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Subsurface Cisterns and Lamellar Bodies: Particular Forms of the Endoplasmic Reticulum in the Neurons*

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Summary. Structures identified as subsurface cisterns (SSC's) and lamellar bodies (LB's) have been observed in the neurons, but not in the glial cells, of the rat and cat substantia nigra. The SSC's are most often opposite what appears to be glial cells, but they are also subsynaptic in position. A single, large (0.4–1.5 μ), unfenestrated, usually flattened cistern closely underlies the inner aspect of the plasma membrane of the perikaryon and proximal parts of the neuronal processes at a regular interval ranging about 100–130 Å. They are sheet-like or discoid in configuration and consists of a pentalaminar structure which usually widens at its lateral edges where its membranes are continuous with each other or with rough ER profiles. Filaments, about 70 Å thick, bridge the cleft between the SSC and the overlying plasmalemma. One or more ER cisterns devoid of ribosomes except on their outermost membrane may be stacked up parallel to an SSC and immediately subjacent to it. A dense filamentous network intervenes between the SSC and its closely applied ER cisterns. At higher magnification, it is seen to consist of a finely textured material which is apparently composed of loosely packed tiny particles. These constituent subunits in turn may represent transverse sections of very fine filaments rather than granules. A mitochondrion frequently occurs in the immediate vicinity of an SSC and may be closely applied to its deep surface. Stacks of unfenestrated, parallel, regularly spaced (about 300–400 Å) cisterns, designated lamellar bodies, appear deeper in the karyoplasm. They are most often flattened and appear as pentalaminar structures. These cisterns, as well as the dense filamentous network intervening between them, are structurally similar to those closely applied to SSC's. They are also devoid of ribosomes except on their outermost surfaces. Whorls of similar cisterns are also occasionally observed. Another particular feature of the rough ER consists of the close apposition of two cisterns without any ribosome attached to the inner membranes of the latter structure. It evokes a simplified type of LB's. It is of particular interest to point out that all these cisterns, i.e. the SSC's, their closely applied cistern(s) and those forming the LB's, are connected to the RER membranes, so that a continuous channel occurs between the nuclear membrane and the SSC which closely underlies the plasma membrane. Our observations show that the SSC's and the LB's are structurally related forms of the ER. A parallel may be drawn between the SSC and the lateral element(s) of a dyad (triad). The structural complex consisting of an SSC, the overlying plasmalemma and the cross-bridges linking them, indeed, bears some resemblance to a dyad. It is suggested that membranes which are closely applied may interact, resulting in alterations in their respective properties. These patches of the neuronal plasma membrane associated with SSC's may, therefore, have special properties because of this relationship, resulting in a non-uniform spread of an action potential on the neuronal surface. The possible significance of SSC's in relation to neuronal electrophysiology, as well as of the latter structures and LB's in relation to cell metabolism, is to be discussed.

Key words: Neurons — Subsurface cisterns — Lamellar bodies — Endoplasmic reticulum — Ultrastructure.

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Introduction

Stacks of membranes in close apposition to the plasmalemma of neurons have been first described in detail by Rosenbluth (1962b), who termed them "subsurface cisterns" (SSC's) and found them to be somewhat similar to the endoplasmic reticulum (ER) of neurons. Moreover, continuity between the latter structures has been reported (Rosenbluth, 1962a, b; Herndon, 1963; Siegesmund, 1968), but, unlike the ER, the SSC, which is about 1–2 μ in length, has only been found in very close proximity to the plasmalemma. The SSC's have been reported in a wide variety of vertebrate and invertebrate neurons of both the peripheral and central nervous systems, but never in the surrounding supporting cells (Rosenbluth and Palay, 1961; Pappas and Purpura, 1961; Rosenbluth, 1962a, b; Herndon, 1963; Rosenbluth, 1966; Siegesmund, 1968; Takahashi and Wood, 1970). They have also been observed in neurons grown in tissue culture (Bunge, Bunge, and Peterson, 1965). In addition, Rosenbluth (1962a, b) and Bunge, Bunge, and Peterson (1965) detected stacks of flattened cisterns deep in the karyoplasm, which resemble those associated with the SSC's, but the latter workers could not demonstrate any connection between these structures.

In his investigation of the fine structure of nervous tissue, Herndon (1964) has observed lamellar structures in the Purkinje cells of the rat cerebellum. They have been interpretated as an unusual arrangement of the RER by the latter author who termed them lamellar bodies (LB's) and questioned their significance, inferring a possible mechanism secondary to the technical procedure. More recently, Adinolfi (1969), studying the fine structure of neurons in the cat entopeduncular nucleus, has described "unique lamellar configurations" consisting of smooth ER cisterns which are separated by thin layers of dense granular ground substance and continuous with the rough ER. The latter author considers their functional nature as being obscure. Similar lamellar configurations have also been observed in nerve cells of the immature orbital cortex (Adinolfi, 1969) and in the medium-sized neurons of the rat substantia nigra (Gulley and Wood, 1971). Anzil, Blinzinger, and Matsushima (1971) have also found parallel arrays of membrane-bound cisterns with a dense intercisternal matrix in the perikarya of a few neurons in the rat striatum. On the other hand, Peters, Proskauer, and Kaiserman-Abramof (1968) have reported that the initial segment of the pyramidal cell axon contains an organelle consisting of stacks of flattened cisterns alternating with plates of dense granular material. They have also stressed the fact that these cisternal organelles resemble the spine apparatuses, which occur in dendritic spines of this neuron, and structurally differentiated them from subsurface cisterns.

In the present ultrastructural study of the substantia nigra in the rat and the cat, it is shown that most of the neurons contain one or more SSC's. In addition, our attention has also been drawn to LB's which have not, however, been frequently seen. We do not attempt, here, to present an exhaustive description of the neurons in the substantia nigra, but wish to lay stress on the above-mentioned cytoplasmic structures. Indeed, it is shown that the unfenestrated, flattened cisterns, which compose the LB's and the SSC's, as well as their closely applied stacks of cisterns, are in continuity with the RER membranes thus forming specialized portions in the ER channel which extends from the

nuclear membrane to the SSC underlying the neuronal plasmalemma. The possible significance of SSC's and LB's in relation to neuronal electrophysiology and metabolism is to be discussed.

Material and Methods

Nineteen adult white rats (mixed sexes) of the Holtzmann and Sprague-Dawley strains, weighing 130–460 g, and five male or female cats, weighing 0.925–4 kg, were used in these experiments. All the rats were kept for at least fifteen days in air conditioned quarters and given Purina laboratory chow and tap water ad libitum.

The perfusion apparatus used for these experiments has been previously described (Le Beux, 1971). The solutions were maintained at a constant temperature of either 37° C or 4° C and perfused at a speed of 40 and 70 ml/min for the rats and cats, respectively.

Anaesthesia and surgical procedures differed according to the animals employed. Rats were anaesthetized by an intraperitoneal injection of urethane (ethyl carbamate), 125 mg/100 g. Surgical procedures were initiated one minute later under complete anaesthesia of the animal. Cats were anaesthetized by an intraperitoneal injection of nembutal, 30 mg/kg. A tracheotomy was performed, a Y glass cannula with a bulbous tip fitting as closely as possible was inserted in the trachea and tied down with a previously placed ligature. The cannula, designed as short as possible (2–3 cm), was connected at its external end to a transparent plastic tubing which was in turn connected to the artificial respiration device. The other branch of the Y tube enabled the tracheotomized animal to breathe voluntarily until the artificial respiration was started.

The hearts of the rats were rapidly exposed after cutting the anterior rib cage obliquely from the left costophrenic angle to the first left rib. The left ventricle close to the apex was excised and a Yale B-D-14T needle filled with a heparin solution was introduced into the left ventricle and ascending aorta, A large curved hemostatic forceps was used to clamp the needle at the level of the apex of the heart, slightly above the incision, so that no blood and no perfusate could escape from the wound. Perfusion with the initial washing solution was immediately started, the right atrium opened and the descending aorta clamped. About 10–15 seconds were necessary to perform all the operations from the opening of the chest to the beginning of the perfusion. Before opening the chests of the cats, artificial respiration was started. Perfusion with the washing solution was then immediately initiated through the left ventricle with the right atrium incised. The descending aorta was also clamped.

In all cases, 1000 I.U. of heparin were injected progressively through the plastic tubing of the blood pump administration set to prevent the formation of blood clots. Perfusion with the washing solution was carried on until the liquid pouring out from the right atrium was no longer tinged with blood, that is, at least two minutes. This solution contained a vasodilating agent, procaine, to prevent tissue damage induced by vasoconstriction. Oxygen was bubbled through the washing solution during perfusion. The cardiac muscle in no case stopped beating before the fixative reached the animal's circulatory system.

The initial washing solution consisted of a 10 mM tris (hydroxymethyl) aminomethane buffer to which a mixture of salts was added (Le Beux, 1972). The pH of the solution was adjusted to 7.34–7.38 by adding 0.1 N HCl. The osmolality of this washing solution was measured by an Advanced Instruments osmometer and found to be 309.6 ± 1.6 mOsm. Osmolality was adjusted by adding either distilled water or NaCl.

Glutaraldehyde was obtained from Ladd Research Industries (70% highly purified glutaraldehyde) or from TAAB Laboratories (24.5% distilled glutaraldehyde) and used to prepare various fixative solutions in Sörensen's phosphate buffer at different molar concentrations (0.045 and 0.07 M). The glutaraldehyde concentration was also varied from 1.8 to 3%. The pH of the fixative solutions was adjusted to 7.36–7.38. The osmolality of the various fixative solutions was found to range from 307.1 ± 1.4 to 569.8 ± 6.7 mOsm. Perfusion of the fixatives was carried out for 5–15 minutes. In another series of experiments, the perfusion of the above-mentioned fixative solutions was preceded by a concentrated solution (17.5%) of glutaraldehyde buffered to pH 7.35–7.37 with 0.07 M phosphate buffer

(165.8 ± 1.8 mOsm). The latter fixative liquid was found to have an osmolality of 2225 ± 22.5 mOsm and perfused for 30 seconds at a speed of 40 and 70 ml/min for the rats and the cats, respectively. 20–35 ml of this concentrated glutaraldehyde solution were already perfused when the second above-mentioned fixative penetrated the vessels. The fixative process was then carried out as usual.

Then a rinsing solution was used again for two (2) minutes to eliminate the fixative from the blood vessels. It consisted of the initial washing solution without procaine and heparine.

Following complete perfusion, the whole brain was carefully removed. While the brain bathed in a 307.5 ± 2.4 mOsm mixture of Sörensen's 0.1 M phosphate buffer and of 0.1 M sucrose solution, small pieces of its different areas were dissected out. Those pieces were then rinsed for one to two hours in the same buffer. Postfixation was carried out in 2% OsO_4 buffered to pH 7.35–7.40 with 0.1 M phosphate buffer for 2 hours at room temperature or at 4° C, according to the temperature used for the initial fixation by perfusion. The tissue was then immersed for 10 minutes in the above-mentioned mixture of Sörensen's 0.1 M phosphate buffer and of 0.1 M sucrose solution, dehydrated in increasing concentrations of methyl or ethyl alcohol (70%, 95%, absolute) and rinsed in propylene oxide prior to embedding in a 3:7 Epon 812 mixture according to Luft's method (1961). Some specimens were stained in blocks in a saturated uranyl acetate solution in distilled water for 1 hour before dehydration. Thin sections were cut with a LKB 3 ultramicrotome equipped with a diamond knife and stained with a 3% aqueous uranyl acetate solution for 30 minutes and then with lead citrate for 1 minute according to Venable and Coggeshall (1965). The sections were examined in a Phillips EM 300 electron microscope equipped with a 20 μ objective aperture and operated at 60 KV.

Observations

I. General Observations

Subsurface cisterns (SSC's) and lamellar bodies (LB's) have been identified in the neurons, but not in the glial cells of the substantia nigra in the rat and the cat. The SSC's are, however, usual findings, whereas the LB's have occasionally been observed. In addition, the former structures occur much more frequently in the neurons of the above-mentioned nuclear mass than in those of the medial vascular prechiasmatic gland. Both they have been found in the perikarya, but the SSC's are also present in the large caliber dentritic trunks and the initial segments of axons. The stacks of LB's are usually located deep in the perikaryon among the RER profiles or occasionally apposed to the nuclear membrane, but similar structures may be also closely applied to the SSC's. LB's and SSC's are both connected to the RER membranes and we have, therefore, thought it appropriate to lay stress on the features which characterize them: (1) they usually consist of unfenestrated, flattened, pentalaminar cisterns; (2) a dense, filamentous, finely textured material is found between all these cisterns without any ribosome or any other organelle intervening; (3) the interval between the cisterns of the LB's and between the SSC's and their closely applied ER cistern(s) is about 300–400 Å in width whereas that between the plasmalemma and the underneath SSC is only about 100–130 Å in diameter. The SSC, the overlying plasma membrane and the dense cross-bridges, about 70 Å thick, linking them, thus form a structural complex which is likely to interfere with the electrophysiological properties of the nerve cell plasma membrane.

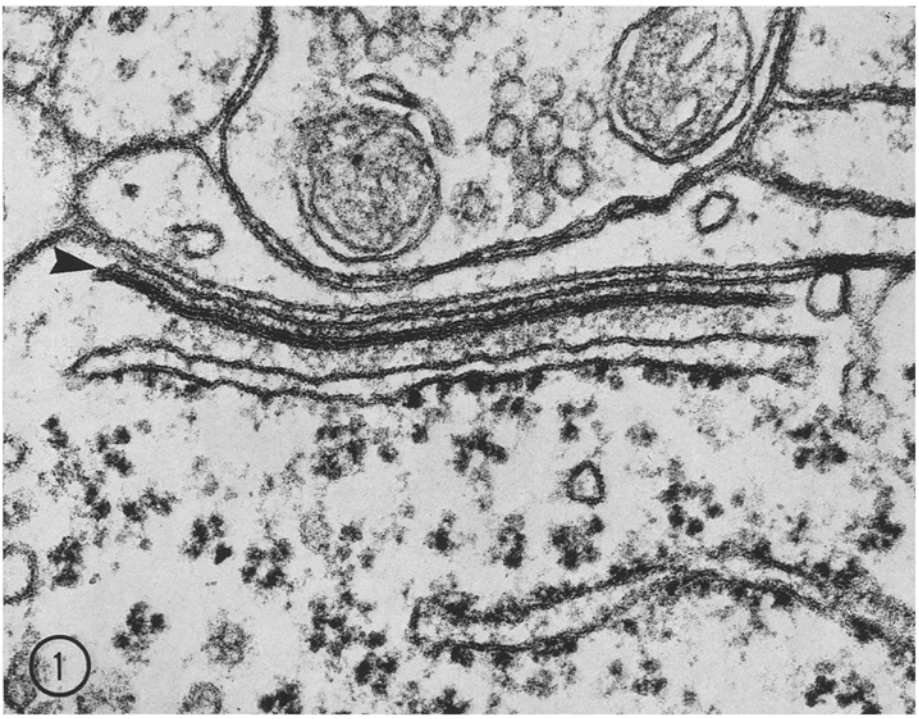

Fig. 1. An approximately 0.8 μ large SSC (arrowhead) closely underlies the perikaryal plasma membrane opposite what appears to be a glial sheet. This pentalaminar structure is separated from the overlying neuronal plasmalemma by a 100–130 Å wide cleft which is cross-bridged by thin filaments. Note the accuracy with which the two structures follow each other as they undulate. The rough ER cistern lying at a distance of about 350 Å beneath the SSC is studded with ribosomes on its deep surface only. Note also that a loose filamentous network intervenes between the two types of cisterns. Rat. Substantia nigra. The fixation procedure consisted of: (1) Perfusion with a Sörensen's phosphate buffered (0.07 M) 17.5% glutaraldehyde solution (2225 ± 22.5 mOsm) followed by a 1.8% glutaraldehyde solution (377.0 ± 6.0 mOsm) in the same buffer, at 37° C; (2) Post-fixation by immersion in Sörensen's phosphate buffered (0.1 M) 2% OsO_4 at room temperature. The following figures, i.e. the Figs. 4, 6, 7, 11 and 12, were identically processed. × 102150

II. Subsurface Cisterns (SSC's)

These structures, varying in length between 0.4 and 1.5 μ, are very closely applied to the plasma membrane of the neurons containing them without any ribosome or other organelle intervening, and follow the undulating course of the plasmalemma, separated from it by a constant interval (Figs. 1–4). One or more elements are commonly observed in the same perikaryon, but the relative abundance of such cisterns appears to be different from one brain area to another. They are, indeed, much more frequently noticed in the neurons of the substantia nigra than in those of the medial vascular prechiasmatic gland. SSC's are usually found in close apposition to cells which could be readily identified as glial cells

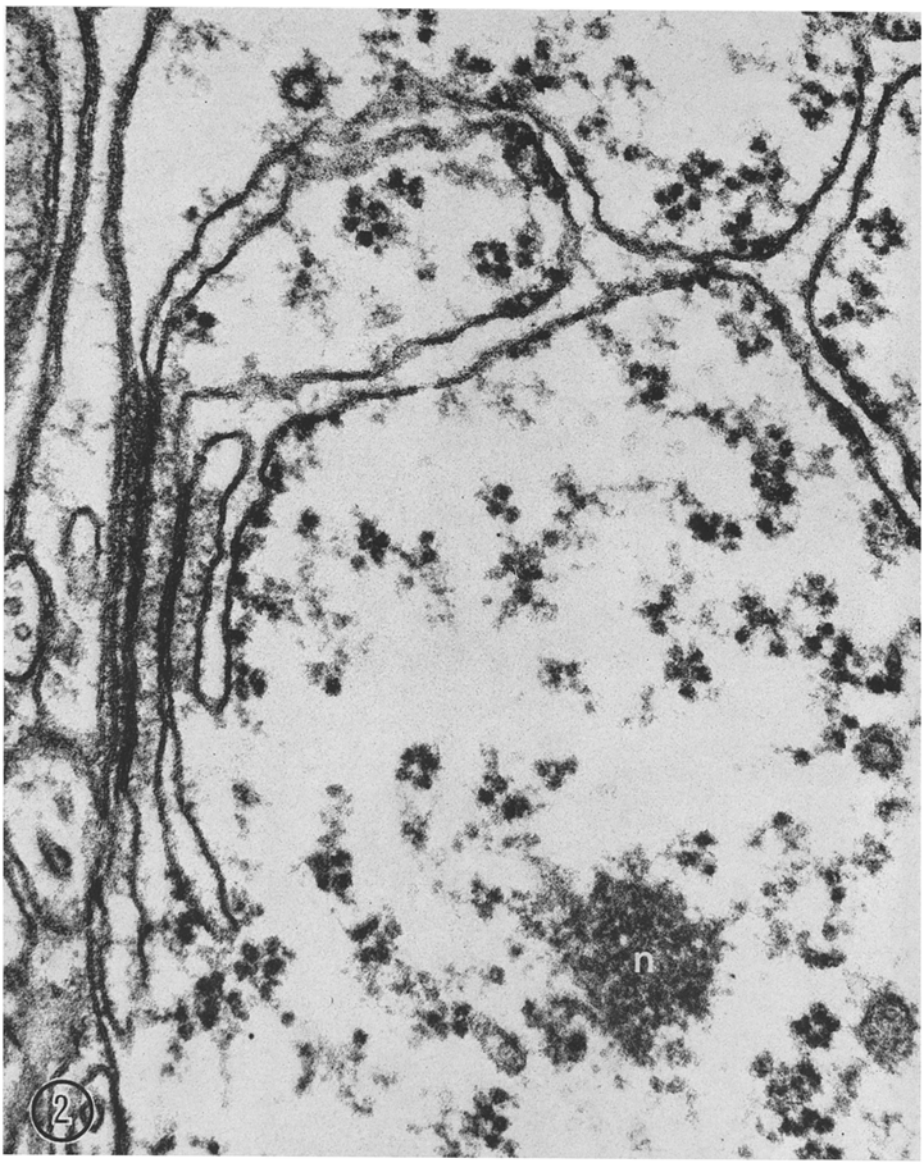

Fig. 2. This electron micrograph depicts another SSC in close apposition to the neuronal plasmalemma. Its lumen is very narrow, but it enlarges at its edges where the deep and superficial membranes are continuous with the rough ER membranes. Two cisterns of the rough ER are closely applied to the SSC. The outer is flattened and appears similar to the SSC, whereas the lumen of the inner one is of the usual depth. The cleft between the SSC and its overlying plasmalemma is 100 Å wide and cross-bridges are seen in the lower portion where the membranes are not tangentially cut. On the other hand, note the loose filamentous network between the outer cistern and the SSC, as well as between the two parallel ER cisterns. Note also a nematosome (*n*) in the lower right quadrant. Rat. Substantia nigra. Fixation procedure: (1) Perfusion with a Sörensen's phosphate buffered (0.045 M) 1.8% glutaraldehyde solution (307.1 mOsm) at 37° C; (2) Post-fixation by immersion in Sörensen's phosphate buffered (0.1 M) 2% OsO_4 at room temperature. The following figures, i.e. the Figs. 5, 8, 9, 10 and 13, were identically processed. × 97800

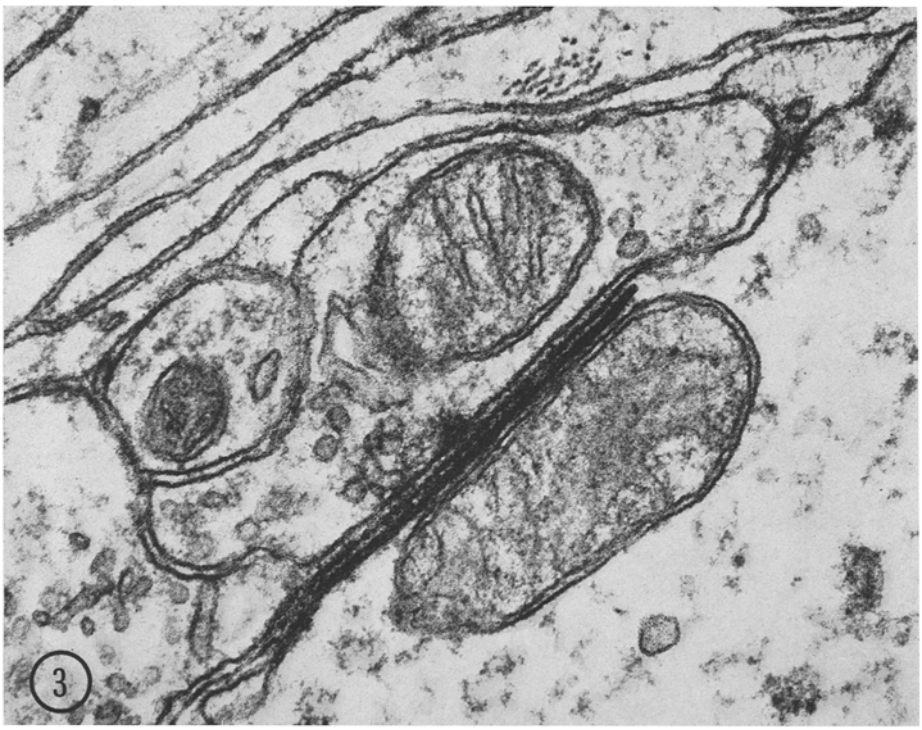

Fig. 3. An SSC underlies a synaptic bouton in which only a few synaptic vesicles are seen. A mitochondrion is closely applied to the deep surface of the SSC and its long axis conforms to that of the former structure. Cat. Substantia nigra. Fixation procedure: (1) Perfusion with a Sörensen's phosphate buffered (0.07 M) 3% glutaraldehyde solution (569.8 ± 6.7 mOsm) at 37° C; (2) Post-fixation by immersion in Sörensen's phosphate buffered (0.1 M) 2% OsO_4 at room temperature. × 75240

(Figs. 1, 2), i.e. sheath cells essentially, but also astrocytes. Processes of dendrites or axons are not infrequently found close to an SSC, but upon careful examination, a thin layer of what appears to be glial cytoplasm is usually seen to intervene between the two structures. The neuronal plasmalemma is separated from the glial cell membrane by an intercellular space of about 100 Å. No cytoplasmic structure or dense material coating the inner leaflet of the plasmalema is seen in the glial cell cytoplasm directly opposite to the SSC's of the neuron. Paired SSC's, such as previously described by Pannese (1968) and Weis (1968), i.e. opposite each other in apposing cells, have never been observed in our investigations. Although the perikaryon of the substantia nigra neurons are not heavily invested with synaptic boutons, SSC's are sometimes subsynaptic in position (Fig. 3). An SSC and its overlying synaptic bouton are lined up one over the other (Fig. 3), each covering about the same length of the perikaryal plasmalemma or underlying only a part of the synaptic bouton with or without any relationship with the active zone. It may also extend for a considerable distance beyond the edge of a bouton.

A single, elongated, flattened cistern underlies the neuronal plasmalemma at a regular interval ranging about 100–130 Å, i.e. slightly wider than that located between the glial and neuronal plasma membranes (Fig. 1). Fine filaments bridge the cleft (Fig. 1). The lumen of the SSC is usually very narrow (Figs. 1–3) except at its bulbous edges where the deep and superficial membranes generally are continuous with each other (Fig. 3) or with rough ER membranes (Fig. 2). The widening of the lateral edges is not, however, constantly found (Fig. 1). It may be inferred from our observations that, in three dimensions, SSC's are sheet-like or discoid in configuration and may anastomose with the surrounding rough ER profiles in another plane of section. Ribosomes are generally absent from their deep cytoplasmic surface, except at their bulbous lateral edges (Fig. 2).

Another type of SSC may be also observed. A ribosome-dotted cistern approaches the plasmalemma, and follows it closely for a distance. Here it is devoid of ribosomes and its lumen may or may not be narrowed. Then it leaves the vicinity of the plasma membrane and, again displaying ribosomes, wanders among the other elements of a Nissl body.

It is common to find one or more ER cistern stacked up parallel to an SSC and immediatly subjacent to it (Figs. 1, 2). These ER elements are unfenestrated, flattened or unflattened cisterns which are spaced at regular intervals of about 300–400 Å. All these membranes facing an SSC are devoid of ribosomes except at their deep cytoplasmic surface (Figs. 1, 2). A dense material intervenes between these apposed cisterns and also between the SSC and the immediately applied cistern of the above-mentioned stacks (Figs. 1, 2). At a sufficient magnification, it appears to consist of a loosely intertwined filamentous material, which apparently differs from the bridges crossing the cleft between the SSC and the opposite patch of plasmalemma (Fig. 1). In addition, no ribosome or other organelle is present in these intervals. All these cisterns, as well as the SSC, anastomose with the RER profiles (Fig. 2).

At a higher magnification (Fig. 4), the 70 Å filaments, bridging the cleft which separates the SSC and the plasmalemma, are seen to consist of tiny dense particles. That these constituent particles represent transverse sections of a fine filamentous material, rather than granules, seems more plausible. In some portions of this interval, the above-mentioned filaments are not, however, so clearly outlined and a rather diffuse material is observed there. A finely textured, electron-opaque material intervenes also between the SSC and its immediately applied cistern, as well as between the other cisterns of the apposed stack. It

Fig. 4. Higher magnification of the SSC shown in Fig. 1. The SSC (large arrow) appears as a pentalaminar structure. Dehiscences between the two closely apposed unit membranes are seen in a few restricted portions. Note that microfilaments (small arrows), about 70 Å thick, link the SSC and the neuronal plasma membranes (*n*). The gap between the SSC and its overlying plasma membrane is 100–130 Å in width. No thickening of the glial cell plasmalemma (*g*) opposite to the SSC is observed. Note also that the interval between the SSC and its applied ER cistern, about 300–400 Å wide, is occupied by a finely textured dense material consisting of tiny particles which are obliquely-, longitudinally- or transversely-sectioned fine filaments and not granules. Rat. Substantia nigra. × 300000

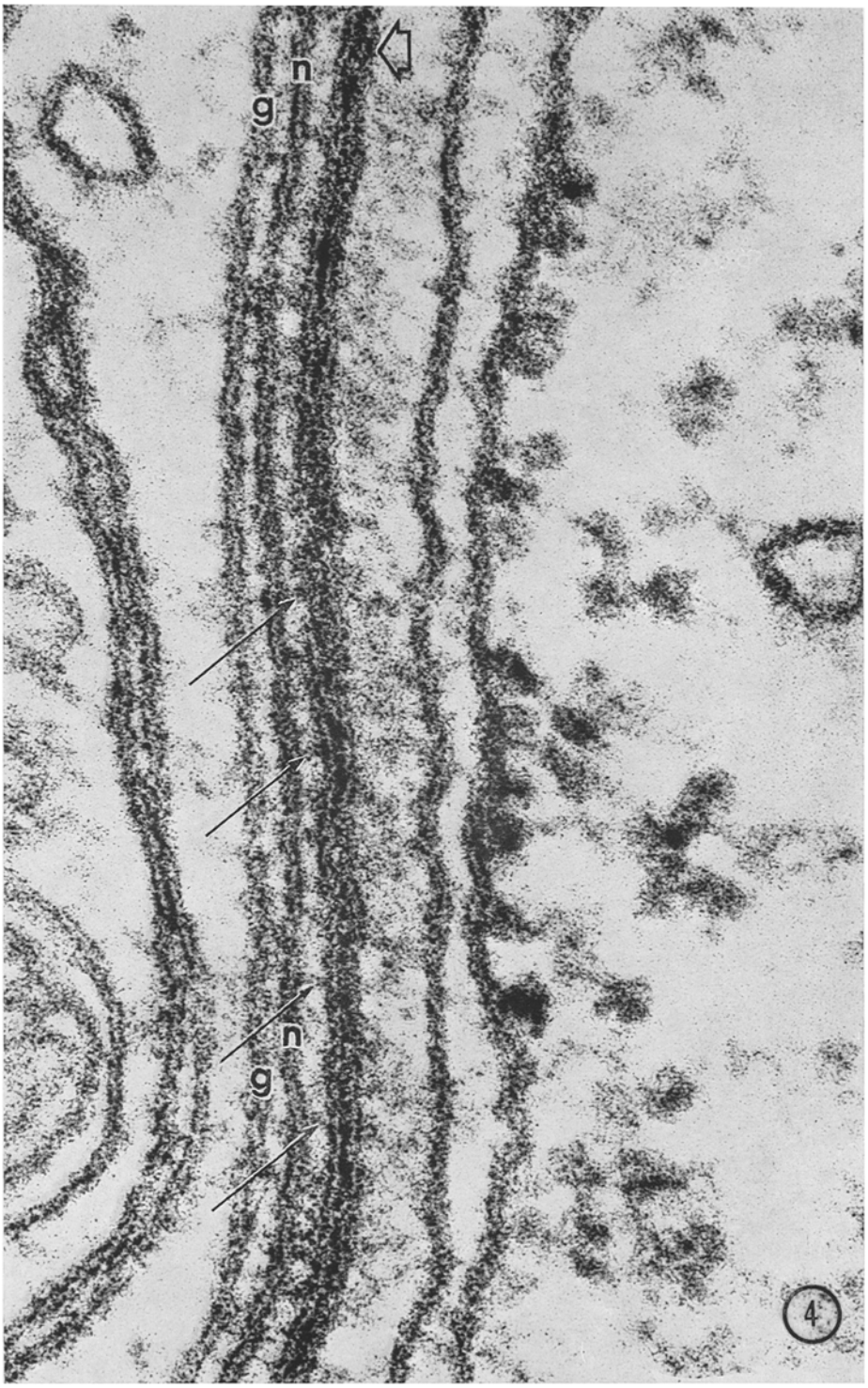

Fig. 4

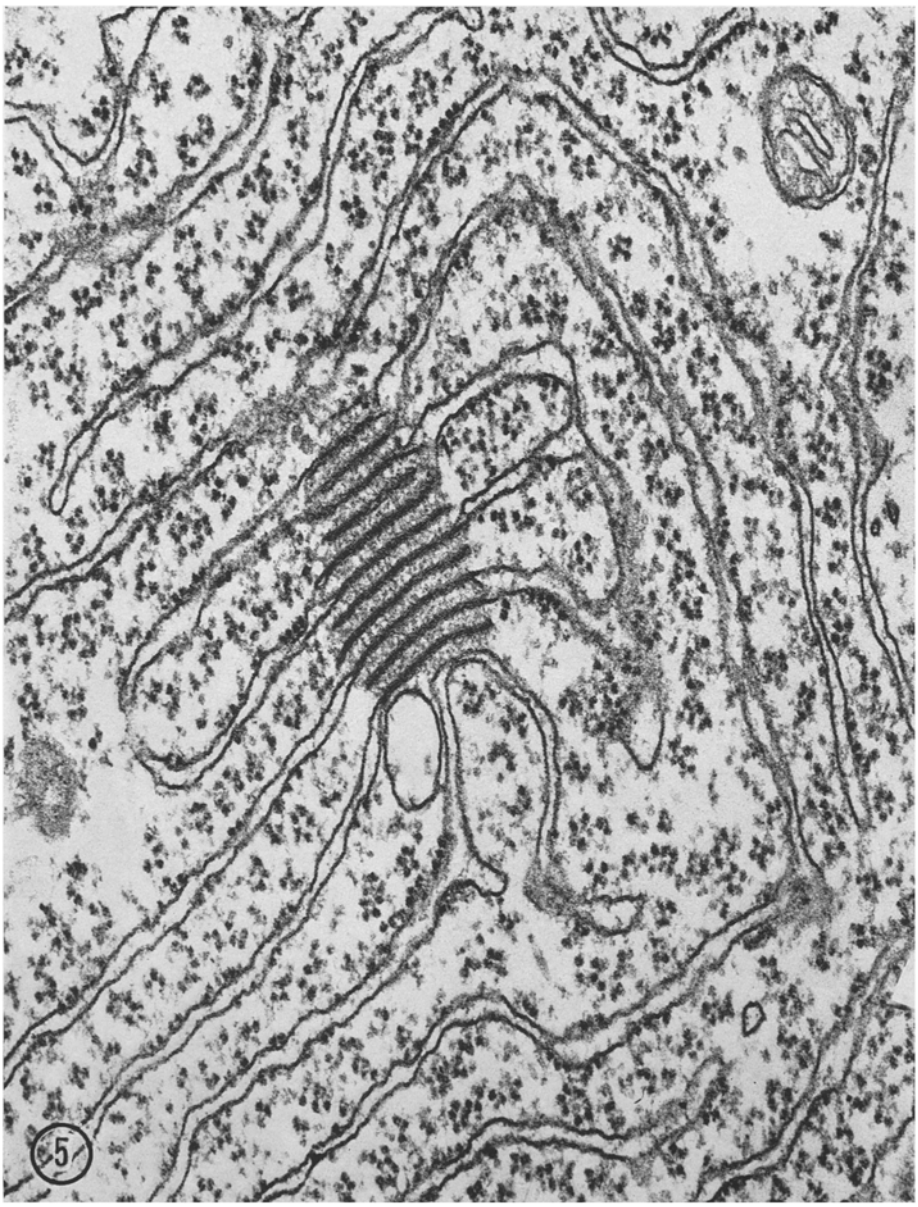

Fig. 5. Neuron from the rat substantia nigra showing a lamellar body. A stack of eleven unfenestrated, parallel, regularly spaced (about 400 Å), pentalaminar cisterns lies deep in its karyoplasm filled with rough ER cisterns, but it may be inferred that they anastomose in another plane of section. A filamentous material intervenes between the successive cisterns. Rat. Substantia nigra. × 66690

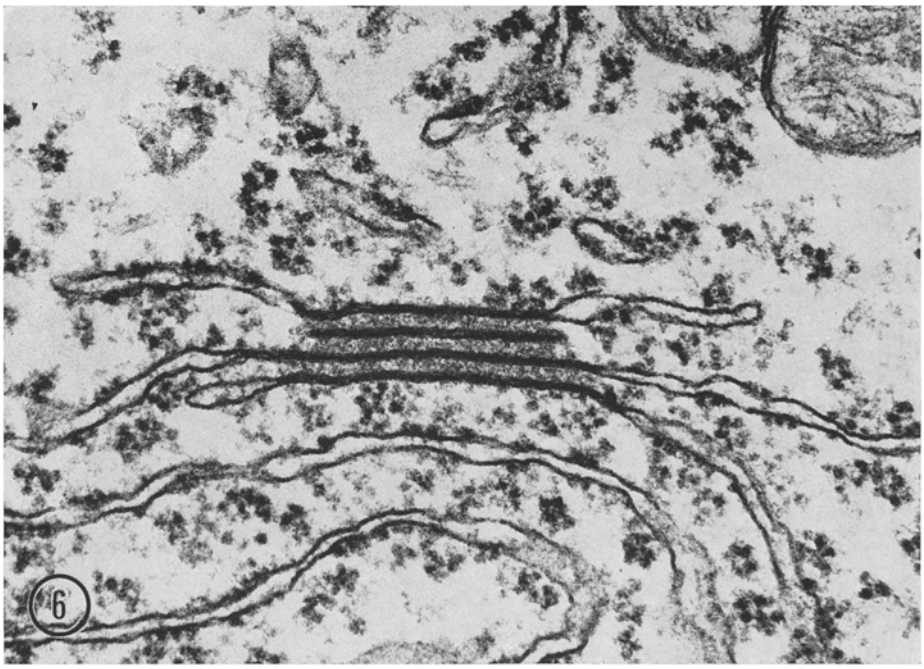

Fig. 6. Four stacked, unfenestrated, highly flattened cisterns lie among the RER profiles to which they are continuous, except one of them. Note that a filamentous material also intervenes between these cisterns which are separated by a constant interval of about 300 Å. Rat. Substantia nigra. × 63950

also consists of particles which appear to be similar to those described above. Their spatial arrangement determines the diffuse appearance of this intercisternal material. It should be pointed out that all these constituent particles may be fine deposits of heavy metals on structural components. A pentalaminar structure (Fig. 4) is observed at the level of the SSC, except in restricted areas where dehiscences are formed between the two closely apposed three-layered cisternal membranes. Whether those ER membranes, such as shown at the edges of the cisterns, are fused, or are separated by minute gaps when pentalaminar structure are seen, needs further investigation.

Mitochondria frequently occur in the immediate vicinity of SSC's. A mitochondrion is occasionally very closely applied to the deep surface of an SSC (Fig. 3). The interval which separates the mitochondrion from the SSC is of the same order of magnitude as that between the SSC and the plasmalemma. The long axis of the mitochondrion exactly conforms to that of the SSC. In addition, a rough ER profile may also closely underlie a mitochondrion.

III. Lamellar Bodies (LB's)

Stacks of several (4–12) unfenestrated, parallel, regularly spaced (300–400 Å) cisterns (Figs. 5, 6) appear deep in the karyoplasm of the substantia nigra neurons

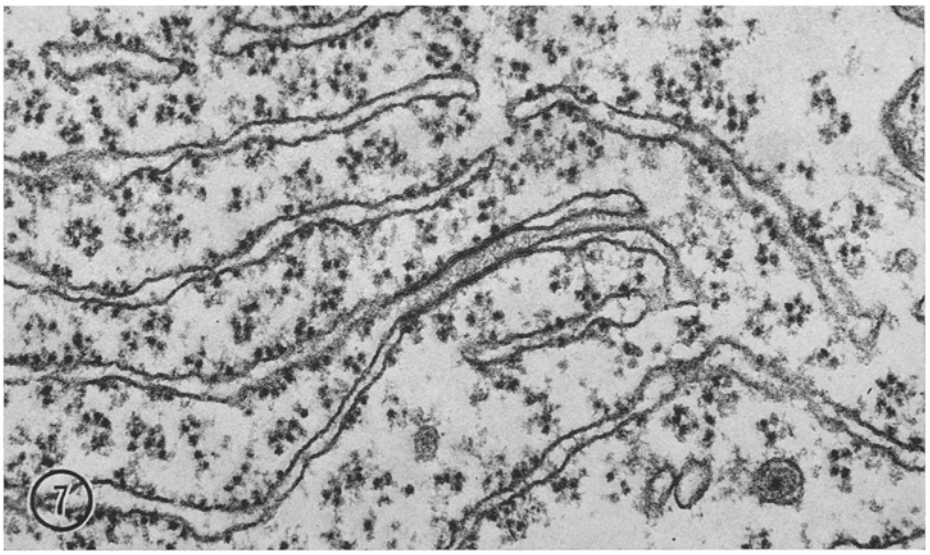

Fig. 7. Two unfenestrated pentalaminar cisterns in continuity with rough ER membranes run approximately parallel for a distance of about 0.4 μ. The interval between these cisterns is also filled with a filamentous material without any ribosome intervening, but some ribosomal particles appear, however, attached to their outer surface. Rat. Substantia nigra. × 57700

among the other organelles especially the RER profiles to which they are connected. In a few cases, this structure consist only of two cisterns applied parallel (Fig. 7). Most of those cisterns are flattened (Figs. 5–7), but their luminal depth may be about 300 Å in a few arrays (Fig. 8). Their average breadth is usually about 0.6 μ but, in a few instances, extends up to 1.2 μ. On the other hand, stacks of parallel cisterns originating from the RER course straight in the cytoplasm and secondarily curve forming whorls of cisterns (Fig. 9). "Nebenkern-like" structures (Fig. 10) also connected to the RER cisterns are occasionally seen in a karyoplasm filled with other organelles. A narrowing of the lumen of the cisterns in the latter structures may occur, but this alteration is more often restricted to some portions of the membranes rather than generalized to all their length. A dense, filamentous material is observed between the adjacent cisterns without any ribosome intervening. Cisternal membranes, indeed, are abruptly devoid of ribosomes as soon as these arrays are formed except at the outermost membranes of the outer cisterns. When concentric whorls of cisterns occur, ribosomes are attached to the innermost and outermost membranes of the inner and outer cisterns, respectively. Some cisterns do not apparently communicate with the RER profiles, but it may be inferred that in three dimensions such cisterns, being sheet-like, are interconnected with rough-surfaced membranes in another plane of section. At higher magnification (Fig. 11), it is seen that a finely textured material, similar to that observed between the SSC and the

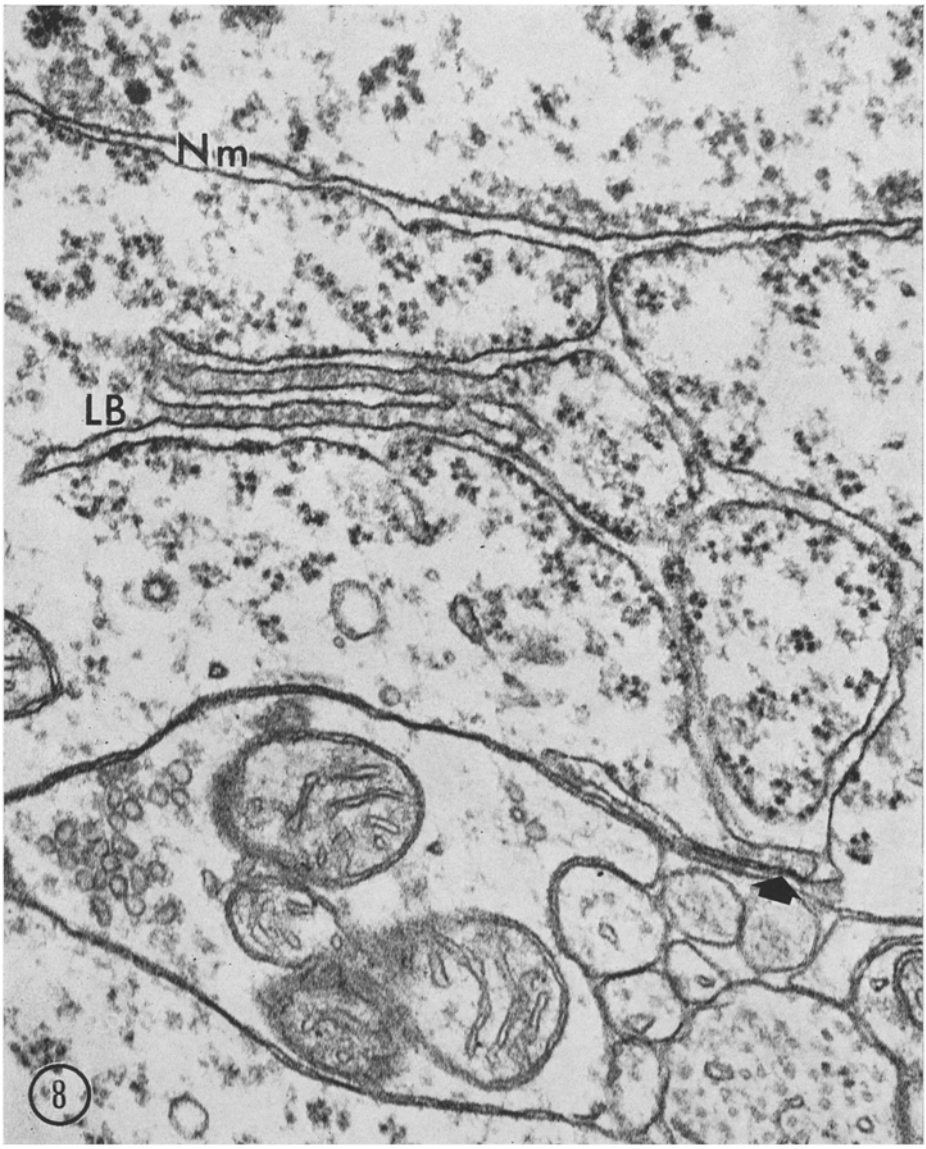

Fig. 8. This electron micrograph depicts a continuous channel between the nuclear membrane (*Nm*) and the peripherally located SSC (arrow). A lamellar body (*LB*) consists of three unfenestrated cisterns which are separated by an interval of about 385 to 450 Å filled with a filamentous material. No ribosome intervenes in these clefts. The latter particles are bound to the outer membranes only. The luminal size of these cisterns is comparable to that of the granular ER cisterns, which are continuous with them. Rat. Substantia nigra. × 65200

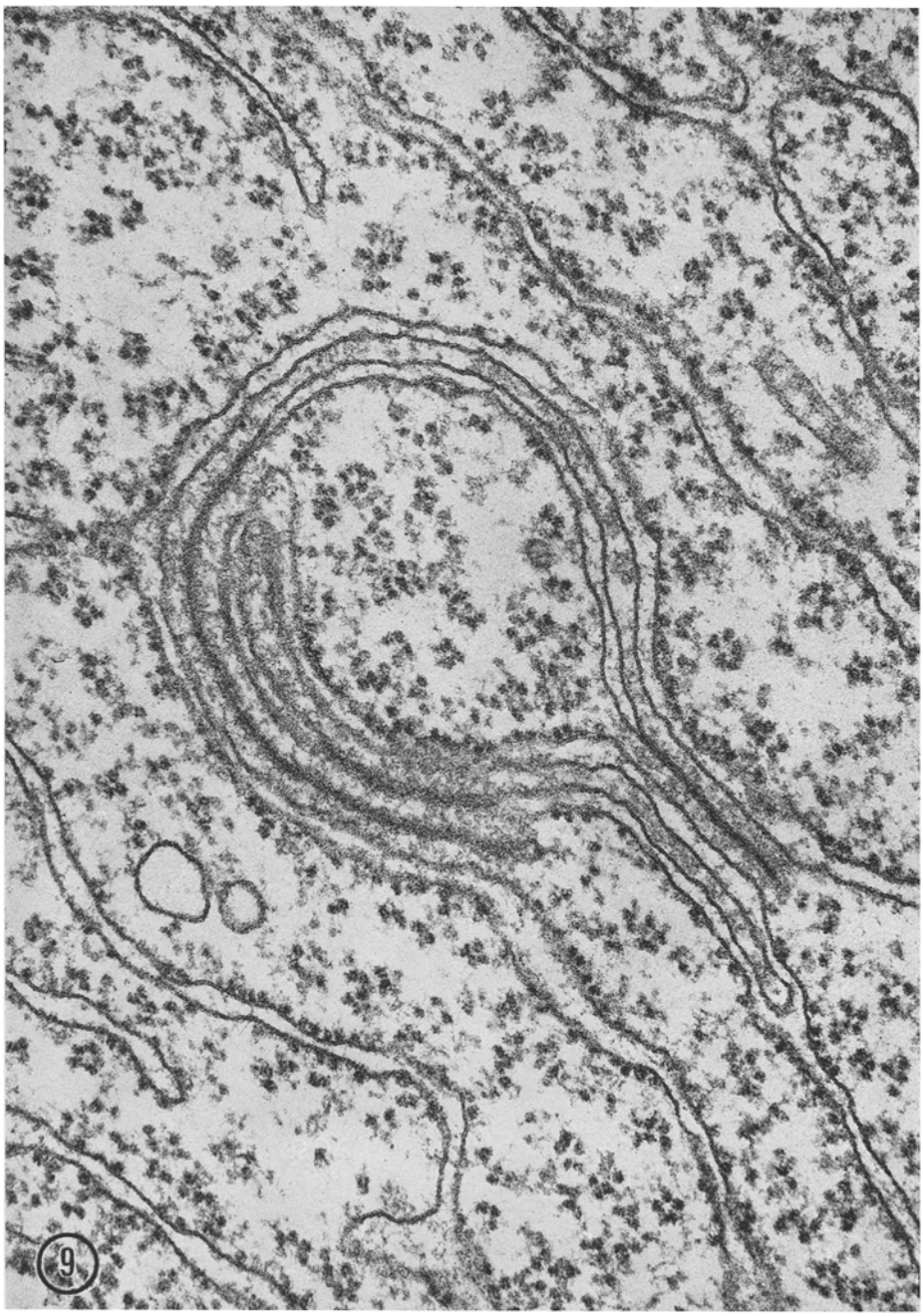

Fig. 9. Parallel, regularly spaced, flattened or unflattened cisterns (lower right quadrant) curve and form whorled arrays. This particular structure lies among rough ER cisterns to which it is in continuity. A filamentous material intervenes between the lamellar body cisterns and is easily disitnguished in portions which are not tangentially sectioned. Rat. Substantia nigra. × 75250

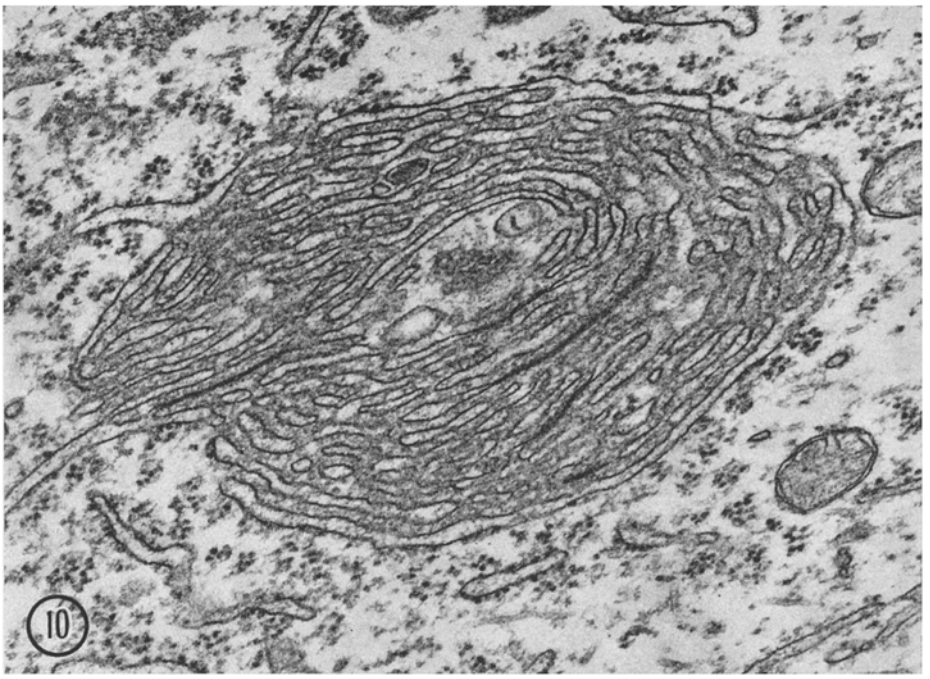

Fig. 10. A "nebenkern-like" structure is shown in this electron micrograph. Note that this body is connected to rough ER profiles. A filamentous material intervenes between these whorled cisterns, which are generally not flattened. Note also that a similar material occurs in the core of this body. Rat. Substantia nigra. × 49250

closely applied ER cisterns, is also found between the flattened or unflattened cisterns of the LB's. Pentalaminar structures are also noticed. Obvious dehiscences between the membranes appear, however, in restricted areas. Another particular feature of the RER has also been observed. Rough profiles become abruptly closer to each other and then parallel applied for a distance of about half a micron (Fig. 12). No ribosome is attached to the innermost membranes of the rough cisterns in this segment. The intermembraneous space is usually crossed by bridges or filled with an electron-opaque material (Fig. 13). Such RER features likely suggest simplified LB's. The role of the fixation procedure is ruled out since these ultrastructural data have been observed whatever the fixation procedure used.

IV. Association of LB's with SSC's and of Both Structures with Nematosomes

Our observations have also demonstrated that the membranes of both the SSC and its applied cisterns are in continuity with the rough ER membranes (Fig. 2). In addition, the SSC's and the LB's are connected to each other by means of rough ER cisterns (Fig. 8), so that a continuous channel is formed between the nuclear membrane and the SSC (Fig. 8). In a preceding section we have

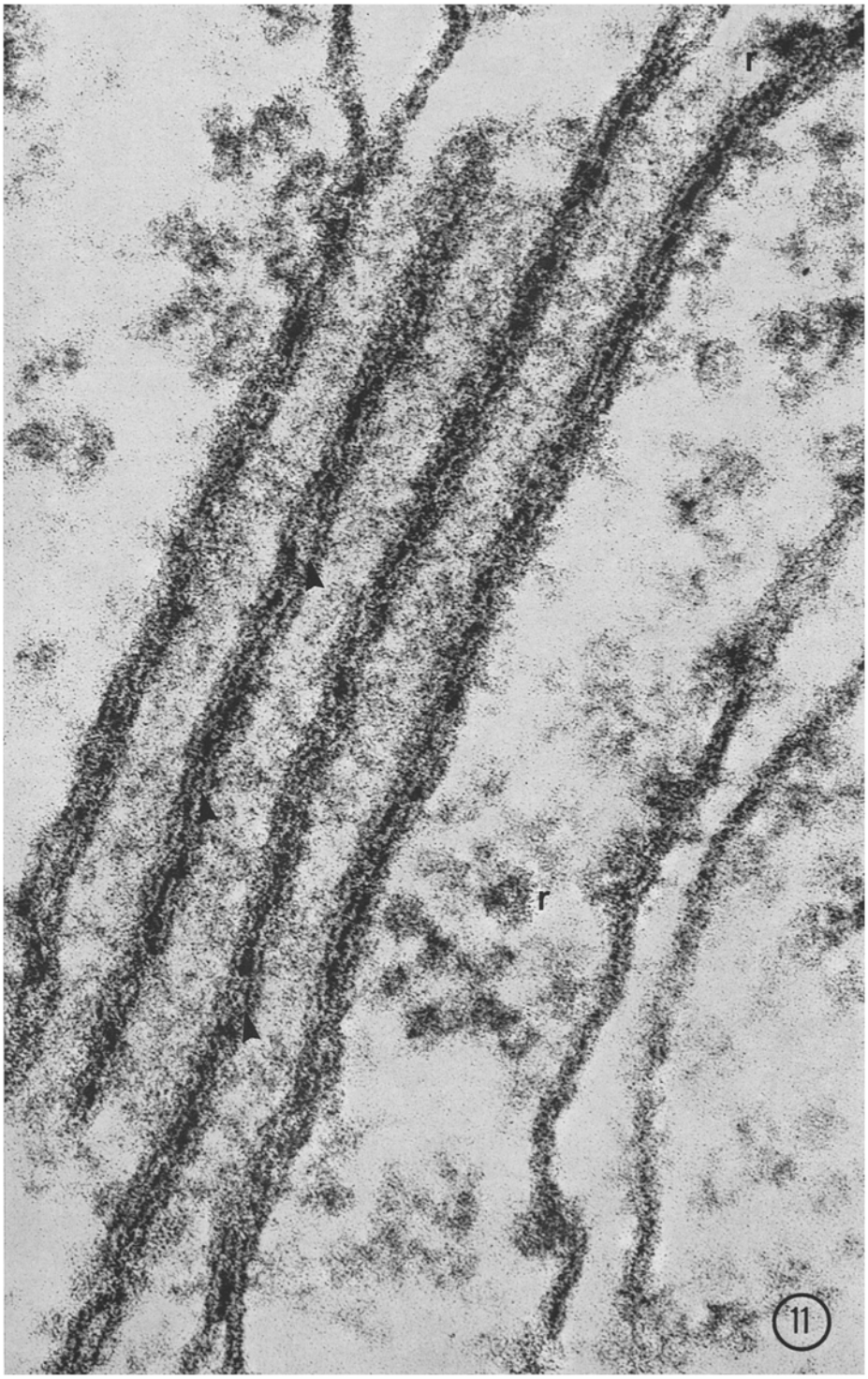

Fig. 11. Higher magnification of the lamellar body shown in Fig. 6. Note the pentalaminar structure of these unbranched, unfenestrated, regularly spaced (about 280 to 350 Å) cisterns.

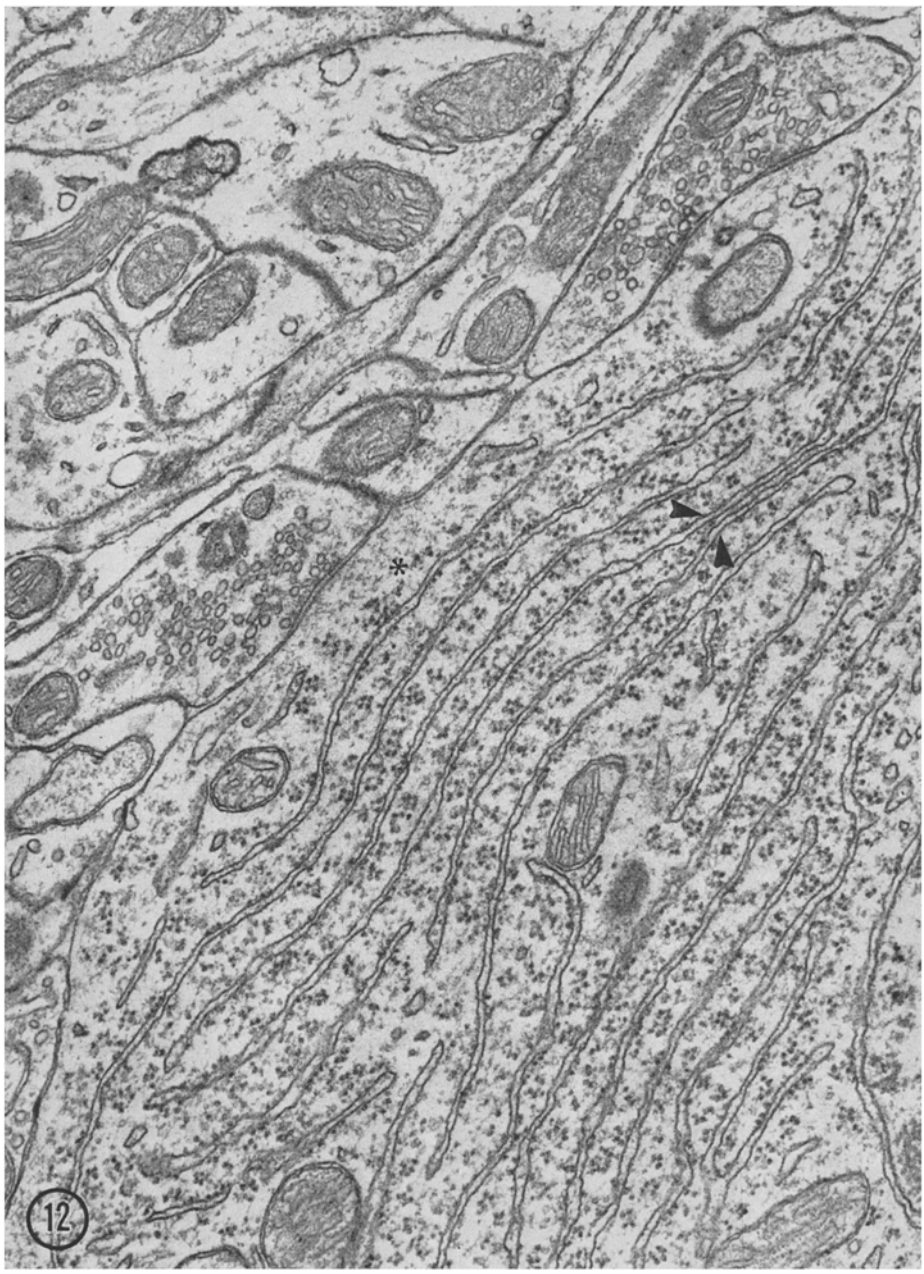

Fig. 12. A small part of the total size of a neuron, represented by a conspicuous Nissl body. Note the two rough ER cisterns (arrowheads) which become closer to each other and then parallel apposed for a distance of about half a micron, where no ribosome intervenes between them. Note also a fluffy material (*) disseminated between the axosomatic synapses and the peripheral RER profiles. Rat. Substantia nigra. × 39100

A dehiscence of the two constituent unit membranes occurs in some portions (arrowheads). Note also that a finely textured material consisting of very fine filaments rather than of granules intervenes between the cisterns. *r* ribosomes. Rat. Substantia nigra. × 300000

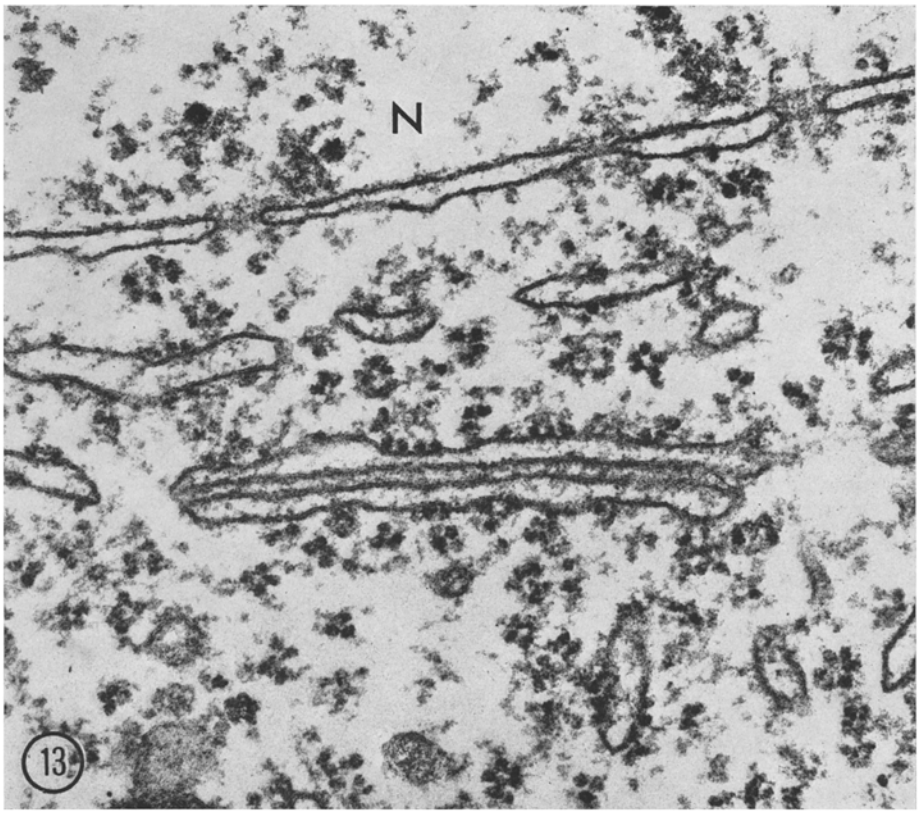

Fig. 13. Two rough ER cisterns are closely applied. A filamentous material cross-bridges the cleft between them. No ribosome intervenes in this interval, whereas many of them are still attached to the outer membranes. *N* nucleus. Rat. Substantia nigra. × 75240

already stressed the fact that both these structures possess common characteristics. They can be considered both as specialized forms of the endoplasmic reticulum of which the function or functions remain to be explained.

Another interesting observation is the association of SSC's (Fig. 2) and of LB's (Fig. 14) with nematosomes in the perikaryon.

Discussion

I. General Organization of SSC's and LB's in Neurons

SSC's are common features of nerve cells. One or more SSC may be closely applied to the perikaryal plasma membrane in the substantia nigra. On the other hand, the modes of formation of the LB's previously put forth by Herndon (1964) in his ultrastructural study of the Purkinje cells are no longer valid. As a matter of fact, the present study has demonstrated that neither a short anoxia,

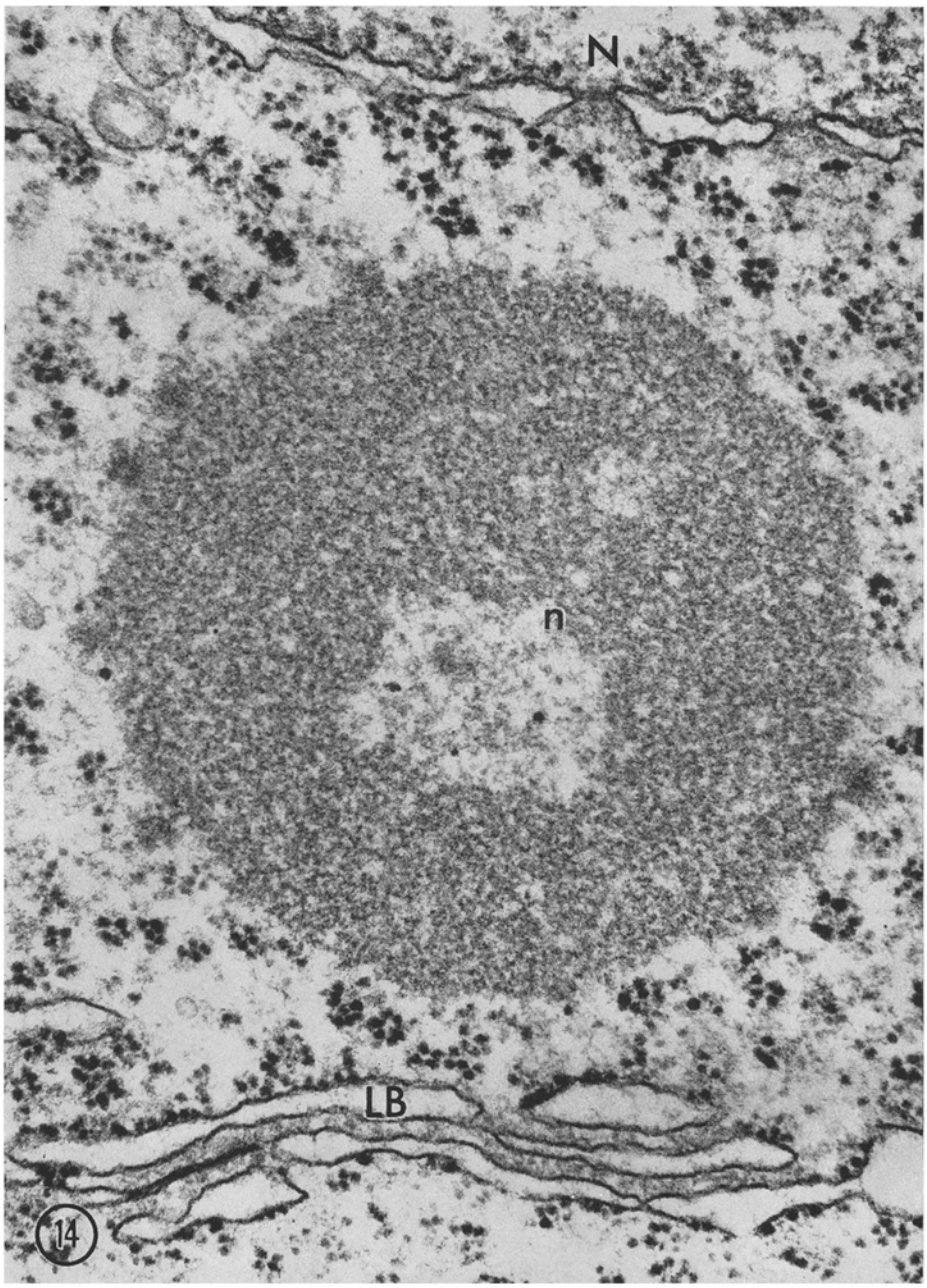

Fig. 14. A lamellar body (*LB*) is shown in the lowest part of this electron micrograph. Note that the electron-opaque material intervening between the flattened or unflattened cisterns appears similar to the filaments which compose the large nematosome (*n*). *N* nucleus. Rat. Medial vascular prechiasmatic gland. The fixation procedure consisted of: (1) Perfusion with a Sörensen's phosphate buffered (0.045 M) 2% glutaraldehyde solution (322.2 ± 0.8 mOsm) at 4° C; Post-fixation by immersion in Sörensen's phosphate buffered (0.1 M) 2% OsO_4 at 4° C. × 63950

nor the effect of a lowered temperature (4° C) can induce the formation of such structures. A comparative study of nervous tissue dissected out from different brain areas, i.e. the substantia nigra, the neostriatum and the medial vascular prechiasmatic gland has been carried out on animals (1) which were given artificial respiration up to the time of fixative penetration into the blood vessels or submitted to a short time (10–15 seconds) of anoxia so as to perform all the operations from the opening of the chest to the beginning of the washing solution perfusion; (2) which were perfused with warmed (37° C) or cold (4° C) buffered glutaraldehyde solutions. Two species, rats and cats, and concerning the rats, two strains, i.e. the Sprague-Dawley and Holtzmann strains, were used. In addition, factors were also varied in the fixative solutions used in our experiments: Concentration of the glutaraldehyde; Molarity of the buffer; Osmolality of the fixative solution. As a result of the present ultrastructural study, LB's, although not frequently observed, have been found in the neurons of the rat and cat substantia nigra and caudate nucleus whatever the fixation procedure used. They are really distinct structures.

On morphological grounds, both the SSC's and the LB's appear to belong to the ER network as shown in the Figs. 2, 5 and 8. They differ from typical rough ER cisterns in that they are usually flattened and devoid of ribosomes except the outermost ones. They are also easily differentiated from smooth ER tubules. Siegesmund (1968) has, however, considered the basic unit of the SSC as consisting only of a short segment of a pentalaminar membrane in close proximity, i.e. about 130 Å, to the neuronal plasmalemma and has not observed any connection between the former structure and the ER profiles. Some SSC's, indeed, do not apparently communicate with the RER membranes (Figs. 1, 3), but it may be inferred that such cisterns being sheet-like structures in three dimensions are plausibly interconnected with the rough-surfaced membranes in another plane of section. The SSC's usually display a pentalaminar structure. Whether two unit membranes have fused at the level of these cisterns in a single intermediary line is, however, questionable. Indeed, a minute gap of about 20 Å, such as observed at the level of a "gap junction" (Revel and Karnovsky, 1967; Revel, Olson, and Karnovsky, 1967; Goodenough and Revel, 1970), may also occur between these apparently fused membranes. Such an hypothesis could be tested by the study of the permeability of this ER membrane "junction" to electron-opaque substances. Extracellular markers, i.e. lanthanum salts or ferritin, cannot be applied, as the SSC's are not connected to the extracellular space, but such is not the case with strontium. Indeed, Somlyo and Somlyo (1971), studying the translocation of calcium from the sarcoplasmic reticulum —the elements which may be in close contact with the surface membrane, as well as those more centrally located—, which has now been shown to accumulate divalent cations, demonstrated that strontium, which has a higher atomic number, and hence higher electron opacity than calcium, may be a suitable marker for identifying sites that accumulate calcium, i.e. the sarcoplasmic reticulum and mitochondria. In addition, it should also be pointed out that an obvious separation of the constituent three-layered membranes is observed in one or more portions of the SSC's. The applied membranes of the LB's also appear as penta-

laminar structures when flattened. It seems likely that a minute gap may also occur at the level of such structures. Therefore, a fusion of the two apposed unit membranes resulting in an occlusion of the intracisternal lumen in some ER portions cannot conceivably occur in nerve cells.

At a sufficient magnification, the electron-opaque substance intervening between the flattened or unflattened cisterns of the LB's, as well as between the stacked cisterns closely applied to the SSC's, appears as a filamentous network (Figs. 1, 2, 5–10) and, at a higher magnification, it is seen to consist of a dense, finely textured material. The cytochemical nature and the function of this material remain unexplained. On the other hand, Rosenbluth (1962b) has previously reported that the SSC's are separated from the overlying plasmalemma by a light zone, 50–80 Å wide, which sometimes contains a faint intermediate line. In the present investigation, the occurrence of filamentous cross-bridges between the above-mentioned membranes has been noticed in nerve cells following perfusions with glutaraldehyde (Fig. 4). A fine granular deposit has also been pictured in a diagram of an SSC previously published by Siegesmund (1968), who used a mixture of acrolein and glutaraldehyde for nervous tissue fixation. Whether subsurface or lamellar body cisterns are studied, all consist of structurally similar pentalaminar membranes, but the complex including an SSC, its overlying plasmalemma and the bridges linking them, forms a particular entity of which the physiological properties are discussed in another section. Indeed, the constancy in the width (100–130 Å) of the cleft between an SSC and its adjacent plasmalemma and the accuracy with which the two structures follow each other as they undulate, as well as the presence of minute bridges traversing this interval, demonstrate that SSC's are structurally joined to the overlying plasmalemma.

II. Endoplasmic Reticulum Counterparts of the LB's and SSC's

The LB's are easily differentiated from multilaminated bodies (Morales, Duncan, and Rehmet, 1964; Smith, O'Leary, Harris, and Gay, 1964; Friedman, Cawthorne, and Bird, 1965; Morales and Duncan, 1966; Peters and Palay, 1966; Kruger and Maxwell, 1969; Herman and Ralston, 1970), which are said to be also derived from endoplasmic reticulum (Morales, Duncan, and Rehmet,1964). The LB's, which consist of unfenestrated, flattened or unflattened cisterns, are also easily distinguished from the annulate lamellae (Kessel, 1968). It has been shown in amphibian and echinoderm eggs that annulate lamellae arise by evagination from the nuclear envelope and that they have also associated ribosomes (Kessel, 1963, 1968). It appears that all these above-mentioned structures, i.e. the multilaminated bodies and annulate lamellae, as well as the SSC's and LB's, are particular portions in the endoplasmic reticulum vacuolar system. The spine apparatus observed in dendrite spines (Gray, 1959) also bears some resemblance to the LB's. Indeed, it consists of smooth cisterns between which intervenes an electron-opaque material. These cisterns may sometimes be flattened, such as in the dendritic spines of the cat caudate nucleus (Le Beux, personal communication). Some cytoplasmic features, described as whorled LB's (Fig. 10)

also bear some resemblance to the membrane arrays of the ergastoplasmic "nebenkerns" (Haguenau, 1958). Closely applied flattened agranular membranes have been described in HeLa cells (Epstein, 1961), but such structures are, however, larger than those figurated in the present study (Figs. 12, 13).

An SSC, unassociated or associated with one or more closely applied ER cisterns, is so remarkable that no other structure can be confused with it. SSC's are, however, akin to a special type of endoplasmic reticulum in the striated muscle cells, the sarcoplasmic reticulum (SR). As mentioned above, the structural complex including an SSC, the overlying portion of the plasmalemma and the electron-opaque bridges associating them bears some resemblance to a dyad rather than to a triad. Indeed, small terminal expansions of the SR in the cardiac muscle here and there may be closely applied to the membranes of T tubes. Similar contacts are also noticed between flattened expansions of the SR and the sarcolemma at the outer surface of the fiber. The junction between the SR and the transverse tubular system at the triad also is formed by flattened surfaces of the SR lateral sacs and the T system tubule, which face each other at a distance of about 120–140 Å. At periodic intervals of about 300 Å, the SR membrane forms small projections, of which the tips are joined to the T system membrane by an amorphous material. The SR projections and the amorphous material have been termed SR feet by Franzini-Armstrong (1970). A parallel may be drawn between the SSC and the lateral elements of the triad (dyad). Indeed the SSC, and the facing membrane of the plasmalemma are separated by a 100–130 Å wide gap (Fig. 4). No projections similar to those found at the level of the SR membrane (the "dimples" of Kelly, 1969) are observed here, but the gap between the SSC and the plasmalemma is crossed by 70 Å wide filamentous bridges which are intensely stained by a combination of uranium and lead. To elucidate the nature of this electron-opaque material, however, needs further cytochemical investigation.

III. Possible Physiological Properties of the SSC's and the LB's

It would be expected that, in the areas where SSC's occur, the properties of the neuronal surface are a function of the complex consisting of the SSC and of its adjacent plasmalemma associated by means of cross-bridges, and not of the plasma membrane alone. The function of this above-mentioned complex should be considered in at least the following two connections: (1) the electrical properties of the neuronal membrane; (2) the metabolic interchanges between the neuron and its environment. The neuronal plasmalemma contain patches occupying a small percentage of the total surface, which may differ functionally, as well as structurally, from the remainder of the neuronal surface. These patches associated with SSC's could be sites of low transneuronal resistance through which direct ion flow may occur. The current flow produced elsewhere on the cell by synaptic depolarization would be channeled selectively through these areas. As regards this hypothesis, it is informative to compare the structure of SSC patches with that of junctions between cells, where a low-resistance pathway has been experimentally established. Rather than put into evidence the possible

similarities, we would like to emphasize the differences. Indeed, the junctions to which low resistance has been attributed are structures in which the membranes of adjacent cells are separated by a very small gap about 30 Å wide (gap junctions). The above-mentioned SSC complex is obviously different from this type of junction because the distance between the SSC and the overlying plasmalemma is relatively wide (about 100–130 Å) over most of the junctional area. Anyhow the effect of an ER membrane in the subsurface position would be to alter the mobility and effective concentrations of ions at the inner surface of the plasma membrane, and this might secondarily affect the electrophysiological behaviour of the neuronal surface in this region. On the other hand, the existence of a vacuolar system separating the cytoplasm in two compartments makes possible the existence of ionic gradients and electrical potentials across these intracellular membranes. On the basis of morphologic observations, it may be inferred that the SSC complex transmits impulses from the surface membrane into deeper regions of the nerve cell. As mentioned above, the resemblance between the configuration of a dyad (triad) and that of an SSC complex is striking. There is now general agreement that, at the level of the triad or equivalent structures, events occur which link the depolarization of the T system with the release of calcium from the adjacent sacs of the SR (Ebashi and Endo, 1968). It is also of interest to point out that heavy meromyosin (HMM) forms characteristic arrowhead complexes with actin filaments in glycerinated nerve cells from the spinal cord of chick embryos, as well as in glial cells (Ishikawa, Bischoff, and Holtzer, 1969). Similar HMM-reactive filaments were also observed in a variety of cell types, i.e. fibroblasts, blood platelets, smooth muscle, chondrogenic, endothelial and epithelial cells, in addition to skeletal and cardiac muscle cells, by means of sectioning and negative staining techniques (Ishikawa, Bischoff, and Holtzer, 1969; Behnke, Kristensen, and Nielsen, 1971; Rostgaard, Kristensen, and Nielsen, 1972). In addition, myosinoid filaments were also identified in negatively stained and glycerinated preparations of blood platelets (Behnke, Kristensen, and Nielsen, 1971). All these data demonstrated that a contractile protein apparatus has been found in a wide variety of cells. The SSC complex bears a structural resemblance to a dyad, but on morphological grounds alone, it is not, however, possible to assume that the physiological role of the SSC to the adjacent patch of plasmalemma is similar to that of the SR to the T system. Further investigations are necessary to determine a possible role of the SSC in calcium release or uptake.

It was also noticed that a mitochondrion is sometimes closely applied to the deep surface of an SSC (Fig. 3), particularly when the SSC is subsynaptic in position. Whether the close association of mitochondria with the SSC's may be transient rather than permanent cannot be inferred from electron micrographs. A metabolic function involving the supply of high energy ATP produced by mitochondria to the SSC's, which may be concerned in the active transport of ions into or out of the nerve cell, is plausible.

As mentioned above, our observations suggest that the LB's and the SSC's are structurally related forms of the endoplasmic reticulum and that a continuous channel exists between the nuclear membrane and the SSC, which is closely

applied to the plasmalemma. It is possible that, like plasma membranes, the ER membranes also exhibit selective permeability and that differences in ionic concentration and electric potential occur between the inside and the outside of these intracellular membrane-limited conpartments. Whether a small gap, about 20–30 Å wide, occurs between the two unit membranes forming the pentalaminar membrane of the LB's or of the SSC's has not been solved in our studies. The role of these pentalaminar membranes located in restricted portions of the endoplasmic reticulum could consist in a modification of permeability between the intracellular compartments, i.e. that a direct ion flow may occur at their level. We would like to stress that in the present paragraph of the discussion the pentalaminar membrane of the SSC itself is concerned, but not the SSC complex.

It should be pointed out that the dense, finely textured material intervening between the cisterns of LB's, as well as between the stacked cisterns closely applied to an SSC, bears some resemblance to that composing the nematosomes (Fig. 14), but it cannot be inferred from these ultrastructural data that they are identical. Indeed, cytochemical investigations are needed to solve this problem.

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