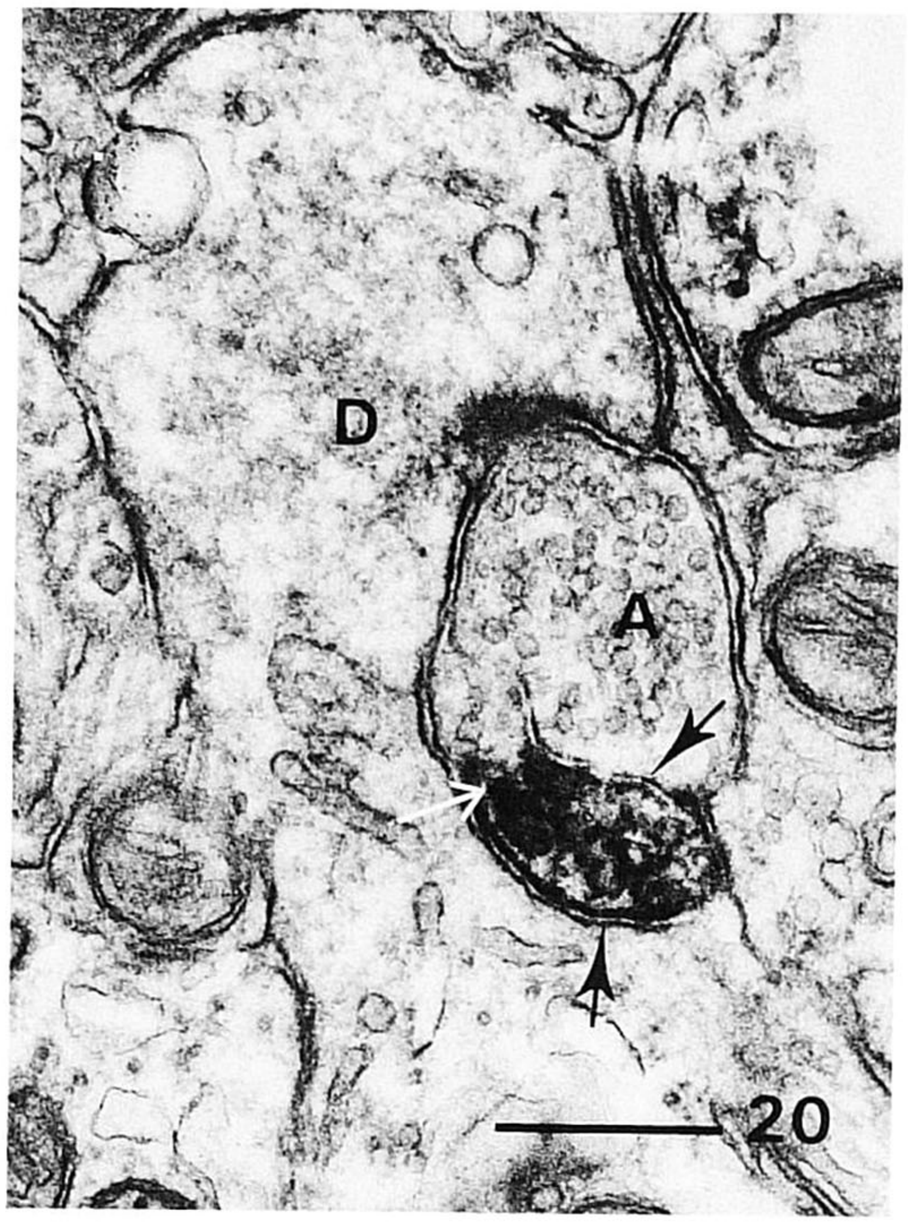


Fig. 20. DA-containing axon in the nucleus accumbens filled with vesicles that are either surrounded or filled (white arrow) with DAB precipitate. Lines of DAB precipitate (arrows) in the plasmalemma apposing a dendrite (D) and an axon (A) suggest the presence of membrane specializations. Bar = 250 nm.

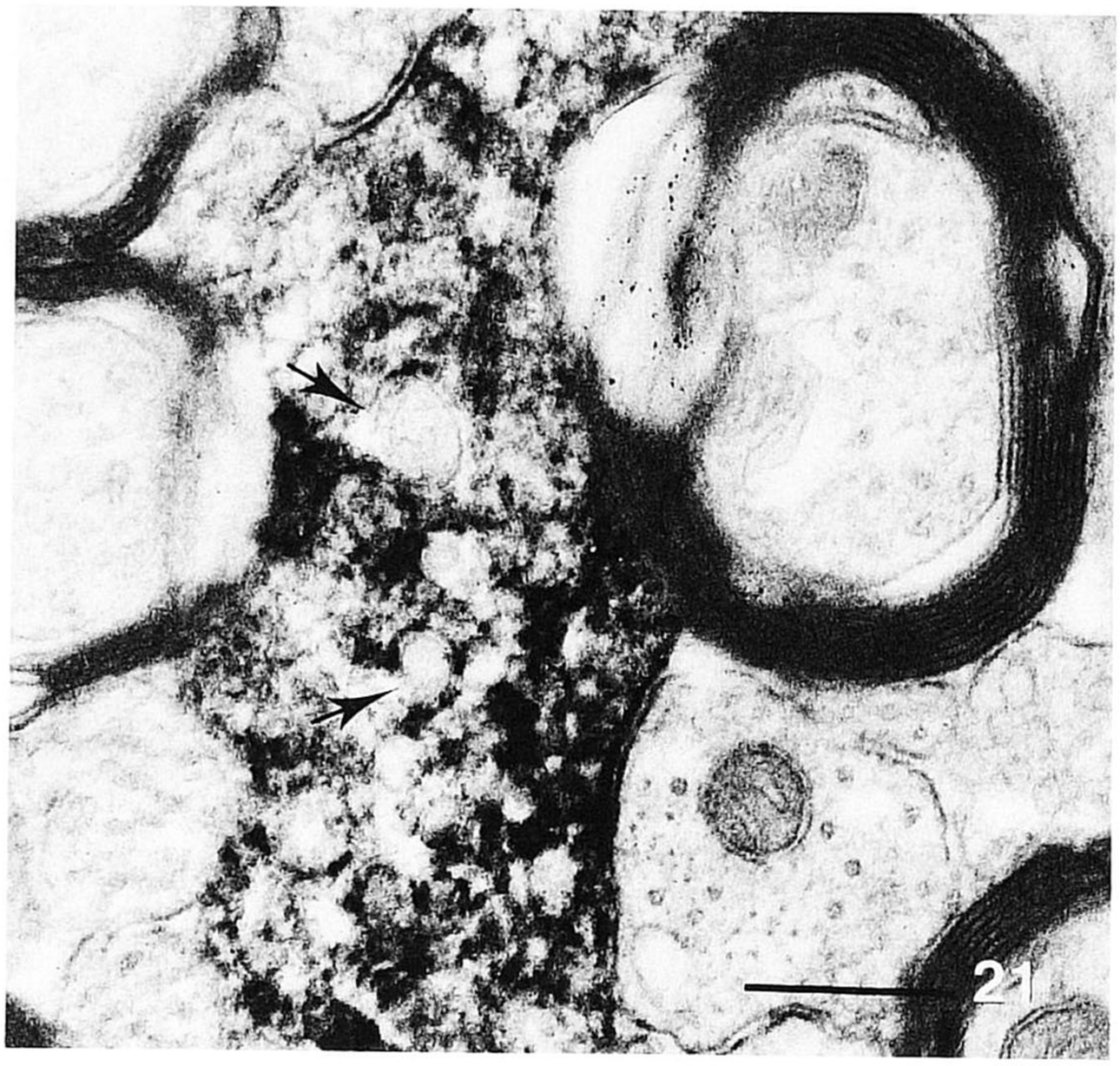


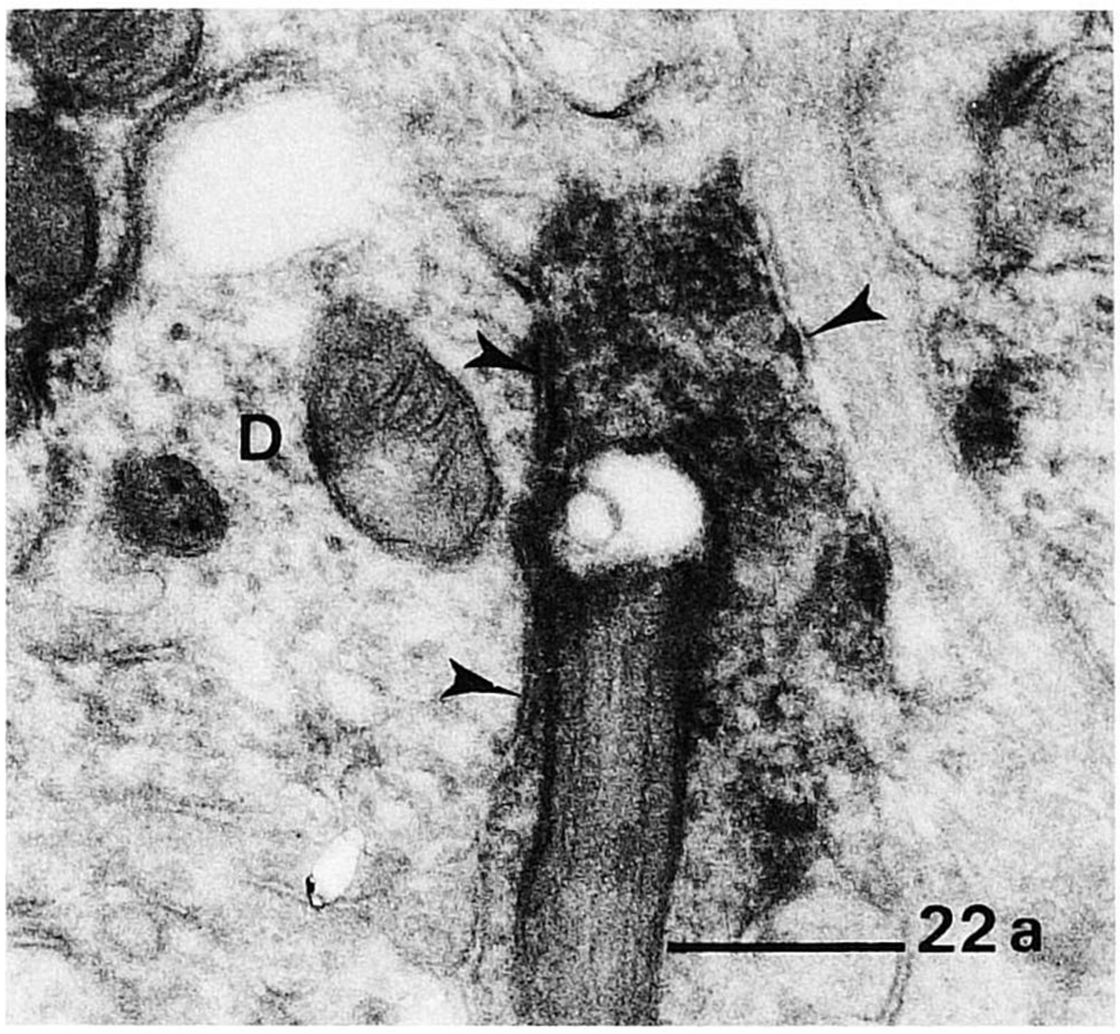
Fig. 21. Unlabeled, large, cored vesicles (arrows) in a DA varicosity in the nucleus accumbens. Bar = 250 nm.

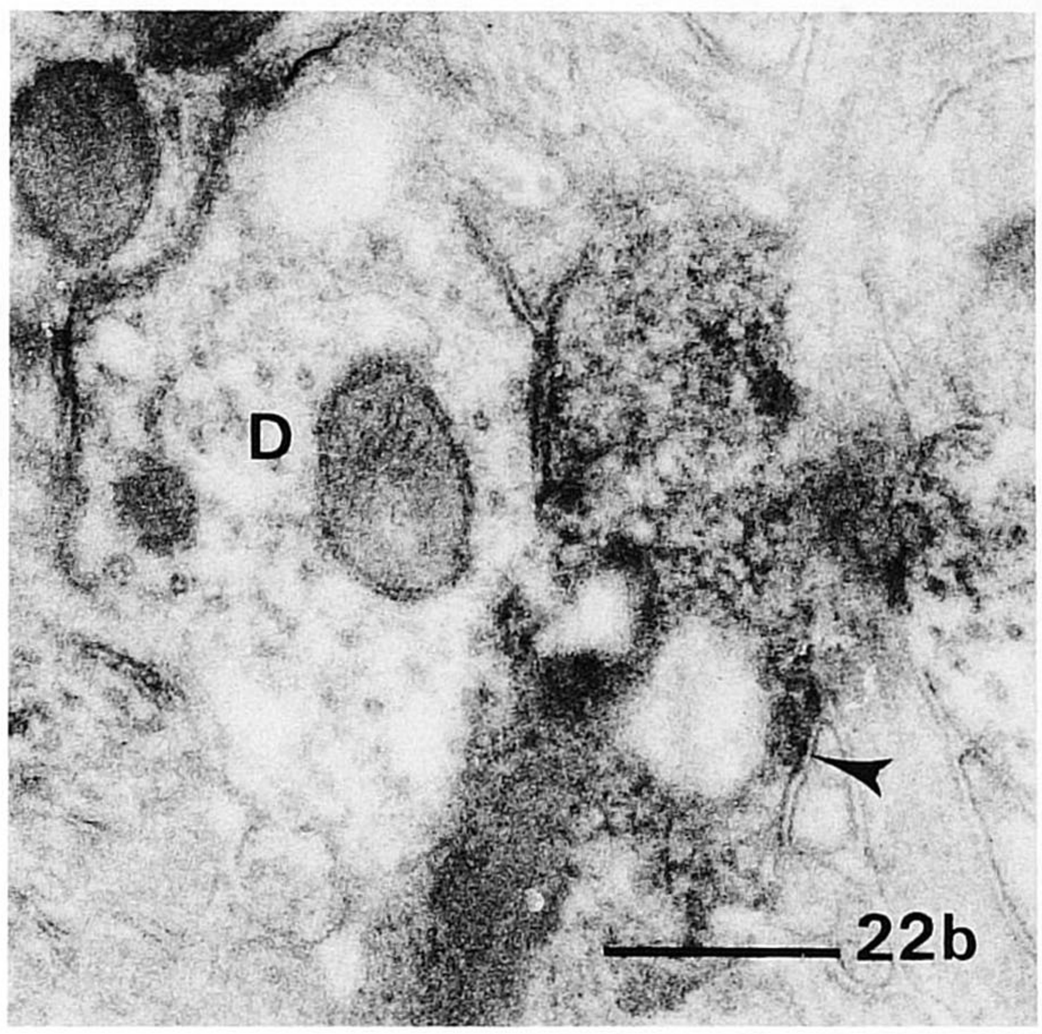
DISCUSSION

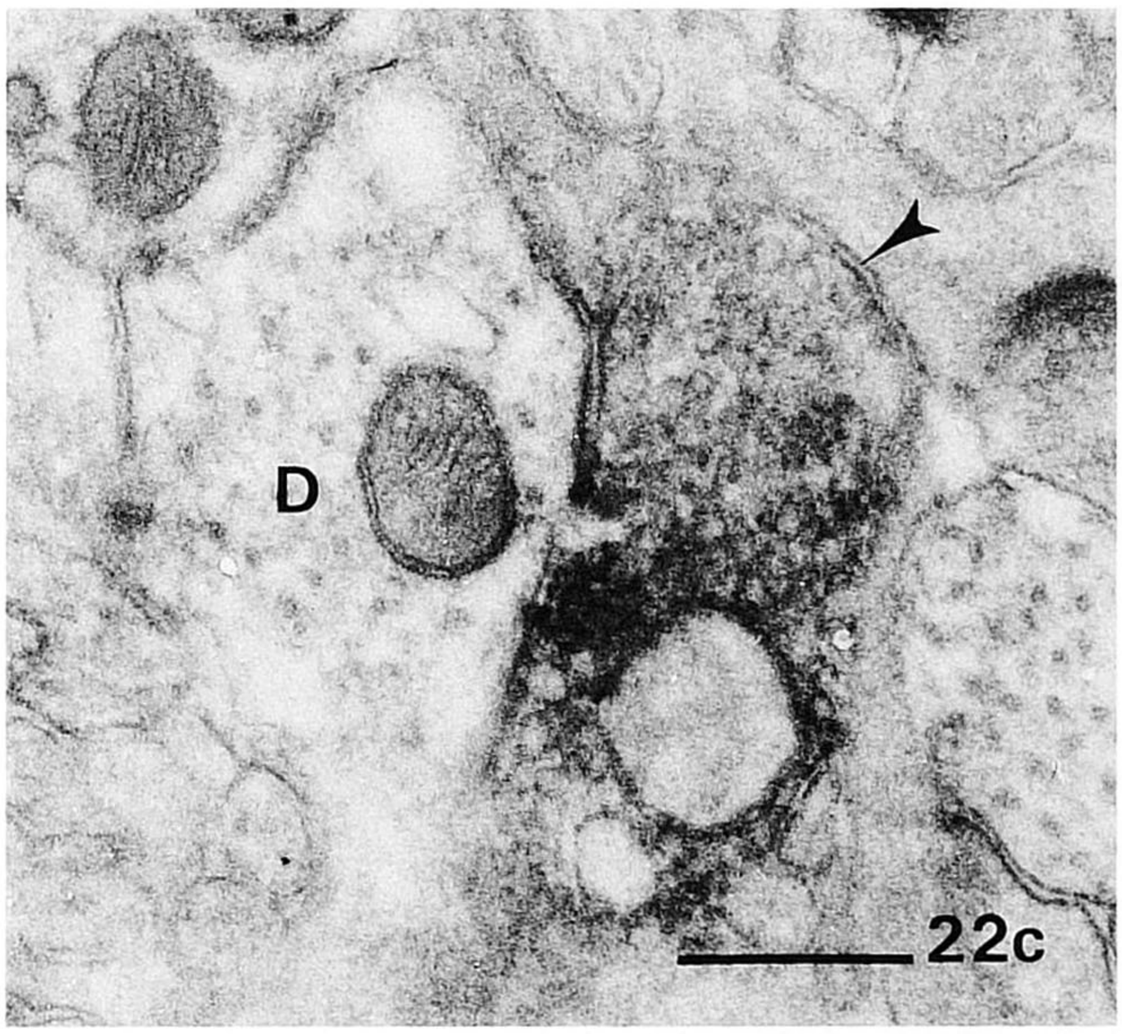
A major advantage of the dopamine (DA) antiserum used in the present study is that it offers a direct and relatively simple procedure for specific histological detection of the transmitter (Buijs et al., '84), so that a differentiation between the dopaminergic and noradrenergic innervation of the striatum is greatly facilitated. Until now the intermingling axonal networks of dopamine and noradrenaline fibers, the latter of which are especially found in the caudal striatum, could be unraveled only after selective lesions of the dopamine or noradrenaline cell groups of origin (Björklund and Lindvall, '84; Brownstein and Palkovits, '84; Moore and Card, '84).

The distribution of DA, as described in the present study, is in good accordance with the biochemical data of Brownstein and Palkovits (Brownstein et al., '74; Brownstein and Palkovits, '84), who reported very high concentrations of DA in the nucleus accumbens and olfactory tubercle. Moderate concentrations of DA are present in the area bordering these two regions (Brownstein and Palkovits, '84), where application of DA antiserum revealed only a dense innervation of the cell bridges connecting the nucleus accumbens and the olfactory tubercle. More caudally, DA fibers were seen in the ventral pallidum, coursing in a rostrocaudal direction. As this area is considered to be devoid of DA (Brownstein and Palkovits, '84), the DA content of these fibers is probably not detected with the micropunch technique.

A comparison of the presently described distribution of immunoreactivity (DA-IR) in the ventral striatum with the patterns established with histofluorescence methods or tyrosine-hydroxylase immunohistochemistry shows that there is considerable agreement among the various data (Hökfelt et al., '77; Fallon and Moore, '78; Lindvall and Stenevi, '78; Fallon et al., '83; Björklund and Lindvall, '84). However, immunostaining of DA appears to reveal more details. In all regions of the nucleus accumbens compartments with high or low DA immunoreactivity DA-IR could be demonstrated. The most striking compartment is the cone-shaped patch of high DA immunoreactivity in the septal pole of the nucleus accumbens. This particular patch is situated in a region with low cell density and clearly avoids the surrounding cell-dense areas. A similar arrangement is present in the ventromedial nucleus accumbens. However, since the top of the "septal pole" of the nucleus accumbens has both a massive cell population and a dense innervation of DA fibers, it appears that the negative correlation between dense innervation and cell density is not a general phenomenon of DA target sites in the ventral striatum. The present findings thus partly confirm previous reports in which an islandlike distribution of catecholamine fluorescence in the same area was shown to correlate negatively with so-called cell clusters (Hedreen and Chalmers, '72; Herkenham et al., '84). Although the patch in the septal pole of the nucleus accumbens can be easily detected in sections treated with a tyrosine-hydroxylase antiserum or in histofluorescence material, with the same techniques other parts of the nu-







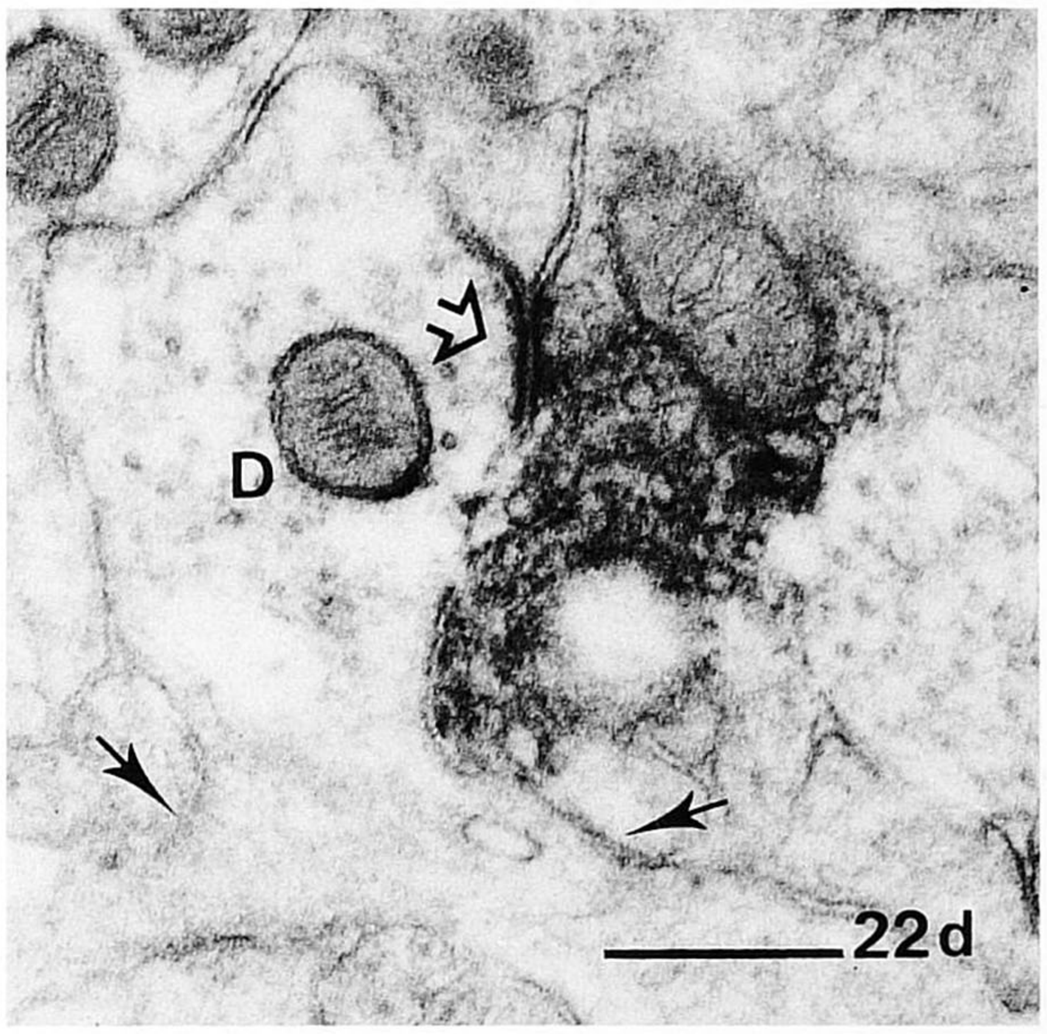


Fig. 22. Four consecutive sections through a DA terminal in the nucleus accumbens establishing a synapse with a dendrite (D) at a point where spines are issued from the shaft (arrows in d). Although lines of DAB precipitate are located at different sites in the plasmalemma of the DA profile (arrowheads), a synapse is only established at one position (large arrow in d). Bar = 250 nm.

cleus accumbens do not display a differentiated staining pattern comparable to that observed in the present material (Hökfelt et al., '77, '84; Bouyer et al., '84b). This holds also for the dorsal striatum where sharply outlined DA islands are present in early postnatal stadia, but subsequently disappear in the evolving diffuse matrix of DA fibers, until they are no longer visible in adult animals (Olson et al., '72; Tennyson et al., '72; Fuxe et al., '79; Graybiel, '84). Surprisingly, in the DA-immunostained sections of the caudate-putamen of adult rats some of these islands are readily detected. It has been suggested by Olson and co-workers ('72) that this may be caused by metabolic differences between particular groups of DA fibers. On the other hand, in the caudate-putamen and the nucleus accumbens islandlike projection patterns have been demonstrated of fibers anterogradely labeled after injection of radioactive tracer or WGA-HRP in the substantia nigra or ventral tegmental area, respectively (Chronister et al., '80, '81; Wright and Arbuthnott, '81; Herkenham et al., '84; Beckstead, '85). Therefore, the pattern of DA patches might be produced also by differences in fiber densities.

In conclusion, the present study demonstrates that the dopaminergic innervation of the ventral striatum is highly compartmentalized. In the caudomedial part of the nucleus accumbens some DA compartments are related to cytoarchitectonic differences, involving areas with low cell den-

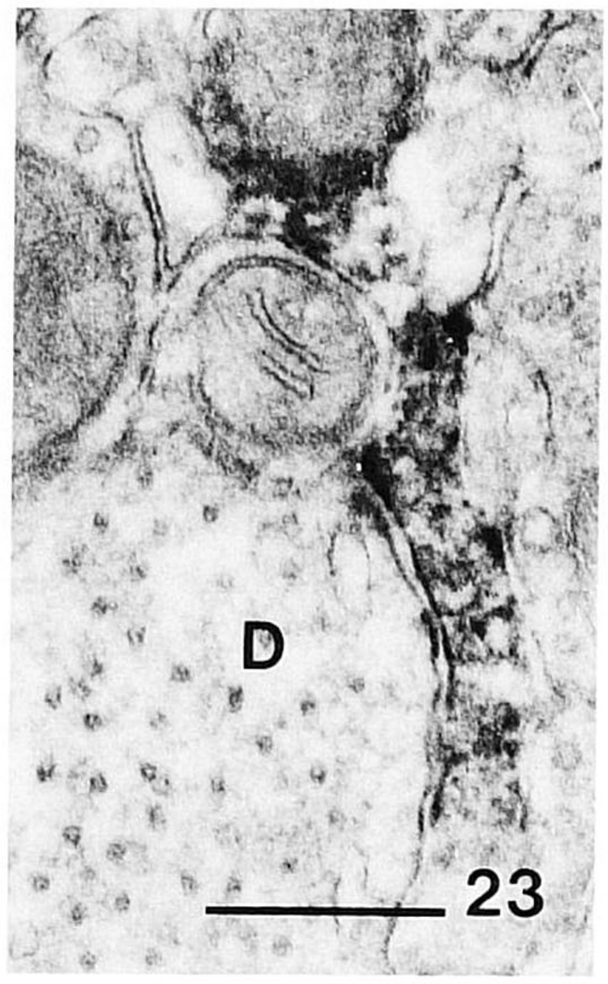


Fig. 23. Thin DA fiber of passage in the nucleus accumbens contacts a dendritic shaft (D). Bar=250 nm.

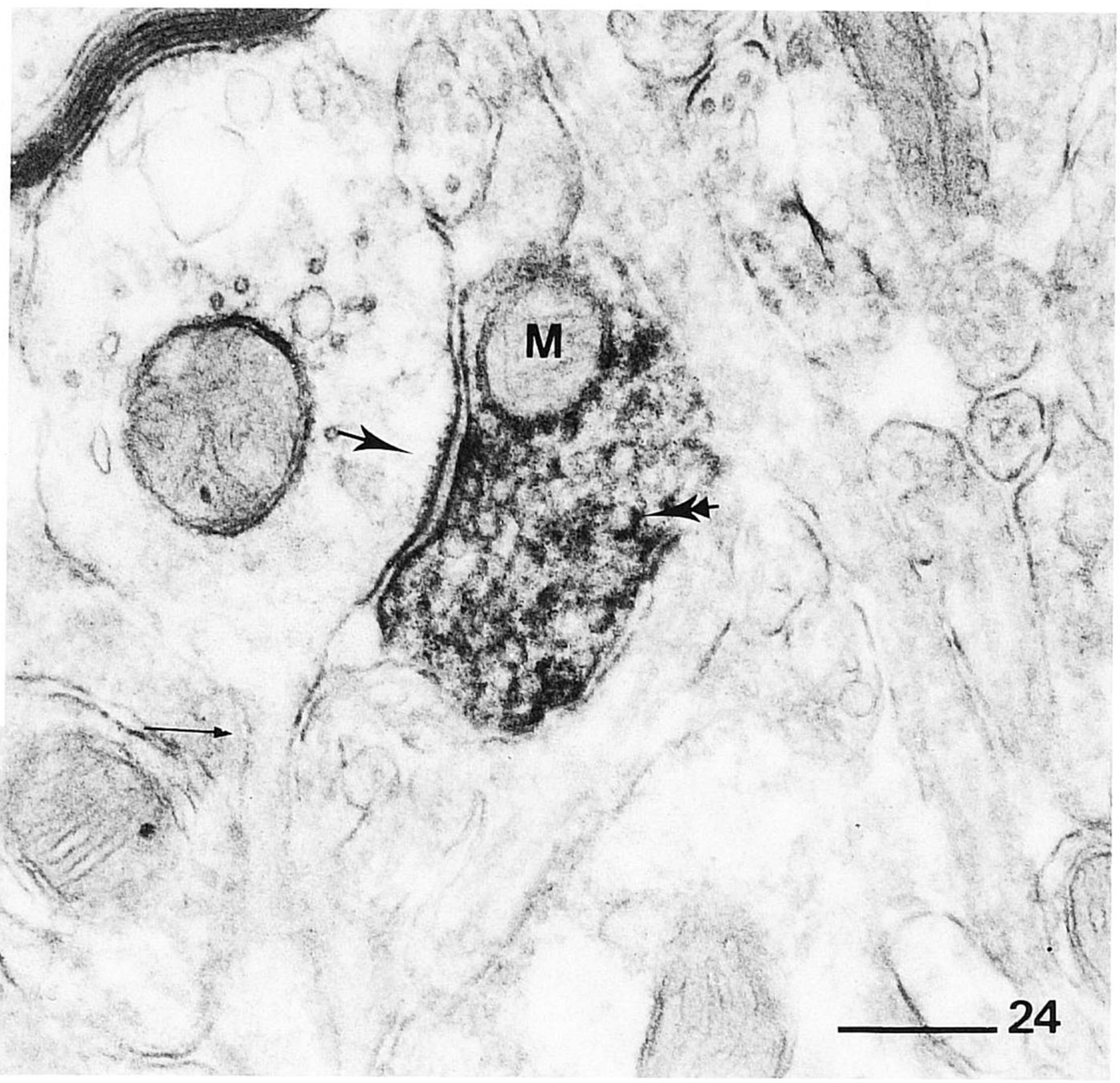


Fig. 24. DA terminal in the olfactory tubercle establishes a synaptic contact (large arrow) with a dendritic shaft at a site where a spine is issued from the shaft (small arrow). DAB precipitate is present around small vesicles (double arrow) and around a mitochondrion (M). Bar=250 nm.

sity or more densily populated areas, which are called cell clusters. Cell clusters are found in nearly all regions of the nucleus accumbens (Chronister et al., '81; Domesick, '81; Herkenham et al., '84). Other histological features such as the distribution of opiate receptors, acetylcholinesterase activity, and thalamic afferents have been shown to be in register with compartments consisting of cell clusters (Herkenham et al., '84). Therefore, whether DA patches are related to cytoarchitectonics and distributional patterns of other neurotransmitters throughout the ventral striatum is currently under investigation (Voorn et al., '85).

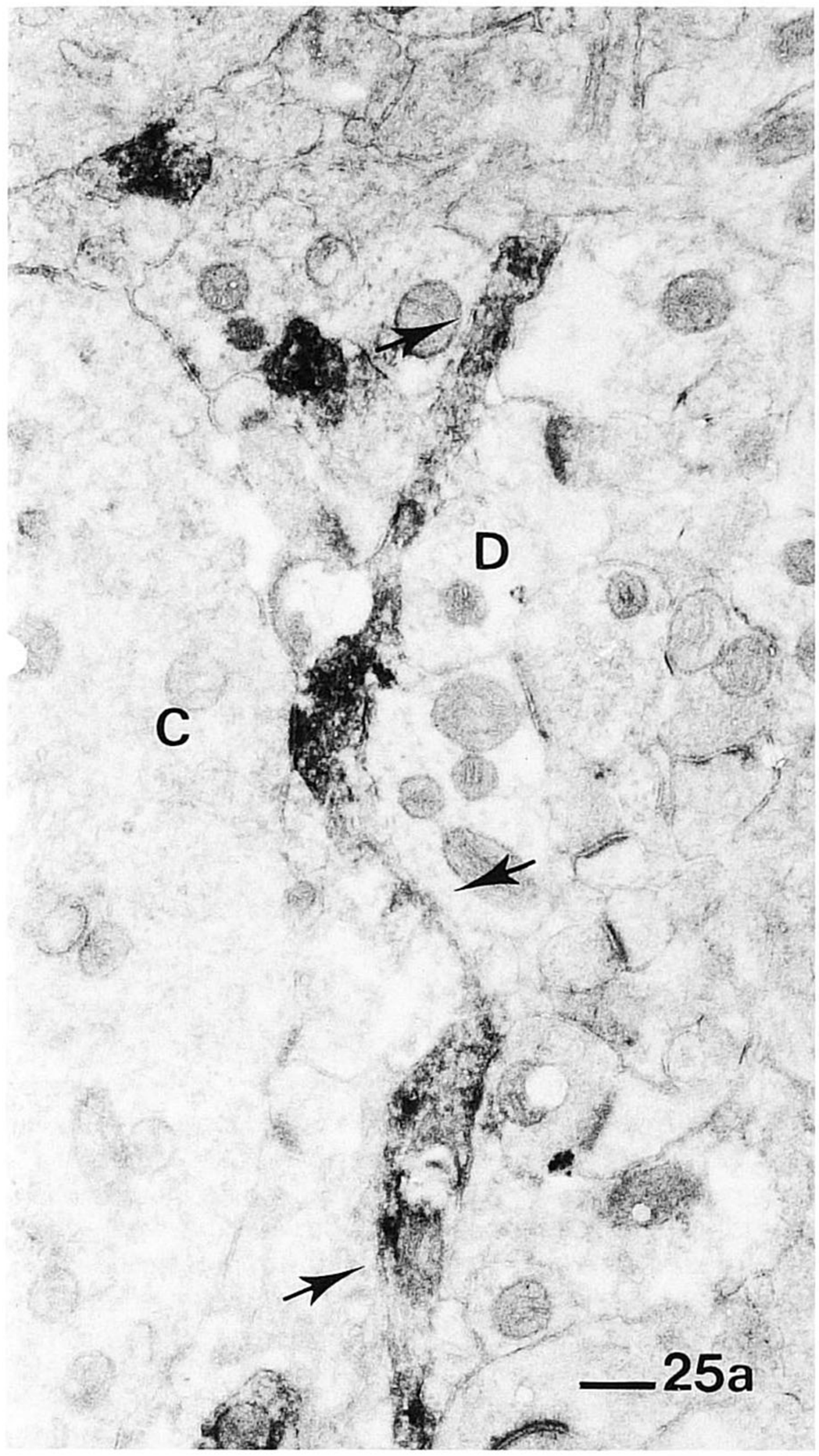
The fact that the dendritic arborizations of cells belonging to the morphological unit of a cell cluster or histochemical compartment (as defined by the presence of leu-enkephalin, Penny et al., '84) are detained within the boundaries of the respective compartments suggests that the segregated character of incoming information is preserved during processing (Herkenham et al., '84; Penny et al., '84). In this respect it is of great interest to note that the vast majority of the observed DA terminals in the ventral striatum make synaptic contacts with dendrites.

At the electron-microscopic level it is quite difficult to discern whether membrane specializations are actually present in DA profiles, because DAB precipitate on the plasmalemma of immunopositive profiles often obscures or, conversely, suggests the presence of synaptic features. This is a specific problem with respect to the rather inconspicuous symmetric specializations. Although several previous reports and the present one agree that, with few exceptions, DA terminals in the striatum display symmetric membrane specializations, the identification problems may account for the discrepancies in quantitative data on the frequency of the occurrence of synaptic relations and features (cf. Arluison et al., '84; Bouyer et al., '84a; present study). Serial sectioning may to some extent provide a more solid basis for objective identification criteria (see also Freund et al., '84). It can be inferred from the results of the present experiments in which this procedure was employed that heavily labeled membranes do not necessarily indicate the presence of a synapse. In single sections only clear-cut morphological features may qualify.

Fig. 25. DA axon in the nucleus accumbens (arrows in a) synapses on a dendrite (D, arrow in b) and a cell body (C, arrow in c). Bar=250 nm.

Fig. 26. DA terminal in the nucleus accumbens establishes two asymmetric contacts. Bar=250 nm.

Fig. 27. DA terminal in the nucleus accumbens establishes a symmetric synaptic contact (large arrow) with a dendritic spine, which also receives unlabeled, asymmetric input (small arrow). Bar=250 nm.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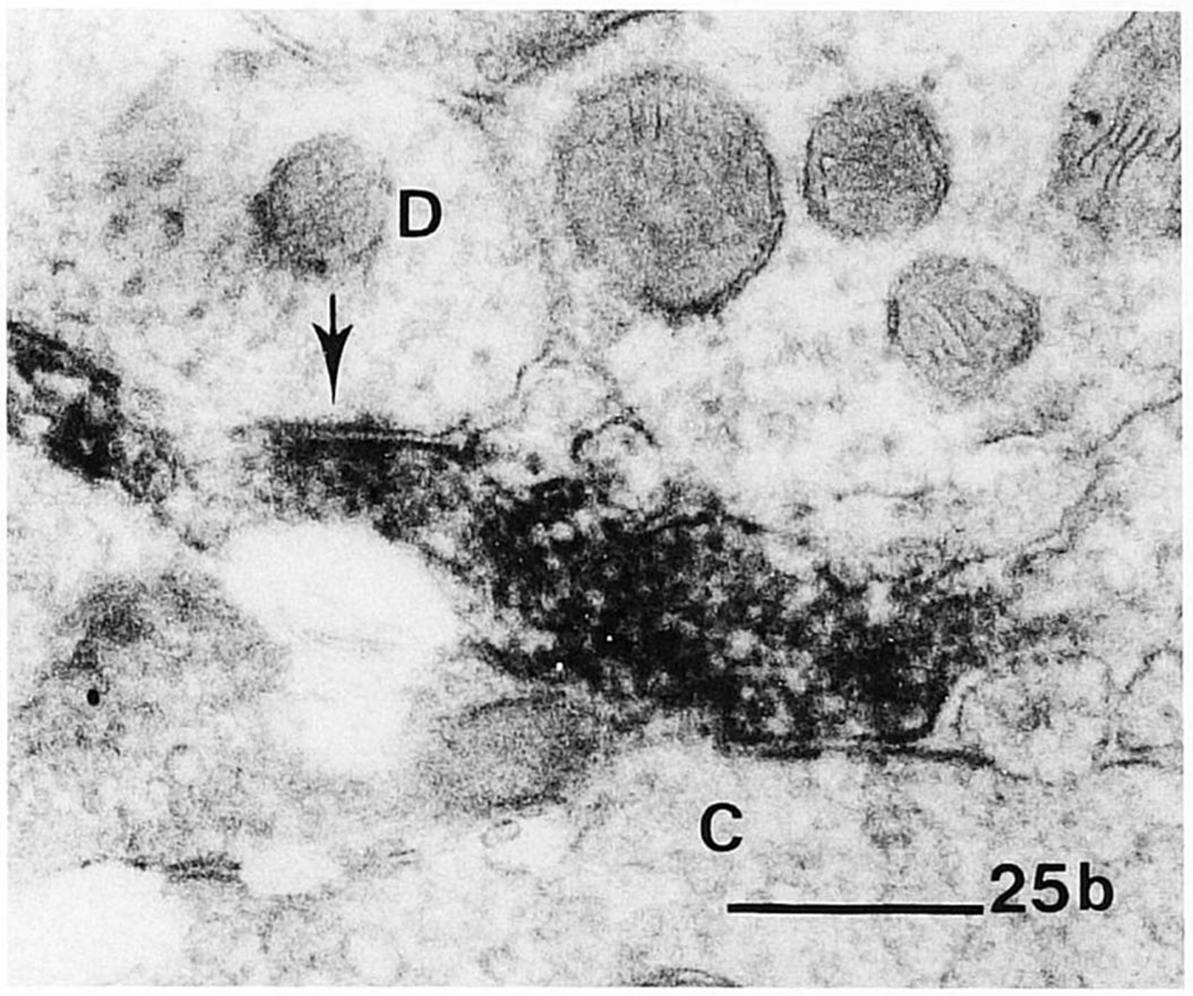
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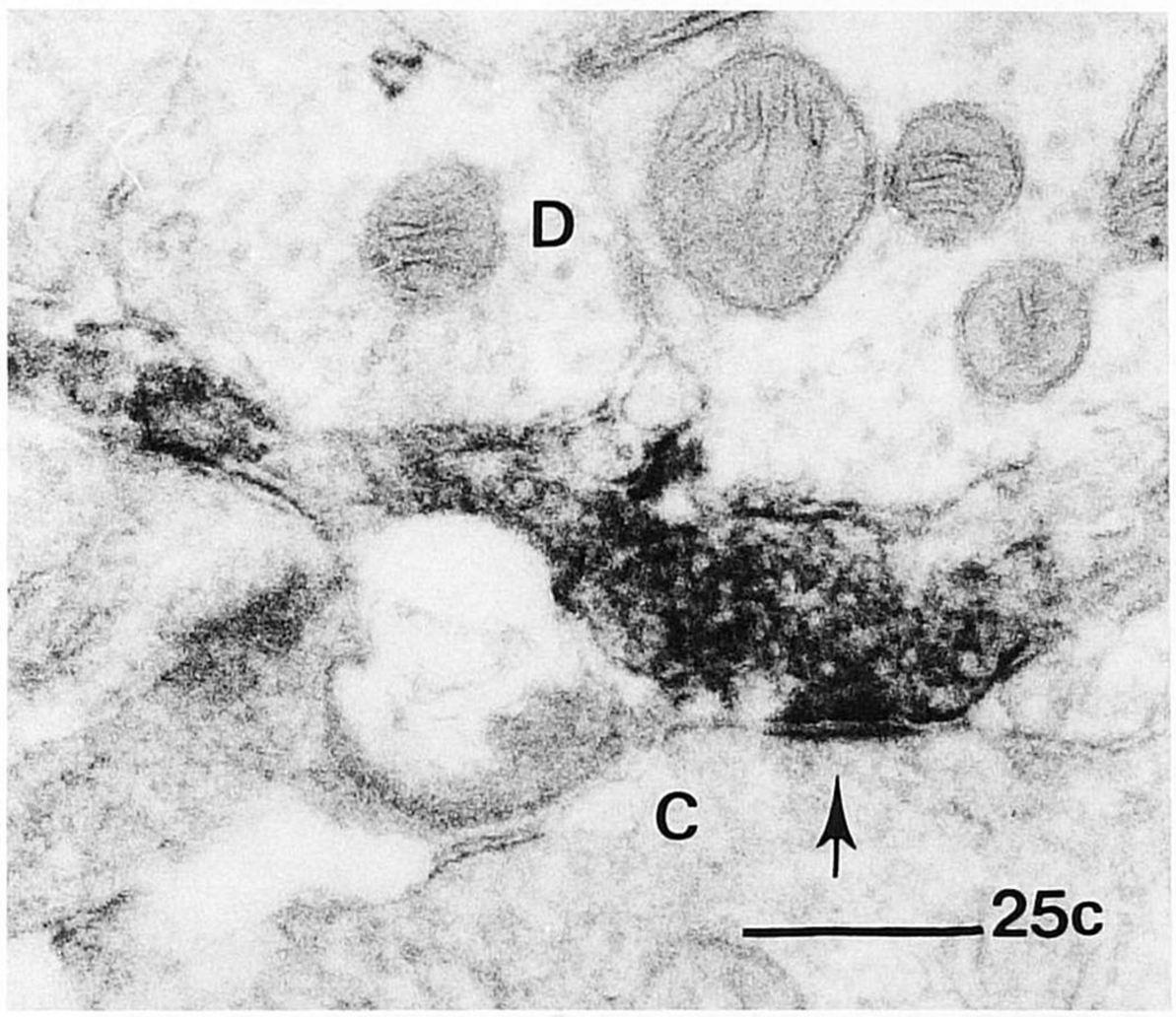
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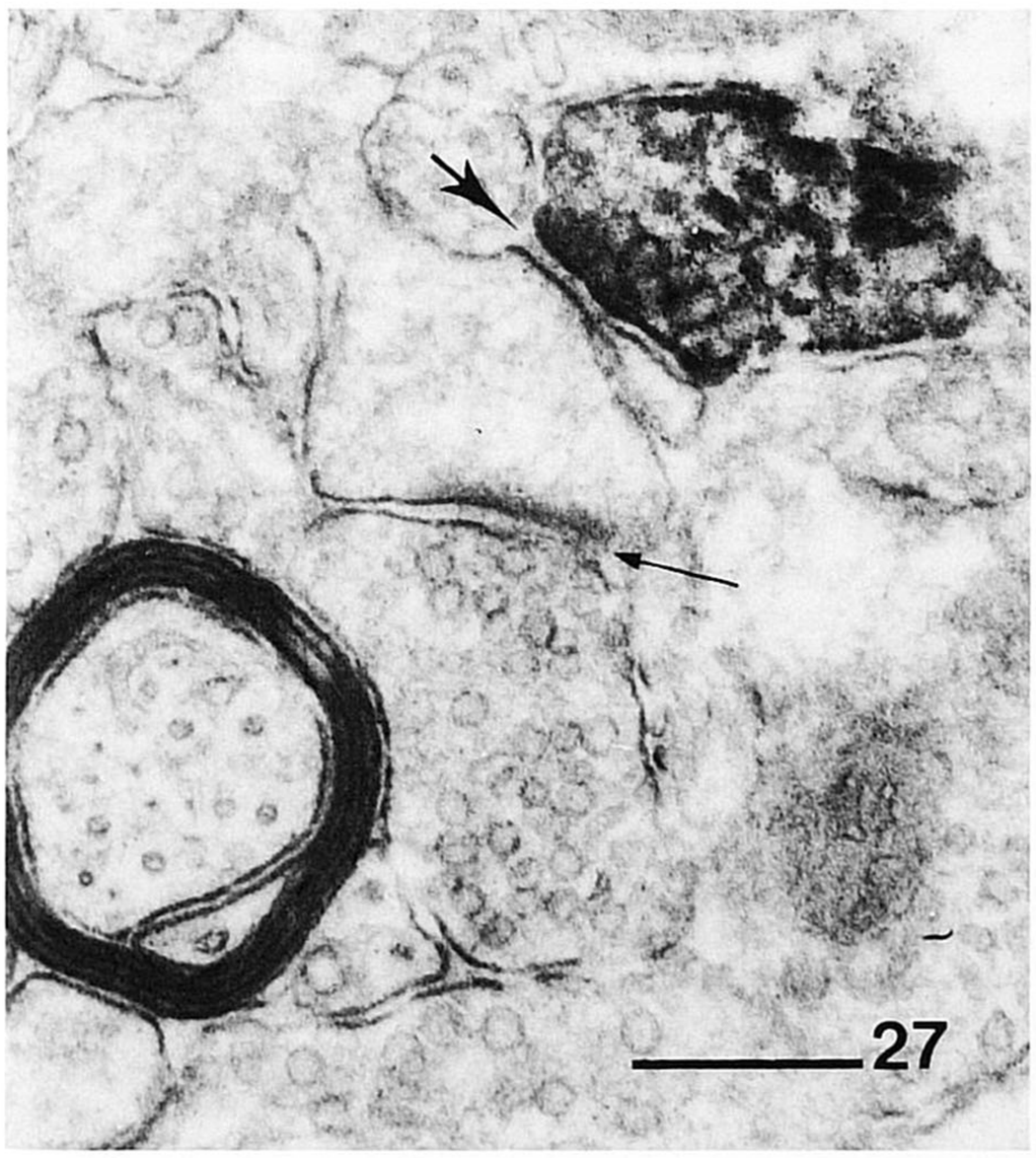
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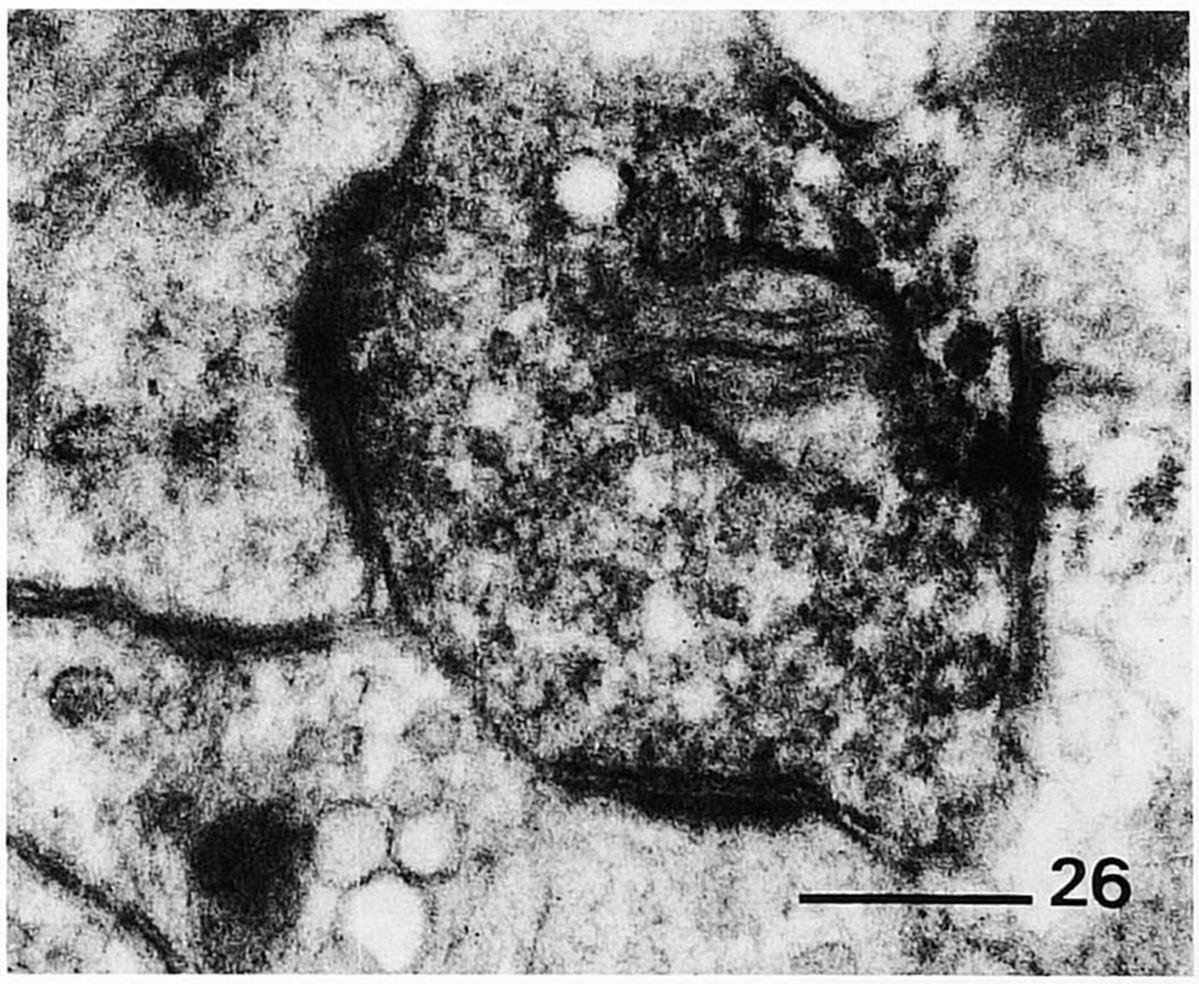
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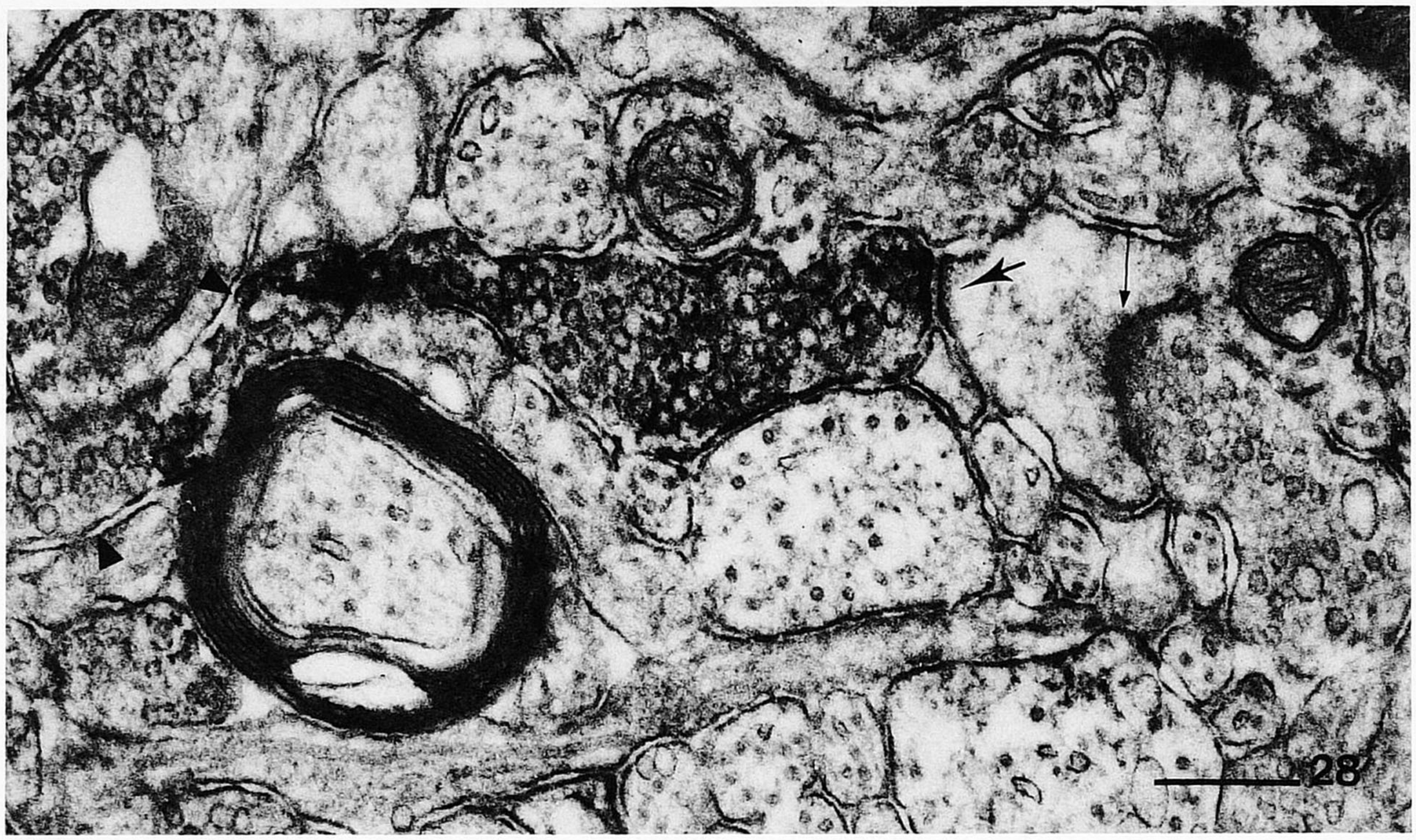

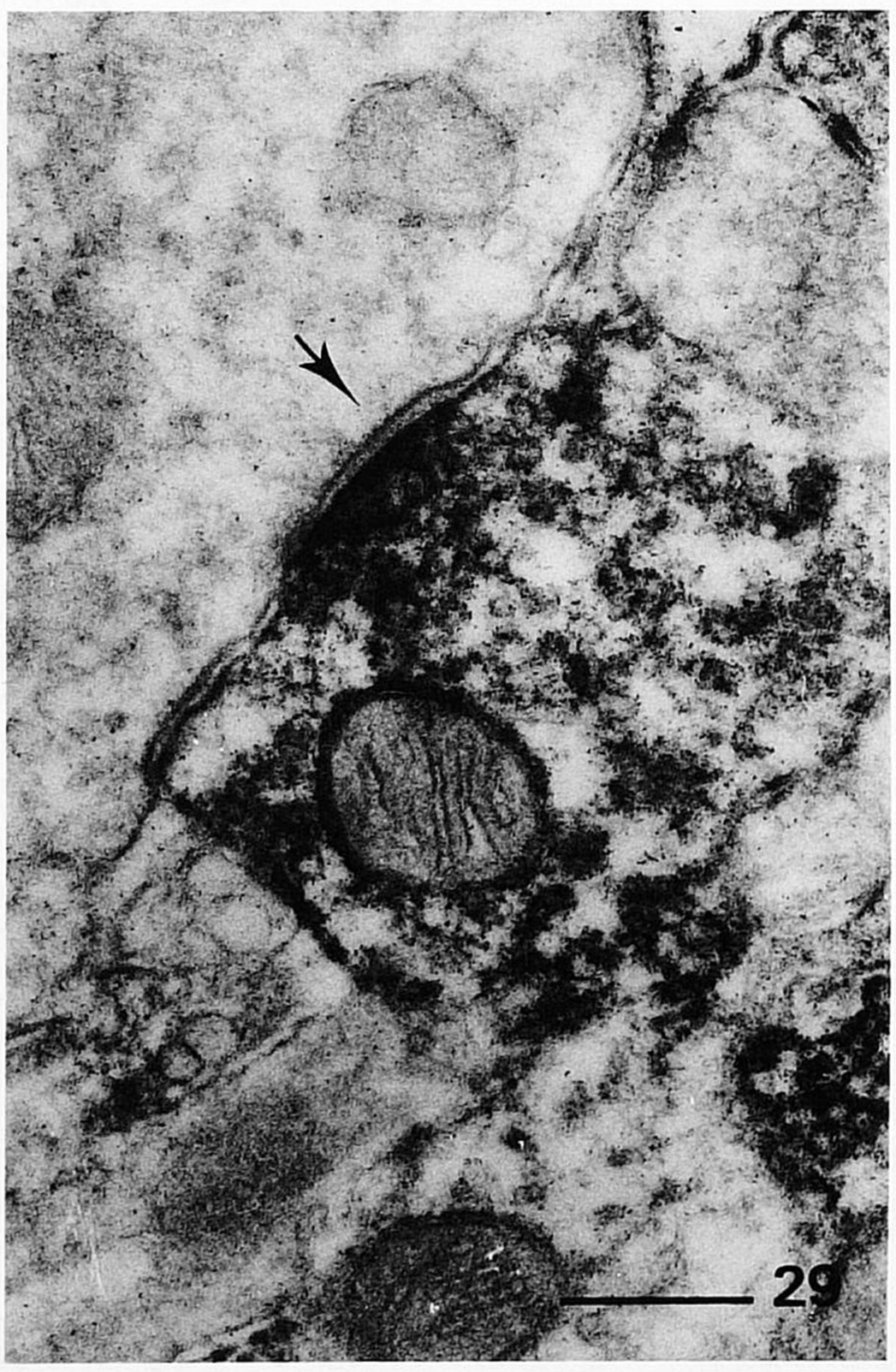

Fig. 28. DA terminal in the olfactory tubercle contacts a dendritic spine (large arrow) that also receives unlabeled input (small arrow). Note that the DA axon is only partly labeled (arrowheads). Bar=250nm.

Fig. 29. DA terminal establishes an axosomatic contact (arrow) in the polymorph cell layer of the olfactory tubercle. Bar=250 nm.

In contrast to the observation that tyrosine-hydroxylase-immunoreactive fibers in the caudate-putamen mainly establish synaptic contacts in thin fiber parts (Freund et al., '84), it was presently found that in the nucleus accumbens thin fibers as well as varicosities exhibit synaptic specializations. Another difference between the dorsal and the ventral striatum is noted at the light-microscopic level. The ventral striatum contains DA fibers with more and larger varicosities compared to the delicate, finely varicosed fibers in the caudate-putamen (Lindvall and Stenevi, '78; present study). The aforementioned discrepancies may reflect a different origin of the fibers in the two striatal regions.

The DA fibers and varicosities generally exhibit the same ultrastructural features as described for tyrosine-hydroxylase-immunoreactive profiles in the dorsal and the ventral striatum. The terminals are packed with round or flattened vesicles, whereas large, labeled granula are only occasionally encountered (Pickel et al., '81; Arluison et al., '84; Bouyer et al., '84a; Freund et al., '85; present study). Yet, some of the DA profiles contain several large, unlabeled vesicles. A plausible explanation for this phenomenon might be that these fibers constitute a DA fiber contingent in which the amine is colocalized with a neuropeptide, possibly cholecystokinin, located in the large granula (Hökfelt et al., '80; Arluison et al., '84; Bouyer et al., '84a). However, the fact that the ultrastructure of cholecystokinin-positive fibers in the nucleus accumbens does not resemble that of the aforementioned profiles does not seem to support this hypothesis (Baali-Cherif et al., '84). The present technique does not allow precise recording of antigenic sites because of the diffusion of the reaction product (Novikoff et al., '72). It is, therefore, not permitted to claim that DA is actually absent from either large or small granula. This restriction of the technique is reflected also in the minor differences in the subcellular localization of DAB precipitates in profiles immunostained for such various substances as amines, amino acids, or peptides (e.g., Ribak et al., '81; Voorn and Buijs, '83; Buijs et al., '84).

The ultrastructural localization of reaction product found in the present study is similar to that described in previous experiments with DA antisera (Buijs et al., '84; Onteniente et al., '85). However, large granular vesicles, present in some DA profiles in the hypothalamus or the lateral septum, are absent from DA profiles in the ventral striatum. Besides, in the former regions DA synapses on dendrites are of the asymmetric type, which is rarely found in the striatum. Although symmetric synapses are present on cell bodies in all three mentioned regions, the frequency of occurrence of perikaryal input is much higher in the hypothalamus and the septum than in the ventral striatum. It may be concluded that DA input to the hypothalamus and the septum is more strongly directed to cell bodies, whereas in the striatum dendrites are the main postsynaptic targets. This arrangement may reflect the modulatory function of DA on the cortical input to the striatum and its output to motor-associated areas, as opposed to a more direct influence of DA on neurosecretion (e.g., Moos and Richard, '82).

From the results of studies on the ultrastructural morphology of tyrosine-hydroxylase-containing profiles in the dorsal and the ventral striatum it can be concluded that there are no essential differences in synaptic features and relations between the two regions (Arluison et al., '84; Bouyer et al., '84a; Freund et al., '84). The present results corroborate these findings: DA input to the dorsal and the ventral striatum converges predominantly upon dendrites, mainly through symmetric synapses. Moreover, it was frequently observed that postsynaptic dendritic spines are simultaneously contacted by unlabeled axons. Similar configurations have been noted to occur in relation to identified striatonigral cells in the caudate-putamen by Freund and co-workers ('84). These authors hypothesize that the unlabeled axons originate from cortical areas (Somogyi et al., '81; Bouyer et al., '84b). Such an arrangement would allow dopaminergic modulation of excitatory cortical input, using glutamate or aspartate as a neurotransmitter (Fonnum et al., '81). Electrophysiological studies demonstrate that iontophoretically applied DA in the striatum inhibits the spontaneous and cortically evoked firing of striatal cells (Herrling and Hull, '80; Brown and Arbuthnott, '83). Furthermore, pharmacological studies indicate that neocortical regions influence extrapyramidal motor behavior, associated with dopaminergic neurotransmission in the striatum (Glick and Greenstein, '73; Scatton et al., '82). The same lines of evidence exist concerning the ventral striatum and allocortical regions (De France et al., '81; Walaas, '81; Isaacson, '84; Lopes da Silva et al., '84; Yang and Mogenson, '84). In view of these similarities it is tempting to entertain the possibility that the morphological substrate for DA modulation in the ventral striatum resembles to some extent that in the dorsal striatum. However, more anatomical evidence will be needed to substantiate this notion.

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