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# The ultrastructure of the central nucleus of the inferior colliculus of the Sprague–Dawley rat

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## Summary

Previous studies from this laboratory have indicated that increased numbers of GABAergic neurons, as well as total neurons, occur in the central nucleus of the inferior colliculus (IC) of genetically epilepsy-prone rats (GEPRs) as compared to non-seizuring Sprague–Dawley rats. Since electron microscopic studies of the IC have not been reported for rats, we wanted to determine the ultrastructure of neurons and their processes in this brain region to serve as a basis for future studies on neuronal circuitry in the GEPRs. Both disc-shaped and stellate types were found for each of three size categories: large, medium and small. Thus, six types of neuron were distinguished by differences in somatic size, shape, organelles and dendritic orientation. Large neurons (longest diameter greater than 25 μm), which are the least frequent cell type, contained vast perikaryal cytoplasm, eccentrically located nuclei and abundant granular endoplasmic reticulum (GER) adjacent to the nucleus as well as clustered in the cytoplasm; many axosomatic, symmetric synapses were present. Medium-sized neuronal somata (15–25 μm in diameter) had smooth as well as infolded nuclear membranes and clusters of GER in their cytoplasm but no GER adjacent to the nucleus; synapses were sparse along the surface of their somata. Small neurons (10–15 μm in diameter), which are the most frequent cell type, had scant perikaryal cytoplasm, usually infolded nuclei, frequently two nucleoli, and few or no stacked cisternae of GER in the perikaryal cytoplasm; only infrequent axosomatic synapses were found.

Based on previous retrograde and immunocytochemical studies, most large disc-shaped and stellate cells project to the medial geniculate body and are probably excitatory, but some large stellate neurons have been shown to be GABAergic and it is doubtful that such neurons participate in this projection. A dense plexus of terminals that form symmetric synapses covers the soma and proximal dendrites of large neurons, and may provide a strong GABAergic inhibition of this type of projection neuron. Small and medium-sized disc-shaped cells also project to the thalamus but they lack this dense axosomatic plexus. The stellate cells from these same two size categories probably do not project to the thalamus and may be GABAergic local circuit neurons.

Other ultrastructural features of IC neurons that were analysed include dendrites, dendritic spines, axon hillocks, initial segments and terminals, as well as the laminae of myelinated axons. Dendrites were either beaded or smooth and few spines were observed. The features of axon hillocks and initial segments of IC neurons were similar to those reported in other brain regions. Axon terminals were classified into five basic categories and some types may correlate with certain projections. The fibrous portions of laminae were composed of parallel fascicles of myelinated axons, some unmyelinated axons and a few dendrites. This description of the central nucleus of the inferior colliculus will provide a basis for future studies on neurotransmitter localization in normal rats and cytological changes in GEPRs.

## Introduction

The inferior colliculus (IC) is an obligatory relay nucleus in the midbrain for ascending auditory information *en route* to the medial geniculate body. It is generally considered to have three major subdivisions based on studies of cytoarchitecture, Golgi staining and distribution of afferents: (1) the central nucleus (ICCN), (2) the external nucleus and (3) the pericentral nucleus or dorsal cortex (Cajal, 1911; Van Noort, 1969; Geneic & Morest, 1971; Rockel & Jones, 1973a, b; FitzPatrick, 1975; Ryugo & Killackey, 1975; Harrison, 1978; Willard & Ryugo, 1983; Aitkin *et al.*, 1984; Morest & Oliver, 1984; Oliver & Morest, 1984; Faye-Lund & Osen, 1985). The ICCN of the rat is a laminated structure which receives afferents from most brainstem auditory nuclei (Faye-Lund & Osen, 1985). The layers of neuropil are formed by dendrites of disc-shaped neurons and certain axons (for details see Oliver & Morest, 1984). In addition to these disc-shaped neurons, stellate (multipolar) neurons are found throughout the ICCN, and both cell types vary in size from small (< 15 μm) to large (> 25 μm). The dendrites of disc-shaped neurons have their long

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axes parallel to the laminae, while the dendrites of multipolar neurons radiate in many directions and may cross laminar boundaries.

Although previous electron microscopic studies have been conducted in the IC, these accounts have been limited to the cat (Rockel & Jones, 1973b; Oliver, 1984, 1985). Rockel & Jones (1973b) described two cell types: (1) a principal cell, which probably corresponds to the disc-shaped neurons of light microscopic studies (Oliver & Morest, 1984), has a large soma, stout spiny dendrites and many synaptic contacts on both the soma and the dendrites; and (2) a small multipolar cell, which may correspond to the stellate neuron of light microscopic studies, has spineless, thin dendrites and receives very few axosomatic terminals. Oliver (1984) has studied these two cell types as well as two others in his analysis of neurons in the ICCN which project to the medial geniculate body, i.e. three subclasses of disc-shaped cell and one type of stellate cell. Briefly, he showed that small and medium-sized disc-shaped neurons had few axosomatic synapses and smooth nuclear envelopes, whereas large disc-shaped and stellate neurons had many axosomatic synapses and infolded nuclei. In addition, both groups of investigators have described axon terminals within the ICCN and demonstrated that certain types are associated with specific projections. These studies provide important information, but do not include a complete description of all six cell types described in light microscopic accounts.

The ICCN is the focus of the present study because our previous work has indicated a large increase in both the number of GABAergic neurons and the total neuronal number in the genetically epilepsy-prone rat (GEPR) (Roberts *et al.*, 1985c). This increase ranged from 100 to 200%, and was not a result of the audiogenic seizures because young pre-seizure GEPRs showed a similar increase to age-matched Sprague–Dawley rats (Roberts *et al.*, 1985a). The neuronal types that were increased in number were the small and medium-sized somata, i.e. the same sizes of most GABAergic neurons in the ICCN (Roberts *et al.*, 1985c). The increase in neuronal number in the IC of the GEPR appeared to be specific for this brain region because other regions in the brainstem that were examined did not show any differences from Sprague–Dawley rats. The region in the IC that displayed the most dramatic difference was the ventral–lateral portion of the ICCN. Our long-term goal is to determine whether there are synaptic changes in this particular region of the IC in the GEPR as a result of the increased numbers of neurons. Since ultrastructural analyses of the IC have not been reported for rats, we have described the neurons and their processes in the ICCN of normal rats to provide a basis for future studies of the GEPR.

## Methods

Six adult Sprague–Dawley rats obtained from Simonsen Laboratories (Gilroy, CA) were used in this study. All animals were deeply anaesthetized with Nembutal and transcardially perfused with one of two fixatives. Fixative A contained 4% paraformaldehyde, 1% glutaraldehyde and 0.0002% $CaCl_2$ in 0.15 M phosphate buffer (pH 7.4, 25° C). Animals were perfused with 30–50 ml of 0.9% NaCl to clear all vessels of blood cells, and this was followed by 300 ml of fixative A. Other rats were perfused with fixative B (Friedrich & Mugnaini, 1981) which has three stages: (1) 100 ml per rat of Ringer's solution containing 0.85% NaCl, 0.25% KCl and 0.02% $NaHCO_3$ (pH 7.3, 37° C); (2) 500 ml per rat of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.12 M phosphate buffer (pH 7.3, 25° C); (3) 500 ml per rat of 3% glutaraldehyde in 0.12 M phosphate buffer (pH 7.3, 25° C). Following the perfusions, animals were placed in the refrigerator and the brains were removed the next day.

The inferior colliculi were dissected out and sectioned on an Oxford Vibratome in the coronal plane at a thickness of 50 μm. Blocks were cut from the ventral–lateral portion of the central nucleus from sections obtained from the middle of the rostrocaudal axis of the IC. This region consists of the entire ventral half of the ICCN shown in the middle diagram of the frontal series in Fig. 11 from Faye-Lund & Osen (1985). These tissue blocks were postfixed in 1.0% $OsO_4$ for 30–60 min, dehydrated in ethanol and embedded in Epon 812. Semithin (1–3 μm) sections were cut from embedded specimens and stained with 0.05% toluidine blue. Serial thin sections were cut on a Sorvall ultramicrotome, mounted on Formvar-coated slot grids, stained with uranyl acetate and lead citrate and examined with the electron microscope. Our analysis was based on the examination of at least 25 small, 25 medium-sized and 10 large neurons per animal.

## Results

### LIGHT MICROSCOPIC OBSERVATIONS

Semithin sections of the ICCN were analysed with a light microscope to determine the different types of neuronal somata and the organization of laminae. Three size categories were easily discerned (Figs 1, 3–5, 10, 14–16, 18) and they corresponded to those described in our previous studies (Roberts *et al.*, 1985a, c). The quantitative data from these studies indicated that the mean numbers of small, medium-sized and large neurons in an area of 62 500 $\mu m^2$ in the ventral–lateral portion of the adult ICCN were 26.6, 15.9 and 6.7, respectively, as determined from Nissl-stained paraffin sections, 10 μm thick. Data from semithin sections used in the present analysis are consistent with these data in that small neurons were the most frequent and large neurons were the most infrequent.

Large neuronal somata with diameters greater than 25 μm were homogeneously distributed in the ICCN.

These somata were either fusiform or round and probably correspond to the disc-shaped and stellate neuronal types, respectively. Both types of somata had Nissl bodies scattered in the abundant perikaryal cytoplasm and adjacent to their slightly infolded, eccentrically located nuclei (Figs 1, 4, 14).

Medium-sized and small neuronal somata form the vast majority of the neurons in the ICCN. The diameters of medium-sized neurons range from 15–25 μm whereas the small neurons are less than 15 μm in diameter. Medium-sized neurons had relatively more perikaryal cytoplasm than the small neurons and they often displayed a Nissl body (Figs 3, 4). In contrast, small neurons lacked distinct Nissl bodies and often contained two nucleoli (Fig. 18). The size of the dendritic processes that arose from these two types varied from thick to very fine, narrow processes (cf. Figs 3, 10, 15, 16).

The neuropil spaces between neuronal somata were occupied by dendrites, myelinated axons, neuroglia and blood vessels. The myelinated axons often formed parallel fascicles (Fig. 5) that resembled the laminae described in Golgi preparations (see Faye-Lund & Osen, 1985). Intervening between these fascicles were neuronal somata and dendritic processes.

## ELECTRON MICROSCOPIC OBSERVATIONS

The only apparent differences between the somata of probable disc-shaped and stellate neurons were the shape of their somata and the orientation of their dendrites. Thus, the ultrastructural characteristics of both types of neuron in a given size category are discussed together. However, many features distinguish neurons in the three size categories, and thus each size category will be discussed separately. Axon initial segments (Figs 2, 7, 11) and some of the cellular organelles, such as the Golgi apparatus, mitochondria and polysomes were typical and did not aid us in distinguishing between the various cell types. Therefore, these features will only be mentioned briefly.

### *Large neurons*

Large neurons were observed in all thin sections of the ICCN. The shapes of their somata were of two basic varieties. One was a typical multipolar shape with several dendritic processes emerging from the soma (Fig. 2). The other type of profile had a rectangular shape and probably corresponds to disc-shaped neurons (Fig. 6). Dendrites emanated from the corners of these somata and had either large or small diameters.

The nuclei of large neurons were eccentric and displayed a prominent nucleolus and some infoldings of the nuclear envelope (Figs 2, 6). The nuclei were usually euchromatic with only occasional condensations of chromatin. Intranuclear inclusions, such as rods or sheets, were not observed.

The perikaryal cytoplasm of these neurons was extensive. It was rich in both quantity and variety of cellular organelles. The granular endoplasmic reticulum (GER) had a unique distribution with numerous cisternae adjacent to the nucleus and others assembled into distinct clusters that were scattered in the perikaryal cytoplasm (Figs 2, 6). Those cisternae of GER adjacent to nuclei were found on the nuclear surface that faced the centre of the soma rather than adjacent to the cellular membrane. Another characteristic feature of these somata was the abundance of lysosomes, both pale-staining and electron-dense types (Figs 2, 6).

The pattern of axosomatic synapses for the two different types of large neuron was essentially the same. The terminals that formed these synapses covered the vast majority of the somal surface (Figs 2, 6, 8, 9). Each terminal formed at least one synaptic site with many forming multiple sites (Figs 8, 9). However, active zones were not observed over the entire length of the apposition between terminal and soma (Fig. 8). The terminals frequently contained flattened synaptic vesicles and formed symmetric synaptic junctions. Some terminals arose from small diameter preterminal axons that were oriented parallel to the surface of the soma. The number of axosomatic terminals varied. In any particular section obtained through the centre of a soma that exhibited a nucleus, there were 30 to 40 axosomatic synapses. This plexus of axosomatic synapses was continuous with the dense plexus of axodendritic synapses found on the proximal dendrites of these neurons.

### *Medium-sized neurons*

These neurons were commonly observed and displayed centrally located nuclei (Figs 11, 17). Both disc-shaped and stellate types had infolded nuclear envelopes. These nuclei often displayed a prominent nucleolus but intranuclear rods and sheets were not observed.

The perikaryal cytoplasm of the medium-sized neurons was less extensive and organized differently from that of the large neurons. Some of the medium-sized neurons had clusters of GER that formed distinct Nissl bodies, but they were not found adjacent to the nuclei as was the case for the large neurons (cf. Figs 6, 11). The multipolar somata were more likely to display stacked cisternae of GER than the fusiform somata (Figs 11, 17), but otherwise both displayed similar intracellular features. Lysosomes were often encountered in the perikaryal cytoplasm, but not with the same frequency as in large neuronal somata. Other organelles, such as the Golgi complex, mitochondria, microtubules and neurofilaments were typical as well as the axon initial segments.

The somata of these neurons were contacted by a few axon terminals that formed mainly symmetric synapses (Fig. 12). In contrast to the somata of large neurons, most of the somal surface of medium-sized neurons was not apposed by axon terminals. Thus, these neurons often had astrocytes and oligodendrocytes adjacent to their surfaces as well as myelinated fibres. In any section through a soma that displayed a nucleus, there were as few as 2–3 axosomatic synapses. The total number of axosomatic synapses observed for this cell type was usually about 10.

The dendrites of medium-sized neurons extended from the soma to enter the laminae between axon bundles (Fig. 13). These dendrites were of small calibre with few, if any, spines found along their length. Axon terminals contacted these dendritic shafts and formed either asymmetric or symmetric synapses. Some of these dendrites displayed varicosities. Such dendrites had more axodendritic synapses located on the thin portions of the dendrite than on the bulbous portions.

*Small neurons*

The somata of small neurons were less than 15 μm in diameter and were fusiform or round (Figs 19, 20). These somata had a relatively large nucleus to cytoplasm ratio. The nuclei were often infolded and, when the infolding was severe, a nucleus appeared to be separated into two parts (Fig. 20). Two nucleoli were often found within the nuclei of small neurons and occasionally a nucleolus contained infoldings of karyoplasm (Fig. 24). The organelles of these neurons were not well developed in their scant perikaryal cytoplasm. For example, the GER was commonly scattered into single cisternae around the nucleus (Figs 19, 20). A Golgi complex was infrequently observed and lysosomes were rarely found. Other organelles were present in the small neurons, such as mitochondria, microtubules and neurofilaments. The surface of small neurons was contacted by only a few axon terminals. Usually, between one and four synapses were observed (Figs 19, 20). These terminals were small and formed symmetric synapses. The processes of small neurons were usually of small diameter.

*Neuropil*

The spaces between the somata of the six major cell types were organized into two types of compartments. One of these contained the fascicles of myelinated axons while the other had dendritic and

**Fig. 1.** Photomicrograph of a semithin section showing a typical large stellate-shaped neuron from the rat inferior colliculus. Note the eccentrically located nucleus (arrow) and the aggregation of Nissl substance adjacent to the nucleus (arrowheads). × 700.

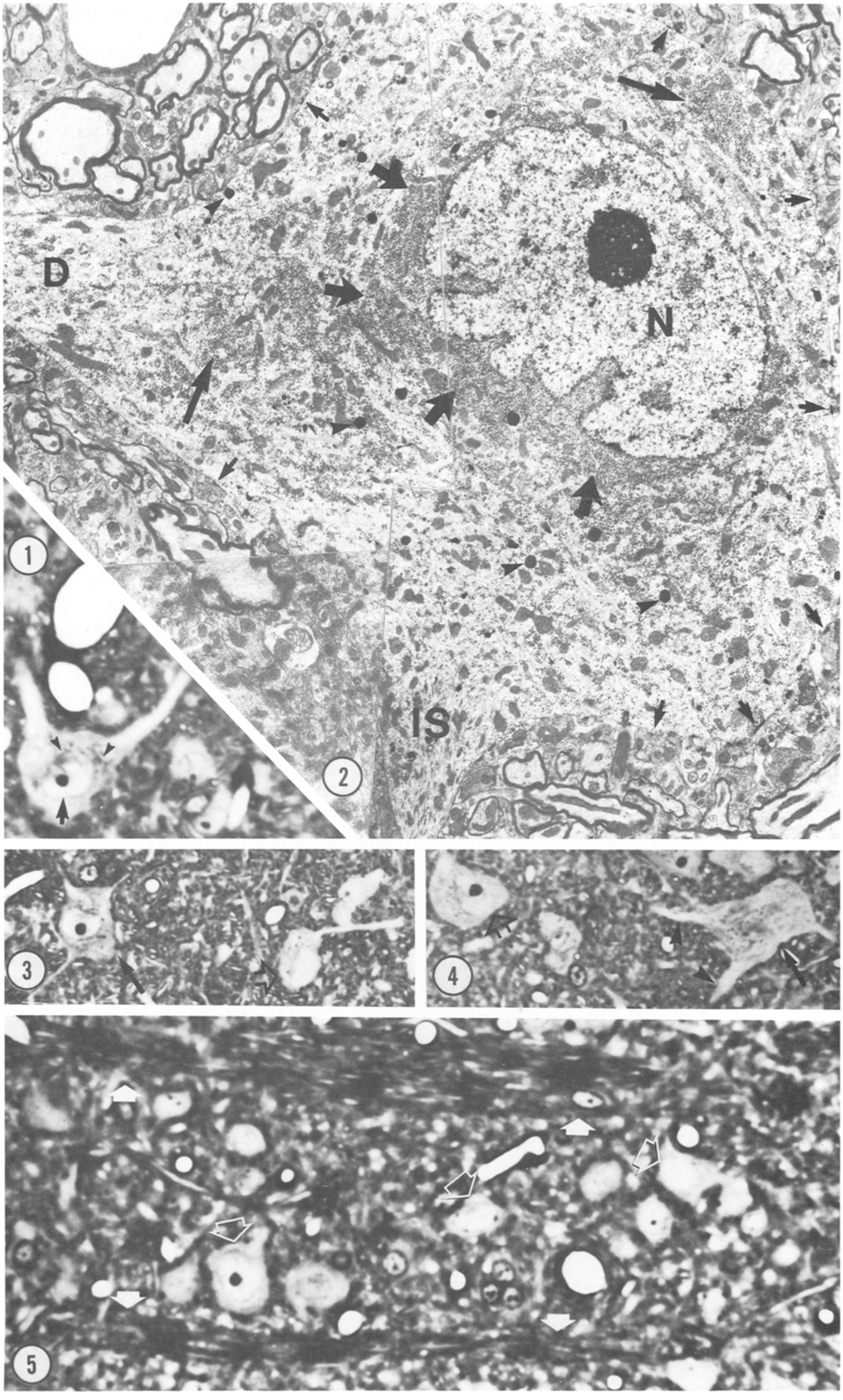
**Fig. 2.** Electron micrograph of a large stellate-shaped neuron similar to the neuron in Fig. 1. Two processes emerge from its soma; a thick dendrite (D) and an axon initial segment (IS). Note the presence of an aggregation of numerous cisternae of granular endoplasmic reticulum (short, stout arrows) adjacent to the nucleus (N) as well as other cisternae (long arrows) and an abundance of lysosomes (arrowheads) in the remaining cytoplasm. Note that there are many axosomatic synapses (small arrows). × 4300

**Figs 3–5.** Photomicrographs of plastic-embedded, semithin sections (1 μm thick) of the ventral–lateral portion of the central nucleus of the inferior colliculus of the rat showing several different cell types. Fig. 3 shows a large disc-shaped neuron (arrow) and a medium-sized neuron (open arrow). Note that the processes in both these neurons are very thin. × 640. Fig. 4 shows an example of a large, disc-shaped neuron (arrow) with dendrites (arrowheads) extending from either end of its long axis. The nucleus of this type of neuron is usually small and eccentric but is absent in this particular section. Note another soma of a large neuron is also present (open arrow). × 640. Fig. 5 shows two laminae of myelinated axons (arrows) that are characteristic of the ventral–lateral portion of the inferior colliculus. Several neurons are observed between the laminae (open arrows). × 7700.

**Fig. 6.** Electron micrograph of a soma of a large fusiform or disc-shaped neuron. This type of soma is distinguished from the multipolar type by its rectangular shape. Other features include an eccentrically-located nucleus (N), prominent perinuclear GER (large arrows), Nissl bodies (NB), abundant lysosomes (open arrows) and many axosomatic synapses (small arrows). × 700.

**Fig. 7.** An enlargement of the axon initial segment (IS) from the neuron in Fig. 2. It displays the characteristic features of an IS, i.e. (1) fasciculation of microtubules (arrow), (2) dense undercoating (arrowheads), (3) lack of ribosomes, and (4) various types and sizes of vesicles (open arrows). Note that the axon hillock region (AH) also displays an absence of ribosomes and fasciculation of microtubules. An axon terminal (T) forms a synapse with the IS. × 13 600.

**Figs 8, 9.** Enlargements of regions of the soma in Fig. 6 (indicated by boxed areas) to show examples of the dense plexus of axon terminals that form axosomatic synapses. In Fig. 8 the terminal in the centre extends for a few microns along the surface of the soma but displays only one restricted active zone (arrow). On either side of this long terminal are two smaller terminals and one of these contains numerous infoldings of axolemma (arrowheads). × 34 000. Fig. 9 shows many terminals, some of which form symmetric synapses (arrows). × 20 000.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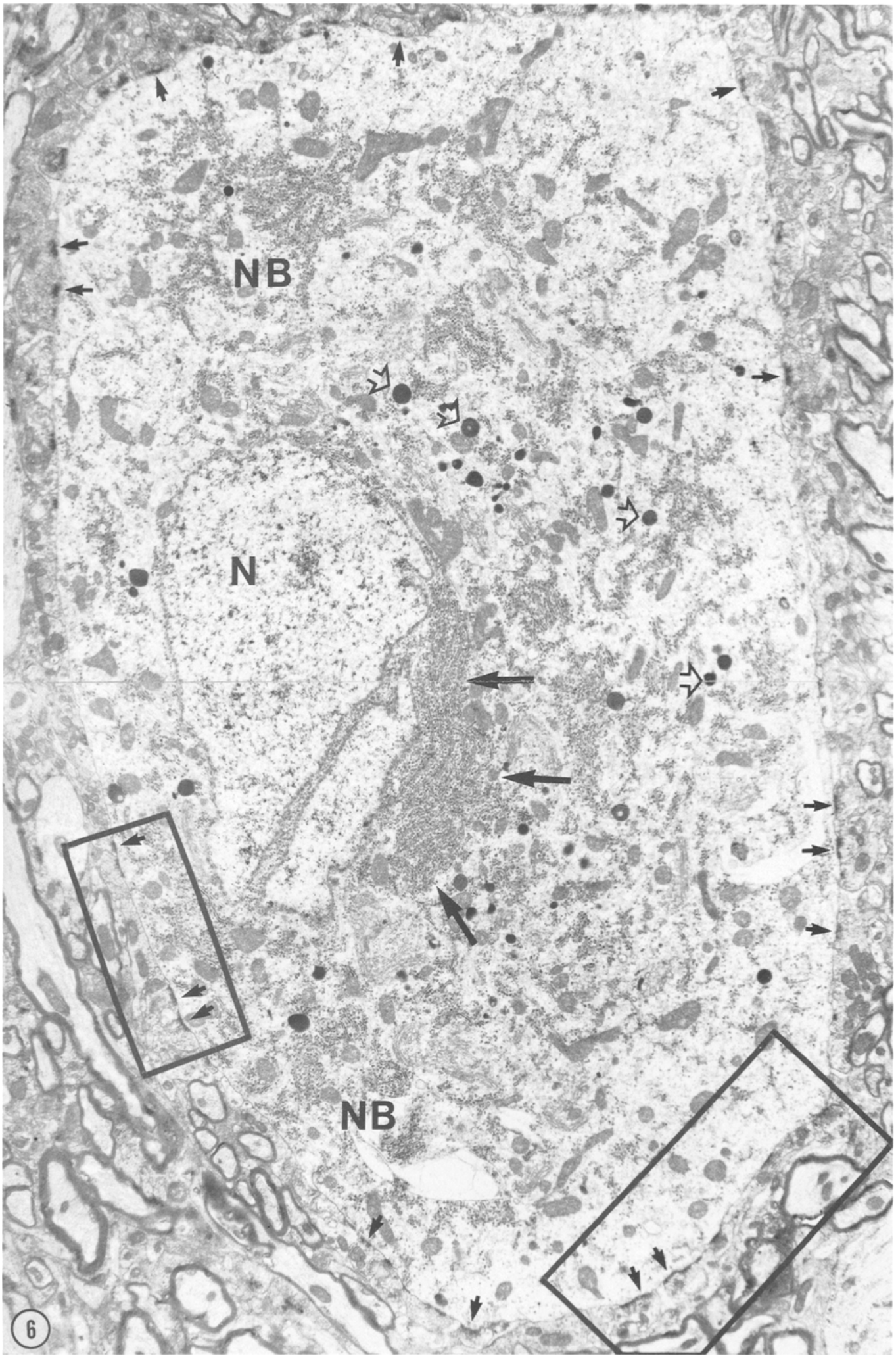


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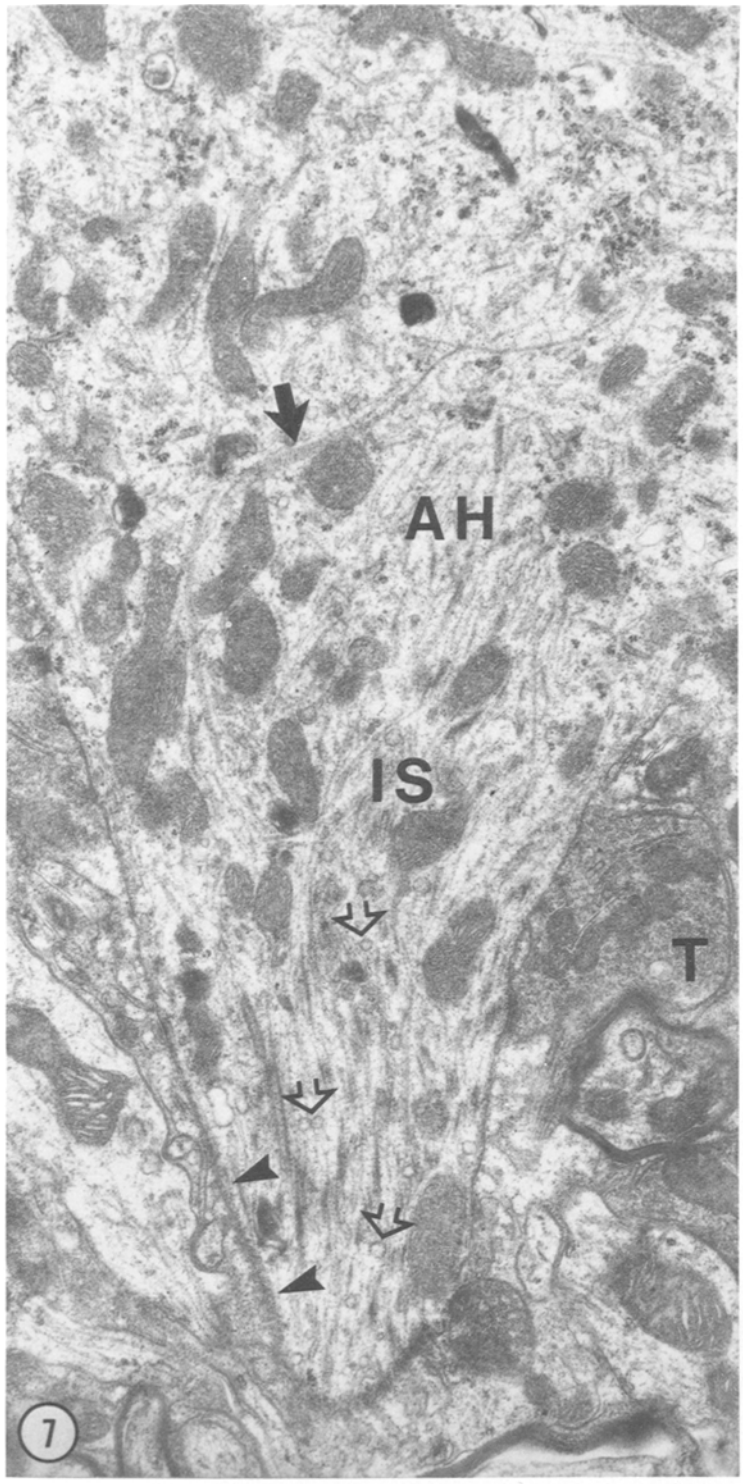
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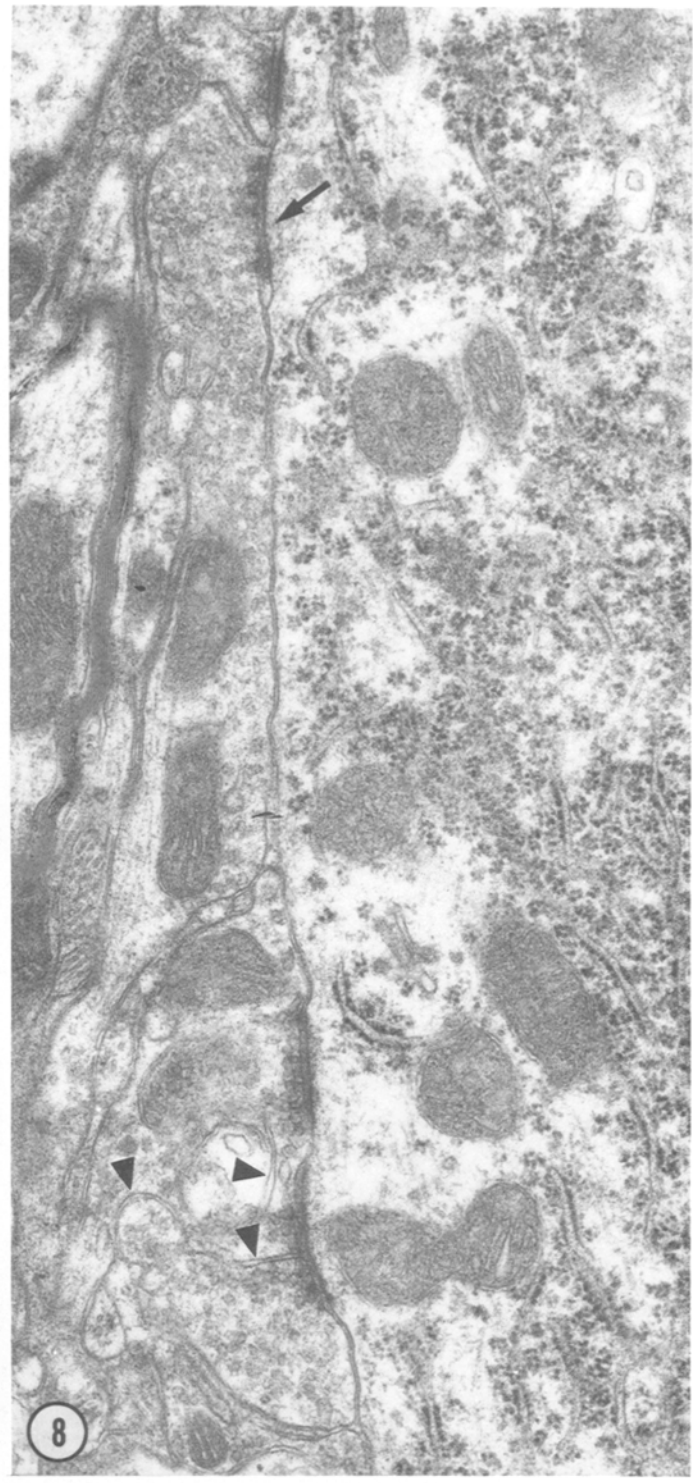
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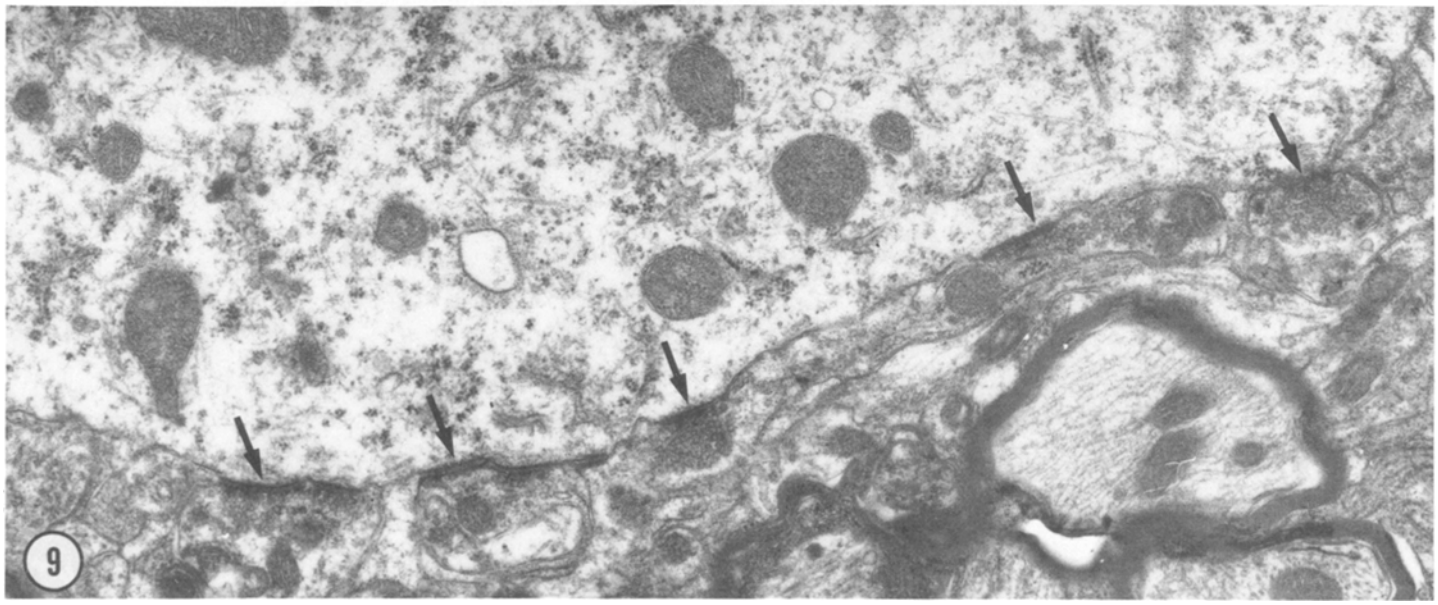
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axonal processes together with neuroglia. These compartments appeared to form alternating bands that resembled the laminae previously described for the ICCN (Oliver & Morest, 1984; Faye-Lund & Osen, 1985).

Most, if not all, of the axons that formed fascicles were myelinated. They were oriented in bundles from dorsomedial to ventral–lateral. Occasionally, a dendrite was observed to enter into these bundles (Fig. 23). However, the portion of the dendrite within the bundle was not contacted by any axon terminals; instead, it was apposed by the external lamellae of the myelin sheaths from the adjacent axons. Since the internodal length of myelin for these axons was long, dendrites within the bundles of myelinated axons were not readily identified unless they were followed in serial sections back to their cell body of origin.

The other compartment of the neuropil contained an assortment of dendrites and axons. Most dendrites were aspinous, although a few spines were occasionally observed (Figs 13, 28). The frequency of these spines was not great in comparison to other brain regions where spiny dendrites are common. Dendrites were also found to have two types of shape: beaded or smooth cylindrical shapes (Figs 21–23, 25–27). Both types contained the same typical organelles for dendrites, but it appeared that the beaded dendrites (Figs 21, 22) were contacted by more axon terminals per unit length of dendritic surface than the smooth cylindrical dendrites.

Five different types of axon terminal were observed. The first two types had round synaptic vesicles, a clear matrix, were either large or small and formed asymmetric synapses mainly with dendrites (Fig. 22). The next two types of terminal had flattened synaptic vesicles, a clear matrix, either a small or large cross-sectional area and formed symmetric synapses with both somata and dendrites. (Figs 8, 9, 12). The last type was similar to the large type with flattened vesicles except that it had a dense cytoplasmic matrix (Fig. 22). This latter type contacted mainly dendrites. The remaining structures observed in the neuropil were neuroglia and their processes which had the typical characteristics as observed in other brain regions.

## Discussion

The results of this study have provided an electron microscopic description of the types of neurons in the ventral–lateral portion of the ICCN of the rat. The morphological differences between the various cell

**Fig. 10.** Photomicrograph of a semithin section showing a medium-sized stellate neuron (arrow) with two dendrites (arrowheads). × 640.

**Fig. 11.** Electron micrograph of a medium-sized, multipolar neuron that displays two proximal dendrites (D) and an axon initial segment (IS). Note that the cisternae of GER that form Nissl bodies (NB) are not adjacent to the nucleus but are dispersed in the perikaryal cytoplasm. Note their absence from the axon hillock region (AH). The number of synaptic contacts (arrows) is much less than that for the large neurons (cf. Figs 2, 6). × 7600.

**Fig. 12.** An enlargement of a terminal that forms axosomatic contacts with the soma in Fig. 11 (indicated by the boxed area). The terminal (T) contacts the soma for quite a distance and makes two active zones (arrows). It contains flattened vesicles in a dense matrix and forms symmetric synapses with the soma. × 26 800.

**Fig. 13.** A longitudinal section through a portion of the dendrite that extends from the lower left-hand portion of the neuron in Fig. 11 and is approximately 25 μm from the cell body. Several terminals form synapses (arrows) with the dendrite. Note that this dendrite lacks spines but a small spine (arrowhead) was found to arise from a neighbouring dendrite. × 10 100.

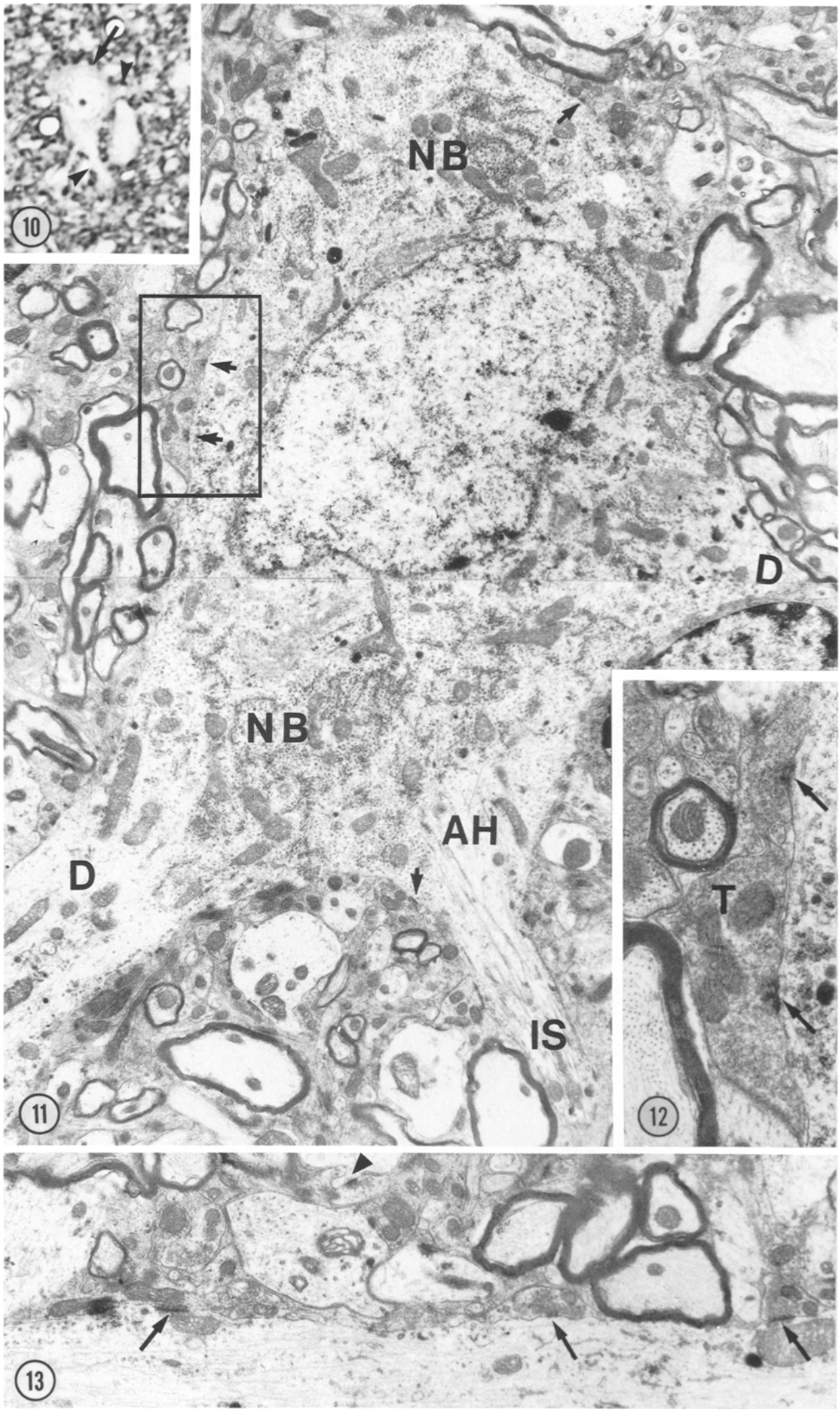
**Figs 14–16.** Photomicrographs of semithin sections of disc-shaped neuronal somata. Fig. 14 shows a large disc-shaped neuron with a stout dendrite extending for several microns from one pole of the cell body. Note the Nissl substance aggregated close to the nucleus (arrowheads) and the prominent nucleolus. Fig. 15 shows a medium-sized, disc-shaped cell (arrow) with thick dendrites extending from either pole of the cell body (arrowheads). Note that the perikaryal cytoplasm is not as abundant as in the large neurons (cf. Fig. 14). Fig. 16 displays several small neurons including a fusiform, disc-shaped neuron (arrow) as well as a round soma (open arrow) from a probable stellate neuron. Dendrites (arrowheads) arise from each neuronal type. Note that only a thin rim of cytoplasm is present in several of these small somata × 640.

**Fig. 17.** Electron micrograph of a medium-sized, disc-shaped neuron with stout dendrites (D) that extend from each pole of the soma. This type of neuron has several features in common with the medium-sized stellate neuron, i.e. few synaptic contacts (arrow) and cisternae of GER (arrowheads) scattered in the perikaryal cytoplasm but not adjacent to the nucleus (N). × 8250.

**Fig. 18.** Photomicrograph of several small neurons, some of which contain two nucleoli (arrows). × 640.

**Fig. 19.** Electron micrograph of a small, fusiform-shaped neuron that displays a smooth nuclear envelope (N), scant cytoplasm, an axosomatic synapse (arrow) and a process extending from the cell body. × 9300.

**Fig. 20.** An example of a small, round soma that is probably a multipolar neuron. It displays a highly infolded nucleus (N), a thin shell of perikaryal cytoplasm, a few scattered cisternae of GER and a paucity of axosomatic synapses (arrow). × 16 000.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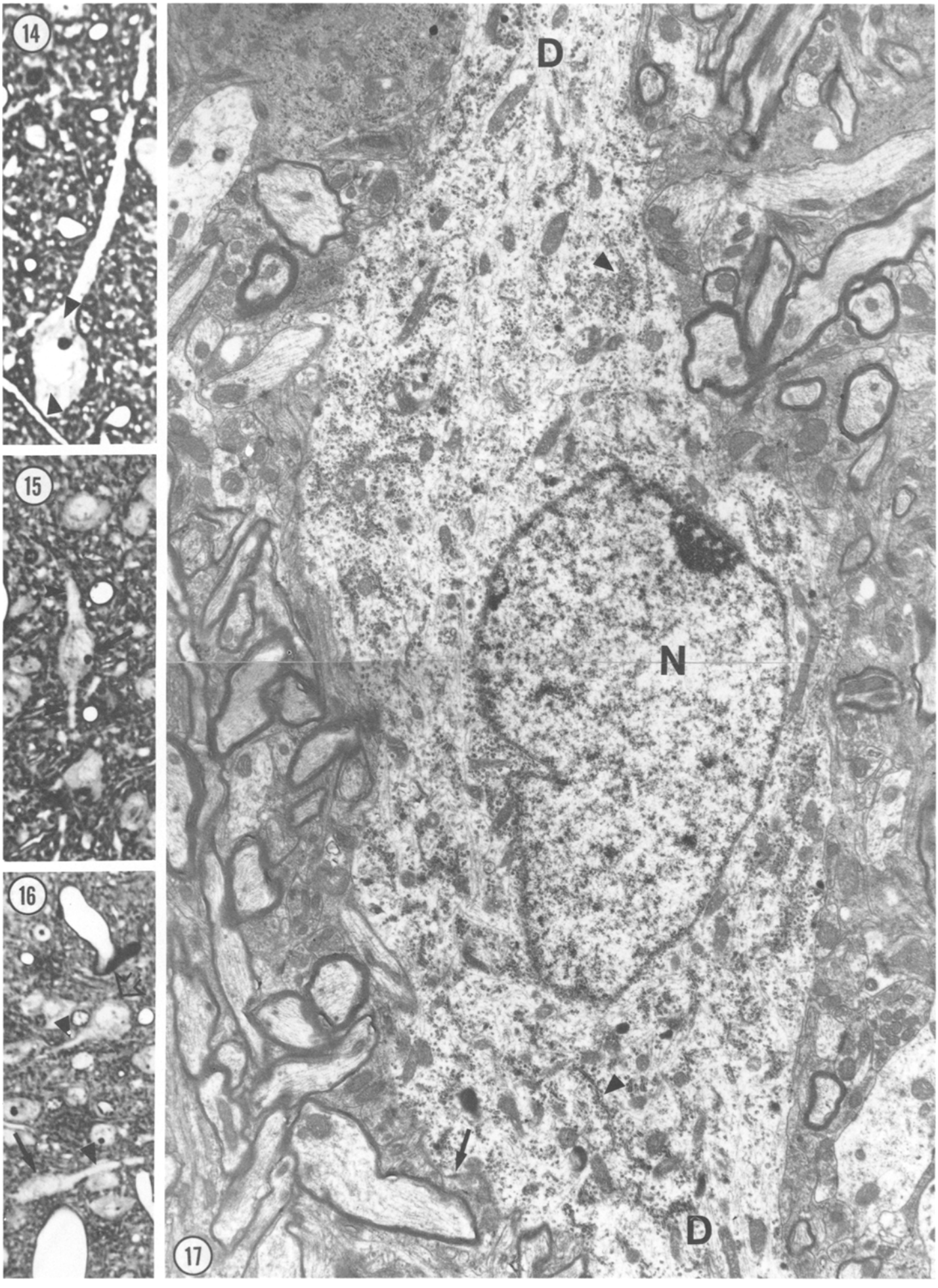
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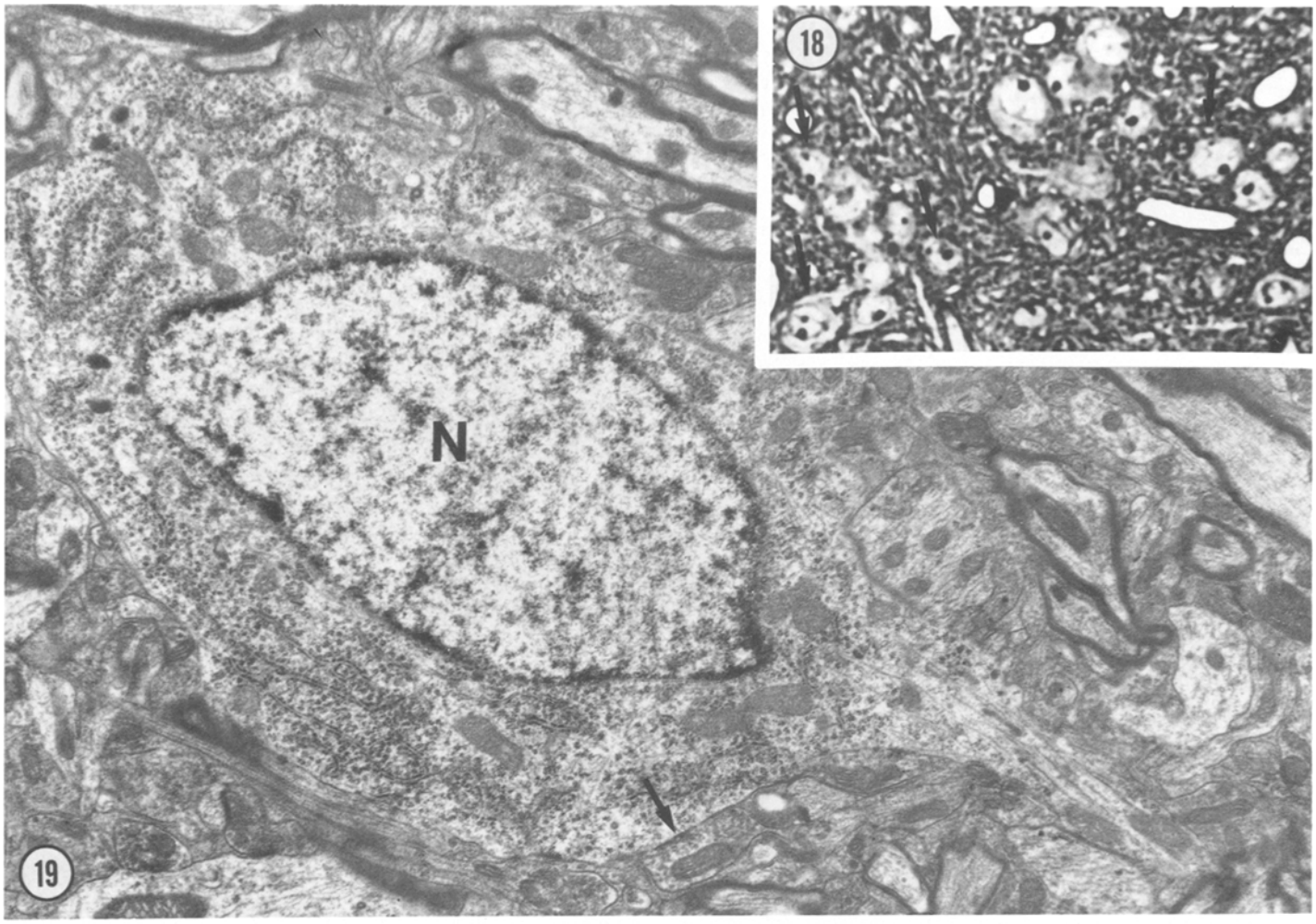
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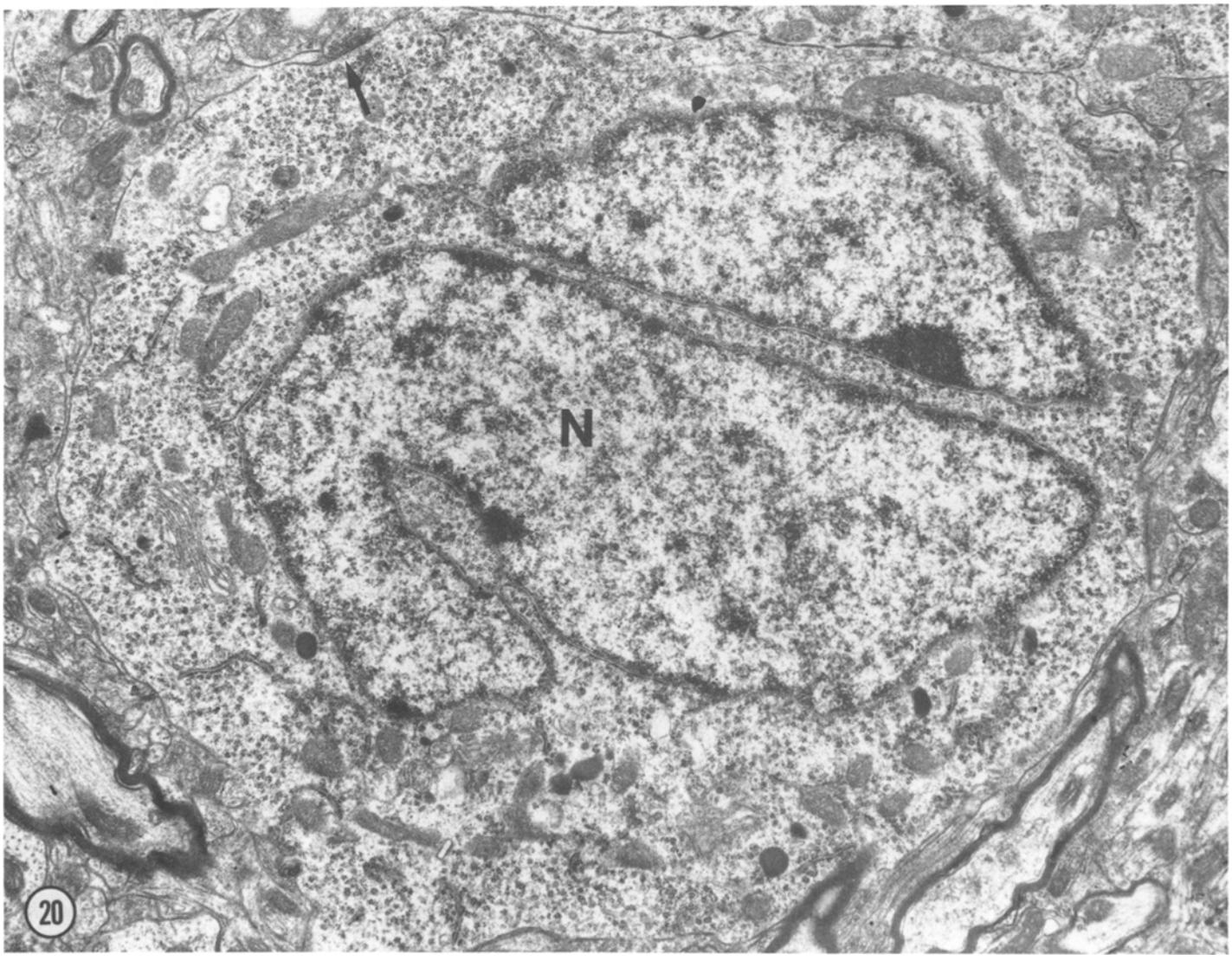
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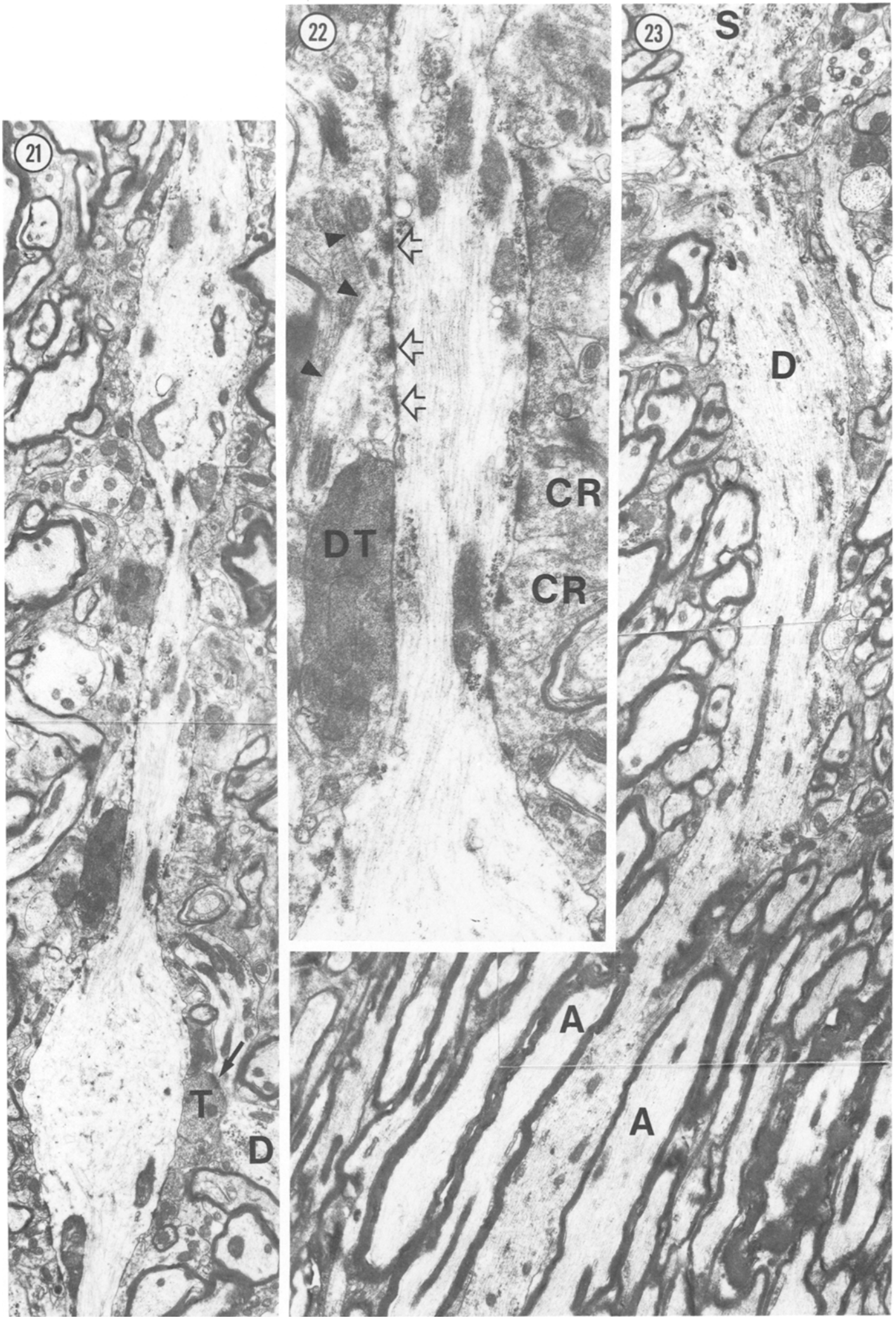
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types and terminal types can be used to distinguish these neurons and terminals in future ultrastructural studies of the ICCN of the rat.

*Somata*

This combined light and electron microscopic study has demonstrated six different cell types in the ICCN based on the size and shape of the soma. Both disc-shaped and stellate neurons were observed in three different size categories that were either small, medium or large. These results are consonant with previous studies in the rat (Faye-Lund & Osen, 1985; Roberts *et al.*, 1985c). In addition, this classification scheme agrees with the findings of Oliver & Morest (1984) for the cat.

The ultrastructural features of these six cell types display many differences as well as some similarities. For the most part, the types of cellular organelles and amount of cytoplasm are similar for the two different shaped cell types in any given size category. The differences between cell types are found when comparing somata from different size categories. This finding is consistent with the data of Oliver (1984) on the ultrastructure of projection neurons to the medial geniculate body (MGB). However, it is inconsistent with the results of Rockel & Jones (1973b) who have stated that they were unable to detect any significant differences between the large principal or projection neurons and the small multipolar neurons in regard to cellular organelles in the perikaryal cytoplasm. Although their study was of cats and the present study involves the rat, differences in the amount and description of organelles in the perikaryal cytoplasm in the cat have been observed by Oliver (1984). For example, the large neurons that he has described have eccentrically located nuclei, many lysosomes and numerous Nissl bodies, including a Nissl body adjacent to the nucleus. These features are similar to those for the large neurons in the ICCN of the rat and distinguish this size class from both the small and medium-sized neurons. Although the medium-sized neurons in the rat have Nissl bodies, they are not found adjacent to the nucleus and the nucleus is always centrally located in these neurons. In contrast, the small neurons rarely display any stacked cisternae of GER in their thin shell of perikaryal cytoplasm. These features are helpful for the identification of the three size categories of the ICCN neurons.

The shapes of the neurons in the ICCN are identified in sections which contain the nucleus and at least two dendrites in continuity with the soma. Such sections are imperative for distinguishing between disc-shaped and stellate neurons in electron microscopic preparations because the quantity and distribution of organelles and the size and shape of nuclei are similar for the same size category. Therefore, serial sections have been used to ensure an adequate identification of somata.

In many other brain regions, the anatomical differences between cell types often reflect their functional differences. For example, the pyramidal neurons in the cerebral cortex have a distinctive multipolar shape and are known to project to subcortical structures (Peters & Jones, 1984). In contrast, the bipolar cells of the cortex are considered to be local circuit neurons (Peters, 1984). Furthermore, these anatomical and connectional differences are also correlated with neurochemical differences in that pyramidal neurons may utilize an excitatory neurotransmitter, such as glutamate or aspartate (Otterson & Storm-Mathisen, 1984), whereas the bipolar cells often contain the peptides cholecystokinin or vasoactive intestinal polypeptide within them (Peters, 1984).

The morphological distinctions between the six cell types in the ICCN may provide only a small indication of their connections or functions. This conclusion is partially based on retrograde horseradish peroxidase (HRP) labelling of neurons in the ICCN following HRP injections into the MGB (Oliver, 1984). This study demonstrated four different cell types in the cat ICCN that project to the MGB. Three of these were different size subclasses of disc-shaped cells and one was the large multipolar cell. We have recently shown that all six cell types in the ICCN display immunoreactivity for glutamate decarboxylase (GAD), the synthesizing enzyme for GABA (Roberts *et al.*, 1985c). This latter study utilized

**Fig. 21**. An example of a beaded dendrite sectioned in the longitudinal plane. This dendrite is contacted by many terminals that form synapses, particularly on the narrowed region. Although several terminals are found on the beaded portion of the dendrite, some do not form synaptic contacts with this dendrite. For example, one of these (T) contacts an adjacent dendrite (D) where it forms a synapse (arrow). × 7500.

**Fig. 22**. An enlargement of the dendrite in Fig. 21. Several types of terminal form synapses with the thin, non-varicose portion of the dendrite: (1) small clear terminals with round vesicles (CR) that form asymmetric synapses; (2) large terminals with a dark matrix filled with several mitochondria (DT) and only one or two active zones; and (3) large clear terminals with round synaptic vesicles (outlined with arrowheads) that have many active sites (open arrows). × 17 500.

**Fig. 23**. A dendrite (D), extending from the soma (S) of a small neuron, enters into the lamina of myelinated axons (A). The dendrite travels in the lamina for several microns (not shown) and does not receive any synapses from the adjacent axons. × 6400.

quantitative methods to show that most of the GABAergic neurons were small and medium-sized multipolar cells. Taken together, these data suggest that individual neurons from the same cell type in the ICCN may have different connections and transmitters, rather than having the same set of characteristics. However, certain trends may allow for general statements about these cell types. (1) Most large disc-shaped cells project to the MGB (Oliver, 1984) and are probably excitatory based on the observations that few of this cell type are GABAergic (Roberts *et al.*, 1985c) and axon terminals in the ventral division of the MGB that originate in the ICCN form exclusively asymmetric synapses (Morest, 1975). (2) Many large stellate neurons project to the MGB, but some have been shown to be GABAergic and it is doubtful that the GABAergic neurons from this subcategory participate in this projection. (3) Many small and medium-sized disc-shaped cells project to the MGB, but only a few members of these two cell types are GABAergic and they most probably project elsewhere, such as to the contralateral IC. (4) Most small and medium-sized stellate cells do not project to the MGB, but are GABAergic and are probably local circuit neurons or give rise to descending projections.

*Axosomatic synapses*

The three sizes of somata each display a distinct pattern of axosomatic synapses. The somata of both types of large cells in the ICCN are almost completely invested with axon terminals that form symmetric synapses. A similar dense plexus of terminals is found on their proximal dendrites. These findings are consistent with the description given for the large somata in the previous ultrastructural studies of the cat ICCN (Rockel & Jones, 1973b; Oliver, 1984). The medium-sized somata are contacted by several terminals but they lack the density of the plexus associated with the large somata. In contrast, the somata of small neurons display only occasional axosomatic synapses. These findings are also in agreement with the data from the cat ICCN.

The significance of these findings is related to the function of the symmetric synapse which has been associated with inhibition. Many previous studies from diverse brain regions, including the superior colliculus, have shown that the terminals which form symmetric synapses contain GABA and GAD immunoreactivity (Ribak *et al.*, 1981; Oertel *et al.*, 1981; Houser *et al.*, 1983; Somogyi & Hodgson, 1985). The presence of GABA and GAD in these terminals and the data from physiological studies support the notion that these synapses are probably inhibitory. Our preliminary immunocytochemical findings are consistent with this general notion in that the terminals which form symmetric synapses in the ICCN of rats contain immunoreactivity for both GABA and GAD (Roberts & Ribak, 1986). Therefore, the numerous terminals that form symmetric synapses with the large somata of the ICCN probably provide a strong, GABAergic inhibition of this type of projection neuron. The medium-sized and small neurons are probably not dominated as strongly by this axosomatic inhibitory plexus if we assume that the number of terminals adjacent to a soma is related to the total amount of inhibition because they have relatively fewer axosomatic synapses.

The dense plexus of axon terminals associated with the cell bodies of large neurons does not continue onto the surfaces of their axon hillocks and initial segments. Instead, only occasional terminals were observed in these locations. Yet, they also formed symmetric synapses similar to the axosomatic synapses. These observations may indicate that inhibitory synapses occur with axon initial segments, but the number of similar synapses with the soma is usually far greater.

*Axon terminals*

Axon terminals were classified into five different types based on size, density of the matrix and the shape of the vesicles within them. Our observations are similar to those described previously in ultrastructural analyses in the cat (Rockel & Jones, 1973b; Oliver, 1985). However, all of the classification schemes are somewhat different. Oliver (1985) classified the terminals according to vesicle size and

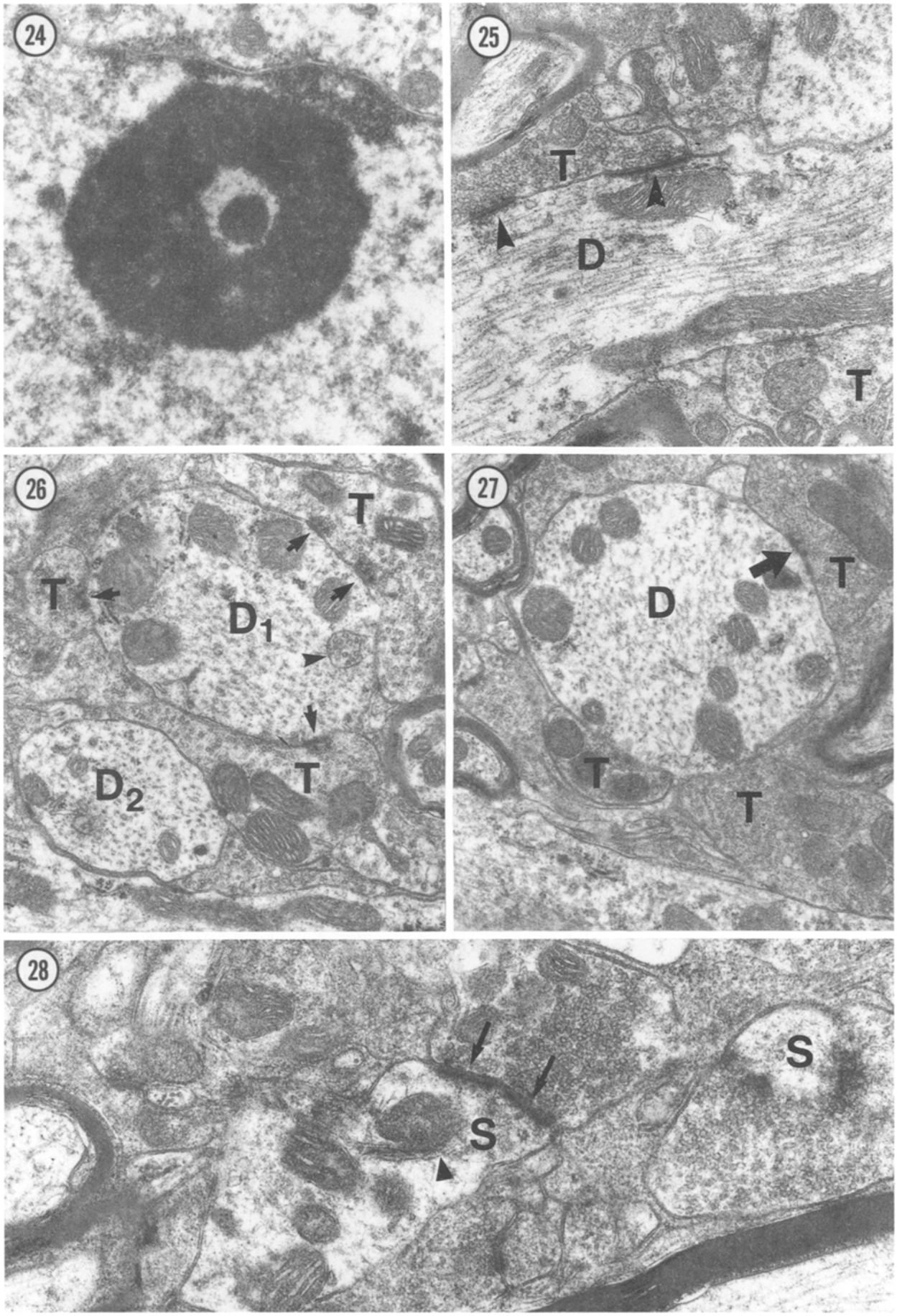
**Fig. 24.** Electron micrograph of a nucleolus which appears to be infolded. This type of nucleolus is common and is found most often in the small cell types. × 25 000.

**Fig. 25.** A dendrite (D) is contacted by two clear axon terminals (T) that contain round vesicles (arrowheads). One of the terminals forms two active zones that are both asymmetric. × 25 000.

**Fig. 26.** Two dendrites cut in cross section ($D_1$, $D_2$). $D_1$ is almost completely surrounded by axon terminals (T) and forms several synapses (arrows) with these clear terminals. Note its multivesicular body (arrowhead). × 21 700.

**Fig. 27.** A cross-sectioned dendrite (D) is contacted by three terminals (T) that have a dark matrix. Only one of these forms a synapse (arrow) with the dendrite. × 21 400.

**Fig. 28.** This section of neuropil contains at least two spines (S). One of these spines is contacted by a terminal that forms two active zones (arrows) which are asymmetric. Note the possible spine apparatus (arrowhead). × 31 250.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shape, while Rockel & Jones (1973b) categorized the terminals according to terminal size, vesicle size and shape. Although each of these classification schemes utilizes different criteria, the common conclusion is that several types of terminal exist and they may arise from different sites. For example, injections of [$^3$H]leucine and/or [$^3$H]proline into the dorsal cochlear nucleus of the cat yielded labelled terminals in the ICCN which had small, round synaptic vesicles and formed synapses mainly on dendrites (Oliver, 1985). More recent data (Oliver & Krevolin, 1985) indicate that similar axon terminals arise from the ventral cochlear nucleus. In addition, Rockel & Jones (1973b) lesioned the lateral lemniscus to produce degeneration in the ICCN and found degenerating terminals with large, round synaptic vesicles that formed synapses mainly on the proximal dendrites of disc-shaped neurons. Therefore, specific projections from lower brainstem auditory nuclei terminate with different characteristics.

We have observed three different types of terminals that form symmetric synapses and contain flattened vesicles, i.e. small and large terminals with clear matrices and another type of terminal with a dark matrix. These terminals may arise from intrinsic neurons of the IC or from somata located in lower brainstem auditory centres. Data that support the notion that some of these terminals may originate from intrinsic neurons include the presence in the IC of many GABAergic neurons (Roberts *et al.*, 1985c) that have morphological features similar to previously described interneurons (Rockel & Jones, 1973b). Adams & Mugnaini (1984) have found that most, if not all, of the neurons in the dorsal nucleus of the lateral lemniscus contain GABA, which suggests that some of the terminals that form symmetric synapses may arise from lower brainstem auditory centres. Since most neurons in this structure project to the IC, some of the GABAergic terminals in the IC must arise from this nucleus. Other lower brainstem auditory nuclei display large populations of GABAergic neurons and they may also project to the IC. These regions include the lateral superior olive, ventral nucleus of the lateral lemniscus and ventral cochlear nucleus (Adams & Mugnaini, 1984; Moore & Moore, 1984; Roberts *et al.*, 1985b). To verify the existence and extent of these proposed GABAergic ascending pathways, double labelling studies must be conducted.

*Dendrites*

Many dendrites of neurons in the ICCN that were analysed in electron microscopic preparations displayed frequent axodendritic synapses. For example, the smooth or beaded dendrites of large and medium-sized neurons were contacted by a large number of axon terminals, whereas spines were not often found adjacent to these dendrites. In the cerebral cortex where pyramidal cells are extremely spiny (Feldman, 1984), most synaptic contacts are made with the spines of these dendrites and not the dendritic shafts. Thus, the dendrites of large and medium-sized neurons may probably have few spines because their shafts are contacted by many terminals. This conclusion is consistent with results from Golgi studies that indicate that the dendrites from large and medium-sized neurons in the ICCN are sparsely-spinous (Oliver & Morest, 1984; Faye-Lund & Osen, 1985). In contrast, the dendritic shafts of small neurons infrequently formed synapses and it is possible that most synaptic input arrives onto spines for this cell type. The Golgi studies of the ICCN (Oliver & Morest, 1984; Faye-Lund & Osen, 1985) have shown that the small neurons have more densely spinous dendrites than the medium-sized and large neurons. The significance of this difference in dendritic spine density for the subclasses of neurons in the ICCN is unclear at the present time. More detailed studies with a combined Golgi–electron microscopic method may provide a better understanding of axospinous synapses in the ICCN.

Dendrites were occasionally observed to enter a lamina of myelinated axons. Their presence within such axon bundles may indicate a simple guidance function of axons for sending certain dendrites to distant laminae in rigid structural networks. The fact that such dendrites are not contacted by terminals in these bundles suggests that they will probably be more easily influenced by a distal lamina of neuropil. The functional organization of these laminae has been suggested from the tonotopic organization obtained from physiological data (Semple & Aitkin, 1980; Webster *et al.*, 1985). Future studies may show the differences in the physiological properties of disc-shaped and stellate neurons with the use of intracellular labelling methods following their recording.

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