SPECIALIZED MEMBRANE JUNCTIONS BETWEEN NEURONS IN THE VERTEBRATE CEREBELLAR CORTEX

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ABSTRACT

"Gap" junctions, the morphological correlate for low-resistance junctions, are demonstrated between some mossy fiber terminals and granule cell dendrites in some lower vertebrate cerebella (gymnotid and frog). Most of the gap junctions (GJs) seen in the gymnotid-fish cerebellum exhibit an asymmetrical configuration, the electron-opaque cytoplasmic material underlying the junction being more extensive in the dendritic than in the axonal side. In the frog cerebellum, the GJs have a symmetrical distribution of such electron-opaque material. In both species the GJs are encountered at the same synaptic interface as the conventional synaptic zone (CSZ), constituting "mixed synapses" in a morphological sense. The axonal surface covered by CSZs is larger than that covered by GJs. In mammalian cerebellum, GJs are observed only in the molecular layer, between perikarya, dendrites, or perikarya and dendrites of the inhibitory interneurons. These GJs are intermixed with attachment plates and intermediary junctions interpreted as simply adhesive. In the mammalian cerebellum, a new type of junction which resembles the septate junctions (SJs) of invertebrate epithelia is observed between axonal branches forming the tip of the brush of basket fibers around the initial segment of the Purkinje cell axon. It is suggested that such junctions may be modified forms of septate junctions. The physiological implications of the possible existence of high-resistance cross-bridges between basket cell terminals, which may compartmentalize the extracellular space and thus regulate extracellular current flow, must be considered.

INTRODUCTION

Morphological studies of the ultrastructure of the cerebellar cortex have provided a well-established basis for the recognition of most of the neuronal profiles found in single, thin sections. The work of several groups of investigators (15, 16, 23, 30) has made possible a detailed description of the synaptic arrangements present in the mammalian cerebellar cortex. The correlation of structural features with the electrophysiological data (for instance the monograph by Eccles et al. [8]), has allowed the postulation of a functional role for most of the chemical synapses encountered in this cortex; however, many points remain still to be understood. Nevertheless, and in general for excitable cells, a correlation has been consistently demonstrated between specific intercellular junctions and low-resistance pathways (4, 11). Given the well-known ultrastructural features of the low-resistance junctions, the cytologists may attempt generalizations to guide the physiological investigation of particular junctions. In some instances, and particularly when the material allows a direct

electrophysiological study, the actual existence of electrotonic coupling may be demonstrated. This was the case with the low-resistance junctions described by Hinrichsen and Larramendi (19) between neuronal perikarya of the mouse mesencephalic fifth nucleus. Thus, Baker and Llinás (2) have recently presented physiological evidence suggesting the existence of electrontonic coupling between similar neurons in the rat. In other instances the morphological description has not been followed by the physiological study (for example, in the lateral vestibular nucleus of the rat where GJs have been observed [46]).

The specialized junction generally accepted as the morphological correlate of a low-resistance pathway for direct intercellular spread of electrical excitation is the gap junction (GJ). Morphological features of these junctions have been described previously (38). Direct evidence that the GJs are sites of electrotonic coupling has been provided by Bennett and coworkers (1, 32) in the lateral giant fiber of the crayfish. In these studies, experimentally induced changes in coupling resistance were associated with separation of the junctional membranes at the GJs. Another specialized junction so far encountered between invertebrate cells exclusively is the septate desmosome or septate junction (SJ). These junctions were claimed to represent low-resistance pathways between cells. Electrophysiological studies by Loewenstein and Kanno (26, 27) and morphological studies by Gilula et al. (13) on different SJs suggested that they could represent small cytoplasmic channels between the two adjacent cells, which could serve as the basis for intercellular communication. More recently, however, GJs have been encountered in some instances in close proximity to the SJs and subserve the electrotonic coupling between these cells (20, 40).

The present paper describes comparative results in cerebellar cortices of gymnotid fish and frogs, with emphasis on the ultrastructural features and the localization of specialized membrane junctions between neurons (probably corresponding to low-resistance junctions). Similar GJs are also described in the molecular layer of the rat and the cat cerebellar cortices. A new kind of junction, resembling the SJs of invertebrate cells, is described as occurring between axonal branches forming the final portion of the brush of basket fibers around the initial segment of the Purkinje cell axon in rat and cat cerebella. Some of the physiological implications of these morphological junctions are discussed.

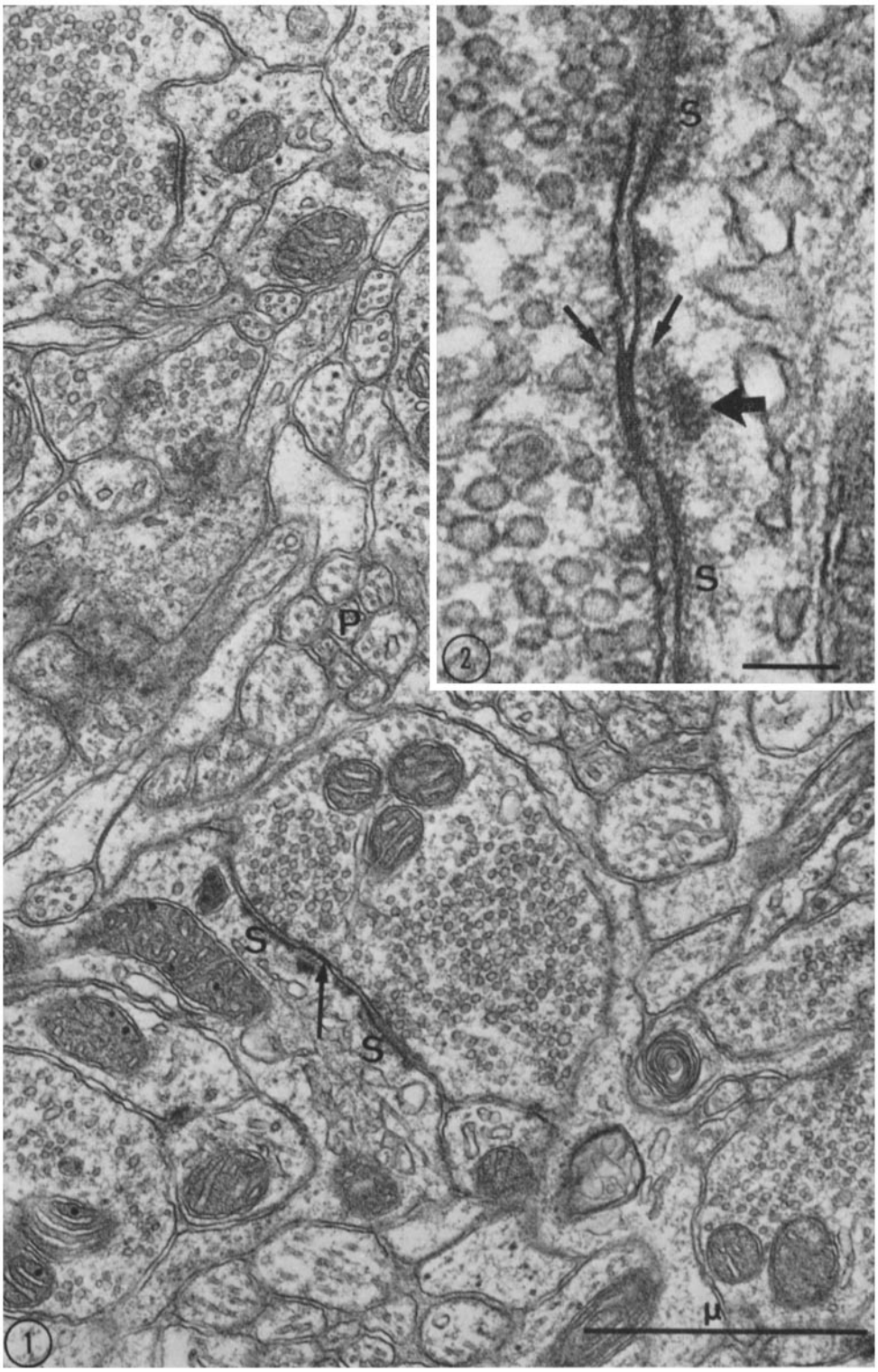
MATERIALS AND METHODS

Adult gymnotid fish (*Gymnotus carapo*) about 12–16 cm in length, adult frogs (*Rana esculenta*) about 20–40 g in weight, adult rats of the Wistar strain about 100–300 g in weight, and adult cats weighing 2–3 kg were fixed by perfusion through the heart. A variety of fixatives were employed. The aldehyde fixative was the most commonly used. This fixative, a mixture of glutaraldehyde and formaldehyde, has given satisfactory results in previous studies (43, 45) and consists of a solution of 0.12 M phosphate buffer (pH 7.4) containing the following compounds in each 100 ml: paraformaldehyde, 1 g; 50% glutaraldehyde, 2 ml; calcium chloride, 2 mg. Also, in a few instances, after washing the blood vessels with McEwen's saline, primary perfusion with 2% OSO_4 solution in phosphate buffer or with 1.8% $KMnO_4$ solution in phosphate buffer was done. Blocks taken from the cerebellar cortex of aldehyde-fixed animals were postfixed by immersion in phosphate-buffered 2% osmium tetroxide and treated for 2 hr with a 2% solution of uranyl acetate in sodium maleate at 4°C (22).

The blocks were dehydrated in graded ethanol solutions and embedded in Araldite.

FIGURE 1 Mossy fiber terminal in synaptic contact with a granule cell dendrite. One gap junction (arrow) and two active zones (*S*) are present at the synaptic interface. Most of the mossy terminal surface is covered by glial cytoplasm. In this upper region of the granule cell layer, the neuropil contains many small axonal profiles belonging to parallel fibers (*P*). Gymnotid fish. Scale, 1 μm. × 42,000.

FIGURE 2 Enlargement of the synaptic interface between the mossy terminal and the granule cell dendrite illustrated in Fig. 1. The gap junction is surrounded by the active zones (*S*). Besides the two semidense layers undercoating both sides of the junction (small arrows) a denser triangular layer is evident in the dendritic cytoplasm (large arrow), giving to the whole junction an asymmetrical aspect. Scale, 0.1 μm. × 140,000.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OBSERVATIONS

A. *Gymnotid Fish and Frogs*

In these vertebrates, GJs are present in the cerebellar cortex at the granule cell layer. The junctions occur between the mossy fibers and the dendrites of the granule cells but are much more frequent in the gymnotid-fish cerebellum than in that of the frog.

In the gymnotid-fish cerebellar cortex, there is no well-defined border between the Purkinje cell layer and the elements of the superficial granule layer. Moreover, in this case the Purkinje cells may be intermixed with elements belonging to the deeper molecular layer (i.e., mossy axon terminals can be found in a neuropil crowded with parallel fibers [Fig. 1] and even with some profiles of Purkinje cell dendrites).

In this animal the mossy fibers may establish two kinds of synaptic arrangement. In one instance the mossy fiber terminal contacts only a few dendritic profiles belonging to granule cells, and the remainder of the axonal surface is covered by glial processes (Fig. 1). This type of arrangement, which is the least frequent, cannot be regarded as a true glomerulus. True glomeruli, resembling simplified forms of those in the mammalian cerebellar cortex (15, 29), constitute the second and more common synaptic arrangement of the mossy fibers. In this latter instance, the center of the glomerulus is occupied by the mossy terminal, which is encircled by numerous granule cell dendritic tips, linked one with another by means of small attachment plates (Fig. 5). In the peripheral zone of the glomerulus small axon terminals containing flattened synaptic vesicles and belonging to Golgi cells are in synaptic contact with the same granule cell dendrites (43, 50).

In both kinds of mossy fiber synaptic arrangement, two types of specialized junctional zones can be found at the synaptic interface between the mossy terminals and the granule cell dendrites. The first type, comprising the large majority of these junctions, is identical with the synaptic complex or "active" zone (Figs. 1, 2, 4, and 5) described as type I by Gray (14). The second type consists of small plaques from 0.08 to 0.2 μm long (Figs. 1–5), where the apposing plasma membranes converge to produce a narrow gap 15–20 A wide. The latter junctions, exhibiting a seven-layered structure (Fig. 2 and 4), correspond to the GJs described by Revel and Karnovsky (38) between heart muscle cells and liver cells. The over-all thickness of these junctions falls in the range of 150–170 A. Even if synaptic vesicles are present in the presynaptic axoplasm, they are not clustered near the GJ. On the other hand, the cytoplasm subjacent to both sides of the contact zone contains a diffuse band of semidense cytoplasmic material (Fig. 3). A peculiarity often encountered in these electrotonic junctions is the presence of a second dense, triangular layer beneath the diffuse band of semidense material in the dendritic cytoplasm. This second layer was found to be always shorter than the first one, arranged in such a way that its base is parallel to the material subjacent to the junctional membrane (Figs. 1, 2, 4, and 5). This cytoplasmic differentiation is not homogeneous, but exhibits a finely granular aspect (Fig. 4). According to this differentiation, two kinds of electrotonic junctions can be encountered in the gymnotid-fish cerebellum: an asymmetrical one (Figs. 1, 2, 4, and 5), bearing the dendritic differentiation, and a symmetrical one (Fig. 3) lacking this dendritic material. The first kind is by far the most common.

From a strictly morphological viewpoint, such a synaptic junction, where different specializations are combined, has been called a "mixed synapse" (35). In most mossy terminal profiles having mixed synapses, the number of active zones was found to be two to six times higher than the number of GJs; three was the largest number of GJs encountered in a single mossy terminal profile. Planimetric measurements of the linear surface occupied by the active zones and by the GJs, made on 20 glomeruli, have shown that the linear surface occupied by the active zones can be 8–12 times larger than that occupied by the GJs.

A detailed description of the granular layer of the frog cerebellum has already been published (18, 43). In this animal, real glomeruli are practically absent and the large varicosities of the mossy fibers, constituting "terminaisons en passant," establish synaptic contacts with a few granule cell dendrites, leaving most of their surfaces surrounded by neuroglial processes. In some instances, GJs can be observed between the mossy terminal and the granule cell dendrite, but chemical-type synapses and their morphological correlates, the active zones, are by far the most commonly found. Fig. 6 illustrates one of the mixed synapses present in the frog cerebellum. The material has not been stained with uranyl acetate before the embedding, and for

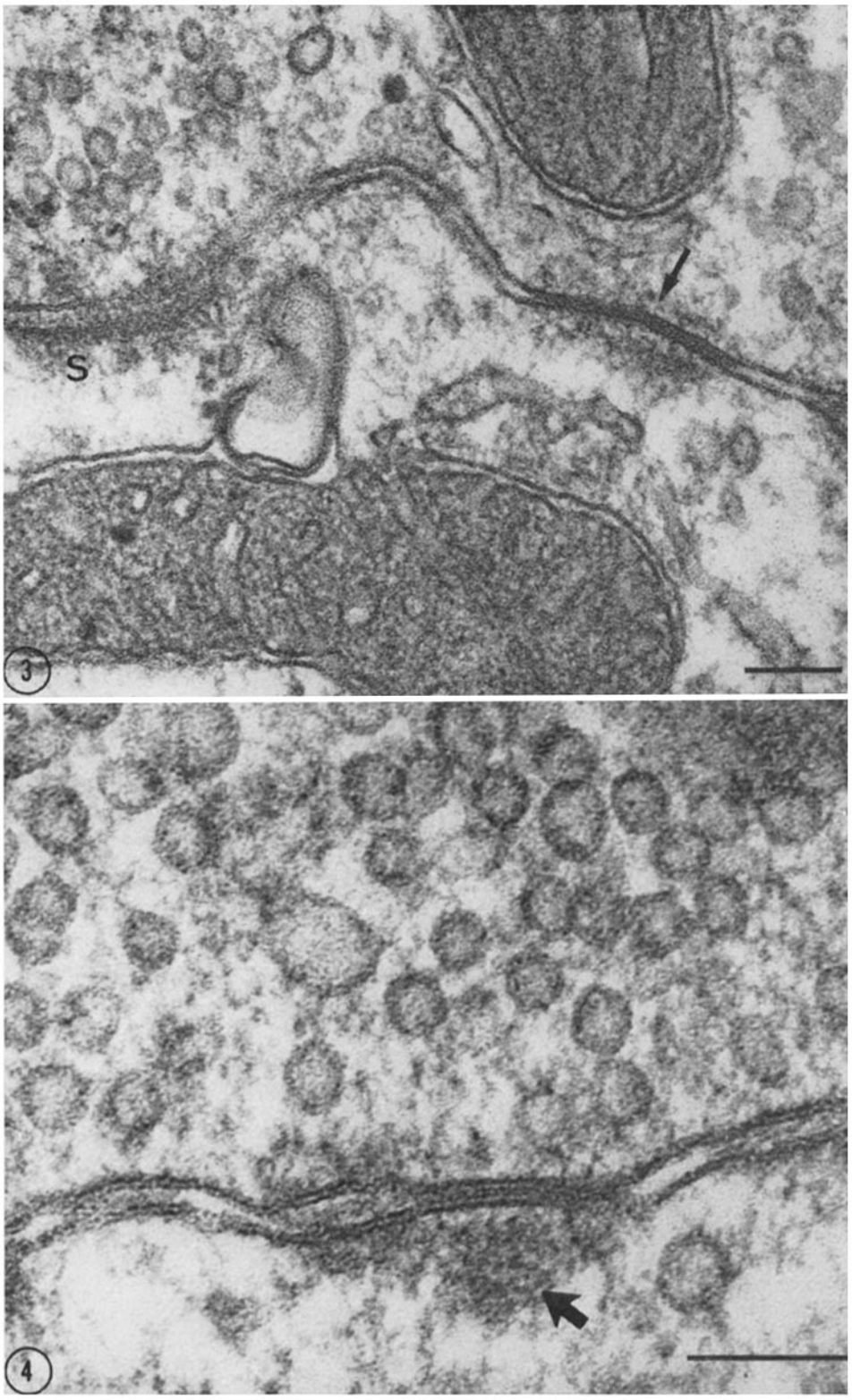


FIGURE 3 Mixed synapse between a mossy terminal and a granule cell dendrite. The synaptic vesicles are clustered at the active zone (*S*) region. The gap junction (arrow) has a symmetrical structure. Gymnotid fish. Scale, 0.1 μm. × 140,000.

FIGURE 4 Asymmetrical gap junction between a mossy terminal and a granule cell dendrite. The seven-layered structure of the junction is evident. The arrow points to the triangular dendritic differentiation with an alveolate aspect. Gymnotid fish. Scale, 0.1 μm. × 242,000.

this reason the GJ does not exhibit the characteristic seven-layered structure. These junctional zones measure about 0.3 μm in length, with an over-all thickness of about 160 A. In all the GJs observed in this cerebellum, the band of semidense cytoplasmic material overlying the whole length of the contact had roughly the same thickness on the presynaptic side as on the dendritic side of the junction. Asymmetrical junctions such as those described in the gymnotid-fish cerebellum were not observed.

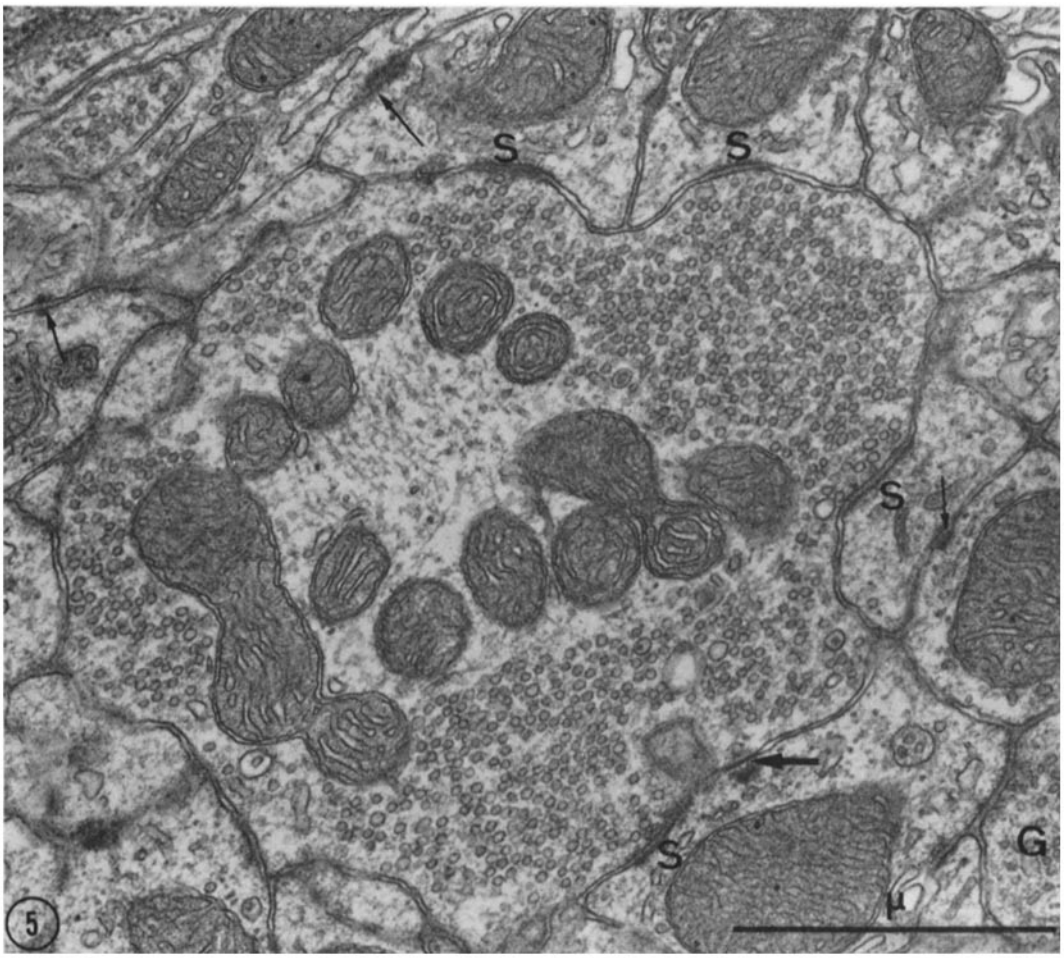
B. *Rats and Cats*

In the cerebellar cortices of cats and rats the only GJs observed were located in the molecular layer between neuronal perikarya and dendrites (Figs. 10 and 11), between two perikarya (Fig. 13), or between two dendritic profiles (Fig. 8). From the classical work of Ramón Y Cajal (37) it is known that the only neuronal perikarya encountered in the molecular layer are those of the molecular layer interneurons. These neurons are called superficial stellate cells and lower stellate cells or basket cells, depending on their localization in outer or deeper regions of the molecular layer. However, both cells belong phylogenetically (43) and functionally (9, 25) to the same category. This fact simplifies the identification of neuronal perikarya in the molecular layer. On the other hand, the identification of isolated dendritic profiles is much more difficult since dendrites belonging to Purkinje cells, Golgi cells, and stellate cells are interspersed in the molecular layer. It is, however, very easy to differentiate primary and secondary branches of the Purkinje cell dendrites from those of the interneurons, due to their intrinsic features and to their synaptic inputs (6). Purkinje cell dendrites are invested with a neuroglial sheath and their shafts are free of synaptic contacts with parallel fibers. The only axon terminals establishing synaptic contacts with the shafts of these dendrites belong to stellate cells or basket cells. The most peculiar organelle present in Purkinje dendrites, despite the microtubules, consists of anastomosing slender tubules and narrow cisterns of endoplasmic reticulum; the latter are mostly located at the periphery of the dendritic profile. All these features allow the identification of Purkinje dendritic profiles in any plane of section. The dendrites belonging to the different types of inhibitory interneurons are, however, much more difficult to differentiate. This difficulty stems from the fact that stellate, basket, and Golgi cell dendrites are free of a neuroglial sheath. Furthermore, their shafts are commonly covered with synaptic boutons, most of them arising from parallel fibers, and the flattened cisterns of endoplasmic reticulum peripherally disposed are almost entirely lacking. In some instances, however, due to their size, their location and orientation in the molecular layer, and the shape of their spines, dendritic profiles belonging to these interneurons may be identified (6, 24).

Generally, the perikarya of basket and stellate cells are in direct apposition to neuroglial processes, synaptic terminals, and nonsynaptic portions of axons (for a quantitative analysis see the paper by Lemkey-Johnston and Larramendi [24]). Dendritic profiles in the proximity of these neuronal perikarya are separated from them by a thin layer of astrocytic cytoplasm. Occasionally, dendritic profiles (Figs. 7, 10, and 11) and even perikarya (Fig. 13) belonging to the inhibitory interneurons can be in direct apposition to basket or stellate cell bodies. In these instances, specialized junctional zones occur between the plasma membranes of both neuronal elements in a sequence of macular structures. At these sites the plasma membranes, lying nearly parallel to each other, are lined with symmetrical accumulation of

FIGURE 5 Typical glomerular arrangement of a mossy fiber terminal. At the synaptic interface between the mossy terminal and the granule cell dendrites, four active zones (*S*) are present. An asymmetrical gap junction (large arrow) can be observed. Note that the granule cell dendrites are linked one with another by attachment plates (small arrows). An axonal profile belonging to a Golgi cell axon (*G*) is in synaptic contact with one of the granule cell dendrites. Gymnotid fish. Scale, 1 μm. × 42,000.

FIGURE 6 Mixed synapse between a mossy fiber terminal and a granule cell dendrite. Synaptic vesicles are clustered at the active zone (*S*) region. The close apposition (arrow) representative of the gap junction in this material (nonpretreated with uranyl acetate) exhibits a symmetrical structure. Frog. Scale, 0.1 μm. × 152,000.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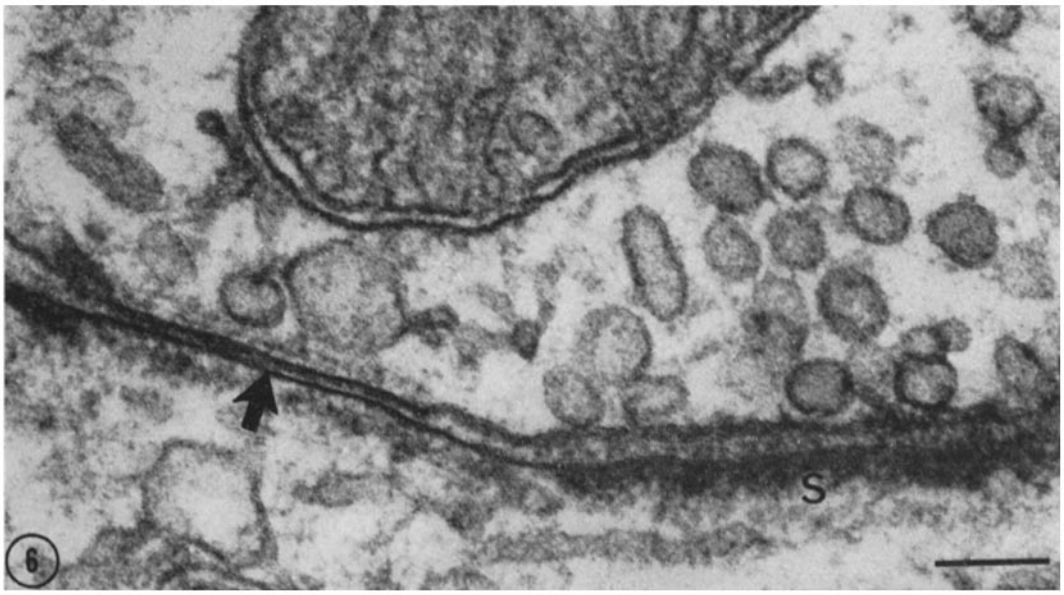
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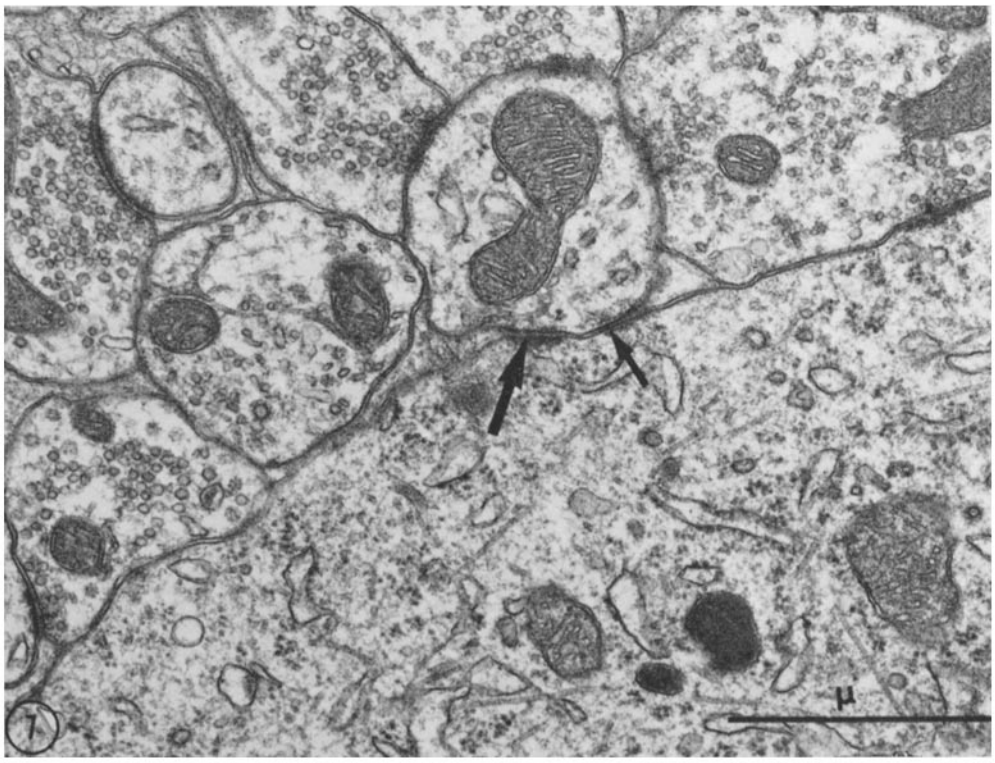


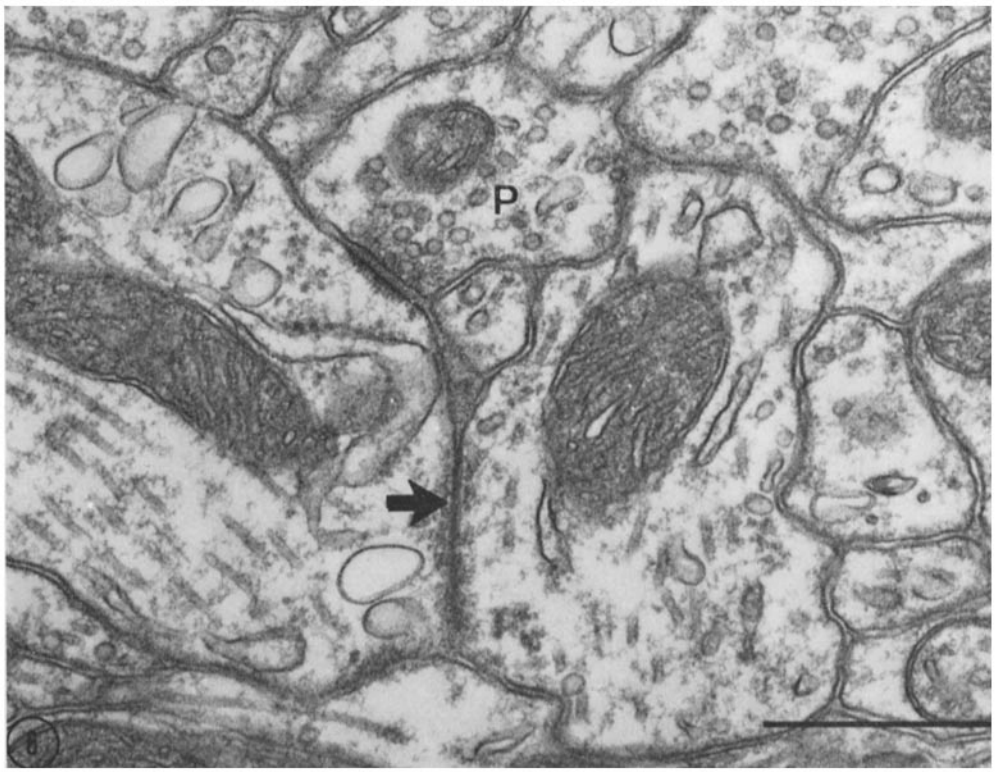


FIGURE 7 Basket cell body in direct apposition to a stellate cell dendrite. At the junctional zone between the neuronal cell body and the dendrite, two kinds of junctions can be observed: an attachment plate (large arrow) and an intermediary junction (small arrow). Deep region of the molecular layer of the cat. Scale, 1 μm. × 35,000.

FIGURE 8 Gap junction (arrow) between two basket cell dendrites. One of the dendritic profiles is in synaptic contact with a parallel fiber axon terminal (*P*). Deep region of the molecular layer of the rat. Scale, 0.5 μm. × 60,000.

electron-opaque material in both adjacent cytoplasms. The neural elements that have been recognized as forming part of these specialized junctional zones are:

(*a*) perikarya of basket cells, illustrated in Fig. 13;

(*b*) a basket cell perikaryon and a dendritic profile, which on the basis of orientation may belong to a Golgi cell (Fig. 11);

(*c*) a basket cell perikaryon and a dendritic profile probably belonging to another basket cell;

(*d*) a stellate cell perikaryon and a dendritic profile belonging to a basket cell or to another stellate cell (Fig. 10).

(*e*) two dendritic profiles probably belonging to basket cells (Fig. 8).

Two main types of junctional zones have been observed; they are illustrated in Figs. 7, 10, 11, and 13. The first type consists of small zones where the plasma membranes are separated by a cleft about 200 A wide. The material accumulated in the cleft is more electron-opaque than the nonjunctional extracellular space. This type of junction (Figs. 7 and 10) corresponds to an attachment plate and is identical with the "punctum adhaerens" described by Palay (31). The second type is formed by zones where the apposing plasma membranes converge to produce a narrow cleft about 20 A wide; they are identical with the GJs already described in nonmammalian cerebellar cortex. The over-all thickness of these seven-layered junctions is 140–150 A (Figs. 12 and 15). In material primary-fixed with $KMnO_4$ the total width of the GJs was 130–140 A, somewhat less than in material primary-fixed with aldehydes. This difference has already been described by Brightman and Reese (5) for the GJs encountered between ependymal cells of the mouse. When in the $KMnO_4$-fixed material the GJs are sectioned obliquely, they have the appearance of a scalariform formation, where the transverse dense lines are repeated at a period of about 90 A. Fig. 9 illustrates one dendro-dendritic GJ present in the rat molecular layer; in this oblique section the ladder-like structure of the junction is evident. The attachment plates and the GJs may be placed in an alternating fashion (Figs. 10 and 13) in such a way that the GJ is surrounded by attachment zones. Intermediary junctions, where the parallel plasma membranes are separated from each other by a cleft approximately 100 A wide (Figs. 7 and 14), may be found at the same interface between a dendrite and a cell body or between two perikarya. Figs. 13, 14, and 15 illustrate the junctional zones between two basket cell perikarya. In Fig. 14 the intermediary junction can be seen whereas, in Fig. 15, corresponding to the same interface but three to four sections beyond, the GJ exhibits all its characteristics. Thus, the intermediary junctions are considered as the transition zone between the attachment plate and the GJ.

In rats as well as in cats, a new type of junction has been observed in the vicinity of the complex nest formation established by the final portion of the brush of the basket fibers as they surround the initial segment of the Purkinje cell axon.[1] This new type of junction has been encountered only between profiles belonging to basket terminals in the area of the synapse between these fibers and the initial segment of the Purkinje cell axon. The junction is of variable length, from 0.5 to 0.1 μm. The extracellular space between the basket fibers, in the junctional region, has its normal width of about 150 A. Along the whole length of the contact, the intercellular cleft contains periodic electron-opaque densities, disposed perpendicular to both plasma membranes attached to their outer leaflets, and almost regularly spaced about 140 A apart (Figs. 16–18). In face view, these junctions have a honeycomb appearance (Figs. 19 and 20) due to the presence of closely packed polygons of about 170 A diameter; that is, about twice the size of the hexagons described in GJs. Using optical diffraction and the Markham rotation techniques, it seems that the subunits or polygons encountered in the face views of these septate-like junctions are assembled in an hexagonal pattern (Fig. 21). All these features resemble those of the SJs of invertebrate epithelia (13, 51, 52). However, a distinction

[1] The actual synapse between the basket terminals and the axon of the Purkinje cells has already been described by Palay (30) in rats and by Hámori and Szentágothai (16) and Fox et al. (10) in cats. Both descriptions coincide with regard to the distribution of the great concentration of basket fibers surrounding the Purkinje cell axon which form a broad vortex-like arrangement where the scarceness of contacts between the basket cell terminals and the Purkinje cell axon seems to be the rule. Nevertheless, the loose neuropil described by Hámori and Szentágothai (16) as surrounding peripherally this complex axo-axonal synaptic formation has neither been described by Palay (30) in rats nor observed in the present paper in cats.

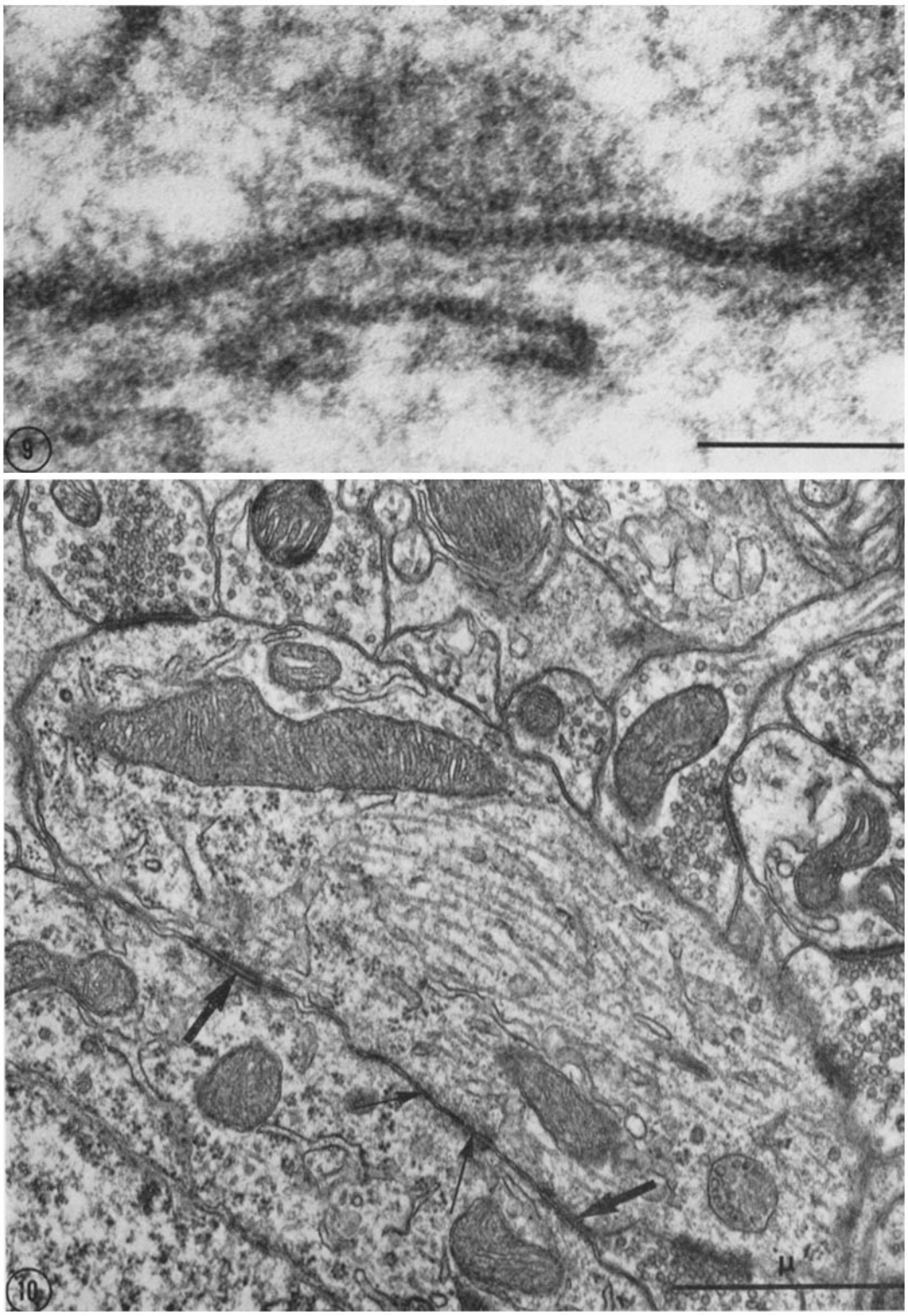


FIGURE 9 Oblique section of a gap junction between two basket cell dendrites. The ladder-like structure of the junction, with the transverse dense lines repeated at a period of about 90 A, is evident. Medial region of the molecular layer of the rat. Primary $KMnO_4$ fixation. Scale, 0.1 μm. × 320,000.

FIGURE 10 Stellate cell body in direct apposition to a basket cell dendrite. At the interface between both profiles, two types of junctional zones can be observed: a gap junction (small arrows) surrounded by attachment plates (large arrows). Outer region of the molecular layer of the cat. Scale, 1 μm. × 36,000.

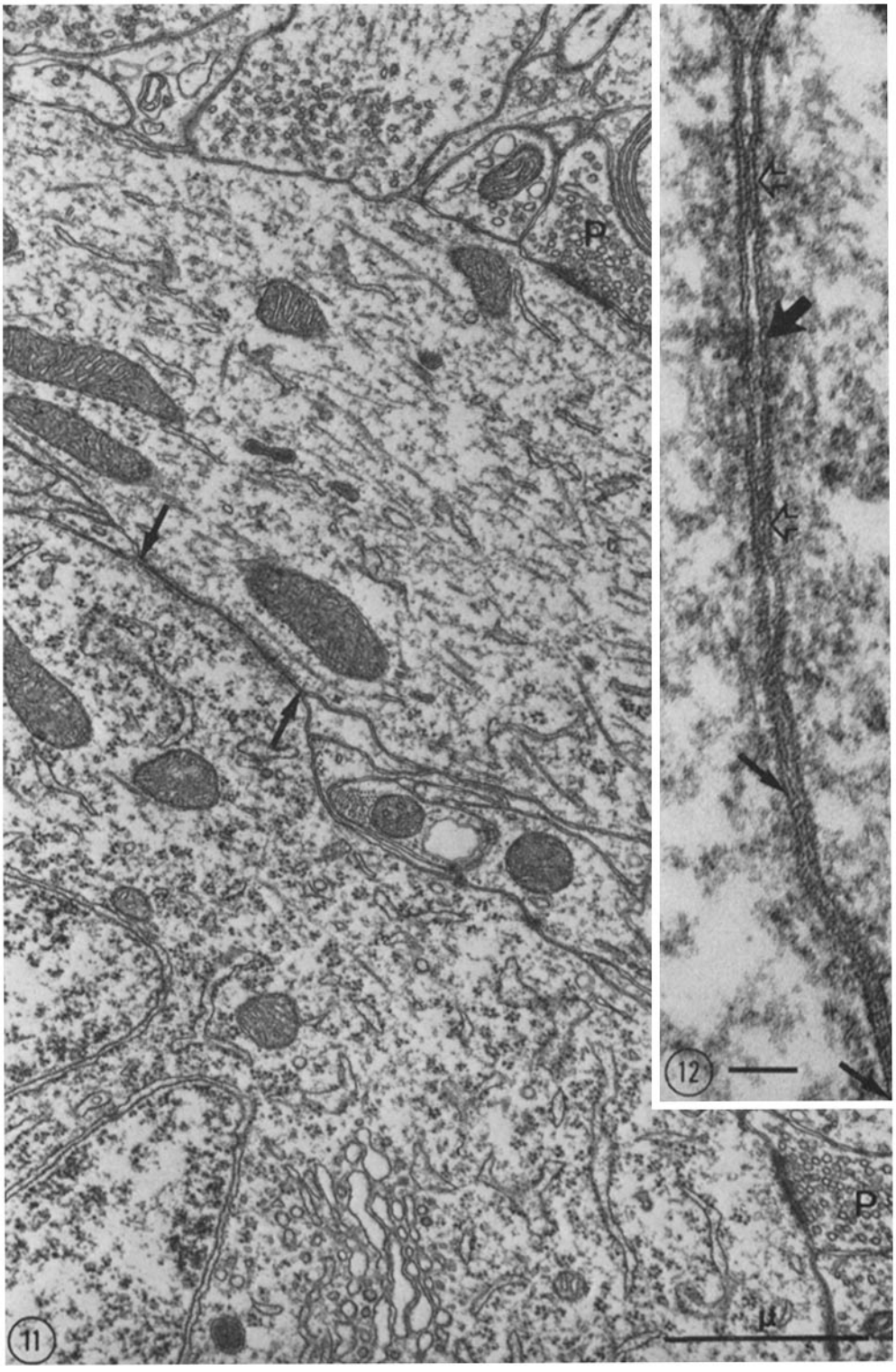


FIGURE 11 Direct apposition between a basket cell body and probably a Golgi cell dendrite (arrows). Axon terminals (*P*) belonging to parallel fibers are in synaptic contact with both neuronal profiles. Deep region of the molecular layer of the cat. Scale, 1 μm. $\times$ 30,000.

FIGURE 12 Enlargement of the zone of direct apposition between the basket cell body and the Golgi cell dendrite illustrated in Fig. 11. This zone is formed by a large gap junction (small arrows) and two small gap junctions (open arrows) separated by an attachment plate (large arrow). Scale, 0.05 μm. $\times$ 200,000.

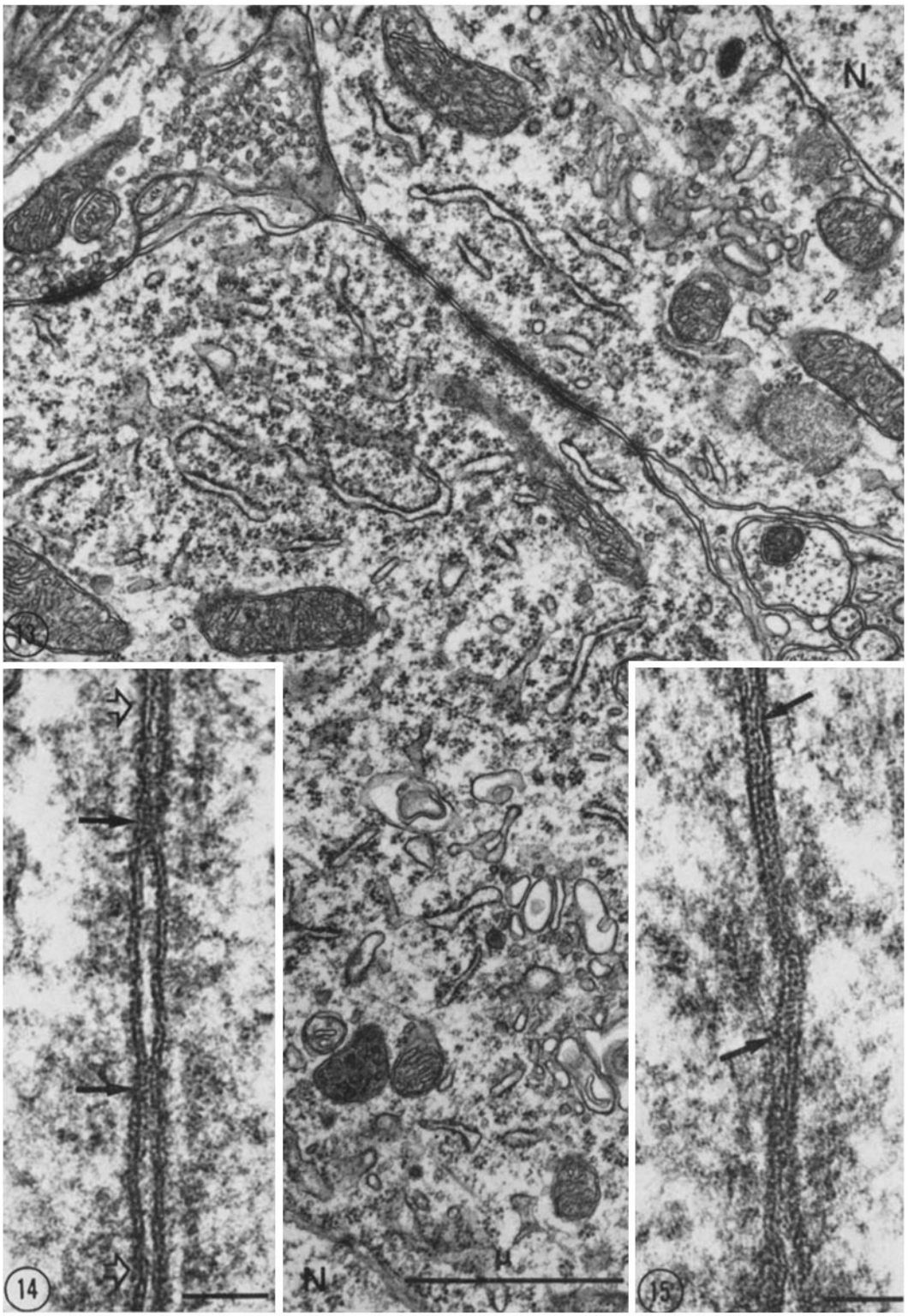


FIGURE 13 Direct apposition between two basket cell bodies exhibiting a series of junctional zones or dense patches. The nuclei (*N*) of both neurons are present in the section. Deep region of the molecular layer of the cat. Scale, 1 μm. × 33,000.

FIGURE 14 Enlargement of part of the direct apposition between the two basket cell bodies illustrated in Fig. 13. Small gap junctions (solid arrows) and intermediary zones (open arrows) can be observed. Scale, 0.05 μm. × 260,000.

FIGURE 15 Semiserial section of the same direct apposition between two basket cell bodies illustrated in Figs. 13 and 14. At this level a large zone of this direct apposition is occupied by a gap junction (arrows). Scale, 0.05 μm. × 260,000.

can be drawn between the SJs and those described here. The electron-opaque densities are not so prismatic as the septa and they often resemble a Greek cross (Fig. 17), giving the impression that the intercellular cleft is bisected by a medial discontinuous line.

DISCUSSION

Two features characterize the GJs. (*a*) The apposed plasma membranes are not really fused, but there is actually a narrow gap 15–20 A wide. This gap can be visualized by the use of aqueous uranyl acetate solution to stain small blocks of the tissue before the dehydration and embedding (22), or by the use of electron-opaque tracer such as lanthanum or peroxidase (5). In the first case (the one employed in the present paper), the gap is visualized as a hollow structure (Figs. 2, 4, 12, and 15), whereas in the second case the gap is penetrated and in perpendicular sections is uniformly filled with the tracer. (*b*) The gap is not an empty space but is occupied by an intermediate lamina of prisms or subunits closely packed in hexagonal pattern (38, 39). This lamina can be visualized in oblique sections (Fig. 9) as a scalariform formation, where the transverse dense lines repeat at a period of 85–90 A. In a frontal view, and in material fixed with $KMnO_4$ or treated with lanthanum as a tracer, the gap exhibits a honeycomb pattern, composed of a system of lines disposed in a hexagonal network. In the center of each hexagon there is a small electron-opaque core about 10 A in diameter which seems to be penetrated by the lanthanum. In the material presented here it has not been possible to observe tangential sections through the junction in the $KMnO_4$-fixed material, but such frontal views have been found in uranyl-pretreated material; in these instances the hexagonal pattern can be recognized but with a more granular and confused aspect. It is of consequence, however, that both features of the GJs have been observed in the present material, since this allows the exclusion of possible nonspecific artefacts and confirms the true nature of these junctions. According to the work of Bennett and Pappas and their collaborators (32, 34) on the lateral giant fiber of the crayfish, it seems evident that the GJ provides intercellular channels for the transeptal passage of tracers such as the dye Procion yellow. For these authors the intercytoplasmic channels could be localized in the center of the hexagons, corresponding to the central electron-opaque spot visualized in $KMnO_4$-fixed material and in lanthanum-treated preparations. Thus, the most characteristic and important feature of the GJs is the existence of an intermediate lamina of subunits with its hexagonal pattern. The sides of the hexagons represent channels in continuity with the extracellular space, and the central electron-opaque spots represent the intercellular channels. The GJs described here demonstrate all of the above, which allows the suggestion that such sites may be regarded as possible low-resistance junctions.

In spite of the large number of ultrastructural studies already published on the vertebrate cerebellar cortex, GJs between neural elements have been only briefly described in a mormyrid fish (21) and in frogs (43). The present findings are the first to relate to the existence of such junctions in the mammalian cerebellar cortex. GJs between mossy terminals and dendrites of granule cells seem to be a peculiarity of nonmammalian cerebellar cortex, since they have been observed in fish, frogs, and chicks. In the last instance they are also frequently found exhibiting a symmetrical cytoplasmic differentiation and are located at the same synaptic interface as the type I active zones (Mugnaini, personal communication).

GJs with an asymmetrical distribution of the cytoplasmic differentiation similar to those described here in the gymnotid-fish cerebellum have already been observed in spinal motoneurons from the swim bladder nucleus of a toadfish (Fig. 5 in reference 33). It is not known whether this asymmetry may underlie a functional characteristic (for instance, rectification). Since rectification is a property of plasma membranes (11), the asymmetry of the cytoplasmic differentiation is not sufficient to explain the asymmetrical current flow in this junction. It is, however, striking that most of the GJs in gymnotid fish are asymmetrical (Figs. 1, 2, 4, and 5), although some are symmetrical (Fig. 3).

In nonmammalian species the junctions between mossy fiber terminals and granule cell dendrites correspond to what has been described as "mixed synapses" where, at the same synaptic interface, adjacent junctions having the morphological features of "chemical" and "electrical" synapses coexist.[2] In these instances, as for those of the lateral

[2] It is important to emphasize, however, that mixed synaptic transmission is an electrophysiological rarity, some of the few examples cited including that of the chick ciliary ganglion (28) and the sea lamprey brain stem (41).

vestibular nucleus of the rat (46), the surface area occupied by the active zones was much larger than that occupied by the GJs. This situation has already been the subject of speculation (44, 46, 47). It has in fact been proposed that such combined junctions, the mixed synapses, underlie a more subtle and refined kind of neuronal interaction than the simple electrotonic coupling.

In the mammalian cerebellum the situation is simpler, since the GJs are only localized between dendrites, cell bodies, or dendrites and cell bodies, and in none of these cases have synaptic complexes or active zones been encountered. Besides the GJ, the only kinds of junction that can be present at the same interface are the attachment plaques and the intermediary junctions. Contacts of these kinds are regarded as adhesive, without any functional role in the nerve cell interaction. In these instances the GJs—morphological correlates of low-resistance junctions—link the two types of inhibitory interneurons present in the cerebellar cortex: the stellate or basket cells and the Golgi cells.

From a morphological viewpoint, it can be concluded that the inhibitory interneurons are coupled by dendro-somatic, dendro-dendritic, and somato-somatic junctions. Electrophysiological studies in other regions of the CNS where these types of junctions exist between similar neural elements (33) indicate that these junctions may have the functional role of synchronization of firing. From a purely speculative point of view, it may be supposed that the presence of such GJs may serve to activate certain groups of inhibitory neurons as closely knit groups. Such group activation can be envisaged as generating patterns of neuronal activity under certain conditions. Although pattern-sensitive properties have been suggested for the complex behavior of Golgi cell inhibition (36), it is probable that the quasi-synchronous activation of neuronal ensembles provided by the GJ may be of considerably more importance in the organization of neuronal nets in the cerebellum.

A rather different view must, however, be expressed regarding the SJs. Although some investigators regard the SJ encountered in invertebrate epithelia as the morphological correlate of a low-resistance junction, this view may be opened for discussion at this time. For instance, Loewenstein and Kanno (27) demonstrated electrophysiologically the presence of electrotonic coupling by means of low-resistance junctions between cells in the salivary gland epithelium of *Drosophila*. These authors originally assumed that the SJs, which are frequently observed between these cells, were in fact the sites of electrotonic contact. Similarly the recent work of Gilula et al. (13) on the gills of freshwater mussels supports this view. Thus, morphological evidence gathered by means of electron microscopy, freeze-etching, and optical diffraction techniques suggests that the SJs provide, at the level of the septal sheet, an intercytoplasmic channel considered to be the structural basis for intercellular communication. In spite of these results, the correlation between SJ and low-resistance

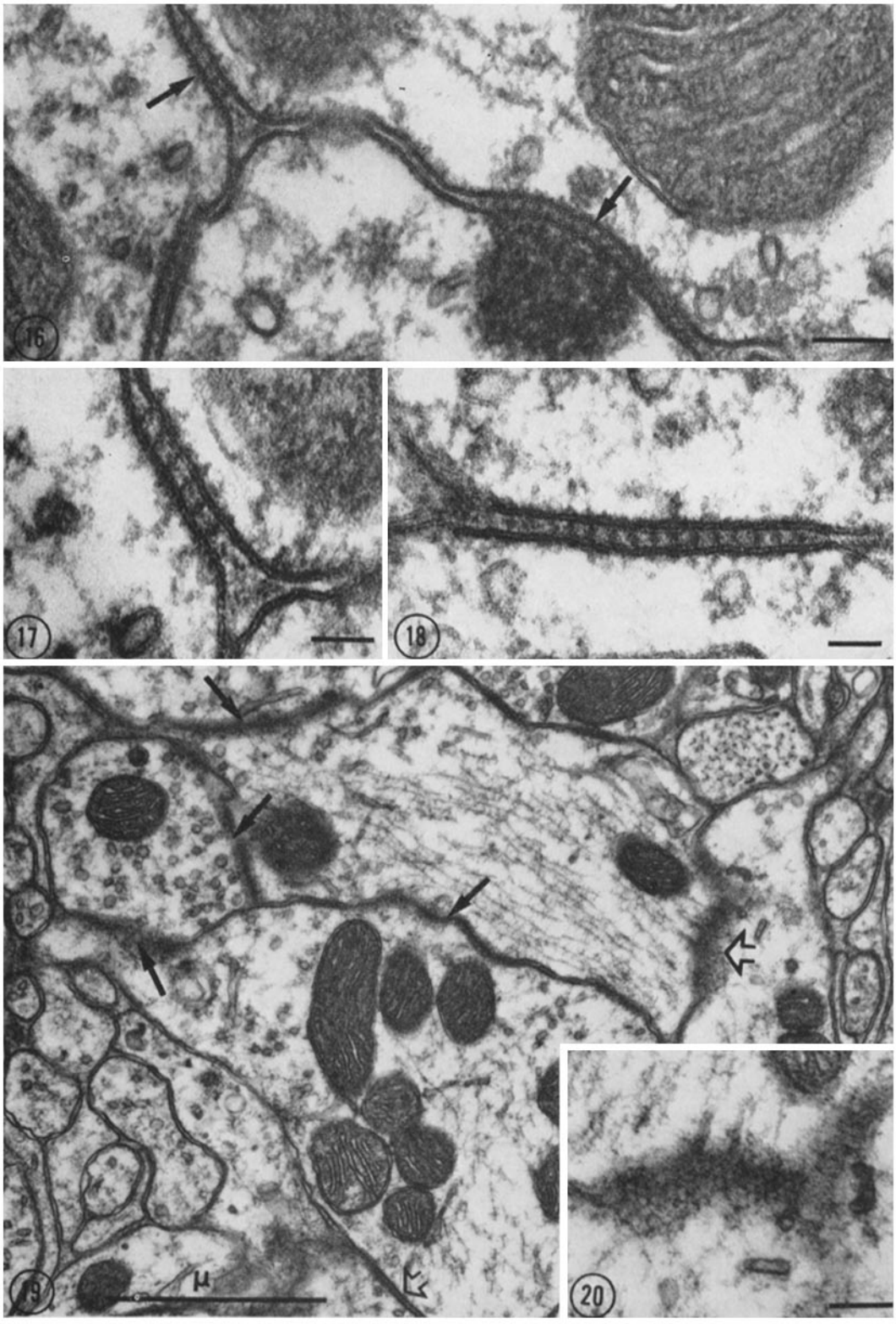
FIGURE 16 Junctions between basket axonal profiles at the tip of the brush surrounding the initial segment of the Purkinje cell axon. At the junctional regions (arrows) the extracellular space, conserving its normal width, is transected by dense cross-bridges perpendicular to the axonal membrane. Cat. Scale, 0.1 μm. × 126,000.

FIGURE 17 Enlargement of one of the junctions illustrated in Fig. 16. At this magnification it can be observed that the electron-opaque material which bridges the extracellular space is not prismatic, but has a Greek-cross shape. Scale, 0.05 μm. × 200,000.

FIGURE 18 Cross-section of another junction between two basket axonal profiles. Note that the electron-opaque material bridges completely the extracellular space, making contact with the outer leaflets of the axonal unit membranes. Cat. Scale, 0.05 μm. × 160,000.

FIGURE 19 This electron micrograph illustrates cross-section (small open arrow), oblique sections (solid arrows), and a tangential section (large open arrow) of the junctions between basket axonal profiles. Cat. Scale, 1 μm. × 35,000.

FIGURE 20 Enlargement of the face view of the junction between basket axons illustrated in Fig. 19. The junction is made up of closely packed polygons, giving to it a honeycomb appearance. Scale, 0.1 μm. × 100,000.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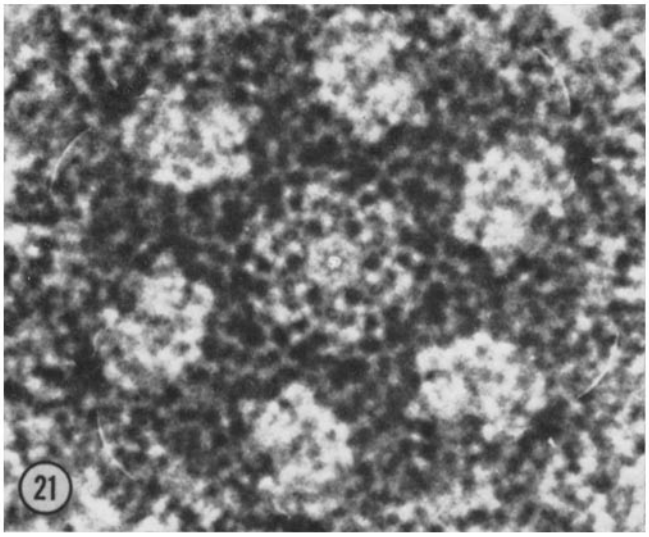

FIGURE 21 Photograph obtained with the Markham rotation technique of the septate-like junction illustrated in Fig. 20. Six axes of symmetry are visible, indicating that the subunits are assembled in a hexagonal pattern. × 2,000,000.

junction is not universally accepted. Furshpan and Potter (11), in their review on low-resistance junctions, mention the unpublished results of C. M. Phillips who has observed, besides the SJs, numerous regions of close membrane apposition between insect salivary gland cells. Similar observations have been made by B. Filshie and D. Smith (reported by Satir and Gilula [42]) on insect epithelial cells where GJs and SJs coexist along the same cellular interface. Recent publications (17, 20, 40), describing intercellular junctions between invertebrate epithelial cells after experiments utilizing lanthanum as a tracer of the extracellular space, also strengthen the postulate that SJs and GJs occur between these epithelial cells. In these instances it is not clear whether the sites of intercellular communication correspond to the SJs or to the macula of GJs.

SJs appear to be a feature of invertebrate epithelial cells, although occasionally these junctions have been reported in vertebrates. Trinkaus and Lentz (49) described them between the enveloping layer cells of a midgastrula in the *Fundulus* embryo, and Barros and Franklin (3) between the sperm and a fertilized egg of the golden hamster. In the first case, the width of the intercellular cleft and the periodicity of the dense bars resemble more those of a GJ than those of an SJ. In the second case, even though the electron micrograph illustrating this junction has too low a magnification to allow its complete description, the junction appears more like an SJ. In this paper, junctions resembling SJs are described for the first time between neuronal elements.[3]

The peculiar junction described here between basket axonal profiles at the tip of the brush surrounding the initial segment of the Purkinje cell axon cannot be considered as a typical SJ, although it exhibits some features of such a junction. For instance: (*a*) the electron-opaque bars cross-bridge completely the intercellular cleft, making contact with the outer leaflets of the axonal membranes (Figs. 17 and 18); (*b*) the electron-opaque bars are regularly spaced, and they are less electron opaque than the dark layers of the axonal membranes (Figs. 16–18); (*c*) in tangential sections of the junctions, polygonal arrays with a honeycomb appearance can be observed (Figs 19 and 20) resembling, for example, the face view of an SJ in the salivary gland epithelium of *Drosophila* (Fig. 9 in reference 51). However, the dense bridges are not as prismatic as in true SJs, being described here as Greek-cross shaped. Further morphological

[3] An article which appeared after submission of this paper for publication (Gobel, S. 1971. *J. Cell Biol.* **51**:320) describes similar SJs between basket axons in the cat cerebellar cortex.

studies, using lanthanum in the fixative in order to permeate the spaces between the dense bars and thus to allow a better analysis of the junction, are planned to achieve a better comparative study of these junctions and the complex structures composing the SJs. It may be suggested that the junctions present between basket axons in the mammalian cerebellum are modified forms of SJs since it seems well established that these junctions can exhibit morphological differences (7).

According to the quantitative Golgi analysis made by Szentágothai (48) in cat cerebellar cortex, there is a considerable convergence of basket axons, arising from different neurons, upon one single Purkinje cell. Szentágothai reached the conclusion that the complete basket formation surrounding one Purkinje cell body is formed by axonal branches arising from at least 50 different basket cells. It seems reasonable to suppose that a great majority of the basket axonal profiles observed at the tip of the basket brush in electron microscope sections belong to different basket cell bodies. It may be postulated that most of the junctions resembling SJs link basket fibers of different neuronal origins. In spite of the fact that there is no definitive proof of the functional meaning of SJs, and although the junctions described between basket axons do not have all the features of SJs, their frequent and constant presence in cat and rat cerebellar cortices does imply a definite functional role. Such a hypothetical role may be related to two different classes of functions: (*a*) electrotonic-like interaction much as is envisaged to occur between basket cell perikarya or basket cell dendrites, or (*b*) extracellular compartmentalization. The latter possibility assumes that the septate-like junction may function as a highly resistive element channeling extracellular current flow, thus serving as a means of generating a highly anisotropic volume conduction. In the case of the basket cell, such compartmentalization may serve to distribute the outward terminal action currents of the basket cell end feet as a vertical sleeve around the Purkinje cell axon. It would thus function as an anodal blocking device similar to that subserved by the axon cap in the electrical inhibitory system of the teleostean Mauthner cell (12).

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