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Dendritic spinules in rat nigral neurons revealed by acetylcholinesterase immunocytochemistry and serial sections of the dendritic spine heads

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Abstract: Dendritic spinules of rat nigral neurons were visualized at electron microscopic level by acetylcholinesterase immunocytochemistry and serial sections of the nigral dendrites. The spinules (at least 150 nm in length and 10-20 nm in width) which protruded from the spine heads are found in extracellular space in the neuropil and particularly between nerve terminals of the presynaptic neurons and fine glial processes. The nigral spinules are, however, not observed as invaginated processes in the nerve terminals. The dendritic spinule may be endowed with synaptic plasticity and metabolic exchange between nerve terminals and glial processes.

Key words: Nigral dendrite - Spine head - Spinule - Glial process - Acetylcholinesterase - Electron microscopy

Introduction

Dendritic spinule was described for the first time in the rat hippocampal pyramidal neurons as fine processes which protruded from the dendritic spine head and invaginated into the presynaptic terminals [16]. Membrane of the dendritic spinules was, however, devoid of the postsynaptic density (PSD) and thus not formed a synapse with the presynaptic nerve terminals. Because of the reduced dimension (75-150 nm in length and 25-100 nm in width), the dendritic spinules escape light microscopic observation and can be visualized only under the electron microscope. Organelles are not found inside the spinule.

Because of this restricted dimension and absence of the organelles, the spinules were difficult to be distinguished from apposed fine glial processes which presented similar morphology. Thus, serial sections of histologically labeled neurons or glial cells [3] were necessary for distinction of the two fine processes which were originally different.

It is known that rat nigral dopaminergic neurons are endowed with intense acetylcholinesterase (AChE) activity [13]. When we examined AChE-positive nigral spine heads under the electron microscope [1], numerous AChE-positive fine processes were observed, though we had not been able to establish their connection to the dendritic spine heads.

In the present study, after AChE immunocytochemistry and observation of serial sections of the nigral dendrites, we confirmed structural continuity between an AChE-positive spine head and AChE-positive fine processes. Continuity with the spine head, small dimensions, and absence of organelles inside the structure allow these AChE-positive fine processes to be considered as spinules of the nigral neurons.

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Materials and methods

Animals and tissue preparation. Research protocols were approved by the Animal Care and Use Committee of Riken and Pierre et Marie Curie University. Wistar rats of both sexes, weighing about 250 g were deeply anesthetized by pentobarbital and fixed through intracardiac perfusion with a fixative containing 4% paraformaldehyde, 0.25% glutaraldehyde, and 0.1% tannic acid in 0.1 M phosphate buffer, pH 7.2. The brains were dissected and 50 μm coronal sections of the region including pars compacta of the substantia nigra were obtained by Vibratome and kept in phosphate buffered saline (PBS: 0.01 M phosphate buffer, pH 7.2 containing 0.9% NaCl).

Immuncytochemistry of AChE. The sections were incubated at 4˚C for 12 h with a polyclonal rabbit antiserum raised against rat brain hydrophobic AChE [11] at the dilution of 1:500 in PBS, pH 7.2, containing 1% milk. After washing in the same PBS solution, the sections were incubated with a biotinylated antibody against rabbit IgG (Vector, Burlingame, CA, USA), 1:100 in PBS containing 1% milk for 2 h at room temperature, washed with PBS, and immersed in the avidin-biotin-peroxidase complex (Vector), 1:100 in PBS for 1 h at room temperature. Then, a diaminobenzidine tetrahydrochloride (DAB)-hydogen peroxide reaction was performed in PBS for 20 min. After washing in PBS, sections were postfixed with 2% osmium tetroxide in 0.1 M phosphate buffer for 30 min at room temperature, dehydrated with a graded ethanol series, and embedded in Araldite (Fluka, Switzerland). Control of the immunocytochemical reaction was performed by replacing the primary specific antiserum with normal rabbit serum. In this case no staining was observed.

Electron microscopy. Both serial and non-serial ultrathin sections were obtained from selected regions of the *substatia nigra pars compacta* where most dendrites come from the somata of dopaminergic neurons. Serial ultrathin sections were mounted on large hole nickel grids with a formvar membrane, while non-serial ultrathin sections were collected on 200 mesh nickel grids without supporting membrane. The observation of the sections was done without counterstaining under JEOL 100 CX electron microscope at 80 kV.

Results

Figure 1 presents a rectangular spine head. Three fine AChE-positive processes are continuous with the AChE-positive spine head labeled with DAB precipitate after the immunoperoxidase reaction. The length of the fine processes is about 500 nm and the width 50 nm. Because of the restricted dimensions, no dendritic organelles could be found inside the fine processes.

The process (1) is flanked by the nerve terminal and a bundle of neurites, while the other two processes (2, 3) are in contact with glial processes which are also devoid of organelles but show no AChE immunoreactivity.

Figure 2 shows a part of AChE-positive dendritic spine head in three serial sections. A fine AChE-positive process protrudes from the spine head. The tip of the process can be seen in its longest section (Fig. 2b).

In Figure 3, another example of AChE-positive spine head and fine process is shown in four serial sections. The dendritic spine head is here close to a blood capillary and the fine process is flanked by the capillary wall and a bundle of neurites. The entire length of the process is shown in Figure 3c. The present observations of serial sections revealed that the fine processes of dendritic spine heads are not plate-like but rather tube-like structures. On the basis of their size and ultrastructural appearance (absence of organelles and continuity with the spine head) we postulate that these processes are spinules of the nigral neurons.

Discussion

During our ultrastructural study on the dendritic release of AChE [1], we observed numerous fine processes labeled either cytochemically for AChE activity or immunocytochemically for AChE immunoreactivity. These processes were found around nerve terminals and in a narrow space between nerve terminal and dendrite. Without careful observation, they could have been taken erroneously as 'synaptic' AChE. However, a close observation revealed that the staining was limited to extremely fine cytoplasmic processes.

We were unable to distinguish these processes from glial processes. However, when we observed that they also revealed tyrosine hydroxylase immunoreactivity (unpublished data), we thought that these processes were neuronal in nature. Comparing our observations with the description on the spinules, fine processes projecting from the spine heads of hippocampal pyramidal neurons, we thought that the processes in our material could be homologous structures. The size of the spinules of rat *substantia nigra* is comparable to that of spinules observed in hippocampal pyramidal cells [16]. In the hippocampal dentate gyrus, Tarrant and Routtenberg [15] described 'synaptic spinules', which were characterised by ultrastructure comparable to that of the spinules demonstrated by Westrum and Blackstad [16] and penetrated into the nerve terminal. According to our observations, the nigral spinules were not found inside a nerve terminal, in contrast to the hippocampal neurons where spinules penetrated into the nerve terminals. In the present study, we confirmed the tubular structure of the spinules by means of serial sections and obtained an electron micrograph showing, in one section, connection between dendritic spine head and three spinules, all of them immunostained for AChE.

The role of the spinules is controvesial. In the hippocampal pyramidal cell, the spinules penetrated into the nerve terminals but apparently did not split them [14]. The hippocampal spinules which penetrated into the nerve terminals, were apparently not in contact with glial processes, while the spinules in the *substantia nigra* were located around the surface of the presynaptic nerve terminals and in close apposition with glial processes.

If the dendritic spine heads move [4, 5, 8, 9, 12] and are sensitive sites of synaptic plasticity [6, 7, 10], the dendritic spinules might be more or less endowed with such functions. In addition, metabolic exchange with glial processes [2] could be performed by the spinules in the *substantia nigra*.

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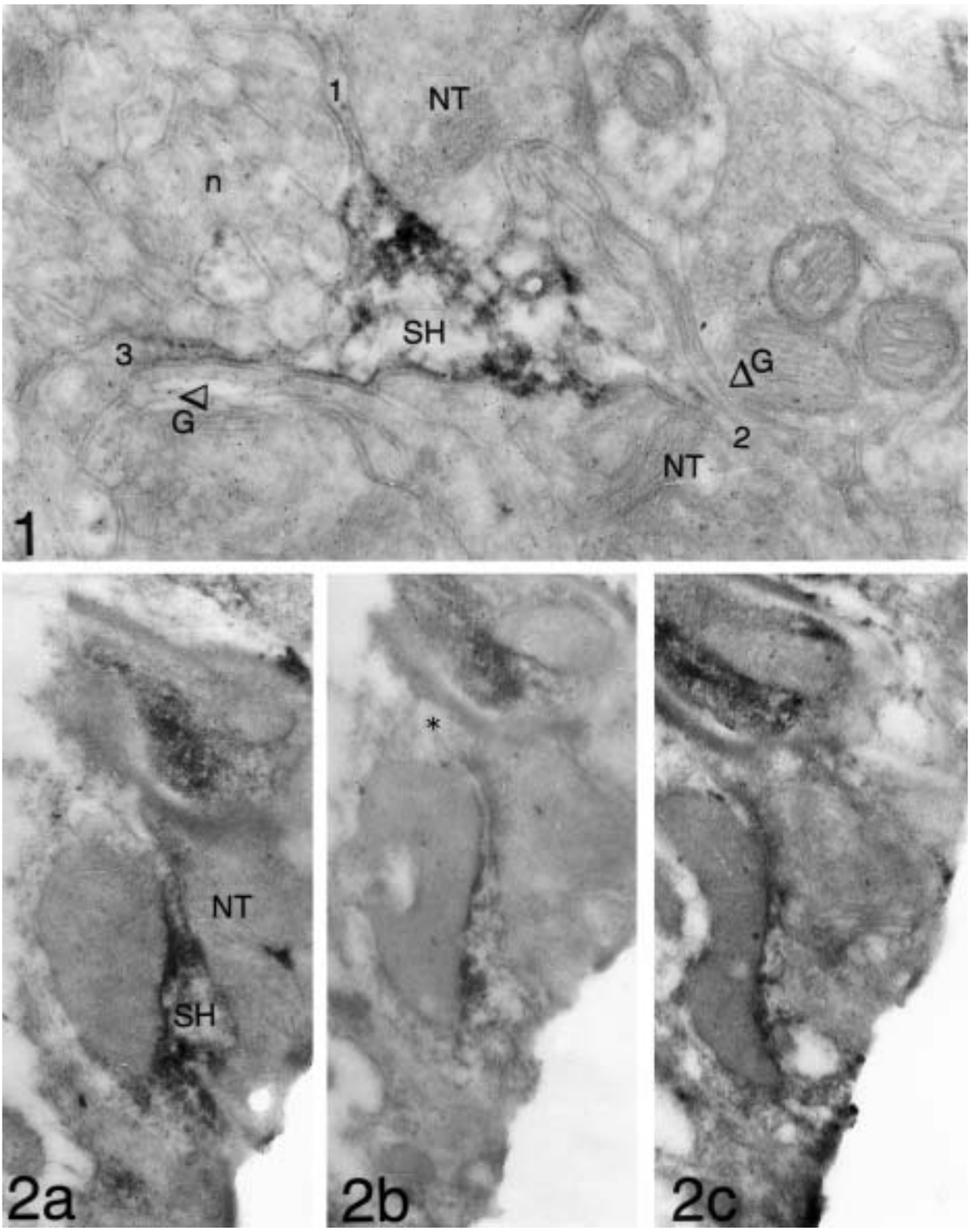

Fig. 1. Dendritic spine head (SH) of rat substantia nigra revealed by AChE immunocytochemistry. The spine head seems to be continuous with three fine processes (1, 2, 3), which are also AChE-positive. Process (1) is flanked by a nerve terminal (NT) and a bundle of neurites (n). Process (2) is flanked by nerve terminal (NT) and fine glial processes (G with an arrowhead). Process (3) is flanked by glial process (G, arrowhead) and a bundle of neurites (n). Glial processes are devoid of AChE. AChE-positive fine dendritic processes are considered to be nigral spinules. × 66 000. **Fig. 2.** Three serial sections (**a, b, c**) of AChE-positive dendritic spine head (SH) and spinule of rat substantia nigra. The spinule is flanked by nerve terminal (NT) and non-identifiable process. Its entire length (tip indicated by asterisk) can be seen in (**b**). × 40 000.

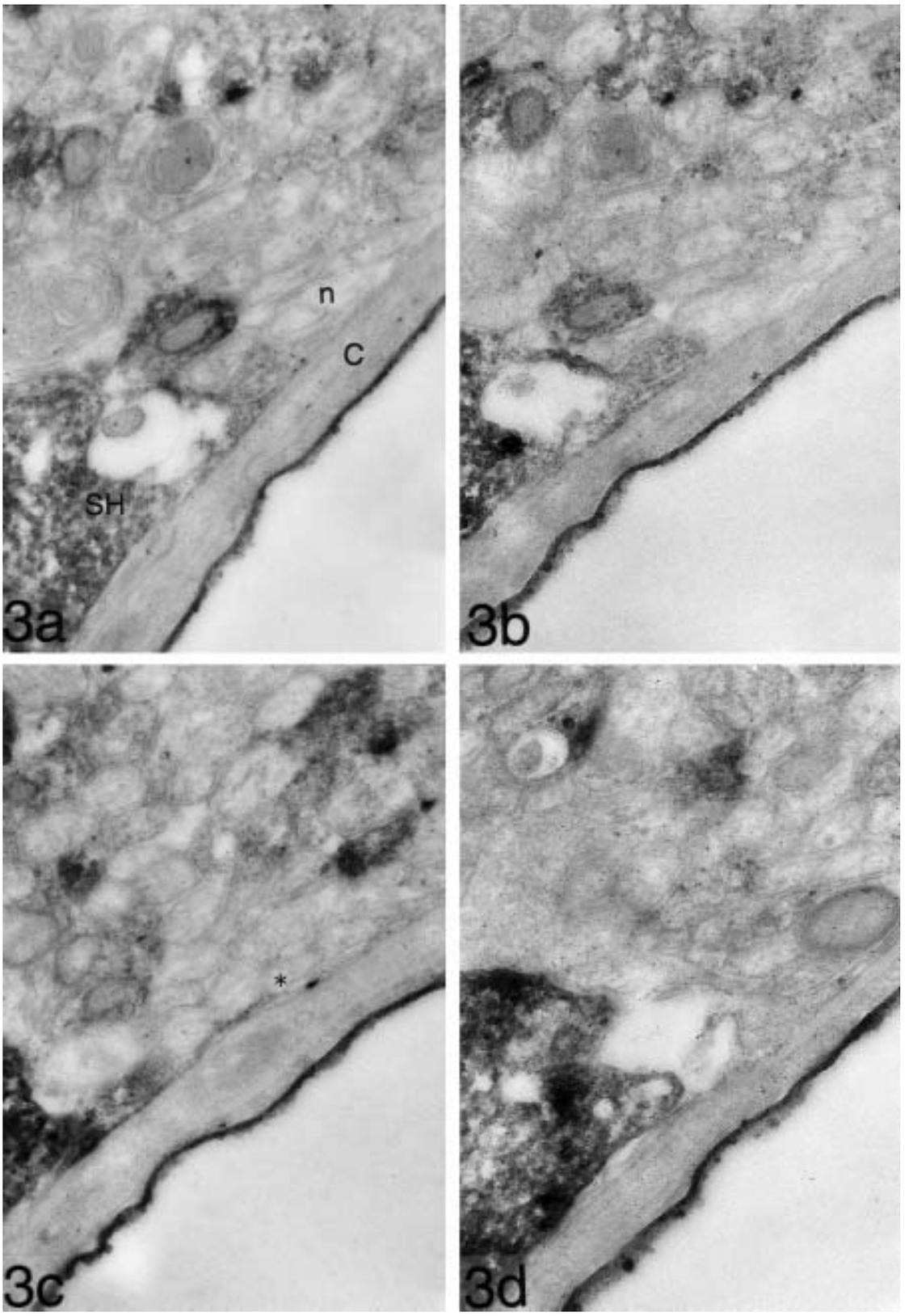

Fig. 3. Four serial sections (**a, b, c, d**) of AChE-positive dendritic spine head (SH) and spinule of rat substantia nigra.The spinule is flanked by a capillary wall (C) and a bundle of neurites (n). The longest section of the spinule (asterisk) is shown in (**c**). × 54 000.

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