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Ultrastructural Study of the Synaptic Glomeruli in the Substantia Gelatinosa of the Rat Spinal Cord

(substantia gelatinosa/pain)

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The substantia gelatinosa contains a number of mechanisms of particular interest to neurobiologists. With the introduction of new techniques, new data are being acquired, however, as the ultrastructural investigations have been few, we carried out such studies on the synaptic glomeruli in the substantia gelatinosa from the sagittal, frontal, and horizontal planes. The sagittal plane proved to be the most suitable for the observation of synaptic glomeruli.

A glomerulus is a network of nerves or capillaries. When used alone, the term refers to a tuft of capillaries at the beginning of each uriniferous tubule in the kidney (1). The glomerulus is composed of a capillary network lined by endothelial cells, a central region of mesangial cells with their surrounding matrix material, the parietal layer of Bowman's capsule with its basement membrane, and the visceral layer of Bowman's epithelium and its associated basement membrane (2). In the nervous system, the acronym glomerulus is given to indicate a complicated ball-like formation of neuropils that are visible to the naked eye or with slight magnification in the superficial layers of the olfactory bulb (3). The meaning of the term "glomerulus" has more recently been extended to encompass other closely related groups of neuronal processes not recognizable under the light microscopy (3). The synaptic glomerulus is a synaptic complex consisting of a large central synaptic bouton filled with numerous synaptic vesicles and mitochondria around a core of neurofilaments and microtubules, a number of small and medium sized dendrites and dendritic spine heads, and axon terminal boutons (4).

The synaptic glomeruli scattered throughout the densely packed neuropil of the substantia gelatinosa are characterized by a peculiar axoplasmic electron density of the large central synaptic bouton (5). Using the electron microscope, the acid phosphatase (AcPase) activity, a marker enzyme for lysosomes, was demonstrated mainly on the plasma membrane of a large central synaptic bouton with an electron dense axoplasm (6). In 1979, Inomata and Ogawa attempted to demonstrate thiamine monophosphatase (TMPase) activity using thiamine monophosphoric acid chloride, another phosphomonoester, as a substrate (7). The intense TMPase activity in the plasma membrane of synaptic glomeruli coincided with findings related to AcPase activity. We reported that the AcPase activity reflected that of TMPase. Such findings are of

considerable interest to researchers studying the functional role of the synaptic glomeruli, however, ultrastructural investigations concerning the synaptic glomeruli in the substantia gelatinosa have not adequately elucidated the problem. In the present investigation, the ultrastructures of synaptic glomeruli in the substantia gelatinosa were demonstrated from the sagittal, frontal, and horizontal planes.

MATERIALS AND METHODS

Male Wistar rats weighing 250 to 350 g were anesthetized with Nembutal (0.1 ml/100g i. p.). The spinal cord tissues were fixed with saline solution by vascular perfusion via the left ventricle, at room temperature for 30 sec followed by 1 % glutaraldehyde (GLA) and 2 % paraformaldehyde (FA) in 0.1 M phosphate buffer, pH 7.3, 900 mOsm at room temperature. After perfusion for 30 min, the spinal cord, at the lumbar level, was removed and immersed in the same fixative for several hours at room temperature. For the purpose of selection of substantia gelatinosa, the spinal cord was sectioned with a Vibratome at approx. 300 μm (8, 9). The sections were rinsed for several hours with several changes of cold 0.1 M phosphate buffer, pH 7.3, containing 2.5 % sucrose, 290 mOsm. After rinsing, the sections were osmicated in cold 1 % osmium tetroxide in Caufield's buffer, dehydrated in graded concentrations of ethanol, treated with propylene oxide and embedded in Spurr's medium (Polyscience) (10). Thin sections prepared using a Porter-Blum MT-2B ultramicrotome were poststained with uranyl and lead citrate and examined under a HS-9 (Hitachi) electron microscope at 75 kV.

RESULTS

It was difficult to discern the outline of substantia gelatinosa, except in the Nissl preparations. According to Ralston (4), the lamina II of Rexed (11) superimposed unstained with osmium in Araldite-embedded tissue. Also in Spurr's medium-embedded tissue, the same finding was obtained. Hence, it was easy to obtain sections of substantia gelatinosa for electron microscopy.

The present investigations were focused on the ultrastructural aspects of peculiar synaptic glomeruli. The criteria for identification of such glomeruli in the substantia gelatinosa of the rat spinal cord have been discussed by several authors. According to Rethelyi and Szentagothai (5), the central terminals of the glomeruli are dark, of a sinusoid structure, and the central terminals are presynaptic to all of the surrounding neural profiles which have been identified as small afferent terminals, small dendrites and dendritic spines. Along the lines of such characteristics of peculiar synaptic glomeruli, the ultrastructural criteria in Table I were prepared.

TABLE I. *Morphological Criteria for the Glomerulus of the Substantia Gelatinosa*

1. Central large axon
High electron density in the cytoplasm.
Synaptic vesicle-filled profile with sphenoid-shape.
Dense core vesicle.
Synaptic formation with surrounding
a. axon
b. dendrite or dendritic spine head.
2. Surrounding process
Ordinary electron density in the cytoplasm of axon and dendrite.
Synaptic vesicle with a flat-shape.
Dense core vesicle.

Sagittal Plane (Figs. 1—4)

The synaptic glomeruli in the substantia gelatinosa are characterized by a peculiar electron density of the central axon terminals. All the synaptic glomeruli observed in the sagittal plane were composed of central axon terminals with a high electron density and the surrounding neural profile by the usual electron density. The central axon terminal contained the synaptic vesicle and the mitochondria. In particular, the synaptic vesicles showed at tendency toward aggregation. Synaptic formations between the central terminals and the surrounding axon or dendrite were evident. In many cases, synaptic vesicles in the central axon terminals clustered against the synaptic membrane. Upon the axo-axonic synapse, the surrounding axon with or without the cluster of the synaptic vesicles against synaptic membrane were intermingled. The dense core vesicle was mainly observed in the dense sinusoid axon terminal (DSA), composing of central part in the synaptic complex. These vesicles randomly appeared within the synaptic vesicles, packed the axoplasm of the DSA, and were also found around the DSA. The morphology of the synaptic vesicle in the DSA was spheroid, while the synaptic vesicle in the surrounding axon was flattened.

Frontal Plane (Fig. 5)

The tendency torward aggregation of the synaptic vesicles is one of the characteristics of the synaptic glomeruli of the substantia gelatinosa. However, axoplasma of DSA with or without a high electron density were intermingled.

With synaptic formation, the vesicles in the axoplasm of the surrounding axon showed a tendency toward clustering against the synaptic membrane under the axo-axonic synapse. The synaptic vesicles in the DSA were spherical, while the synaptic vesicles in the surrounding axon were more or less flat.

Horizontal Plane (Fig. 6)

The central axon terminals with both high and ordinary density were intermingled. With synaptic formation, axo-axonic synapse between central axon terminals and the surrounding terminals, axo-dendritic synapse between synaptic central axon terminal and the surrounding dendrite, and axo-axonic

synapse between both surrounding axon terminals were evident. Dense core vesicles were occasionally detected in the axoplasm.

DISCUSSION

The substantia gelatinosa, laminae II and III of Rexed superimposed (11), in the posterior horn of the rat spinal cord is morphologically and functionally one of the peculiar regions in the central nervous system. The laminae II and III were termed gelatinosal complex by Scheibel (12). In the monkey, the ratio 1 : 2.1 : 2.8 for laminae I, II and III were seen at a vertical distance between the dorsal surface of the dorsal horn (13).

A prominent feature of the neuropils in the substantia gelatinosa is the synaptic glomeruli which are more abundunt in lamina III. These glomeruli consist of a large central bouton with a characteristic electron dense matrix, which is in synaptic contact with a number of small and medium sized dendrites and dendritic spine heads and with other terminal boutons. The complex is surrounded by thin glial lamellae (5, 14−17).

The "scavenger activity" of the glial system in the substantia gelatinosa is extremely rapid (6, 18). Knyihar *et al.* (6) reported that the phenomenon is due to the rich capillary nets in the substantia gelatinosa. When degenerative experiments related to the substantia gelatinosa are done, the inflammatory reaction, due to the presence of rich vascularity, always has to be considered (18).

It would appear from routine examinations that the high electron density of the central axoplasm indicates axon terminal degeneration. However, this peculiar density upon the synaptic glomeruli was as a normal finding. This high electron density may be due to the presence of neurofilaments and to a homogenous electron density materials (6). According to the Cobb and Pentreath (19), neurofilaments and microtubules were associated with electron density. A qualitative analysis is required for a better understanding of the peculiar electron density (20).

There are differences of opinion with regard to the origin of the central axon terminal. Rethelyi and Szentagothai (5) are of the opinion that these terminals originate from the pyramidal cell whose soma lies in the junction between laminae III and IV, while Gobel (14) considered that they originate from primary afferent fibers. In addition, Knyihar (21) thought that the central axon terminal originates from small dark cells of the spinal ganglia via thin dorsal root fibers. In 1980, the ultracytochemical demonstration of the TMPase activity from the dorsal root to the dorsal root ganglion neuron was performed by our group (22). The reaction products for TMPase activity were found to be localized in the axolemma of the dorsal root and the Golgi saccules and the reticular part of the Golgi apparatus of the dorsal root ganglion neuron, a small and dark neuron containing the well-developed Golgi apparatus. Hence, it was suggested that TMPase activity was transported from the small dorsal root ganglion neuron to the plasma membrane of the

central axon terminal in the synaptic glomeruli (22). The central axon terminal, DSA, probably originates from the dorsal root ganglion neuron, small and dark neuron, via the doral root.

The central axon terminals reportedly contain the dense core vesicle (23). It is generally considered that this vesicle is associated with the transmitter (23). The transmitter is noradrenaline or 5-hydroxytryptamine in case of the dense core vesicle (24—25). At this point, attention must be given the existence of the dense core particles (26). Although the dense core particles are usually associated with astrocytes in human pathological conditions, and large dense core vesicles with neurons in normal mammals, they are morphologically the same. Hence they probably share cellular functions such as intracellualr digestion rather than those related to neurotransmission (27).

The most characteristic of the synaptic glomeruli is the central axon terminal. Second, the central axon terminal makes synaptic contact with the surrounding axon or dendrite. The sagittal plane proved to be suitable for the observation of the above two elements. In a previous series of cytochemical studies (28—32), the same results were obtained using rats.

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LEGENDS

Figs. 1–4. Photos from the sagittal plane.

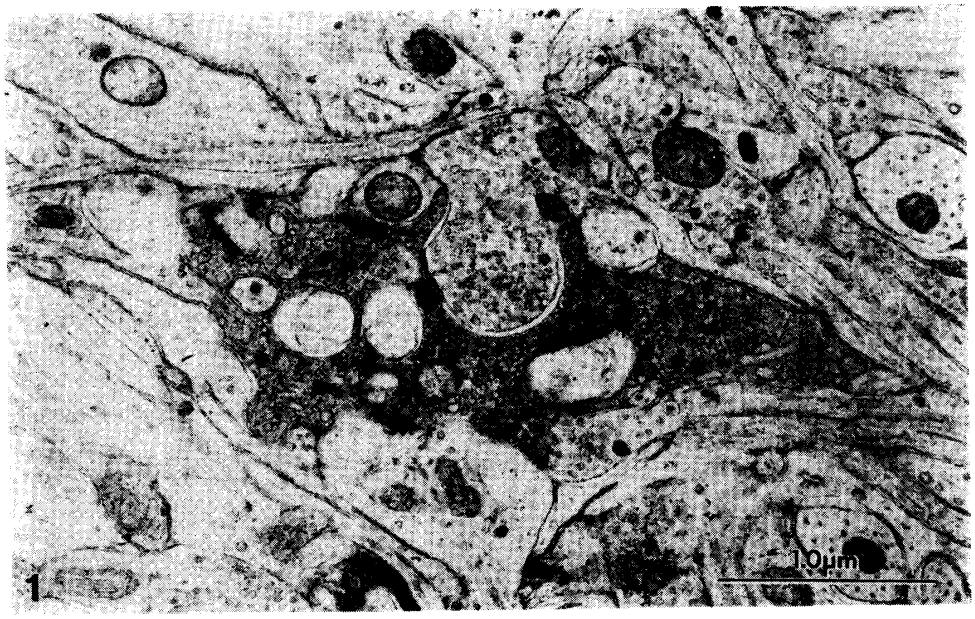
Figs. 1 and 2. Typical synaptic glomeruli. The synaptic glomerulus, a synaptic complex, consists of a dense sinusoid axon terminal filled with numerous synaptic vesicles, dendrites, dendritic spine heads and axons. Note the dense core vesicles.

Fig. 3. Note the number of dense core vesicles in the synaptic glomerulus.

Fig. 4. Axo-dendritic synapses. Synaptic formations between dendrite and axons.

Fig. 5. A photo from the frontal plane. Synaptic glomerulus with ordinary electron dense sinusoid axon terminal.

Fig. 6. A photo from the horizontal plane. Synaptic glomerulus with ordinary electron dense sinusoid axon terminal.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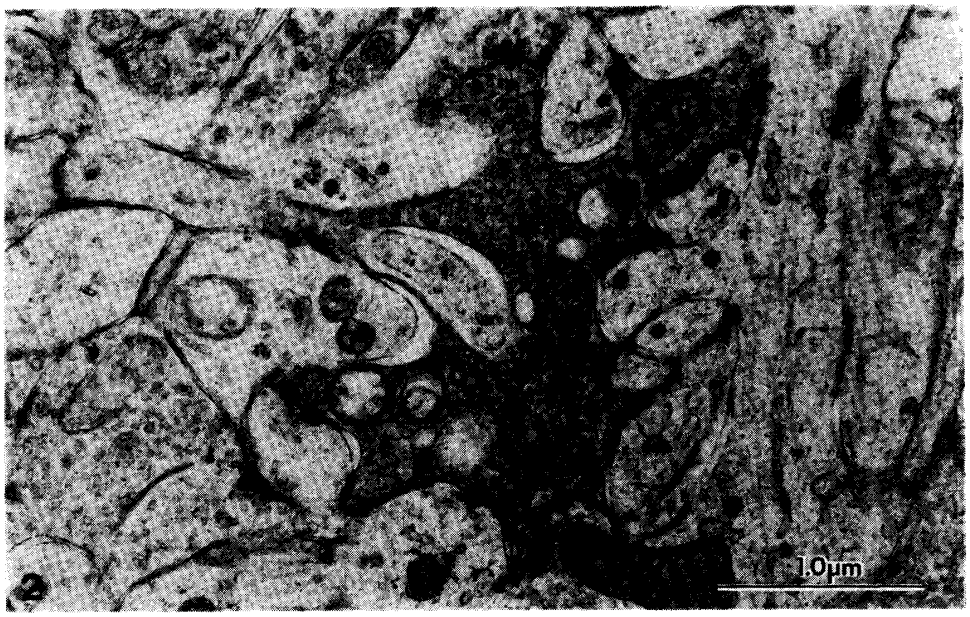
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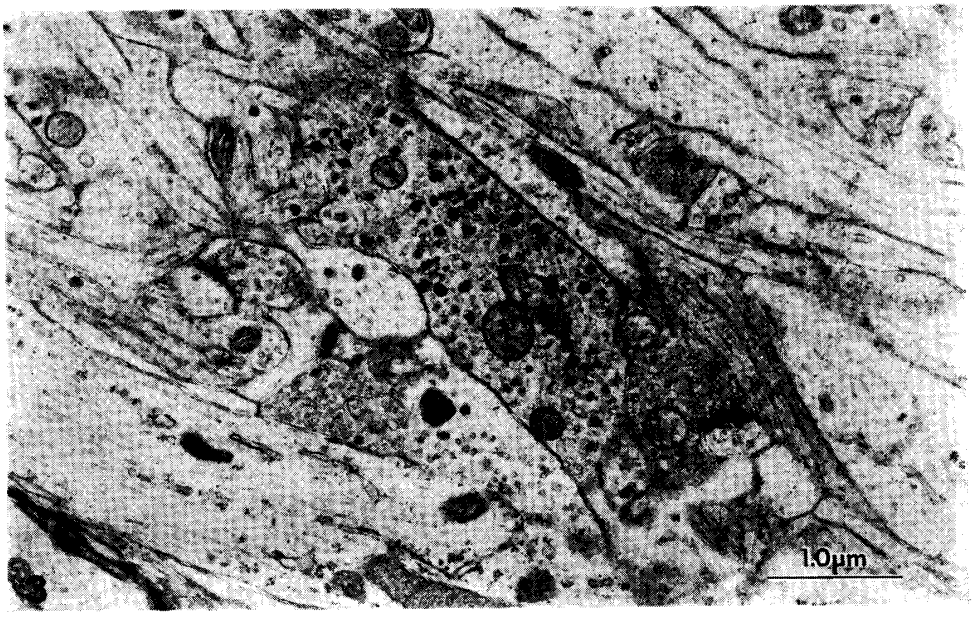
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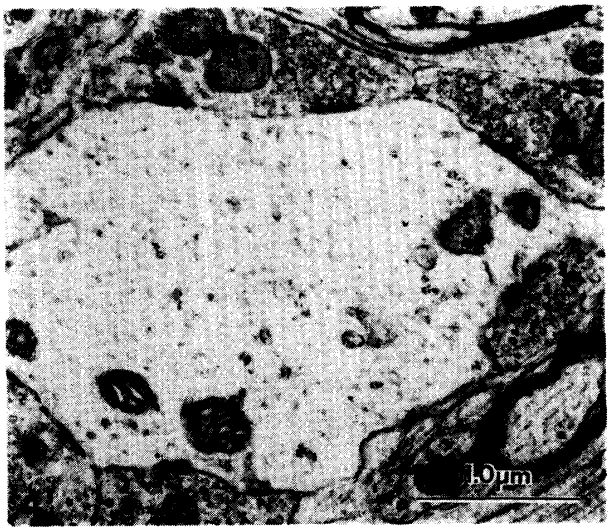
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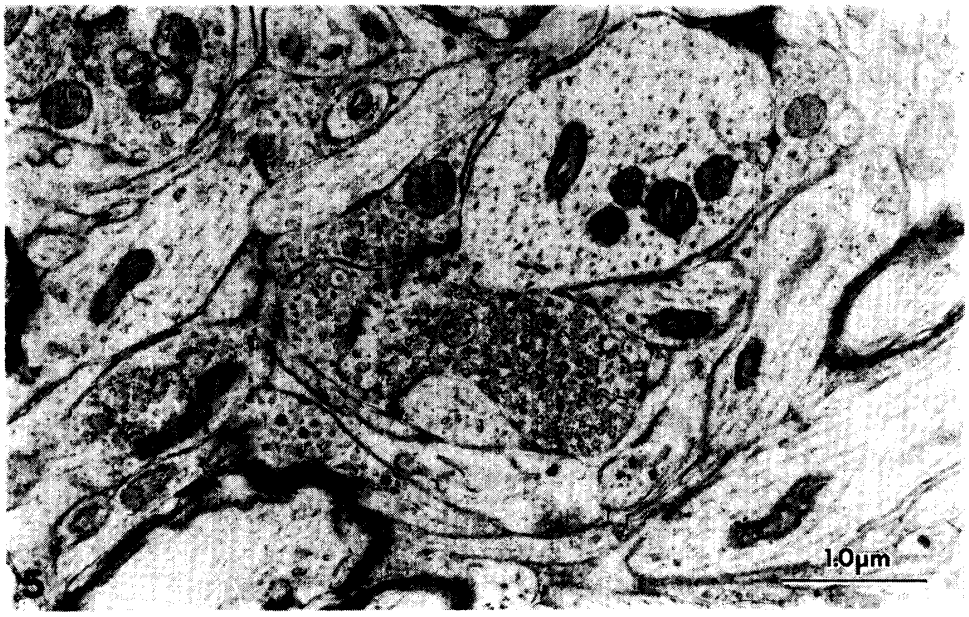
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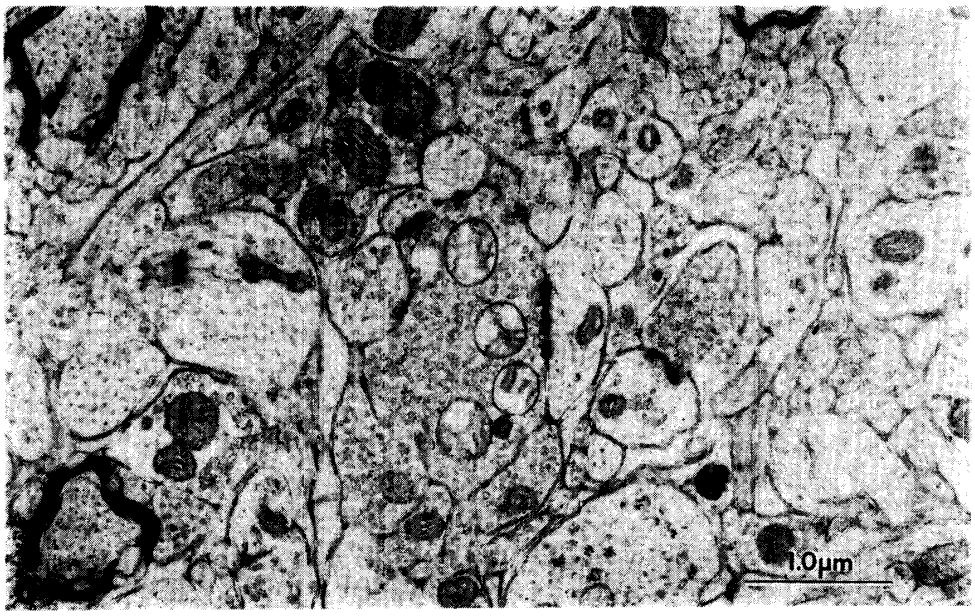
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