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Ultrastructural features of six types of neurons in rat dorsal root ganglia

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Summary

Sections of glutaraldehyde-fixed lumbar dorsal root ganglia of the rat were examined in the electron microscope following impregnation with the uranyl–lead–copper technique or postfixation in potassium ferrocyanide-reduced osmium. Three types of ganglion cells (A, B, C) were identified on the basis of their size and the distribution of their organelles. They were further subdivided into six subtypes according to the arrangement and three-dimensional organization of the Nissl bodies and Golgi apparatus in the perikarya. Type A_1 cells were large, clear neurons in which Nissl bodies, separated from each other by pale narrow strands of cytoplasm containing small stacks of Golgi saccules and rod-like mitochondria, were evenly distributed throughout the perikaryon. In type A_2, the Nissl bodies assumed a similar distribution but were separated by much wider strands of cytoplasm. Type A_3, the smallest of the type A category, displayed densely packed Nissl bodies and long stacks of Golgi saccules which formed a perinuclear ring in the midportion of the perikaryon.

Type B cells were smaller and showed a concentric zonation of their organelles. In type B_1, large Nissl bodies located in an outer cytoplasmic zone were made of long piles of parallel cisternae interrupted by curved Golgi stacks. Type B_2 was characterized by a ring-like Golgi apparatus separating the perikaryon in a cortical zone composed mainly of Nissl substance and a juxtanuclear zone containing mitochondria and smooth endoplasmic reticulum. Type C cells were the smallest of the ganglion cells and contained small, poorly demarcated Nissl bodies and a juxtanuclear Golgi apparatus.

Introduction

The neurons of normal sensory ganglia, when examined in the light microscope, have usually been classified into two main categories: the large pale neurons or type A and the small dark neurons or type B (see review by Scharf, 1958). Using criteria such as the size of the perikarya or the morphology and distribution of the Nissl bodies, these two main categories of neurons were further subdivided into subclasses, the number of which

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varied according to the author (Dogiel, 1908; Clark, 1926; Hirt, 1928; Körner, 1937; Andres, 1961). As pointed out in a recent review (Lieberman, 1976), 'most of these classifications were esoteric, few showed more than a superficial correspondence one with another and none are now of great importance'. Electron microscopy, however, confirmed the existence of two groups of neurons on the basis of ultrastructural features (Dawson *et al.*, 1955; Cervos-Navarro, 1959; Andres, 1961; Novikoff, 1967; Jacobs *et al.*, 1975) and histochemical properties (Tewari & Bourne, 1962; Cauna & Naik, 1963; Droz, 1967a, b; Novikoff, 1967; Kalina & Bubis, 1968; Kalina & Wolman, 1970; Sarrat, 1970; Novikoff *et al.*, 1971; Knyihar, 1971; Boutry & Novikoff, 1975; Spater *et al.*, 1978, 1981). Andres (1961), using both phase contrast and electron microscopy, was able to distinguish from the appearance of the Nissl bodies three subtypes for each of the light (A_1, A_2, A_3) and dark (B_1, B_2, B_3) neurons. Jacobs (1976) suggested that in the pale type A cells the Nissl bodies might assume a wide spectrum of configurations, of which these different subtypes would be extreme examples (Jacobs *et al.*, 1975). Nevertheless, from the aspect of the Golgi apparatus in zinc iodide-osmicated material, Duce & Keen (1977) could divide spinal ganglion cells into six subtypes apparently corresponding to those described by Andres (1961). Furthermore, according to Duce & Keen (1977), prolonged nerve stimulation did not modify the relative numbers of the various cell types, suggesting that none of the differences observed between cell types were due to variations in their state of activity. In the present study, the distribution and three-dimensional configuration of intracytoplasmic organelles in dorsal root ganglion cells were reinvestigated using heavy metal staining techniques, and stereo electron microscopy, which allowed a more precise description of the various types of ganglionic neurons.

Materials and methods

Ten adult rats were perfused through the aorta with a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer. After 15 min of perfusion, spinal ganglia were removed from the lumbar region, postfixed for an additional hour in the same fixative and treated in either of the following ways. In one case, the ganglia were rinsed in cacodylate buffer, immersed in a 5% aqueous uranyl acetate solution for one hour at 42° C, washed in distilled water and poststained for an additional hour at 42° C in a lead and copper citrate solution. After several rinses in distilled water, they were postfixed overnight at 4° C in a 1% aqueous solution of osmium tetroxide. This technique will be referred to as the Ur–Pb–Cu technique (Thiéry & Rambourg, 1976). In the second case, the ganglia were stored overnight at 4° C in cacodylate buffer and postfixed for one hour at room temperature in a 1:1 mixture of 2% aqueous osmium tetroxide and 3% aqueous potassium ferrocyanide. This technique will be referred to as the reduced osmium technique (Karnovsky, 1971). After staining with either method, the tissues were rinsed in distilled water, dehydrated in ethanol and embedded in Epon. Thin and thick (0.5–1 μm) sections were prepared with an MT1 Porter Blum ultramicrotome, mounted on copper grids and examined in the electron microscope (Philips 400). 0.5–1 μm thick sections of Ur–Pb–Cu-impregnated material were examined without counterstaining at 100–120 kV. Thin sections of reduced osmium-stained tissue were counterstained for 2 min with lead citrate and examined at 60 kV. For stereoscopy, thick sections were placed on the goniometric stage of the electron microscope and two photo-

graphs of the same field were taken after tilting the specimen -7° and $+7^{\circ}$ from the original 0° position. A three-dimensional image of the impregnated structures was obtained by looking at properly adjusted pairs of such pictures with a stereoscopic binocular lens.

Results

In toluidine blue-stained semithin sections examined in the light microscope or in Ur–Pb–Cu-impregnated 0.5 μm thick sections examined at low magnification in the electron microscope, three main types of ganglion cells were identified, and referred to as type A, B and C, respectively. The *type A* cells were large neurons (40–75 μm in diameter) containing numerous small, block-like Nissl bodies uniformly distributed throughout the perikaryon except in the area of the axon hillock. Numerous small Golgi bodies and rod-like mitochondria were interspersed in the cytoplasm separating the Nissl bodies. The *type B* cells were smaller neurons (20–50 μm in diameter) which showed a characteristic zonation of their organelles. The Nissl substances formed a continuous web which completely filled the outer third of the perikaryon. The Golgi apparatus appeared as long and thick crescentic bodies arranged in a ring-like fashion in the mid-zone of the perikaryon. In contrast, mitochondria tended to accumulate next to and around the nucleus. The *type C* cells were the smallest of the ganglion cells, i.e. with a diameter of less than 20 μm. Their perikaryon contained small, often poorly individualized Nissl bodies and dispersed mitochondria. The Golgi apparatus formed a perinuclear network in close contact with the nucleus.

Cells of the A and B groups could be further subdivided into a number of subtypes according to the spacial organization of their intracytoplasmic organelles. Each of these subtypes is diagrammatically presented in Fig. 1. *Type* A_1 cells were the largest of the ganglion cells and the most frequent amongst the type A. In ferrocyanide-reduced osmium-stained sections, the Nissl bodies appeared as ill-defined clusters of short and profusely anastomosed cisternae of rough endoplasmic reticulum (ER) (Fig. 2). These ER cisternae were occasionally parallel to one another (Fig. 2, wide arrow), but more commonly assumed a haphazard arrangement. In Ur–Pb–Cu-impregnated 0.5 μm thick sections, the Nissl bodies took the form of much more compact aggregates of densely stained lamellae, interconnected in all directions of space (Fig. 3). Examination of the same material in thin sections (Fig. 3, inset) confirmed that such lamellae corresponded to rough ER cisternae, filled with Ur–Pb–Cu precipitate (compare insets of Figs. 2 and 3). The three-dimensional architecture of the Nissl bodies could be best appreciated by stereoscopic examination of Ur–Pb–Cu-impregnated thick sections (Fig. 12). They were composed of plate-like cisternae, oriented in all directions and interconnected by multiple narrow ribbon-like cisternae. In Ur–Pb–Cu-stained material, the Nissl bodies were separated from each other by narrow bands of poorly impregnated cytoplasm (Fig. 3). Within these strands, short stacks of Golgi saccules, secondary lysosomes and rod-like mitochondria were evenly dispersed amidst a loose meshwork of narrow tubular and/or ribbon-like cisternae. The latter were continuous with and

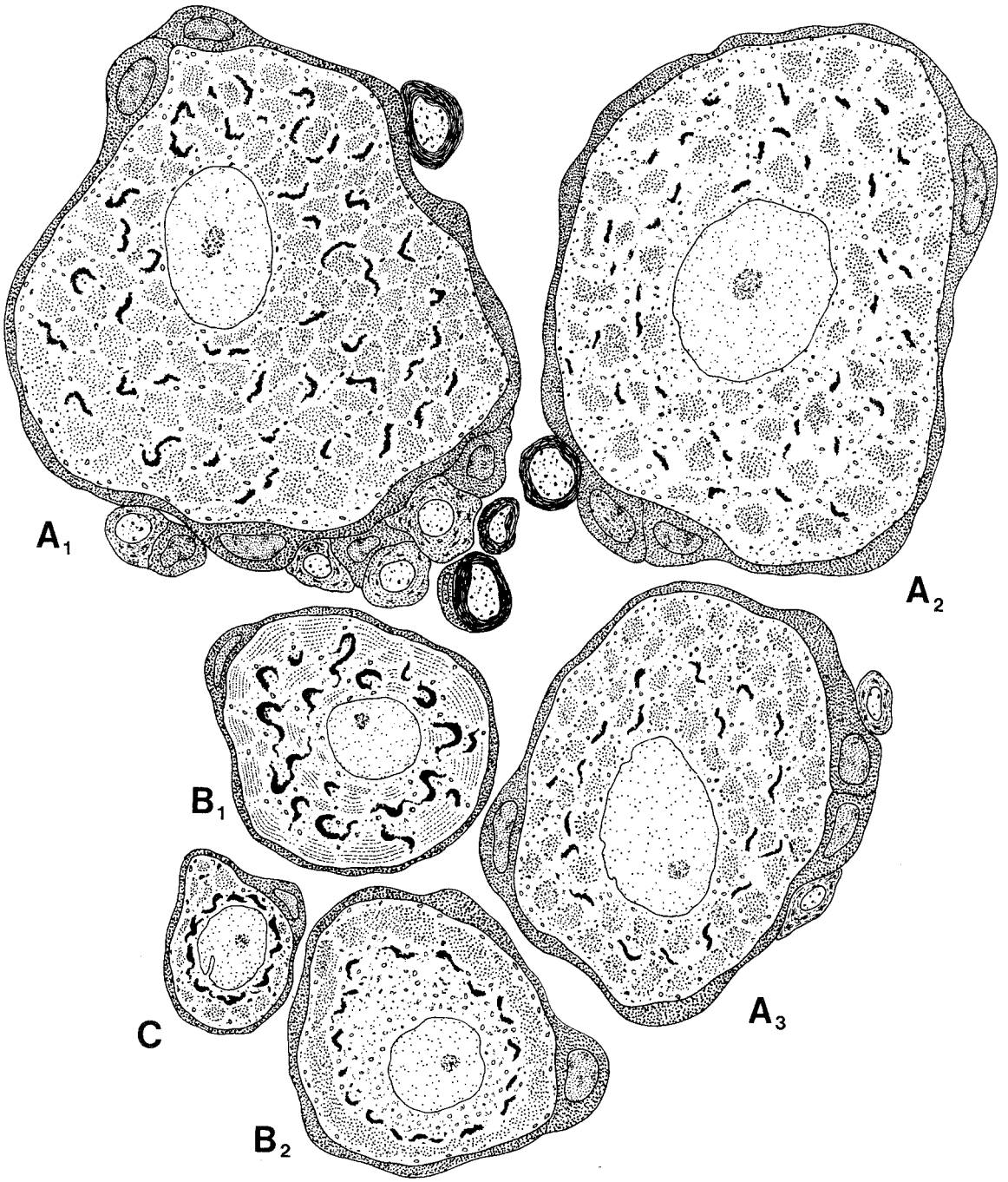
thus appeared to connect the rough ER cisternae forming the Nissl bodies (Figs. 3, 12). These thread-like cisternae were particularly abundant in the area of the axon hillock (Fig. 14), where comparison with thin sections of ferrocyanide-reduced osmium-stained material clearly showed that they corresponded to elongate cisternae of smooth ER.

In *type* A_2 cells, the Nissl bodies showed basically the same three-dimensional configuration as in the type A_1 cells, but were separated from one another by much wider strands of cytoplasm (Fig. 4). These wide channels of cytoplasm were partially occupied by large bundles of neurofilaments, visible in reduced osmium-stained material (Fig. 5). They also contained Golgi bodies, mitochondria, lysosome-like granules and loosely anastomosed cisternae of smooth ER (Figs. 4, 5). The latter were continuous with and bridged the ER cisternae of adjacent Nissl bodies (Fig. 4, arrowheads). The stacks of Golgi saccules were usually smaller than in the type A_1 cells. The membranous tubules and vesicles seen on the transface of the stacks were also less prominent than in type A_1 cells.

Type B_2 cells were the smallest of type B cells and showed the most clear cut zonation of their perikaryal organelles. The outer *cortical zone* extended through the external third of the perikaryon and was almost exclusively occupied, except at the level of the axon hillock, by an intricate meshwork of interconnected rough ER cisternae (Fig. 6). In Ur–Pb–Cu-stained thick sections, these ER cisternae appeared as short lamellae haphazardly oriented and anastomosed in all directions by ribbon-like elements (Figs. 6, 13). Occasionally, collections of 4–6 cisternae formed short parallel arrays (Figs. 6, 7). This homogeneous cortical zone was sporadically infiltrated with mitochondria but only rarely by Golgi bodies. The juxtanuclear zone contained the Golgi apparatus, an abundance of mitochondria, some lysosome-like granules and a loose network of smooth ER cisternae which established numerous connections with the rough ER cisternae of the cortical zone (Fig. 7). Several long and wavy stacks of Golgi saccules occupied the outer aspect of the juxtanuclear zone (Fig. 7).

Type B_1 cells were larger and more frequently encountered than type B_2 cells. The concentric zonation of their cytoplasmic organelles was also less evident than in the B_2

Fig. 1. Diagrams illustrating the characteristic features of six types of neurons in dorsal root ganglia. A_1: Large neuron with Nissl bodies evenly distributed throughout the cytoplasm and separated from each other by narrow spaces; stacks of Golgi saccules distributed throughout the perikaryon. A_2: Large neuron with Nissl bodies distributed throughout the cytoplasm but separated from each other by wide strands of neuroplasm; small Golgi stacks widely dispersed throughout the perikaryon. A_3: Smaller neuron with closely packed Nissl bodies distributed throughout the cytoplasm; stacks of Golgi saccules form a perinuclear ring located in the mid-portion of the perikaryon. B_1: Small neuron with Nissl bodies showing parallel cisternae located mainly in an outer cytoplasmic zone; large, curved stacks of Golgi saccules form a broad perinuclear net. B_2: Small neuron showing a cortical zone composed mainly of Nissl substance; the Golgi apparatus forms a ring separated from the nucleus by a zone of cytoplasm containing mitochondria and smooth ER cisternae. C: Smallest neuron showing poorly demarcated Nissl bodies and a juxtanuclear Golgi apparatus.


A1
A2
B1
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cells. The *cortical zone* was composed of much more orderly arranged rough ER cisternae, which in Ur–Pb–Cu-impregnated tissue appeared as long and flattened lamellae forming regular parallel arrays running in the same direction as the plasma membrane (Fig. 15). At their extremities, the cisternae were interconnected by narrow bridging ER cisternae (Fig. 15). In reduced osmium-stained sections, the parallel arrangement of the

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Fig. 2. A_1 cell: reduced osmium-stained thin section. The mitochondria (M) and short stacks of Golgi saccules (G) are widely and evenly dispersed throughout the perikaryon. They are located in poorly stained bands of neuroplasm separating the Nissl bodies (NI). In each Nissl body (inset), short ER cisternae are disposed in a haphazard fashion in a light cytoplasmic matrix containing free ribosomes. Occasionally, a few cisternae may assume a parallel arrangement (wide vertical arrow). N: nucleus; L: lysosome-like body. × 9000. Inset: × 30 000.

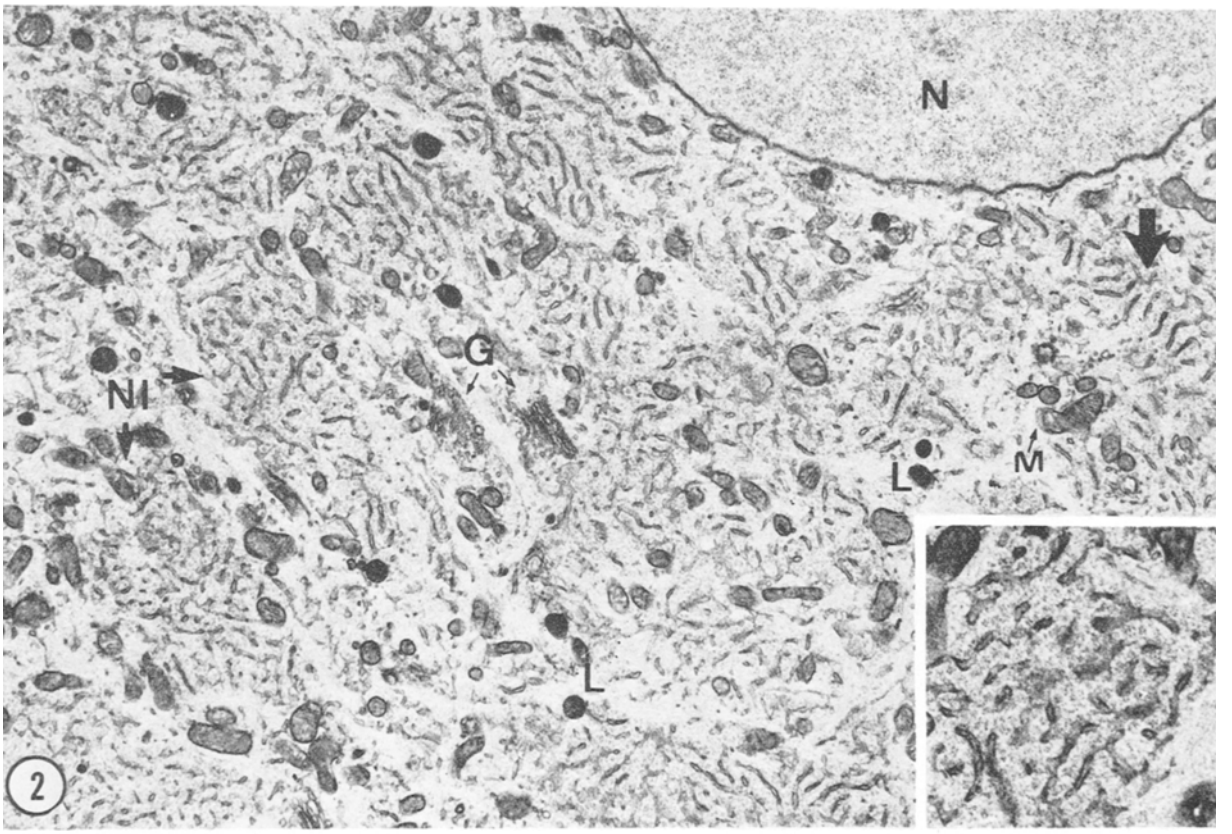
Fig. 3. A_1 cell: 0.5 μm thick section. Ur–Pb–Cu technique. The Nissl bodies (NI) consist of small plate-like cisternae oriented in all directions and interconnected by narrow ribbons. As seen in a thin section at higher magnification (inset), the heavy metals accumulate within the cisternae whereas the ribosomes remain unstained. In the Nissl body located at upper left, a few cisternae are parallel to one another. The Nissl bodies are separated by narrow bands of poorly stained neuroplasm in which short stacks of Golgi saccules (G) and rod-like mitochondria (M) are seen amidst a loose network of tubular or ribbon-like cisternae (vertical arrow). On the concave or transface of a Golgi stack poorly stained membranous tubules are indicated by a horizontal arrow. L: lysosome. × 9000. Inset: × 22 000.

Fig. 4. A_2 cell: 0.5 μm thick section. Ur–Pb–Cu technique. Poorly developed Nissl bodies (NI) are distributed evenly throughout the perikaryon and display a honeycombed appearance. Rod-like mitochondria (M) and short Golgi stacks (G) may be seen in wide channels of poorly stained cytoplasm. Loosely anastomosed cisternae of smooth ER are indicated by arrowheads. L: lysosomes. × 9000.

Fig. 5. A_2 cell: reduced osmium-stained thin section. The Nissl bodies (NI) consist of short cisternae anastomosed in all directions. As in the A_1 cell, these cisternae are embedded in hyaloplasm filled with free ribosomes. Neurotubules and neurofilaments fill the wide spaces separating the Nissl bodies. In these spaces, small Golgi stacks may be seen in cross sections or in front view at G. The mitochondria are labelled M and the tubules of smooth ER are indicated by arrowheads. × 15 000.

Fig. 6. B_2 cell: 0.5 μm thick section. Ur–Pb–Cu technique. Curved Golgi stacks (G) form a perinuclear ring in the mid-zone of the perikaryon, dividing it into an outer cortical and an inner perinuclear zone. In the outer cortical zone, the Nissl bodies (NI) form an almost continuous lamellar structure in which 4–6 cisternae (NI at upper right) are occasionally disposed in short parallel arrays. The inner perinuclear zone is filled with mitochondria (m) and contains a loose network of tubular and ribbon-like smooth ER cisternae. × 9000.

Fig. 7. Thin section of a B_2 cell. Ur–Pb–Cu technique. At the inner surface of the outer cortical zone, the lamellar cisternae of the Nissl bodies (NI) are continuous with the loosely anastomosed tubular or ribbon-like cisternae of the smooth endoplasmic reticulum located in the inner perinuclear zone (vertical arrow). In between the two zones, a Golgi body (G) consists of stacks of intensely stained saccules (S) and of a less heavily stained system of anastomosed tubules (horizontal arrow) located in the concave or transface of the stacks. m: mitochondria; mv: multivesicular body; L: lysosome. × 21 000.



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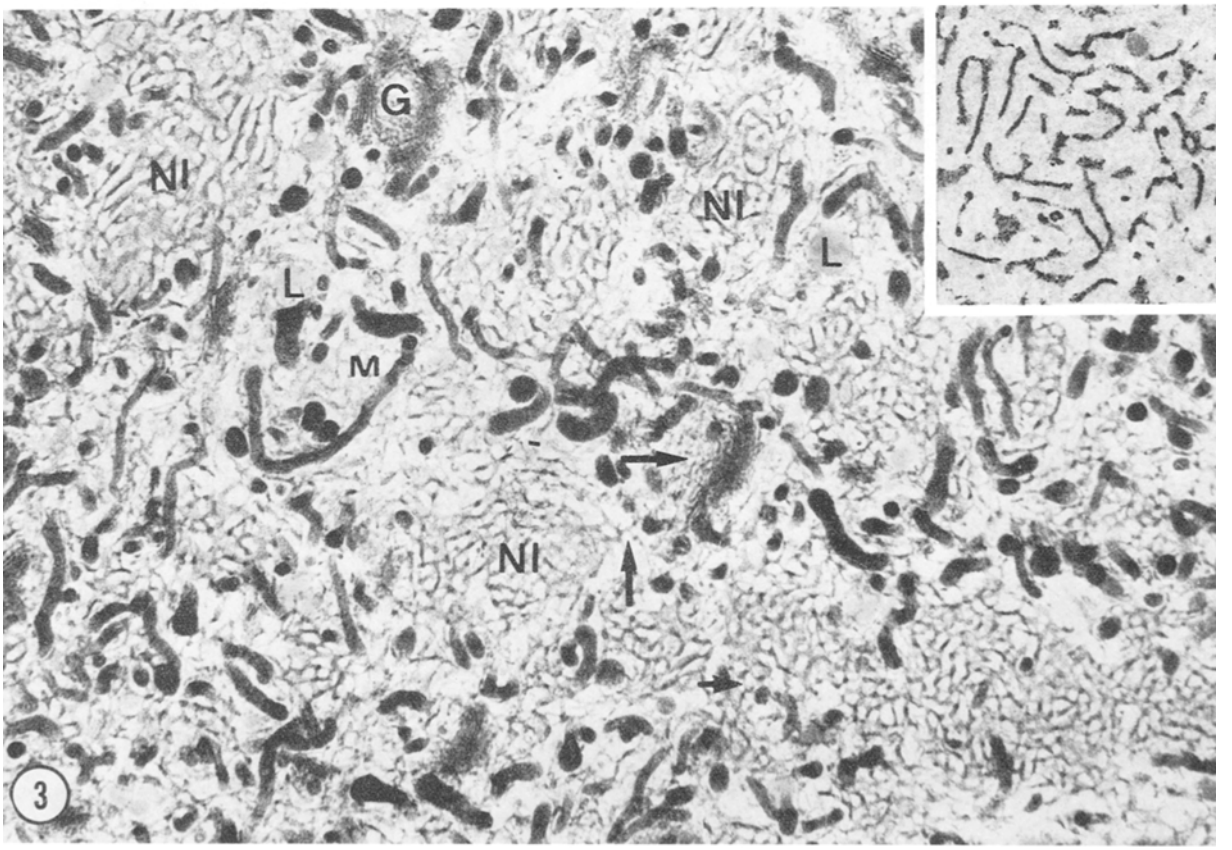

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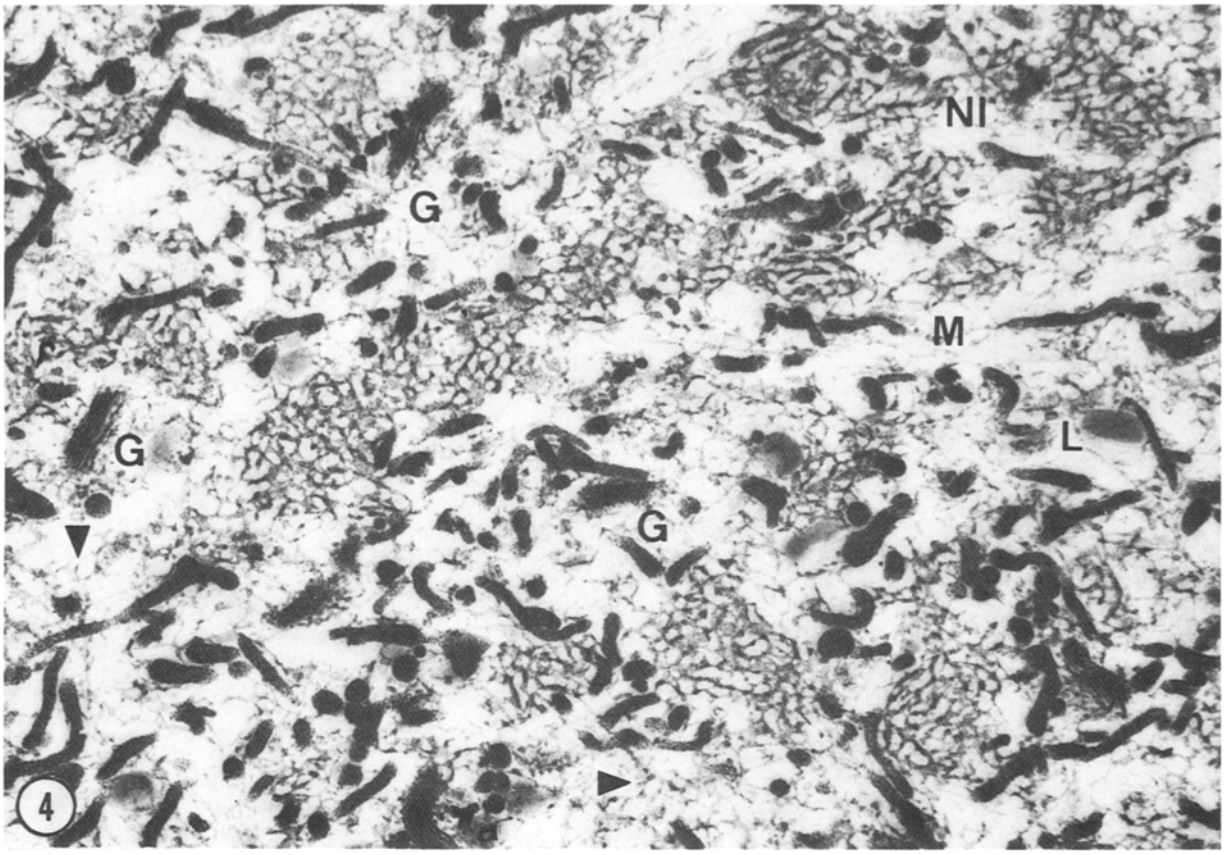
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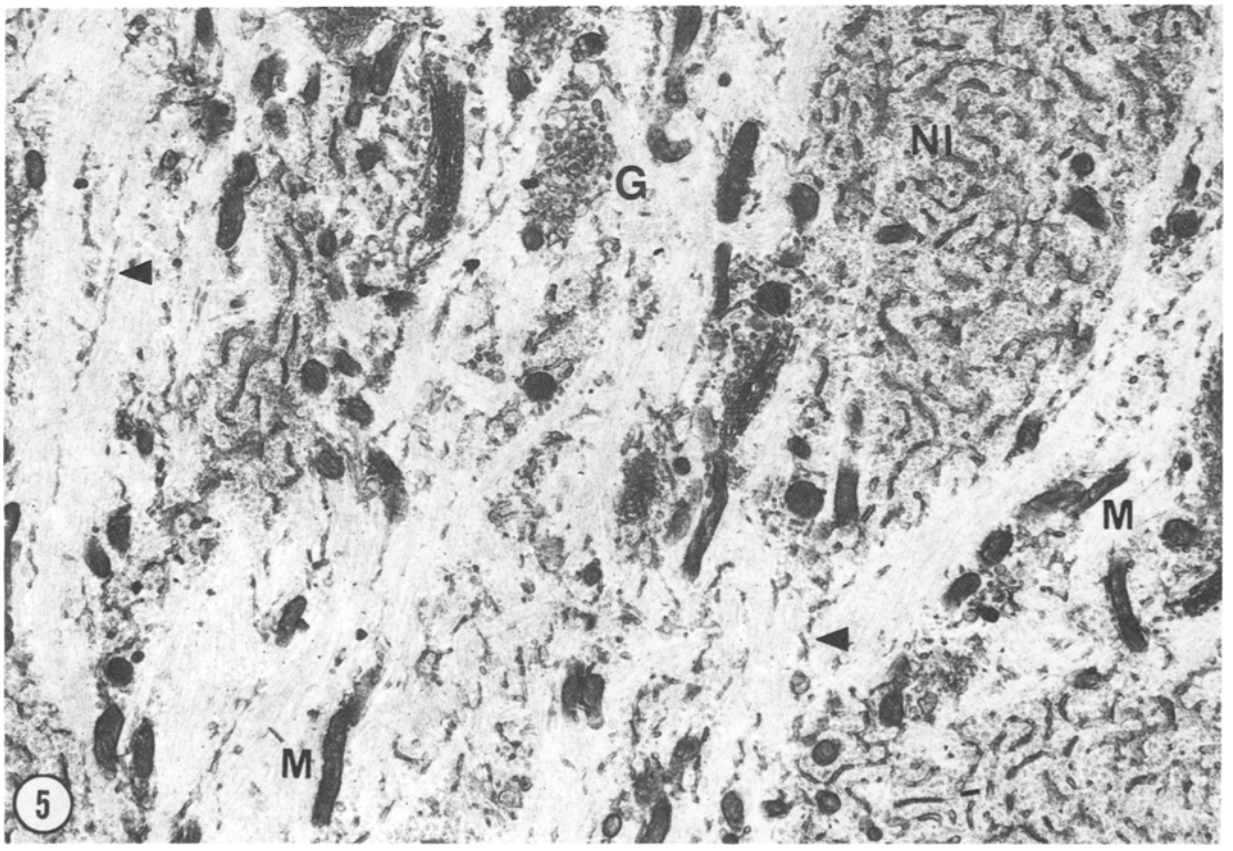
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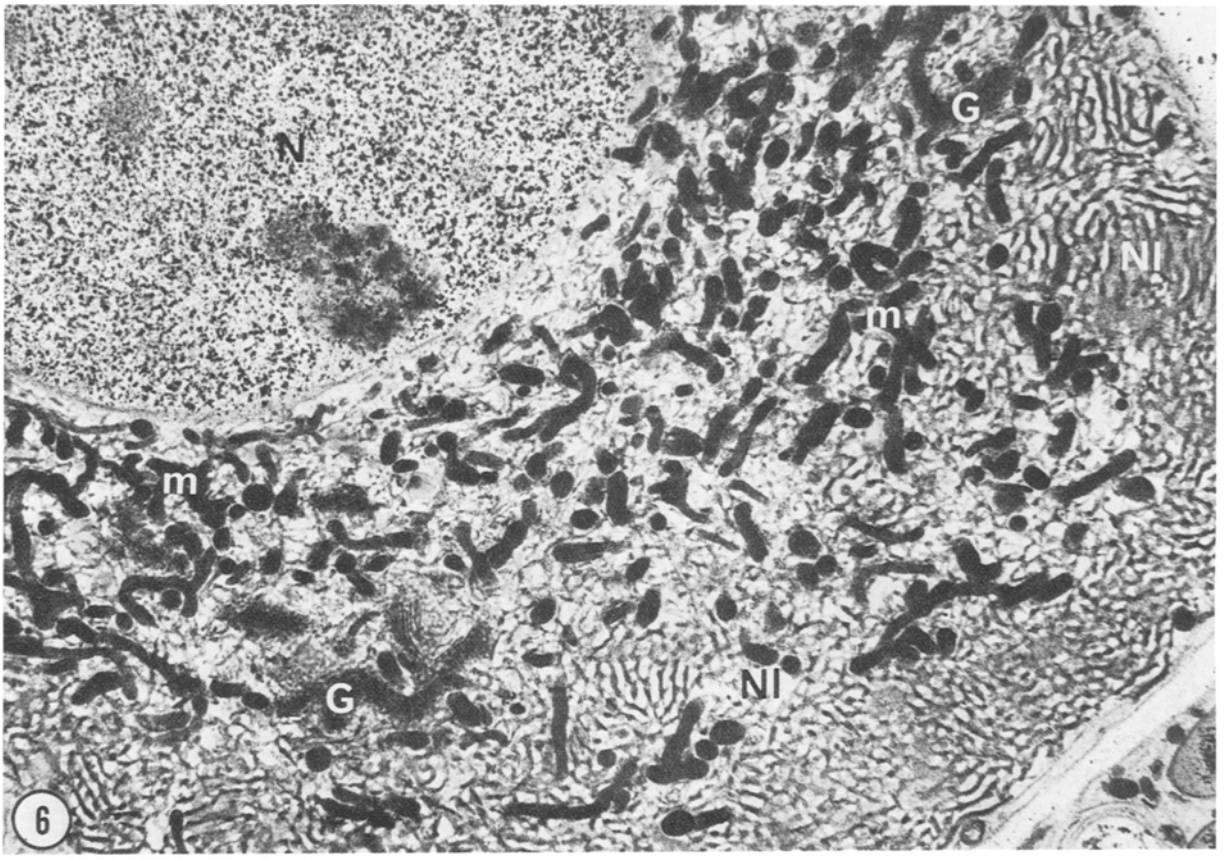

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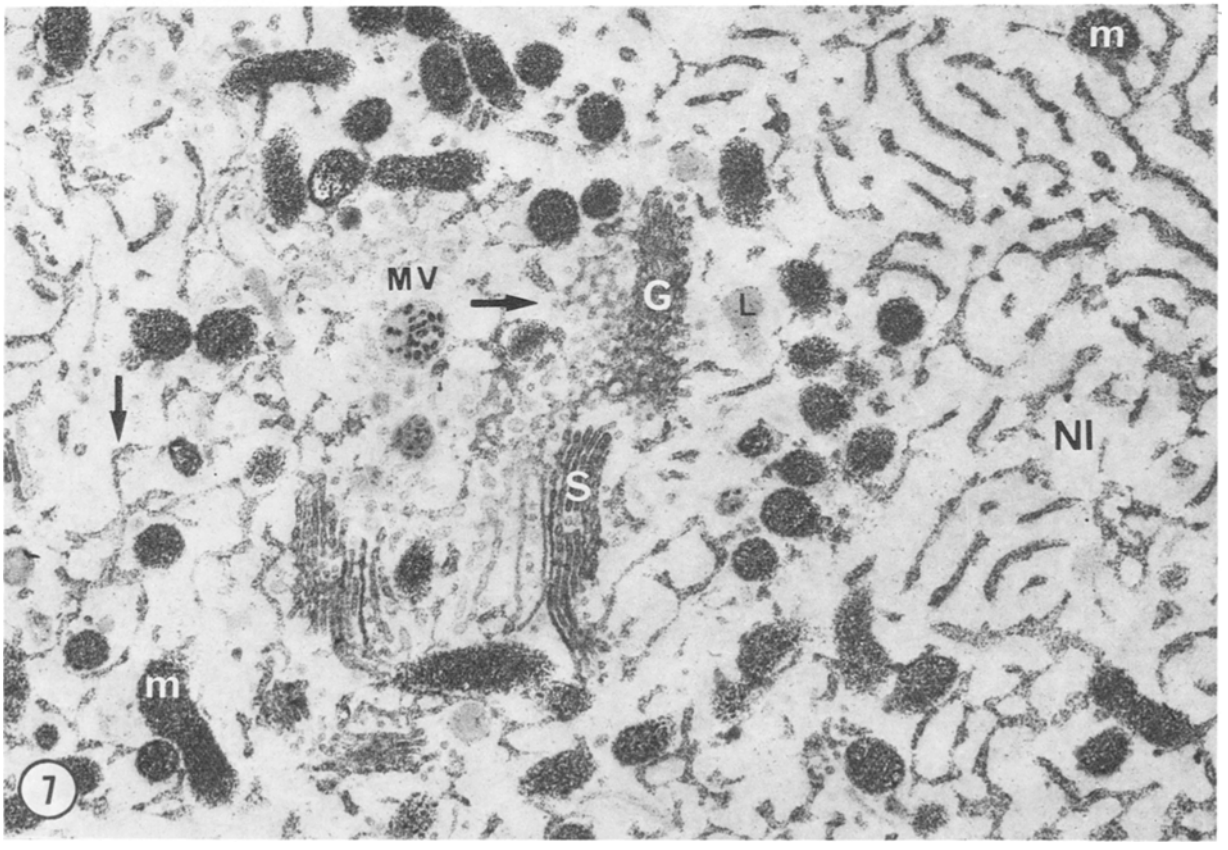

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long cisternae was particularly obvious (Figs. 8, 9). An occasional stack of Golgi saccules and some mitochondria were seen in the narrow gaps separating these Nissl bodies (Figs. 8, 9). The Golgi apparatus was the most conspicuous component of the *juxtanuclear zone* in both Ur–Pb–Cu or reduced osmium-impregnated sections. This apparatus was composed of series of stacks of saccules having the shape of strongly curved scales

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Fig. 8. B_1 and A_3 cells in a reduced osmium-stained thin section. Two cells of approximately the same size are seen in this field taken at low magnification. In the B_1 cell, at left, curved Golgi bodies (G) stand out sharply against a more greyish background and form a conspicuous perinuclear ring. Nissl bodies (NI) are preferentially located in the outer third of the perikaryon and consist of stacks of cisternae mainly arranged in parallel arrays (arrows). As the mitochondria and dense bodies are evenly distributed throughout the cytoplasm, the division of the perikaryon in inner and outer zones is less 'clearcut' than in the B_2 cells. In the A_3 cell, at right, Nissl bodies (NI), Golgi stacks (G) and mitochondria are interspersed throughout the perikaryon. The Golgi stacks and the mitochondria are mostly concentrated in the perinuclear region. bc: blood capillary; nf: myelinated nerve fibre; S: satellite cell. × 3000.

Fig. 9. B_1 cell: reduced osmium-stained thin section. Particularly striking at higher magnification are the long arrays of parallel plate-like cisternae forming the Nissl bodies (NI). The perinuclear Golgi apparatus forms large loops (G) which are sectioned under various incidences. In the concavity of these loops, a system of less intensely stained tubules and vesicles may be seen. M: mitochondria; L: lysosomes; N: nucleus. × 9500.

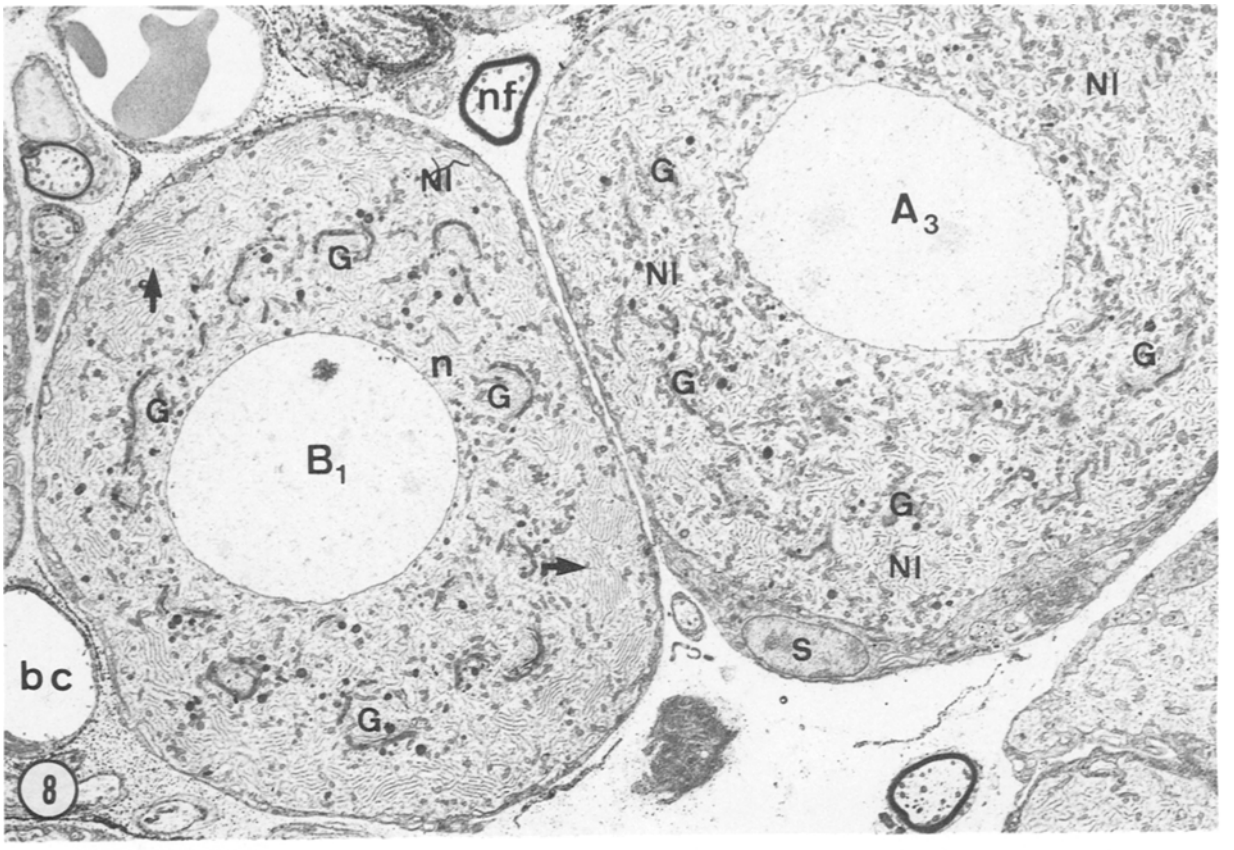
Fig. 10. A_3 cell: 0.5 μm thick section. Ur–Pb–Cu technique. The Nissl bodies (NI) display a honeycombed appearance and are separated from one another by clear strands of neuroplasm. Straight Golgi stacks (G) are found in the inner two-thirds of the perikaryon and form a perinuclear network as in a B cell. Faintly stained lysosomes (L) and rod-like mitochondria are interspersed throughout the perikaryon. N: nucleus. × 9500.

Fig. 11. C cell: 0.5 μm thick section. Ur–Pb–Cu technique. Curved Golgi stacks (G) form a perinuclear ring in the immediate vicinity of the nucleus (N). Small Nissl bodies (NI) consisting of a few narrow plate-like cisternae are loosely scattered through the mid-zone of the perikaryon. The mitochondria and lysosomes (L) are dispersed through the perikaryon. × 9500.

Figs. 12–15 are stereopairs. The two members of each pair are photographs from the same field taken at different angles (± 7°) from the original 0° position of the goniometric stage. A single stereoscopic image of the impregnated organelles may be obtained by using a stereoscopic binocular lens, the distance between the central axes of the two lenses being adjusted to 65–70 mm. All figures were taken from 0.5 μm thick sections of rat spinal ganglia impregnated by the Ur–Pb–Cu technique.

Fig. 12. Perikaryon of A_1 cell. Two Nissl bodies (NI) are seen at the upper left and in the lower part of the figure. They consist of anastomosed plate and ribbon-like cisternae and are interconnected by tubular and ribbon-like cisternae (arrowheads) which are parts of the smooth endoplasmic reticulum. A small Golgi stack is visible at the letter G; on its transface note a system of less intensely stained tubules which is poorly developed in this cell type. A rod-like mitochondrion is indicated by an arrow. L: lysosome. × 7500.

Fig. 13. Nissl body of a B_2 cell. Short plate-like cisternae are interconnected by ribbon-like cisternae to make up a continuous three-dimensional network. × 25 000.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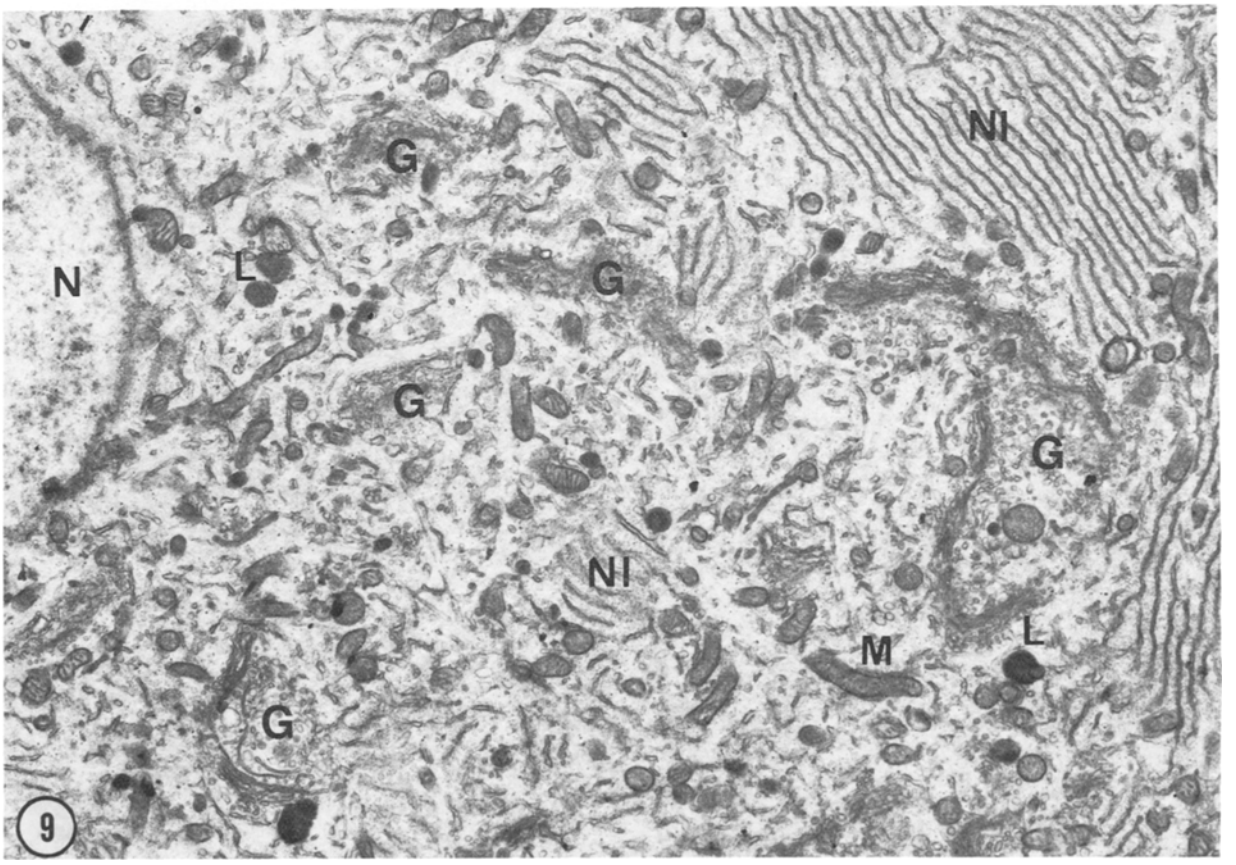
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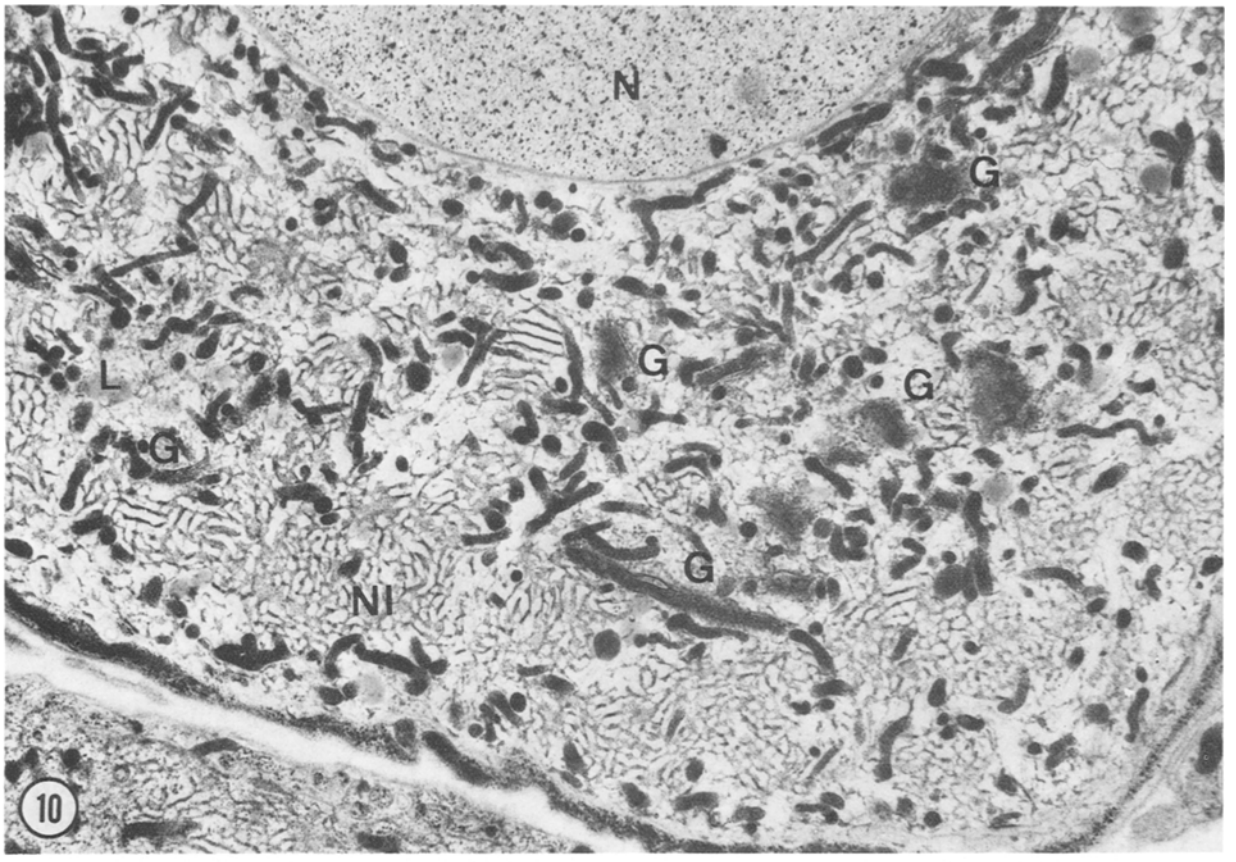

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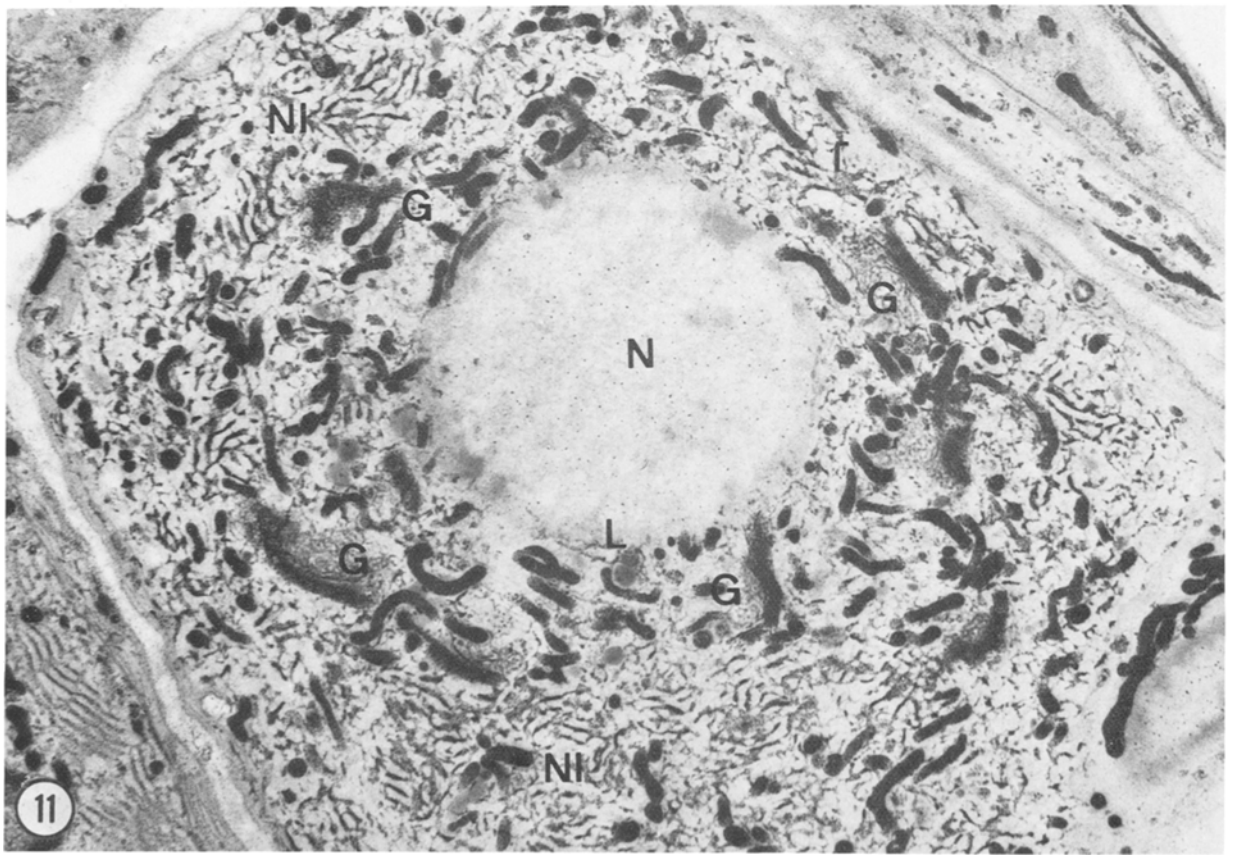

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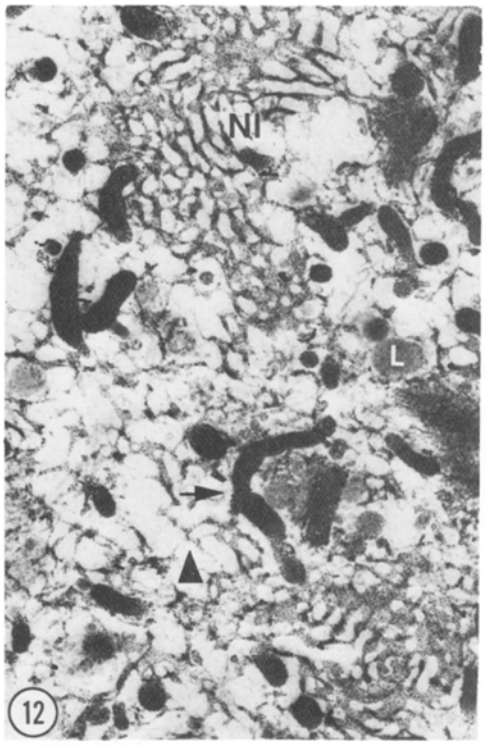

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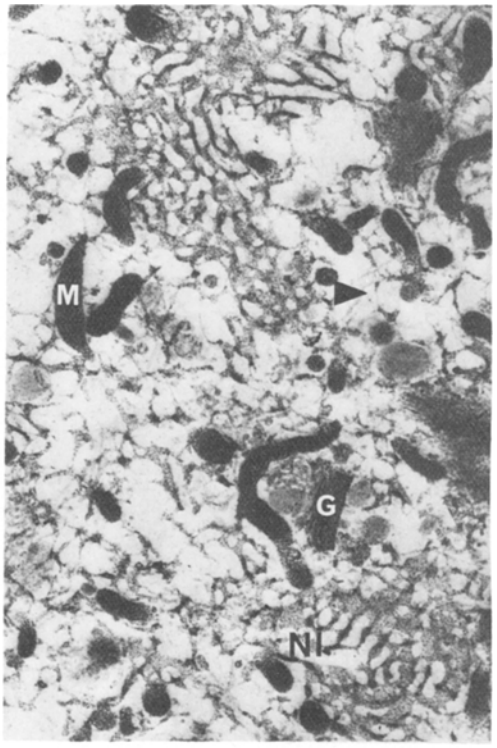

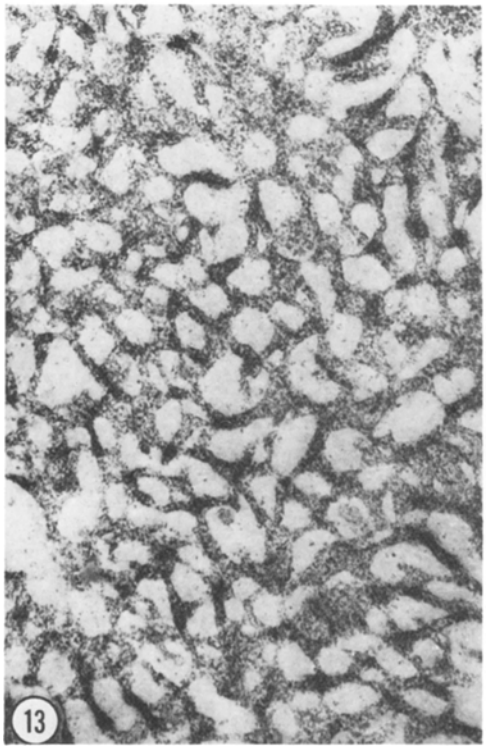

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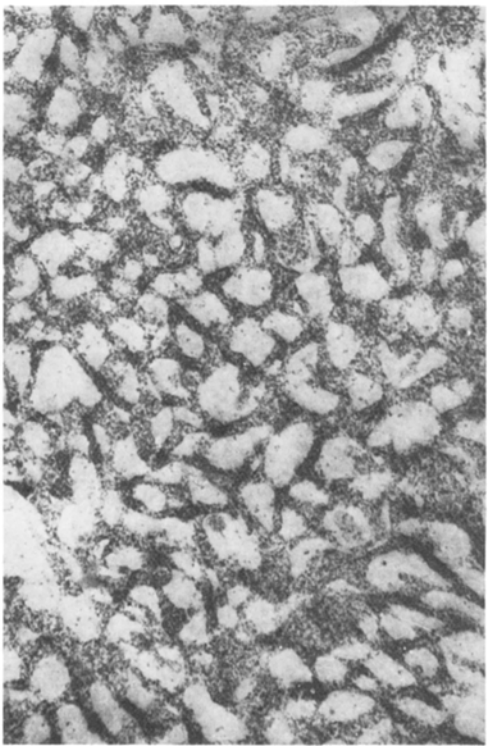

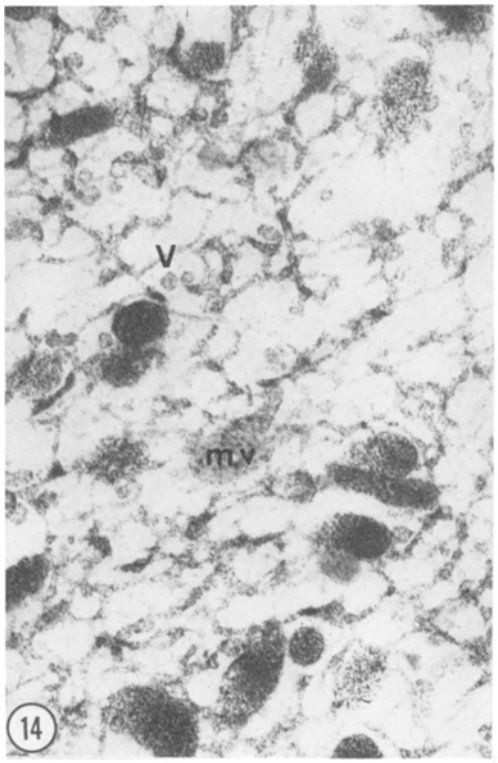

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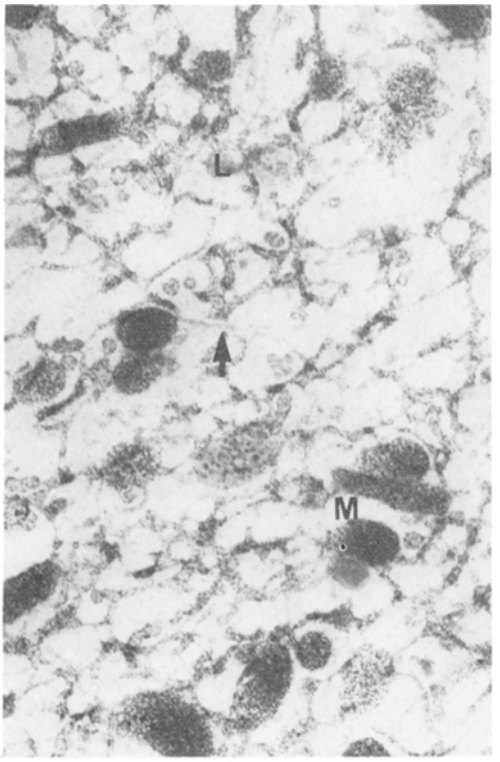

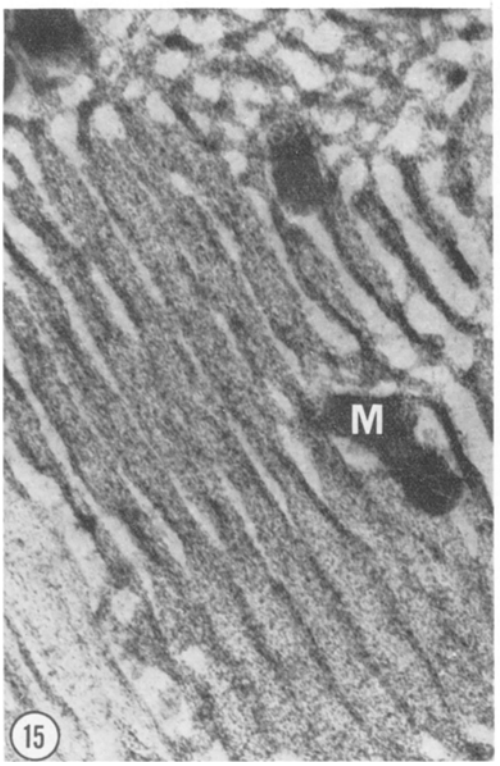

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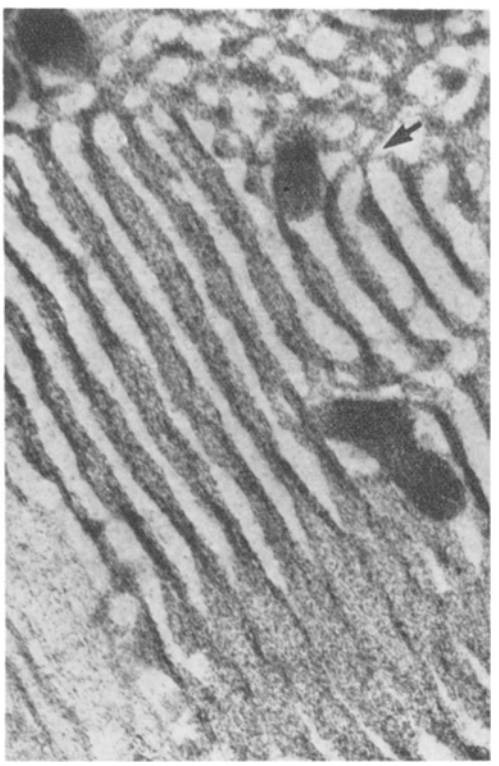

or plates (Figs. 8, 9). In the concavity or transface of these stacks there was an accumulation of tubular or vesicular elements which in Ur–Pb–Cu-impregnated ganglia gave a pale greyish colour to the area. These curved stacks of saccules were all connected to each other and in three dimensions formed a broad perinuclear network which approached the nuclear surface and occasionally extended into the cortical zone (Fig. 8). Intermingled with the Golgi apparatus were mitochondria, lysosomal granules and an extensive network of anastomosed tubular ER cisternae.

Some ganglionic cells (type A_3) exhibited morphological features of both type A and type B cells. Their sizes were closer to those of the type B cells but they showed no concentric zonation of their organelles. As in type A_1 cells, the Nissl bodies were distributed throughout the perikaryon, except in the region of the axon hillock, and were separated from each other by narrow spaces of poorly stained cytoplasm (Figs. 8, 10). The Golgi apparatus, however, was not dispersed throughout the cytoplasm as in the type A cells, but formed a broad network occupying approximately the inner two-thirds of the perikaryon (Fig. 10). The stacks of Golgi saccules, usually wavy, were intermediate in size between those of the type A and B cells. Yet, they showed fewer tubules and vesicles on their transface than the corresponding structures in type B cells and thus resembled the stacks of saccules of type A cells more. Between the Nissl bodies and the Golgi saccules, rod-like mitochondria and numerous lysosome-like bodies were interspersed between a loose network of tubular ER cisternae (Fig. 10). Thus, these neurons showed overall structural characteristics closer to those of type A than of type B cells and were accordingly classified as type A_3 cells.

The *type C* cells were the smallest of all the ganglion cells. In the perikaryon, the Nissl bodies, whenever present, were small and composed of a few flattened ER cisternae occasionally arranged in a parallel manner (Fig. 11). These Nissl bodies were scattered in the middle zone of the perikaryon and were separated from each other by a poorly stained neuroplasm containing mitochondria, lysosome-like granules and a loose network of fine tubular ER cisternae (Fig. 11). The most characteristic cytological feature of these cells was a conspicuous Golgi apparatus located in the immediate vicinity of the nucleus (Fig. 11) and on some rare occasions confined to one of its poles. This apparatus was made of long wavy stacks of saccules which showed some poorly stained membranous tubules and vesicles on the transface (Fig. 11).

Fig. 14. Axon hillock of an A_1 cell. The smooth endoplasmic reticulum consists of thin elongated ribbon-like cisternae which are anastomosed in all directions of space, forming a loose three dimensional network. Small plate-like cisternae are only encountered (arrow) at the intersections of the wide meshes of this network. M: mitochondria; v: isolated vesicle; L: lysosome; mv: multivesicular body. × 22 000.

Fig. 15. Nissl body of a B_1 cell. Long plate-like cisternae are arranged in parallel arrays. They are interconnected at their extremities by short ribbons (arrow). M: mitochondrion. × 25 000.

Discussion

Previous attempts to classify neurons of the sensory ganglia were usually based on the configuration of a single organelle such as the Nissl bodies (Clark, 1926; Andres, 1961) or the Golgi apparatus (Duce & Keen, 1977). Hirt (1928) pointed out that the use of a single morphological criterion could not lead to any comprehensive classification of the nerve cells in the light microscope. Indeed, as stressed by Lieberman (1976) most of the light microscopic classifications did not show more than a superficial correspondence with one another and were thus difficult to reconcile. In the electron microscope, further difficulties arose from the use of conventionally contrasted thin sections that made it difficult to understand the overall organization of the various cell organelles. In this context the use of heavy metal impregnation techniques (Karnovsky, 1971; Thiéry & Rambourg, 1976), by allowing selective ultrastructural visualization of membrane-bound cell organelles in relatively thick sections, facilitated a classification of the ganglion cells based on the overall distribution and three-dimensional configuration of their ER, Golgi apparatus and mitochondria.

The two main categories of cells, or types A and B, obviously corresponded to the two groups of cells previously recognized by most authors: large pale and small dark cells (Dogiel, 1908; Andres, 1961; Droz, 1967a, b; Novikoff, 1967; Kalina & Bubis, 1968; Kalina & Wolman, 1970; Sarrat, 1970; Lawson *et al.*, 1974; Jacobs *et al.*, 1975; Duce & Keen, 1977; Spater *et al.*, 1978, 1981; Lawson, 1979). The type A cells were further divided into subtypes A_1 and A_2 according to the width of the pale channels of cytoplasm separating the Nissl bodies. The same criterion had led Andres (1961) and Jacobs *et al.* (1975) to propose a similar classification on the basis of light and electron microscopic observations, respectively. In keeping with the observation of Jacobs *et al.* (1975), the type A_1 cell was found to contain relatively few neurofilaments whereas the type A_2 showed prominent bundles of neurofilaments, running within the large strands of cytoplasm separating the Nissl bodies. This classification was thus retained despite the different nomenclature proposed by Duce & Keen (1977) in reference to the development of the Golgi apparatus.

The class of neurons referred to here as type A_3 was difficult to classify since it showed characteristics of both type A and type B cells. It displayed Nissl bodies widely dispersed throughout the perikaryon as in type A cells and a well-developed Golgi apparatus which together with the mitochondria showed a tendency to form a perinuclear ring as in the type B cells. A similar type of cell, referred to as type A_3, was observed by Andres (1961) in the light microscope. Furthermore, the *larger* Golgi bodies and the numerous dense bodies described in the electron microscope by Duce & Keen (1977) in their type A_3 cells were also present in our type A_3 cells. Since the type A_3 cells were large and displayed at high magnification ultrastructural characteristics reminiscent of the A group cells, they were classified in the A rather than in the B category.

The type B_1 cells exhibited a prominent Golgi apparatus in which strongly curved stacks of saccules arranged in a perinuclear ring formed long loops extending into the

cortical zone of the perikaryon. The Nissl bodies were peripherally located and consisted of arrays of long parallel cisternae running parallel to the cell surface. This type of cell closely resembled the type B_1 cells of Andres (1961) and was identical to some of the type B_1 cells described by Duce & Keen (1977). In fact these authors, basing their classification on the structure of the Golgi apparatus, further subdivided the B_1 group in two subclasses. While their type $B_1\beta$ was similar to our type B_1, their $B_1\alpha$ cell in which 'Golgi bodies were long, frequently straight and not confined to the perinuclear cytoplasm' seemingly corresponds to our type A_3 cells.

Due to the peripheral location of its Nissl substance and the clearcut concentric zonation of its organelles, the type B_2 cell had been identified in the light microscope by most authors (e.g. Clark, 1926; Körner, 1937) and obviously corresponded to the type B_2 cell of Andres (1961). In contrast, the type B_2 cell described by Duce & Keen (1977) did not correspond to this cell type, since in their osmium-impregnated B_2 cell, the Golgi apparatus and Nissl bodies were evenly distributed throughout the perikaryon.

The type C cell was the smallest of the ganglion cells and had been classified as a type B_3 cell both by Andres (1961) in the light microscope and by Duce & Keen (1977) in the electron microscope. Yet, it failed to exhibit the concentric zonation of organelles characteristic of type B cells. In fact, its Nissl bodies were dispersed throughout the perikaryon, giving the cell a rather light appearance as in a type A cell. The Golgi apparatus admittedly displayed, as in the type B cells, a perinuclear ring-like arrangement but was unvariably closer to the nuclear membrane. In addition, on some rare occasions, it was less developed and exclusively located on one side of the nucleus. For these reasons, the type C cell was considered as a distinct cell category rather than being classified as a type B cell.

As already emphasized, the classification of dorsal root ganglia nerve cell bodies into type A and B cells is generally accepted. A recent statistical analysis of cell numbers and size in mouse dorsal root ganglia even showed that the proportion of neurons in each population did not change with age indicating that cells were unlikely to change from one type into the other (Lawson, 1979). However, their subdivision into *subclasses* on the basis of fine structural features might appear esoteric since, as pointed out by Lieberman (1976), 'the functional differences between light and dark cells, if any, are still obscure'. The morphological criteria chosen for the identification of the various subtypes were nevertheless sufficiently unequivocal to permit the identification of most ganglion cells. In their electron microscope investigation of the L_4 dorsal root ganglion, Duce & Keen (1976) reported that the electrical stimulation of the ipsilateral hind limb did not modify the relative number of the various cell types, which, although affected in their morphology, remained recognizable. These observations suggested that differences in the ultrastructural configuration of the intracytoplasmic membrane system were not likely to be due to variations in the state of activity of neurons but corresponded to true neuronal classes (Jacobs, 1976). It has been postulated as early as 1904 by Warrington & Griffith that various classes of ganglion cells might be involved in the transmission of different sensory stimuli. Light microscopic immunocytochemical

studies have recently emphasized the neurotransmitter heterogeneity of dorsal root ganglion cells. Thus, substance P, somatostatin and vasoactive intestinal peptide (VIP) were found to be present in apparently distinct subpopulations of the small, dark neurons (Hökfelt *et al.*, 1976; Lundberg *et al.*, 1978, 1980; Nagy & Hunt, 1982). Substance P-containing cells, identified by electron microscope immunocytochemistry (Chan-Palay & Palay, 1977), were described as small (10–20 μm) fusiform or stellate neurons which may well correspond to some of our type C cells. Gastrin/cholecystokinin-like immunoreactivity was detected in both small and large neurons (Lundberg *et al.*, 1978, 1980). A small proportion of the large pale cells was also found by light microscope radioautography to be retrogradely labelled, in a selective fashion, following injection of tritiated D-aspartate or tritiated GABA into the spinal cord (Rustioni *et al.*, 1981; Cuenod *et al.*, 1982). Further studies combining these techniques with electron microscopy are obviously needed to determine whether the various subtypes of ganglion cells described in the present study indeed correspond to functionally different neurons.

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