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Dendritic Transport

II. Somatofugal Movement of Neuronal Lysosomes Induced by Colchicine: Evidence for a Novel Transport System in Dendrites[1]

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Abstract

The effect of colchicine injections on the ultrastructural localization of dipeptidyl peptidase II (Dpp II) was studied in the mitral cells of the rat olfactory bulb. In control animals, electron-dense reaction product representing Dpp II activity was observed in lysosomes, lipofuscin granules, short cisternae located close to the granular endoplasmic reticulum, and dense granules. Lysosomes and lipofuscin granules were the most intensely stained organelles. Dpp II-containing organelles were localized mainly to the cell body and were randomly distributed in the perikaryal cytoplasm.

Twenty-four hours after a 100-μg intracerebroventricular colchicine injection, the distribution of Dpp II-containing organelles was drastically altered. Short cisternae and dense granules containing Dpp II reaction product were noticeably absent in these preparations. Lysosomes and lipofuscin granules were depleted from the perikaryal cytoplasm and were concentrated in dendrites. Lysosomes were observed to extend for considerable distances in dendrites where they acquired elongated and dumbbell shapes. The shapes of some of these labeled lysosomes gave the impression that they were actively being "pulled" into the dendrites. These results indicate that microtubules sequester lysosomes to the perikaryal cytoplasm and suggest the presence of a novel transport system responsible for the movement of lysosomes from the cell body to the dendrites.

The sequestration of the protein synthetic machinery to the soma of neurons necessitates a system to transport newly made proteins to distal regions in the axon and dendrites. Cellular organelles also must undergo directed locomotion to reach distal areas in the axon and dendrites. Cytoskeletal elements such as microtubules, neurofilaments, and microfilaments have been recognized as playing important roles in maintaining the integrity and spatial localization of organelles, as well as directing their movements. For example, the axonal transport of neurotransmitter vesicles is blocked when cells are treated with microtubule inhibitors like colchicine (McClure, 1972; Gray, 1975), or when microfilaments are destroyed by microinjections of DNAse (Goldberg et al., 1980; Isenberg et al., 1980). Also, the integrity of the Golgi apparatus is disrupted when cells are treated with microtubule inhibitors (Wehland et al., 1983).

Evidence for the direct association of organelles with cytoskeletal elements has been obtained both by light and electron microscopy. Direct interactions have been observed between microtubules and the following organelles: synaptic vesicles (Smith et al., 1970), mitochondria (Smith et al., 1977; Heggeness et al., 1978; Johnson et al., 1980), cell nuclei (Holmes and Choppin, 1968), coated vesicles (Imhof et al., 1983), and lysosomes (Collot et al., 1984). Microfilaments have been reported to interact with coated vesicles and lysosomes (Moore et al., 1976; Salisbury et al., 1980; Linden et al., 1981; Mesland et al., 1981); and intermediate filaments have been reported to interact with cell nuclei (Blose, 1979).

In the preceding light microscopic study (Gorenstein et al., 1985), we demonstrated with histochemical methods that colchicine produces a paradoxical redistribution of dipeptidyl peptidase II (Dpp II) and acid phosphatase. Normally, these two enzymes are concentrated in the perikarya of neurons with relatively little staining observed in dendrites (Koenig, 1969; Gorenstein et al., 1981). Intracerebroventricular injections of colchicine induce a paradoxical movement of these enzymes from cell perikarya to the dendrites, suggesting that microtubules are involved in sequestering these enzymes to the cell body.

The goals of the present ultrastructural study were to determine: (*1*) the identity of neuronal organelles which contain Dpp II and (*2*) the effect that colchicine has upon the intracellular distribution of Dpp II-containing organelles. The olfactory bulb was chosen for these studies because it has a well laminated structure and an intensely Dpp II-stained cell type, the mitral cell.

Materials and Methods

Male Sprague-Dawley rats (150 to 200 gm) were placed on a stereotaxic apparatus while under deep pentobarbital anesthesia. A microsyringe was placed with its tip in the lateral cerebral ventricle using coordinates obtained from the atlas of Koning and Klippel (1963). A solution containing 100 μg of colchicine dissolved in 10 μl of saline was injected slowly. Control animals received 10-μl injections of saline. Animals were allowed to recover for 24 hr, and their brains were processed for electron microscopic histochemistry as described below.

Histochemical localization of Dpp II. The histochemical protocol which we used to localize Dpp II at the electron microscopic level is a modified protocol from that described by Smith and Van Frank (1975). Briefly, rats were deeply anesthetized with pentobarbital and perfused intracardially at a pressure of 100 mm Hg with normal saline for 2 min followed by ice-cold, freshly made 4% paraformaldehyde-0.4% glutaraldehyde in 50 mM sodium phosphate, pH 7.0, for 30 min. The brains were removed, cut along the midline, and glued to a Vibratome stage with cyanoacrylate glue. A 2% solution of low-temperature agarose (FMC Corporation) at 37°C was poured

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over the brain and allowed to solidify at room temperature. Sagittal sections, 50 μm thick, were cut through the olfactory bulb with a Vibratome. Brain slices were stained for Dpp II activity by incubating them at 37°C in a solution containing 1 mg/ml of lysyl-alanyl-4-methoxy-β-naphthylamide (Lys-Ala-MNA), 0.1 mg/ml of hexazotized *p*-rosaniline (Lojda et al., 1979), 0.1 M sodium acetate, pH 5.0, for 30 min. Sections were rinsed in 2% sucrose, postfixed in 4% paraformaldehyde and 0.4% glutaraldehyde for 30 min and treated with 1% thiocarbohydrazide for 30 min at 37°C. Sections were then postfixed with 2% osmium tetroxide for 20 min followed by dehydration in a series of graded alcohols and embedding in Araldite.

Semithin, 1-μm sections were cut from these embedded specimens with glass knives on a Reichert ultramicrotome. The mitral cell layer was identified in these sections and the block was trimmed for thin sectioning to include this area. Thin sections were cut with a diamond knife, mounted on Formvar-coated 1 × 2 mm slot grids, and stained with uranyl acetate and lead citrate. All grids were examined with a Philips 300 electron microscope. Control tissue included brain sections incubated in the absence of the substrate Lys-Ala-MNA, or in the absence of hexazotized *p*-rosaniline. In addition, a few animals were perfused with a stronger fixative that contained 4% paraformaldehyde and 2% glutaraldehyde and which is typically used to demonstrate the normal distribution of intracellular organelles (Palay and Chan-Palay, 1974).

Results

Ultrastructure of mitral cells. The normal ultrastructure of the mitral cell was studied in tissue sections obtained from animals perfused with a stronger fixative as described by Palay and Chan-Palay (1974). In these preparations, the somata of mitral cells were identified at the interface between the granule cells and the external plexiform layer (Price and Powell, 1970a, b; Shepherd, 1970). Briefly, these cells have large, round centrally located nuclei and an organelle-rich cytoplasm with numerous cisternae of endoplasmic reticulum and many well developed Golgi complexes (Fig. 1). Lysosomes, mitochondria, microtubules, and other organelles are evenly distributed throughout the perikaryal cytoplasm. Proximal dendrites are frequently observed to arise from the portion of the cell body that is adjacent to the external plexiform layer.

Dpp II localization in saline-injected control preparations. The localization of Dpp II reaction product was studied in the mitral cells of the olfactory bulb. A compromise between adequate fixation and maintenance of enzymatic activity was required for these experiments because of the sensitivity of Dpp II to aldehyde fixatives. Therefore, the ultrastructural integrity of neurons in these sections treated for Dpp II histochemistry was generally poorer than that seen for the mitral cells shown in Figure 1. In semithin sections, the granular reaction product was found throughout the large rim of the perikaryal cytoplasm that is characteristic of this neuronal type. All mitral cells in the mitral cell layer displayed this staining pattern as well as some tufted cells in the overlying external plexiform layer. The granule cells as well as neuropil elements were unstained.

In thin sections, the Dpp II reaction product was localized to mitral cells and was found in lysosomes, lipofuscin granules, short cisternae located close to the granular endoplasmic reticulum, and dense granules (Figs. 2 to 6). The most intense staining appeared in lysosomes and lipofuscin granules. Most of these Dpp II-containing organelles were located between the granular endoplasmic reticulum and the cisternae and vesicles of the Golgi complex (Fig. 4). These latter two structures did not contain Dpp II reaction product. By contrast, only a few granule cells displayed a labeled lysosome, whereas most were unstained.

The most unusual organelles that were labeled for Dpp II were the short cisternae (Figs. 4 and 5). Some of these labeled cisternae appeared as C-shaped structures adjacent to the Golgi complex. The area encircled by such short cisternae was unstained. Other labeled cisternae formed ringlet structures that were composed of multiple layers of membranes with associated electron-dense Dpp II reaction product (Fig. 5). The cores of these ringlets were unstained. The sizes of these spiralling lamellae or ringlets were similar to and slightly larger than the size of the C-shaped short cisternae.

The labeled lysosomes were larger in size than the short cisternae and ringlet structures. Most lysosomes had Dpp II reaction product evenly distributed within their borders. However, some lysosomes showed paler stained regions. In some cases, the pale region appeared on the periphery of the labeled lysosome (Fig. 2). The association of the pale regions with the limiting membrane of lysosomes suggests that these were in the process of being extruded from the lysosome.

The dendrites of mitral cells were examined for lysosomes and other Dpp II-labeled structures and were rarely found to contain stained structures. This result is consistent with the light microscopic observation of non-colchicine-treated preparations (Gorenstein et al., 1981, 1985).

Control sections incubated in the absence of substrate displayed the typical osmiophilic, electron-dense material within lysosomes. However, ringlets and other short cisternae were not stained and were noticeably absent in these preparations.

Dpp II localization in colchicine-injected preparations. The localization of Dpp II in mitral cells and proximal dendrites was examined in sections from rats that received intraventricular colchicine injections. Two major findings were observed in these preparations that differed from the normal localization: (*1*) Dpp II-containing organelles displayed a polarity in terms of their distribution and (*2*) the types of labeled organelles were different in these colchicine-treated preparations.

In thin sections from colchicine-treated preparations, mitral cells were identified using the same criteria as described above for the normal preparation. The distribution of most intracellular organelles was unaffected. For example, the cisternae of the granular endoplasmic reticulum remained in their usual position located adjacent to the centrally located nucleus (Fig. 7). In addition, mitochondria were uniformly distributed throughout the cytoplasm and dendrites. In contrast to these unlabeled organelles, the organelles that contained Dpp II reaction product were concentrated at the base of the proximal dendrite (Fig. 7). Many of these labeled organelles were found extending into the dendrites a considerable distance (30 to 70 μm). In contrast, the portion of the mitral cell body that was adjacent to the granule cell layer lacked Dpp II-containing organelles. Thus, a polarity of these labeled organelles was induced in colchicine-treated preparations.

Not only were the Dpp II-containing organelles redistributed in the mitral cells, but some of the types of labeled organelles were different from those found in the normal preparations. Lysosomes and lipofuscin granules contained Dpp II-reaction product, but they were reduced in size and number. In addition, the shapes of many lysosomes were changed from a round or circular shape to an elongated or rectangular appearance. Some lysosomes appeared as tear-drop shapes with their apices directed toward the proximal dendrite (Fig. 9). Others were shaped like dumbbells, and these more elongated Dpp II-labeled organelles were located further along the dendrite (Fig. 10). Some label was also observed in cisternae of the agranular endoplasmic reticulum found in the dendrites.

It is interesting to note that ringlets and C-shaped labeled structures were noticeably absent from these colchicine-treated preparations. Also, the dosage of colchicine (100 μg) that produced the redistribution of Dpp II-labeled organelles did not dramatically depolymerize microtubles because many appeared intact within the dendrites (Fig. 10).

Discussion

Ultrastructural localization of Dpp II. Within the mitral cells of the olfactory bulb, lysosomes are found randomly distributed throughout the perikaryal cytoplasm and are rarely observed in axons or dendrites (Fig. 1; see Holtzman, 1976; Peters et al., 1976). When these cells are stained histochemically for Dpp II activity, most of the reaction product is localized to lysosomes with some Dpp II localized to lipofuscin granules, short cisternae of the agranular reticulum, and dense-core granules. The cisternae of the agranular reticulum which contain Dpp II reaction product often form unusual structures. These

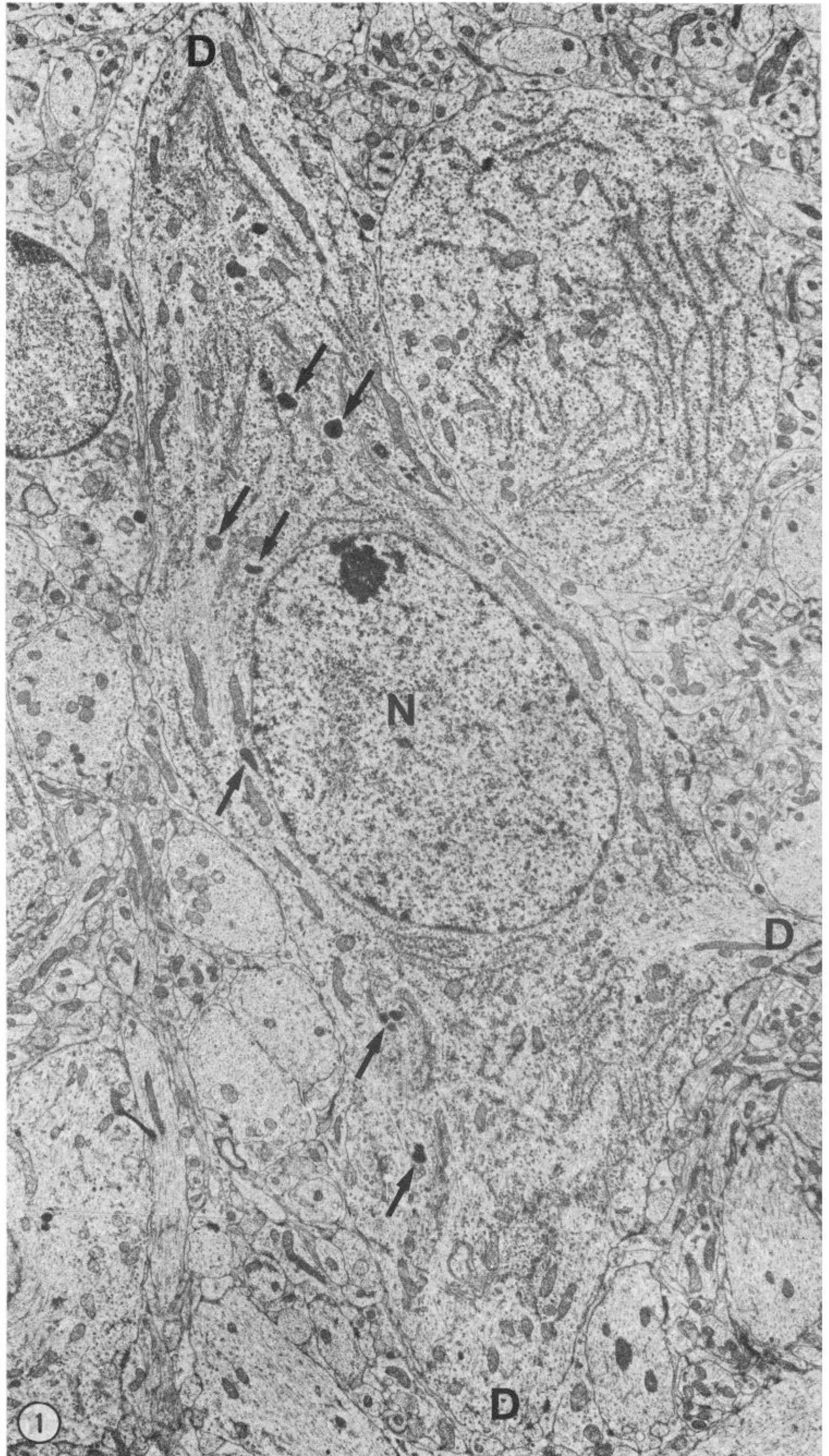


Figure 1. Electron micrograph of a mitral cell from the rat olfactory bulb obtained from a rat perfused with a routine fixative to optimize preservation of tissue. The nucleus (*N*) is centrally located in this triangular-shaped soma. The dendrites (*D*) extend from the soma into the adjoining neuropil of the external plexiform layer. The perikaryal cytoplasm contains the typical organelles, including lysosomes (*arrows*) that are distributed homogeneously. Magnification × 7,000.

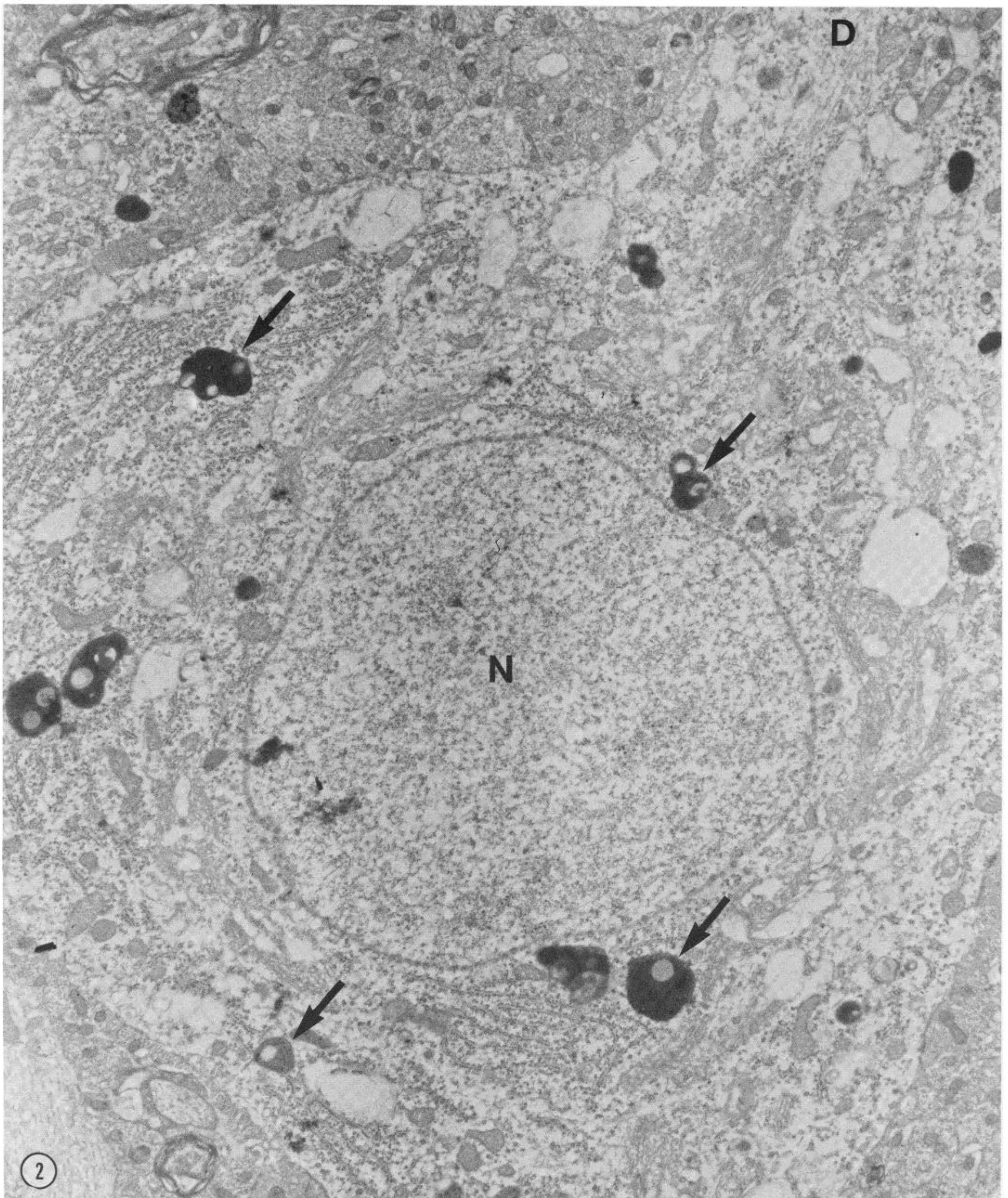


Figure 2. Electron micrograph of a mitral cell from a section treated for Dpp II histochemistry. Reaction product is contained in lysosomes and lipofuscin granules. Some of these (*arrows*) have lucid regions close to the limiting membranes. These Dpp II structures are evenly distributed in the cytoplasm around the nucleus (*N*). A portion of a dendrite (*D*) is also shown. Magnification × 15,000.

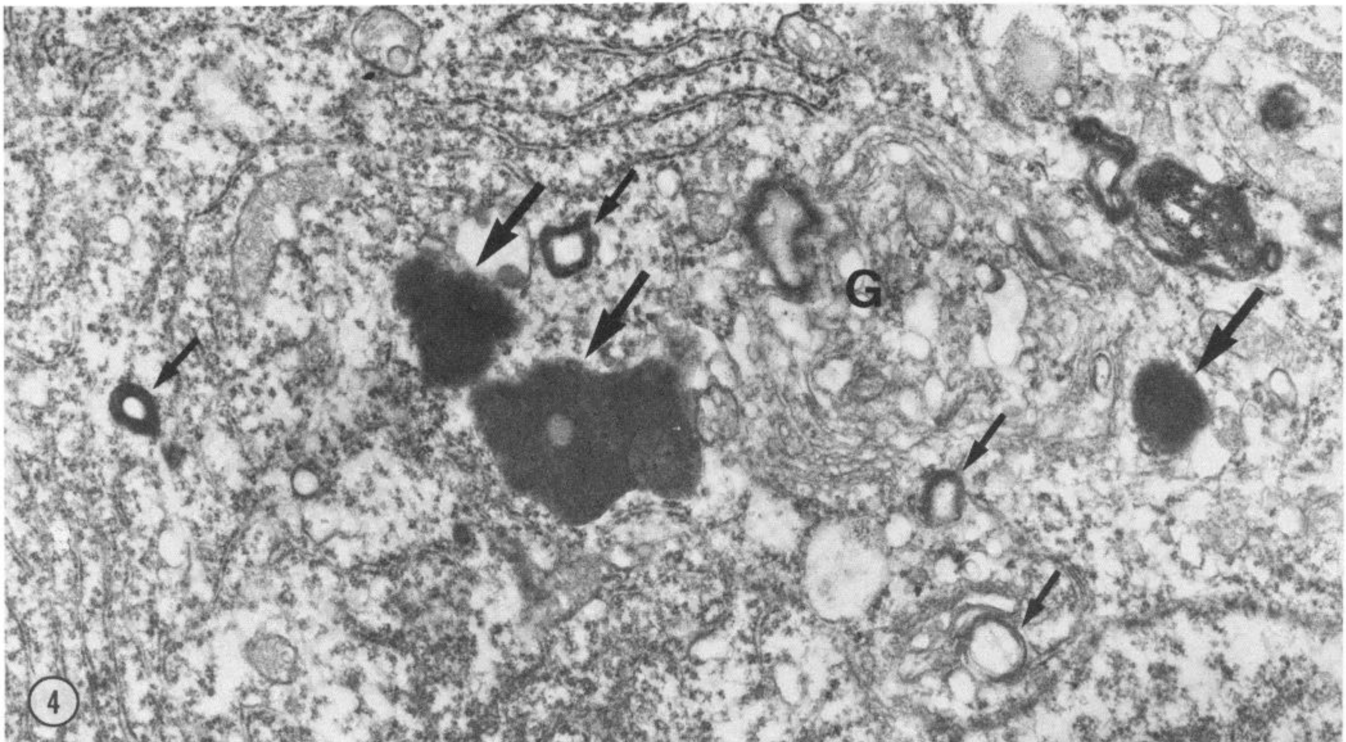
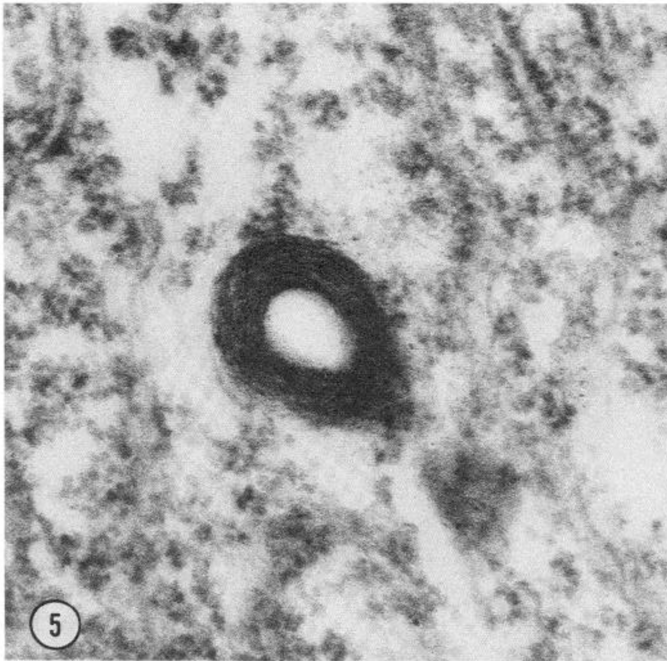


Figure 3. A mitral cell body located near a capillary (*C*) displays Dpp II reaction product in numerous organelles. The nucleus (*N*) is unstained. Magnification × 12,000.

Figure 4. Enlargement of the rectangular area in Figure 3 to show the variety of Dpp II-containing structures located adjacent to an identified Golgi complex (*G*). These structures include typical lysosomes (*large arrows*) as well as spiralling lamellae or ringlets (*small arrows*). Magnification × 26,000.



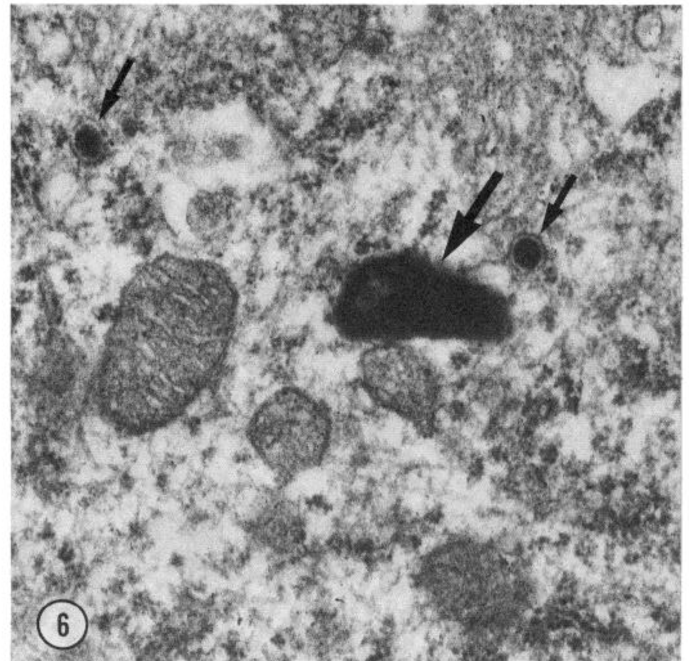


Figure 5. Enlargement of the Dpp II-containing ringlet from the *left side* of Figure 4. A lamellar structure with a hollow core is evident. Magnification × 50,000.

Figure 6. Electron micrograph of Dpp II reaction product within a lysosome (*large arrow*) and two dense-core granules (*small arrows*). Magnification × 36,000.

appear as ringlets or spiralling lamellae containing pale areas in their center, and are closely associated with the Golgi complex. These results confirm and extend previous findings indicating that Dpp II is a lysosomal enzyme (Smith and Van Frank, 1975; Sannes et al., 1979).

Somatofugal movement of lysosomes in the presence of colchicine. The most interesting finding of this study is colchicine's effect on the distribution of Dpp II-containing organelles. In the preceding paper (Gorenstein et al., 1985) we showed that intracerebroventricular injections of colchicine produce a rapid and striking redistribution of two lysosomal enzymes, Dpp II and acid phosphatase, from their normal localization in the perikaryal cytoplasm of neurons to the dendrites of these cells. The present electron microscopic study supports this finding and indicates that the movement of these enzymes is a result of a somatofugal translocation of lysosomes from the cell body to the dendrites (Fig. 11). Since labeled lysosomes were not observed in the axons of mitral cells or in the axons of trigeminal mesencephalic neurons that lack dendrites (see Gorenstein et al., 1985), we conclude that the redistribution of these organelles is directed specifically to the dendrites and not to axons.

In addition to a redistribution of enzymes, colchicine produces an apparent decrease in the total amount of Dpp II reaction product deposited in most brain regions. Biochemical studies indicated that this decrease is not the result of a direct inhibitory action of colchicine on Dpp II activity (Table I in Gorenstein et al., 1985). Instead, this decrease in staining may be accounted for, in part, by the drastic reduction in the observable number of Dpp II-containing ringlets and spiralling lamellae. The close association of these structures with the Golgi apparatus suggests that these ringlets and lamellae are intermediate stages in the formation of lysosomes; their absence in colchicine-treated preparations may indicate that their formation as well as that of lysosomes may be inhibited by colchicine. A related phenomenon has been previously described in other systems where colchicine disrupts microtubule-associated vesicle and enzyme transport from the Golgi apparatus to the plasma membrane (Hindelang-Gertner et al., 1976; Odam and Ikehara, 1981; Redman et al., 1981).

Following colchicine treatment, labeled lysosomes display striking changes in their shapes and sizes. For example, in control tissue, lysosomes are large and have rounded shapes, whereas in colchicine-treated animals they are smaller and assume elongated and dumbbell shapes. On occasion the lysosomes assume a "Hershey kiss" shape (Fig. 9), with the apex directed toward the proximal dendrite. The general impression obtained from these electron micrographs is that of lysosomes streaming away from the cell body and into the dendrites. It is of interest to note that other organelles such as mitochondria, the Golgi apparatus, or cell nuclei were insensitive to colchicine and did not show any drastic alterations either in size, shape, or location.

How does colchicine produce a redistribution of lysosomes? In neurons lysosomes are predominantly concentrated in the cell body, whereas axons and dendrites contain relatively few numbers of these organelles (Peters et al., 1976; Holtzman, 1969, 1976). Clearly, a mechanism exists which maintains this lysosomal asymmetry and at the same time permits small numbers of lysosomes to enter the axon and dendrites.

Previous studies have shown that microtubules are involved in the activity and function of lysosomes. Briefly, the secretion of lysosomal enzymes in polymorphonuclear leukocytes is affected by colchicine (Hoffstein et al., 1977). Also, the saltatory movement of lysosomes is dependent on the integrity of microtubules (Freed and Leibowitz, 1970; Wang and Goldman, 1978). Furthermore, the distribution of lysosomes in mouse macrophages and in fibroblasts overlaps the distribution observed for microtubules (Phaire-Washington et al., 1980; Collot et al., 1984). These data strongly suggest that a specific interaction of lysosomes with cytoskeletal elements regulates the movement and spatial distribution of these organelles in the cytoplasm.

The following hypothesis is proposed to explain the normal distribution of lysosomes in neurons and their somatofugal movement into dendrites following colchicine treatment. We propose that a network of microtubules directly interacts with lysosomes and sequesters them within the cell body, preventing their free diffusion into dendrites. The primary effect of colchicine is to disrupt this

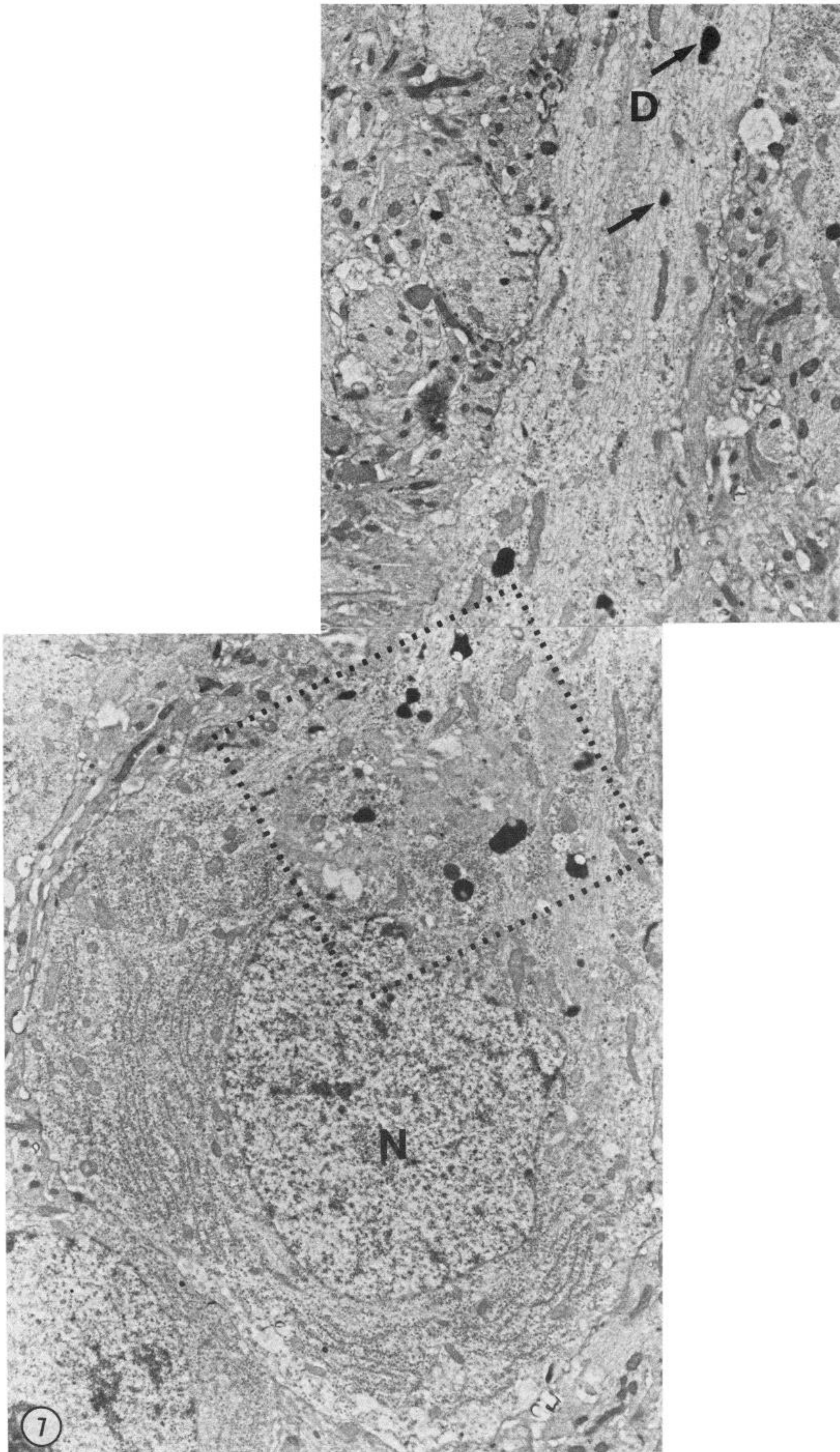


Figure 7. Montage of a mitral cell body and proximal dendrite from a colchicine-treated preparation. The distribution of most organelles in the cytoplasm about the nucleus (*N*) is normal (cf. Fig. 1). However, Dpp II-containing lysosomes and cisternae are concentrated in the soma on the side of the proximal dendrite (*boxed area*) and are absent on the side adjacent to the layer of granule cells (*lower left corner*). In addition, Dpp II-containing structures (*arrows*) appear in the proximal dendrite (*D*). Magnification × 9,000.

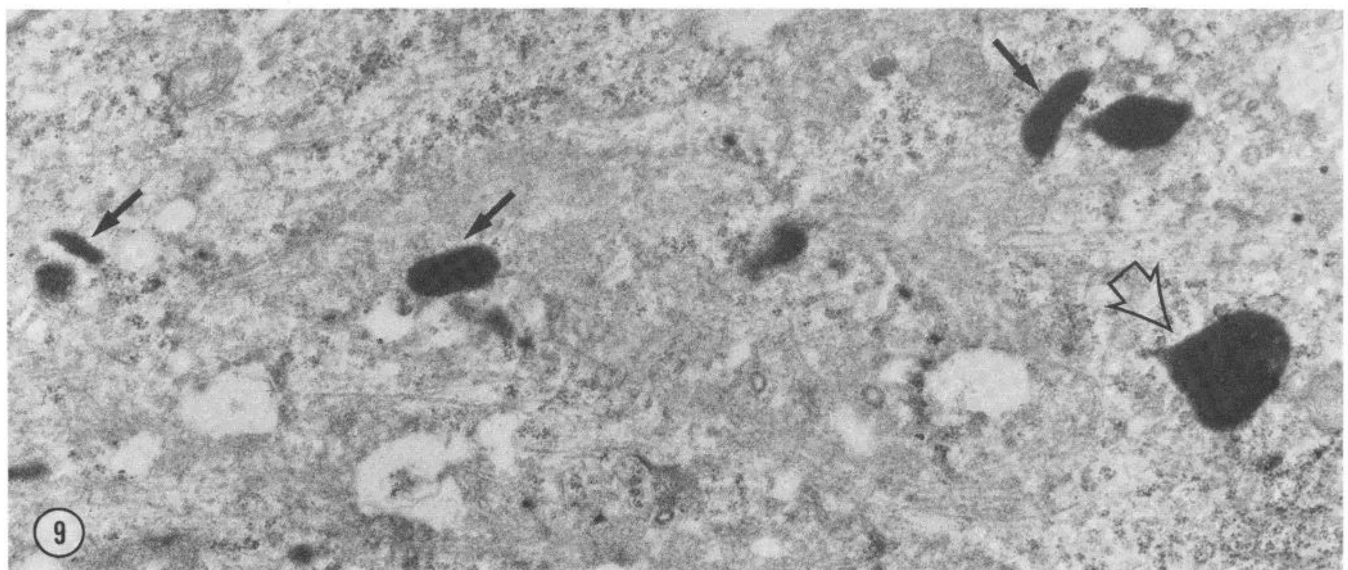
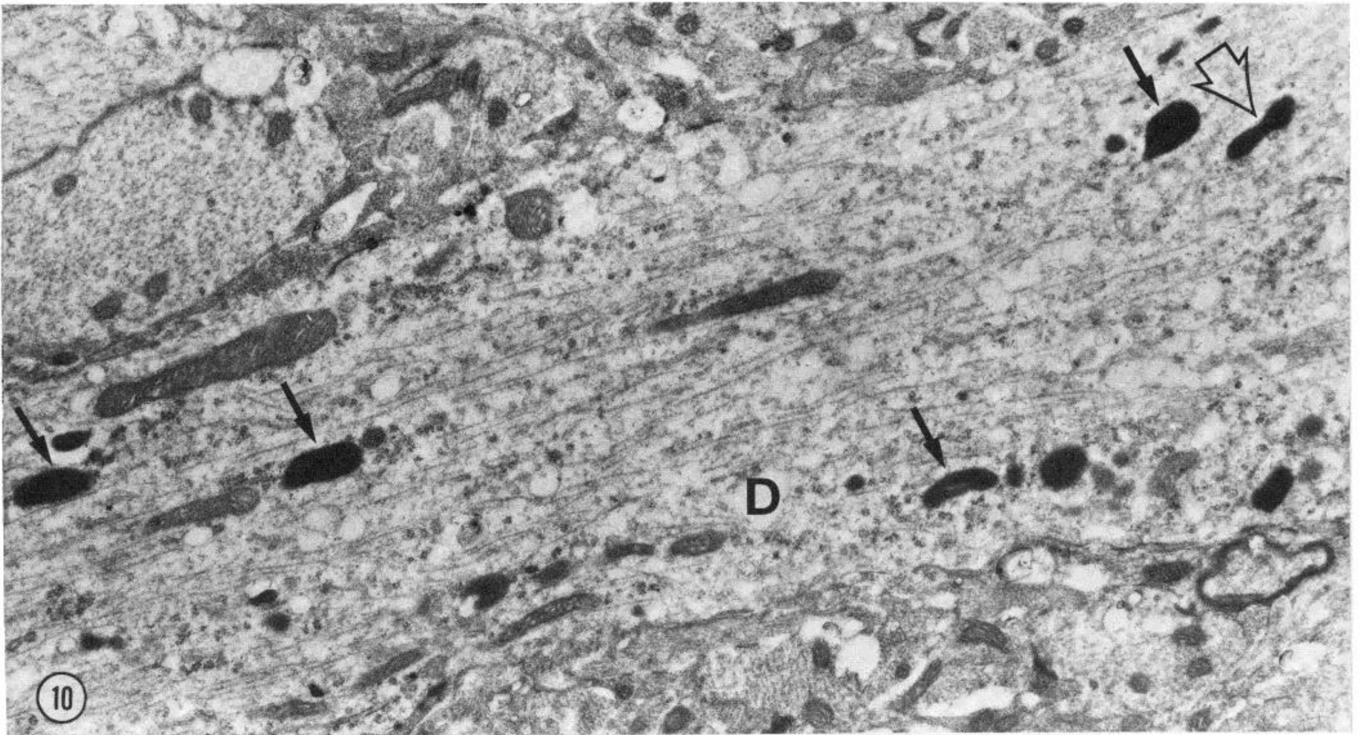


Figure 8. Enlargement of the *boxed area* of the mitral cell body in Figure 7. Dpp II-containing organelles include lysosomes (*large arrows*), dumbbell-shaped lysosomes (*open arrows*), and elongated cisternae (*small arrows*). Ringlets and spiralling lamellae were not observed in the vicinity of the Golgi complex (*G*). Magnification × 27,000.

Figure 9. Electron micrograph of another mitral cell body from a colchicine-treated preparation that shows Dpp II-containing lysosomes and elongated cisternae (*solid arrows*) at the base of a dendrite. One lysosome (*open arrow*) has an unusual shape with its apex pointing toward the dendrite (not shown). Magnification × 23,000.



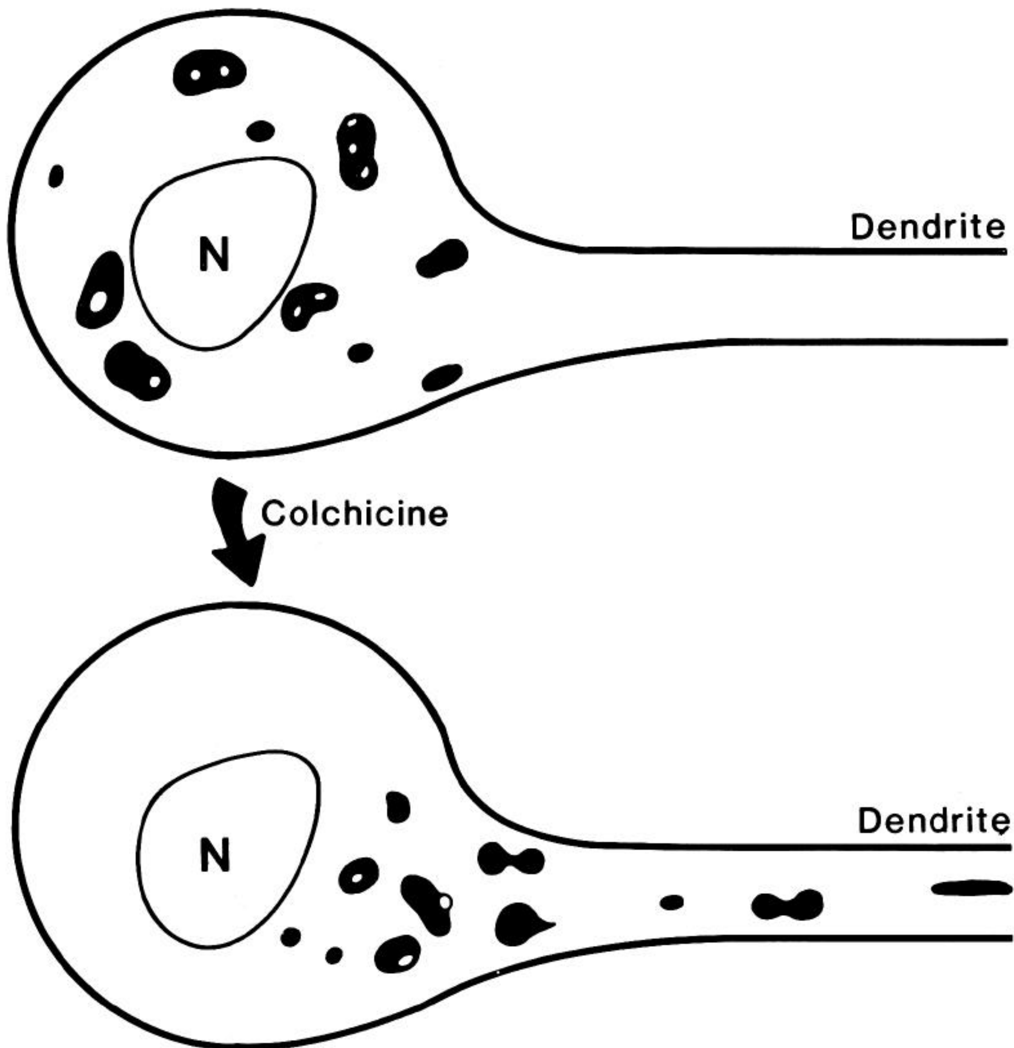


Figure 10. Electron micrograph of Dpp II-containing organelles in the proximal dendrite (*D*) of a mitral cell from a colchicine-treated preparation. Most of these labeled structures are elongated cisternae (*solid arrows*), but one appears as a dumbell shape (*open arrow*).

Figure 11. Schematic diagram of two mitral cell bodies and proximal dendrites to demonstrate the paradoxical redistribution of Dpp II-containing organelles following colchicine treatment. Colchicine changes both the distribution and the types of organelles that contain Dpp II. *N*, nucleus.

network. To account for the specific movement of lysosomes into dendrites once microtubules are disrupted, we also propose that lysosomes are coupled to a unique colchicine-insensitive transport system which specifically directs these organelles into dendrites. When the sequestering effect of microtubules is eliminated by colchicine, the dendritic transport of lysosomes becomes readily apparent. We speculate that this novel transport system is normally responsible for the low level transport of lysosomes into dendrites and could be activated under physiological conditions by endogenous colchicine-like factors (Sherline et al., 1979; Lockwood, 1979).

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