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Aspiny neurons and their local axons in the neostriatum of the rat: a correlated light and electron microscopic study of Golgi-impregnated material

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Summary

Three types of neuron with smooth (aspiny) dendrites could be distinguished in the Golgi-impregnated rat neostriatum. Examples of each type of aspiny neuron were found with local axon collaterals within the neostriatum and these were selected for gold-toning and examination in the electron microscope. One type of aspiny neuron had an elongated, usually spindle-shaped, medium-size soma with two, or rarely three, primary dendrites originating from opposite poles of the cell; one example of this type of neuron had two separate axons. The second type of aspiny neuron had a nearly round, medium-size soma with four primary dendrites that branched profusely quite close to the cell body. A third type of aspiny neuron had a very large polygonal-shaped cell body. Afferent axon terminals were found in synaptic contact with the dendrites and cell bodies of all three types of aspiny neuron.

Axon collaterals of each type of neuron displayed varicosities which, when examined in the electron microscope, were frequently found to be boutons making synaptic contact. All such synaptic contacts had symmetrical membrane specializations and the most common postsynaptic targets were dendritic shafts, sometimes spine-bearing. Dendritic spines themselves also received synapses from each type of neuron. No axosomatic synapses involving boutons of identified axons were found. One example of a synapse between an axon collateral of an aspiny neuron and one of the same neuron's dendrites (an 'autapse') was demonstrated by electron microscopy.

It is concluded that the synaptic terminals of at least four types of neuron, the three aspiny types described here and the medium-size densely spiny neuron, participate in local circuit interactions in the neostriatum.

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Introduction

Many different types of neuron have been distinguished on morphological grounds in the Golgi-impregnated neostriatum (for review see Pasik *et al.*, 1979; Chang *et al.*, 1982). Two main groups of neurons have been defined, spiny and aspiny, according to the presence or absence of numerous dendritic spines. These groups have been further subdivided according to the size and shape of the cell body and the pattern of the dendritic arborization. Another important distinguishing feature is the presence of local axon collaterals within the neostriatum. The most commonly impregnated neuron, the medium-size densely spiny cell, has local axon collaterals (Ramón y Cajal, 1911) which form synapses within the neostriatum (Wilson & Groves, 1980; Somogyi *et al.*, 1981a; Bishop *et al.*, 1982) and this cell was for many years considered to be an interneuron (Ramón y Cajal, 1911; Vogt & Vogt, 1920; Kemp & Powell, 1971; Fox *et al.*, 1971/72). However, it has now been established from combined Golgi and retrograde transport studies (Somogyi & Smith, 1979; Somogyi *et al.*, 1979, 1981a; Freund & Somogyi, 1983) and by intracellular injection of horseradish peroxidase (Preston *et al.*, 1980; Chang *et al.*, 1981; Kitai, 1981) that neurons of this class project to the substantia nigra or globus pallidus. It has been directly demonstrated that some of the medium-size spiny neurons that project to the substantia nigra (Somogyi *et al.*, 1981a) or globus pallidus (Chang *et al.*, 1981) also have local axon collaterals in the neostriatum and that some of these collaterals form synapses within the neostriatum (Somogyi *et al.*, 1981a). It is therefore clear that the medium-size spiny neuron is capable of influencing the activities of neostriatal neurons as well as those in the target areas outside the striatum.

The question arises, is this the only form of local circuit activity in the neostriatum or are there other types of neuron with local axon collaterals that form synapses within the striatum? The purpose of this study was to answer this question. Following reports that some of the less commonly impregnated neurons of the aspiny type have local axon collaterals (Kemp & Powell, 1971; Di Figlia *et al.*, 1976; 1980; Leontovich, 1977; Pasik *et al.*, 1977; 1979; Rafols & Fox, 1979; Danner & Pfister, 1981; Braak & Braak, 1982; Chang *et al.*, 1982) we have examined different types of neuron which exhibit local axon collaterals. Here we describe studies on the ultrastructure of three types of Golgi-impregnated aspiny neurons and their processes in the rat neostriatum, with special reference to their local efferent connections.

Materials and methods

Two albino CFY strain rats (150–200 g) were used in this study. The rats were anaesthetized with intraperitoneal chloral hydrate (350 mg/kg) and perfused through the heart with Tyrode's solution (gassed with a mixture of O_2 and CO_2, pH 7.4) for 1–2 min, followed by approximately 200 ml of fixative consisting of 4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.4) for 30 min, as described previously (Somogyi & Takagi, 1982).

The brain was removed from the skull and immersed in the same fixative for 2–3 h. Blocks containing the neostriatum, 4 mm × 4 mm × 2 mm, were washed in 0.1 M phosphate buffer and impregnated by the rapid Golgi technique as described previously (Somogyi *et al.*, 1979). After

silver nitrate treatment the blocks were embedded in 5% agar. Then 85 μm thick, frontal sections were cut with a tissue chopper, gold-toned and deimpregnated after illumination (Fairén *et al.*, 1977; Somogyi *et al.*, 1981b), followed by dehydration and flat-embedding in Durcupan (Fluka) on glass slides. Illumination was carried out either using the specimen stage of a Zeiss (Jena) microscope equipped with a 6 V, 12 W light bulb, or with a Schott, Mainz KL150B fibre optic device; the purpose was to enhance the gold-toning.

The sections were examined in the light microscope; cells of interest, i.e. gold-toned aspiny neurons with their axon collaterals, were photographed and drawn, using a Leitz light microscope equipped with a drawing tube. For electron microscopic observation, the sections were re-embedded as described previously (Somogyi & Takagi, 1982). Serial ultrathin sections were mounted on single slot Formvar-coated grids and examined using a Philips 201C electron microscope with 20–30 μm objective apertures at 80 kV. In order to improve contrast, the sections were stained *en bloc* with 1% uranyl acetate for 1 h at 70% ethanol dehydration stage and the ultrathin sections were stained with lead citrate (Reynolds, 1963). None of the measurements given in this study has been corrected for shrinkage; they apply to the Golgi sections.

Results

Three morphologically distinct types of aspiny neurons, each with local axon collaterals, could be distinguished at the light microscopic level in Golgi-impregnated, gold-toned material. For convenience, we have defined these cells as follows: (a) spindle-shaped, medium-size aspiny (MA1) neurons; (b) round-shaped, medium-size aspiny (MA2) neurons; (c) giant aspiny neurons. Although this classification emphasizes the differences in the perikarya, other properties of the neurons are also different, as will be described below.

In the present study, a total of 15 aspiny neurons with local axon collaterals was analysed: six were of the MA1 type, seven of the MA2 type and two were giant aspiny neurons. Although the frequency of particular types of impregnated neurons in Golgi material cannot be used as an indication of their frequency within the overall population, we note that the most commonly impregnated aspiny neuron was the type labelled MA1, followed by MA2 and then the giant neuron.

In order to correlate light and electron microscopic studies, we selected two MA1 type neurons and one each of the MA2 type and giant type. To establish whether several types of neuron were involved in local circuits in the neostriatum through local efferent synaptic boutons, we used the following criteria: (i) each bouton studied in the electron microscope should first have been shown unequivocally in the light microscope to belong to an axon that could be traced back to an identified cell body; (ii) synaptic specializations of the membrane contacts were only accepted if they were not rendered ambiguous by heavy deposits of gold or by poor preservation of the fine structure. On the basis of the thickness of the postsynaptic membrane specialization, both the afferent and efferent synaptic contacts were classified into two broad categories: asymmetrical (or Gray's type I) or symmetrical (Gray's type II; Gray, 1959).

In this study, the preservation of ultrastructure was variable and, in general, did not reach the standard that is possible when more conventional fixatives are used. However,

the picric acid-containing fixative has proved very useful in our hands for the impregnation of neurons with their local axons (Somogyi *et al.*, 1983a).

SPINDLE-SHAPED, MEDIUM-SIZE ASPINY (MA1) NEURON

Both of the cells illustrated in Fig. 1 showed a similar morphology of the soma and dendrites, except that cell MA1a had longer lengths of dendrite within the section because its long axis was parallel with the plane of section. The cell bodies were spindle-shaped, being 18 μm long and 11–12 μm wide and gave rise to two relatively

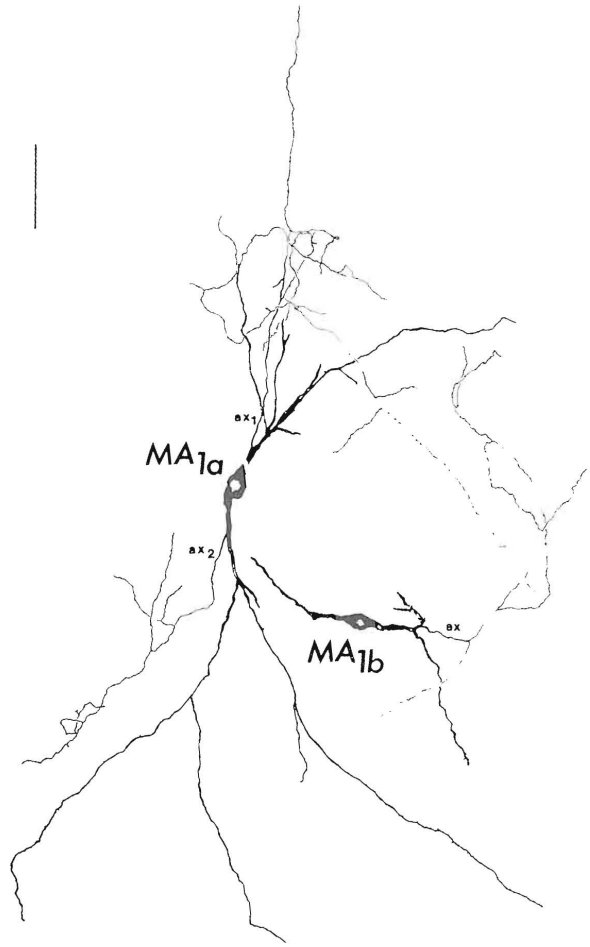

Fig. 1. Camera lucida drawings of two Golgi-impregnated and gold-toned spindle-shaped, medium-size neurons (MA1a, b). The axons (ax, ax_1 and ax_2) of each neuron originate from the base of a primary dendrite as it emerges from the perikaryon. One of the neurons (MA1a) has two axons (ax_1 and ax_2) which project from opposite poles of the cell; note that the arborizations of the local collaterals of these two axons do not appear to overlap. Light and electron micrographs of parts of neuron MA1a are shown in Figs. 2–10 and of parts of neuron MA1b in Figs. 11–15. Scale bar: 50 μm.

thick dendrites which originate from opposite ends of the cell body. The dendrites do not branch very frequently and may extend up to 330 μm from the soma. Occasional spines occur on the dendrites after their first branch point, but varicosities were not seen. The axon originates from the proximal part of the primary dendrite (Fig. 1) and emits thin, delicate collaterals with few varicosities. A feature of cell MA1a was the presence of two distinct axons, originating from dendrites at the opposite poles of the cell (Figs. 1, 2A–C).

Ultrastructural features of neuron type MA1

The ultrastructural features of the two MA1 neurons were found to be very similar, in spite of the fact that one neuron (MA1a) had two axons. Therefore we will describe them together. This neuron type has an almost oval nucleus which appears polylobulated due to the numerous deep nuclear invaginations (Figs. 2D, 9). The apparent degree of indentation of the nucleus depends upon the level of the section: in some sections little or no evidence of indentation could be seen. The nucleus is roughly centrally located in the soma and has a single nucleolus. The cytoplasm is fairly sparse and seems to be concentrated near the base of the dendrites and contains a moderate number of mitochondria and dense bodies, some areas of Golgi apparatus and granular endoplasmic reticulum (Figs. 2D, 11).

Boutons in synaptic contact with the impregnated neurons MA1a and MA1b displayed either symmetrical or asymmetrical membrane specializations and also had distinct morphological features (Table 1). Axosomatic contacts were exclusively of the symmetrical type, but were rare; the boutons forming such contacts were large or medium in size and contained pleomorphic vesicles and large mitochondria (Fig. 3). A similar type of bouton formed symmetrical synapses with both proximal (Fig. 4) and distal (Figs. 5A, 12) dendrites and on neuron MA1b a patch covered densely by boutons

Table 1. Characteristics of afferent synaptic boutons on Golgi-impregnated aspiny neurons.

Type of neuron	*Soma*	*Proximal dendrites*	*Distal dendrites*
MA1	Large boutons: symmetrical	Large or medium boutons: symmetrical; small boutons: asymmetrical	Large boutons: symmetrical; small boutons: asymmetrical
MA2	Symmetrical Asymmetrical	Symmetrical Asymmetrical	Asymmetrical
Giant aspiny	Symmetrical	Symmetrical	—

Unless otherwise stated, boutons were of medium size.

was observed (Fig. 12). Occasionally, dendrites were found to be in asymmetrical synaptic contact with small boutons that contained small round or oval vesicles (Figs. 5B, 13).

The local axons of neurons MA1a and MA1b were examined in some detail. Twenty-five varicosities were studied in serial sections in the electron microscope, but either the poor ultrastructural preservation or the intensity of the gold deposit made it difficult to establish that all of them were synaptic boutons. However, a total of 16 varicosities satisfied the criteria for the identification of synaptic contacts and each of them displayed symmetrical membrane contacts (Figs. 6–10, 14–15) (see also Table 2). Nine of these synaptic boutons were found along the collaterals of both axons (ax_1, ax_2; Figs. 1, 2A–C) of neuron MA1a and seven were along the axon of neuron MA1b. The

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Fig. 2. (A) Photomontage of part of the neuron (MA1a) with two axons (ax_1 and ax_2) that was shown in Fig. 1. (B, C) The origins of the two axon initial segments are shown at higher magnification. (D) Low magnification electron micrograph of the gold-toned cell body of the same neuron MA1a. Note that the oval nucleus appears polylobulated due to deep nuclear invaginations. Scale bars: (A) 25 μm; (B, C) 10 μm; (D) 1 μm.

Fig. 3. Symmetrical axosomatic synapse (arrow), formed by an unimpregnated axon terminal containing pleomorphic vesicles, in contact with neuron MA1a. Scale bar: 0.2 μm.

Fig. 4. Proximal dendrite (d) of neuron MA1a (Fig. 1) in symmetrical synaptic contact (arrow) with an unimpregnated large axon terminal which contains pleomorphic vesicles. Note the asymmetrical synaptic contact formed by another axon terminal (asterisk) with an unimpregnated element. Scale bar: 0.2 μm.

Fig. 5. A distal dendrite (d) of neuron MA1a of Fig. 1. (A) The dendrite is in symmetrical synaptic contact (arrow) with a large unimpregnated axon terminal containing pleomorphic vesicles and a large mitochondrion. (B) The same dendrite is in asymmetrical contact (arrow) with a small axon terminal packed with small round or oval vesicles. Scale bar: 0.2 μm.

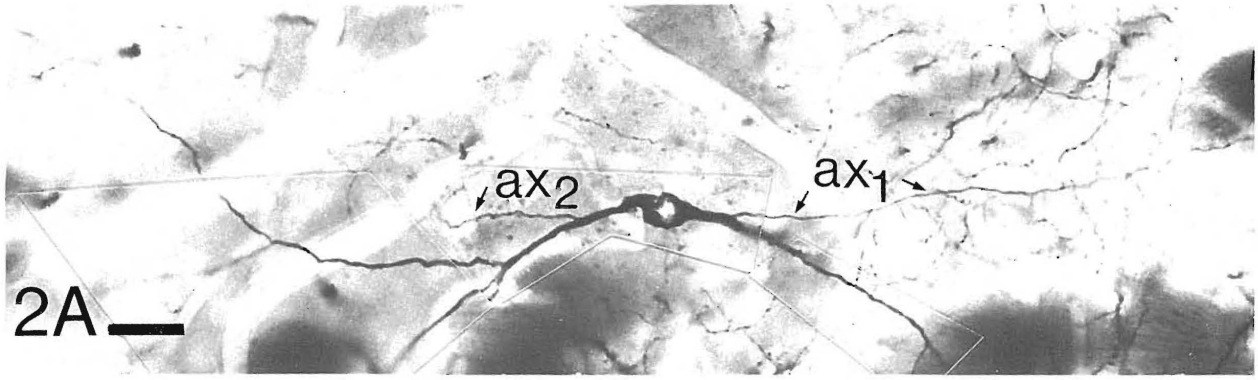
Fig. 6. (A, B) Serial sections showing a symmetrical synapse (arrows) established by one of the axons (ax_2) of neuron MA1a (Fig. 1) with an unimpregnated dendritic shaft (d). 6B is four sections away from 6A. The impregnated bouton contains small round or oval vesicles. Note that in 6A the dendrite postsynaptic to this bouton emits a spine (s) which is in asymmetrical contact (open arrow) with an unimpregnated axon terminal. Scale bar: 0.2 μm.

Fig. 7. (A) Light and (B) low magnification electron micrographs of part of one of the axons (ax_2) of neuron MA1a (Fig. 1). A capillary (c), an unimpregnated neuron (n) and a group of myelinated fibres help in the correlation of the light and electron microscopic studies. (C) The framed area is shown at a higher magnification in Fig. 8. Scale bars: (A) 10 μm; (B) 2 μm.

Fig. 8. Higher magnification electron micrograph of the framed area in Fig. 7C. Scale bar: 0.2 μm.

Fig. 9. Electron micrograph of the section adjacent to that shown in Fig. 8. The bouton indicated by an arrow in Fig. 7A can be seen in Fig. 8 to contain small round or oval vesicles; it is in symmetrical synaptic contact with both a dendritic shaft (d, large arrow in Figs. 8 and 9) and with a spine (small arrow in Figs. 8 and 9). The spine is also in asymmetrical synaptic contact (open arrows in Fig. 8) with an unimpregnated axon terminal. Scale bar: 0.2 μm.

Fig. 10. Another bouton of the same axon (ax_1 of neuron MA1a) as shown in Fig. 7, which is in symmetrical synaptic contact (arrow) with a dendritic shaft (d). Scale bar: 0.2 μm.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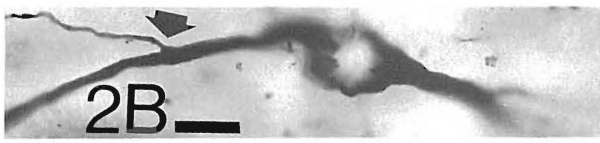
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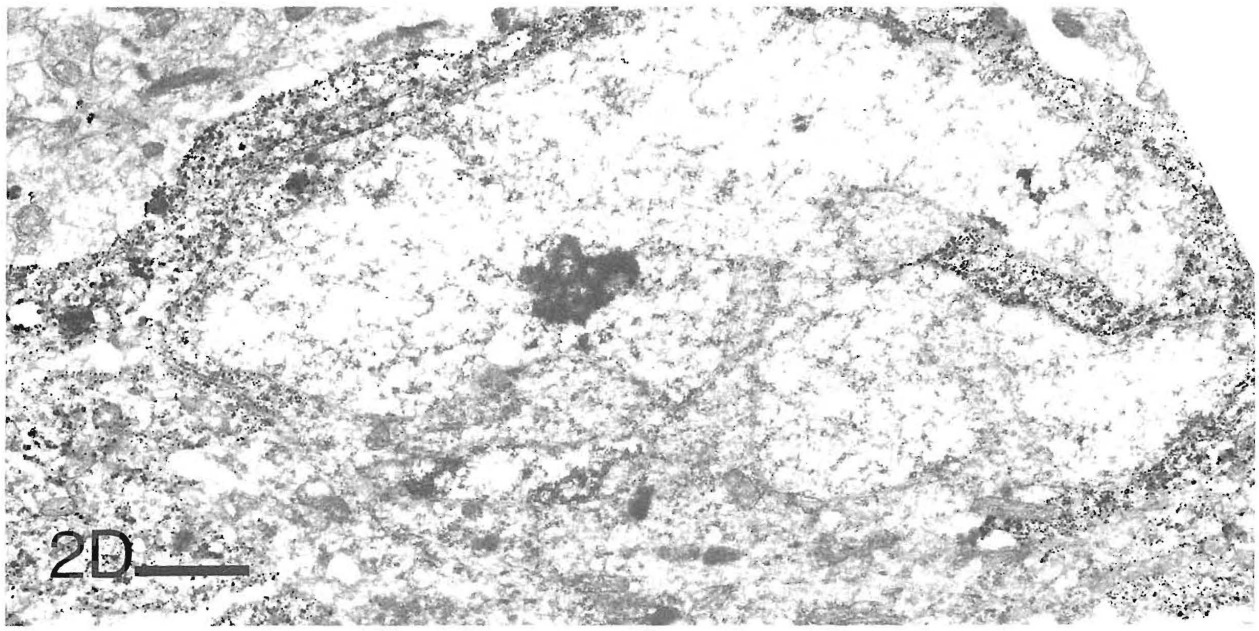
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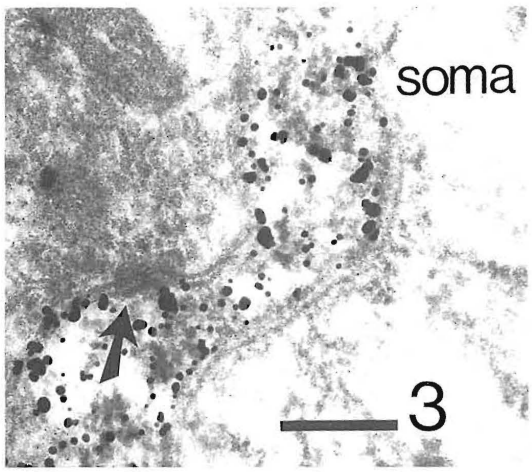
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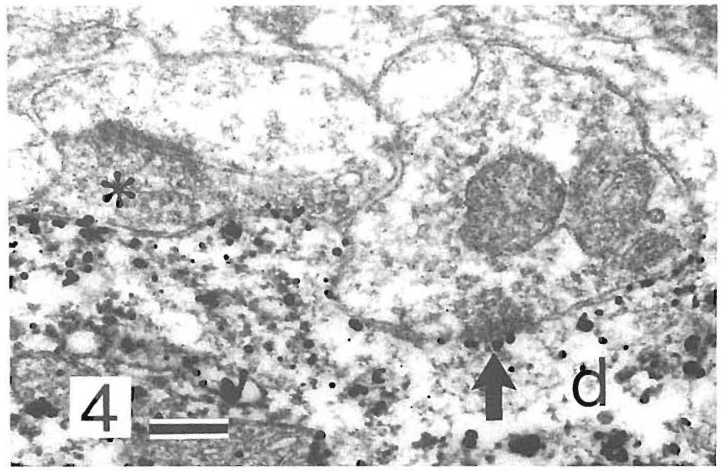
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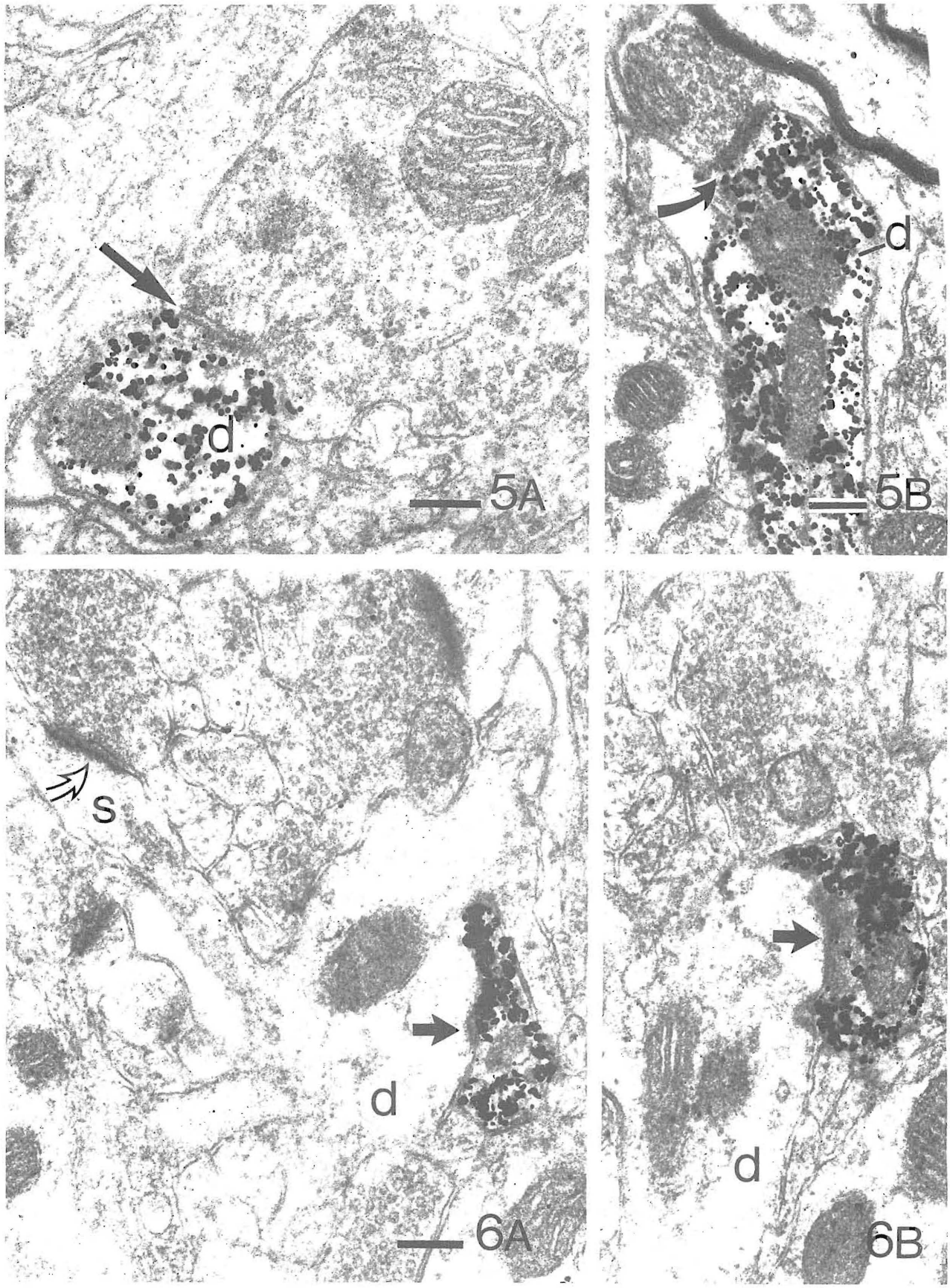
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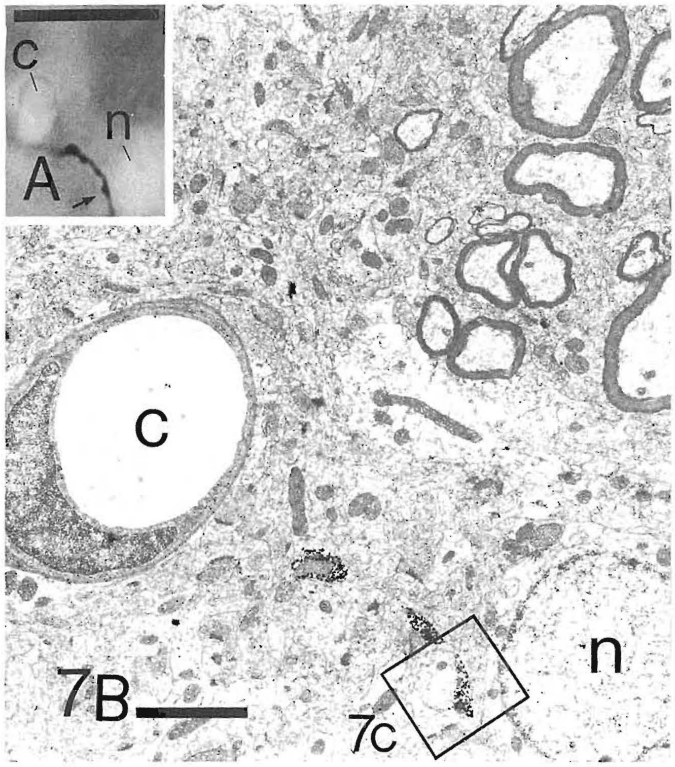
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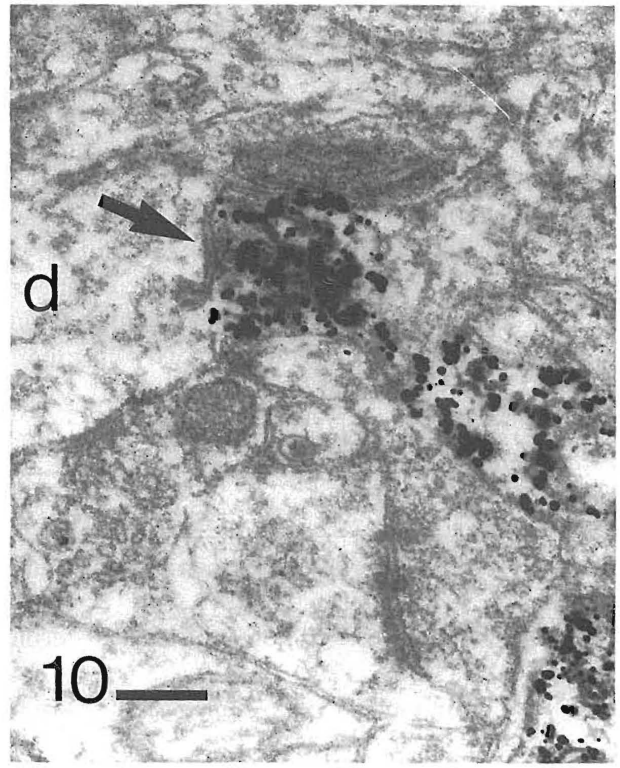
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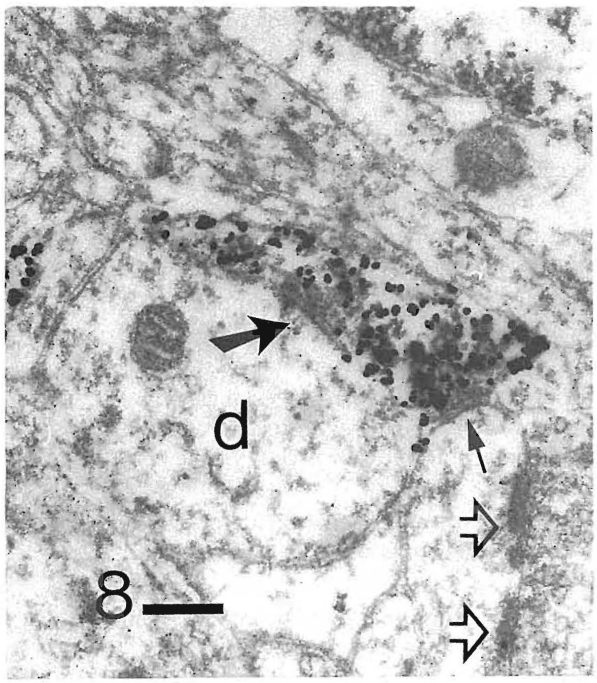
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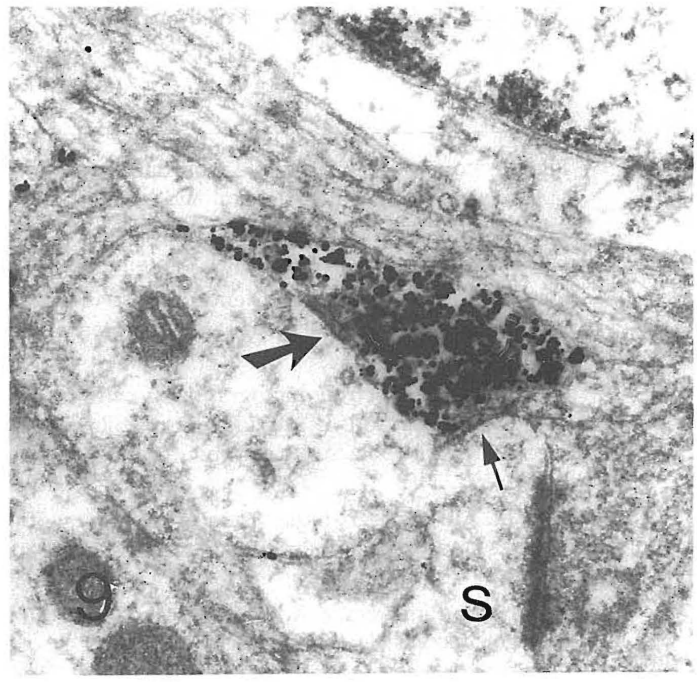
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postsynaptic elements were identified as follows: four were dendritic shafts which displayed spines (Figs. 6, 14); ten were dendritic shafts which did not have obvious spines (Figs. 7–10, 15); and two were dendritic spines (Figs. 8, 9). Dendritic spines were identified using serial sections. A postsynaptic profile was considered to be a spine if it lacked mitochondria throughout the section series and if it became a narrow neck in one direction but did not continue in the other direction. Spine apparatus was found in some, but not in all, spines. One example was found of an impregnated bouton in synaptic contact with two different dendritic elements, a shaft and a spine (Figs. 8, 9). It is noteworthy that the spines emanating from dendritic shafts (Fig. 6) or the spines themselves (Figs. 8, 9) that were in symmetrical contact with impregnated boutons were also in asymmetrical contact with unimpregnated terminals.

Table 2. Characteristics of boutons of local axon collaterals of Golgi-impregnated aspiny neurons.

Type of neuron	*Specialization*	*Size*	*Vesicle type*	*Postsynaptic targets*
MA1	Symmetrical	Medium	Small, round/oval	14 dendritic shafts 2 dendritic spines
MA2	Symmetrical	Medium	Small, round/oval	10 dendritic shafts* 3 dendritic spines
Giant aspiny	Symmetrical	Medium	Large, electron-lucent	3 dendritic shafts 3 dendritic spines

*One of the dendritic shafts originated from the same neuron that gave rise to the local axon collateral and represents an 'autapse'. Note that none of the boutons observed was found in contact with a cell body.

Fig. 11. Low magnification electron micrograph of the cell body of medium-size aspiny neuron MA1b (see Fig. 1). Note the oval and highly indented nucleus. Scale bar: 1 μm.

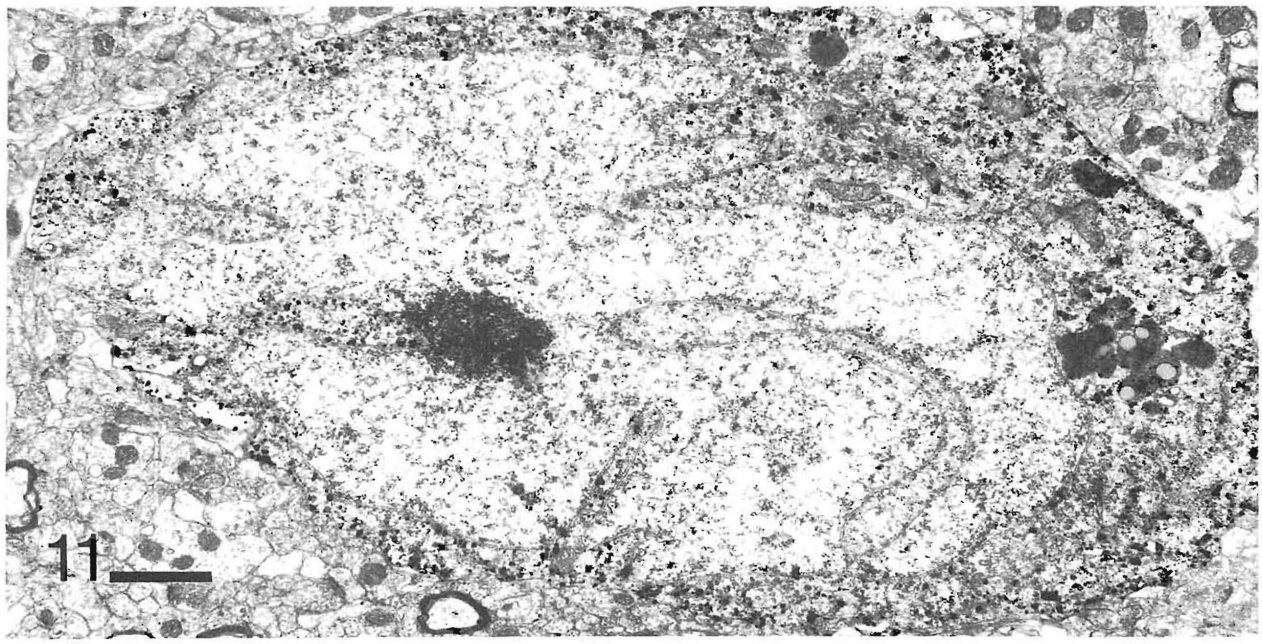
Fig. 12. Proximal dendrite of neuron MA1b (Fig. 1) in symmetrical synaptic contact (arrows) with five axon terminals each of which contains pleomorphic vesicles. Scale bar: 0.2 μm.

Fig. 13. The same proximal dendrite of neuron MA1b (Fig. 1) in asymmetrical synaptic contact (arrow) with a small unimpregnated axon terminal containing small round or oval vesicles. Scale bar: 0.2 μm.

Figs. 14 & 15. Electron micrographs showing symmetrical synapses (filled arrows) established by impregnated boutons of the local axon collaterals of neuron MA1b (Fig. 1) with unimpregnated dendritic profiles. Each synaptic bouton contained small pleomorphic or oval vesicles, visible here in Fig. 15.

Fig. 14. An impregnated bouton is in contact (filled arrow) with a dendritic shaft (d) which receives another symmetrical synaptic contact (open arrow) and emits a spine (s). Scale bar: 0.2 μm.

Fig. 15. The impregnated bouton in synaptic contact (arrow) with a dendrite (d) contains small pleomorphic vesicles. Scale bar: 0.2 μm.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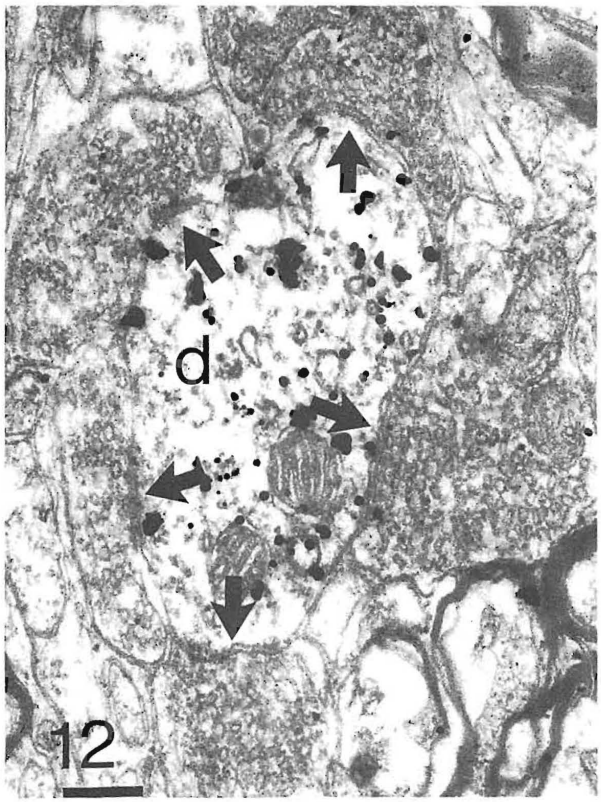
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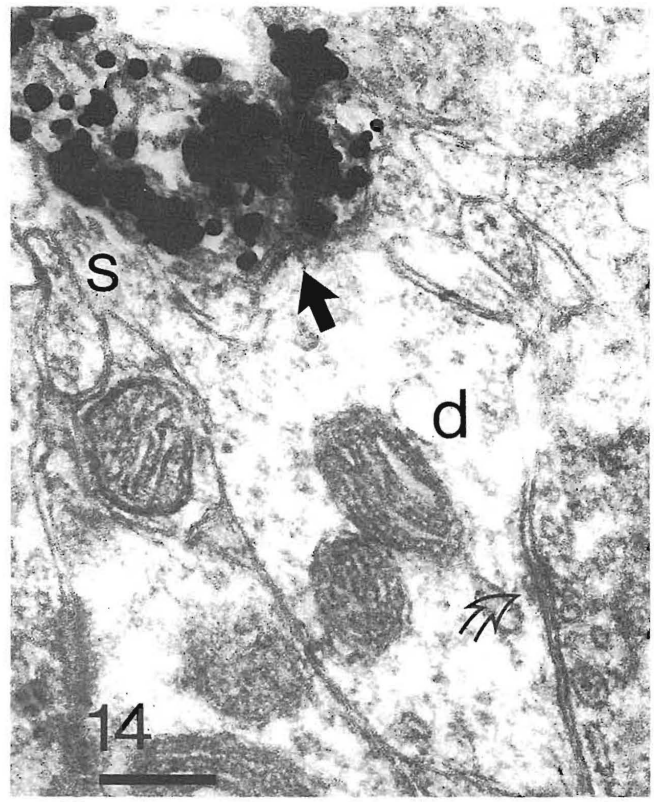
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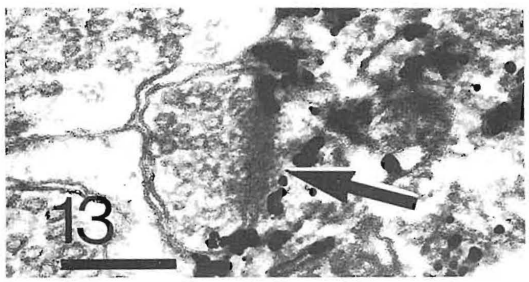
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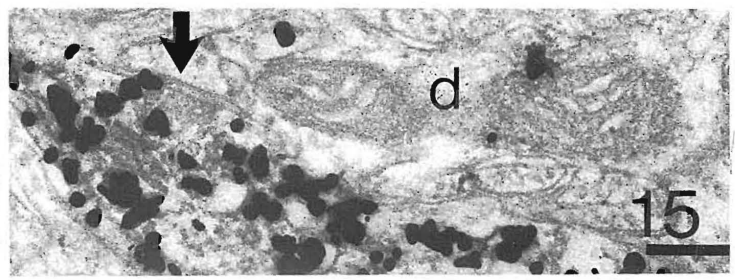
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Although particles of gold sometimes obscured the cytological details within the boutons of axons of MA1 neurons, it was possible in some examples to see that they contained small pleomorphic vesicles and medium-size mitochondria (Figs. 6–10, 14–15).

ROUND-SHAPED, MEDIUM-SIZE ASPINY (MA2) NEURON

The spine-free cell body is nearly round, 12 μm in diameter, and gives rise to four primary dendrites which branch about 15–20 μm from the soma (Fig. 16). Another

Fig. 16. Camera lucida drawing of a Golgi-impregnated and gold-toned round-shaped, medium-size, aspiny neuron (MA2) of the second type. The primary axon is indicated (ax) and the large arrow shows the apparent contact between a varicose part of a local axon collateral with one of the neuron's own dendrites; light and electron micrographs of this region are shown in Fig. 20. Note the many varicose local axon collaterals and the dendrites which display a recurving course. Scale bar: 50 μm.

characteristic feature is the recurving course of the dendrites, with abrupt changes in direction: as a result, the tips of the dendrites usually point in a different direction from that of the original shaft. The dendrites had occasional spines on the more distal parts (Fig. 16). The axon originates from the soma and displays a most elaborate and highly branched local collateral arborization, which extends some way beyond the dendritic field (Fig. 16). In contrast to collaterals of the MA1 neuron (Fig. 1), these collaterals not only branch more frequently, but have much more frequent and larger varicosities.

Ultrastructural features of neuron type MA2

The soma has an irregularly shaped nucleus due to invaginations of the nuclear envelope. A moderate amount of cytoplasm is present, containing some dense bodies and Golgi apparatus (Fig. 17).

Very few axosomatic synapses were found on the impregnated MA2 neuron, but both symmetrical and asymmetrical contacts occurred (Table 1). The boutons forming axosomatic synapses were of medium size and contained small round or oval vesicles. The impregnated dendrites also received boutons forming either symmetrical or asymmetrical contacts; the latter were more frequent on the distal dendrites (Fig. 18).

Thirty-nine impregnated varicosities along local axon collaterals of neuron MA2 were examined in serial ultrathin sections: thirteen (Table 2) were found to fulfil the criteria for synaptic boutons. The boutons that were lightly covered by gold precipitate contained small round or oval vesicles. Each of these boutons was in symmetrical contact with its respective postsynaptic element (Figs. 19, 21). The postsynaptic elements were: three dendritic spines and ten dendritic shafts (Figs. 19, 21), one of which was also impregnated and was shown to belong to the same parent neuron that gave rise to the axon (Fig. 21).

GIANT ASPINY NEURON

This neuron has a large, polygonal-shaped cell body, without somatic spines, about 30 μm long and 13 μm wide; there are three thick primary dendrites, two of which bifurcate soon after leaving the soma (Figs. 22A, B). The nucleus is located in the middle of the soma, with a single nucleolus in its centre. The dendrites extend up to 150 μm from the soma but most of them were cut at the surface of the section and so their real extent is probably much greater. They have occasional spines, but no varicosities. The axon arises directly from the cell body and gives rise to several varicose local collateral branches, mainly within the area occupied by the dendritic tree (Figs. 22A, B).

Ultrastructural features of the giant aspiny neuron

The nucleus is oval, deeply indented and is surrounded by a moderate amount of cytoplasm that displays typical organelles (Fig. 23). Sections cut at different levels of the soma than that illustrated in Fig. 23 showed a well-developed granular endoplasmic reticulum.

Very few synapses were found on the soma of the giant aspiny neuron and those that were observed were all symmetrical and were formed by medium-size boutons containing fairly densely packed small round or oval vesicles (Fig. 24, Table 1). A similar type of bouton was found in contact with the proximal dendrite (Fig. 25); this bouton also contained a large dense-cored vesicle. Other boutons were also found to make symmetrical contact with the proximal dendrite, but in these the vesicles were less densely packed (Fig. 26).

Thirty-four impregnated varicosities along the local axon collaterals were examined in serial ultrathin sections: six of them were positively identified as synaptic boutons in symmetrical contact with their target elements (Table 2). Three of the postsynaptic

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Fig. 17. Low magnification electron micrograph of the cell body of the aspiny neuron MA2 (Fig. 16). Nuclear indentations are indicated by arrows. Scale bar: 1 μm.

Fig. 18. Electron micrograph of an impregnated distal dendrite (d) of aspiny neuron MA2 (Fig. 16) showing asymmetrical synaptic contacts (curved arrows) with two unimpregnated axon terminals, one of which (asterisk) also forms a synapse (broad arrow) with another dendrite. Scale bar: 0.2 μm.

Fig. 19. Electron micrograph of symmetrical synapse (arrow) between a local axon collateral of aspiny neuron MA2 with a dendritic shaft (d). Scale bar: 0.2 μm.

Fig. 20. (A) Light and (B) low magnification electron micrographs of the parts of the local axon and a dendrite of the aspiny neuron MA2 indicated by an arrow in Fig. 16 (reversed with respect to the drawing in Fig. 16). c, capillary. High magnification electron micrographs of the area indicated by arrows are shown in Fig. 21. Scale bars: (A) 5 μm; (B) 2 μm.

Fig. 21. The arrow indicates a symmetrical synaptic contact between part of the impregnated axon and the impregnated dendritic shaft, both of which were shown by light microscopy to belong to the same neuron (Fig. 20A). The impregnated axon terminal contains small round or oval vesicles. Scale bar: 0.2 μm.

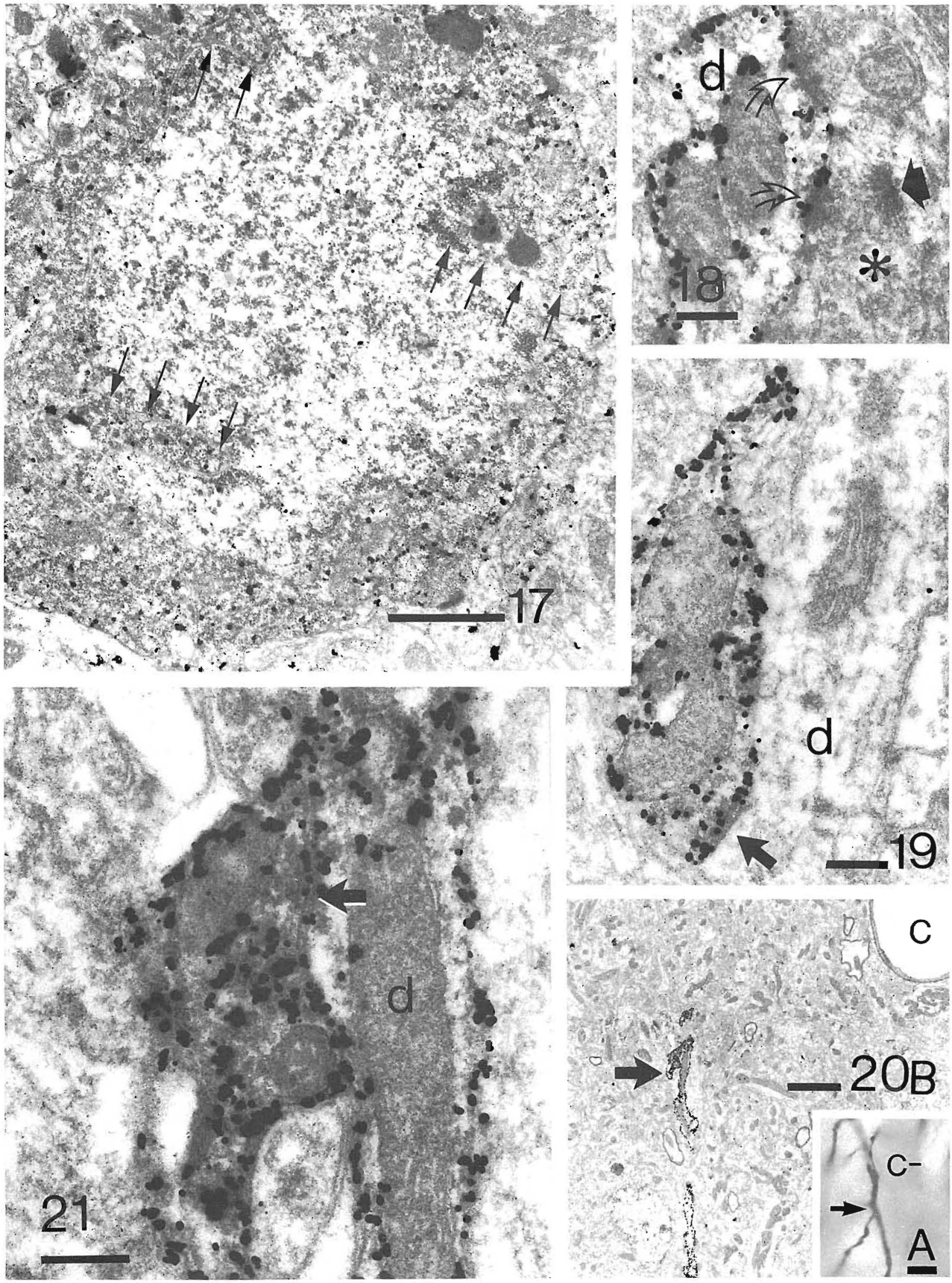
Fig. 22. (A) Camera lucida drawing of a Golgi-impregnated and gold-toned giant aspiny neuron. (B) Photomontage of part of the same gold-toned neuron. ax, axon; d, dendrite. Light and electron micrographs of parts of this neuron are shown in Figs. 23–28. Scale bars: (A) 50 μm; (B) 20 μm.

Fig. 23. Low magnification electron micrograph of part of the gold-toned cell body of the giant aspiny neuron shown in Fig. 22. Indentations of the nucleus are indicated by arrows. Scale bar: 1 μm.

Fig. 24. High power electron micrograph showing part of the perikaryon of the giant aspiny neuron (Fig. 22). A bouton containing fairly densely packed, small round or oval vesicles is in symmetrical synaptic contact (arrows) with the soma of the impregnated neuron. Scale bar: 0.2 μm.

Fig. 25. Part of a proximal dendrite (d) of the giant aspiny neuron (Fig. 22) in apparent synaptic contact (black arrow) with a bouton containing many small round or oval vesicles and a large dense-cored vesicle (white arrow). It is not possible to decide whether the synaptic contact is symmetrical or asymmetrical because the section is tangential to the specialization. Scale bar: 0.2 μm.

Fig. 26. Proximal dendrite (d) of the giant aspiny neuron (Fig. 22) in symmetrical synaptic contact (arrows) with an axon terminal containing small round or oval vesicles. Scale bar: 0.2 μm.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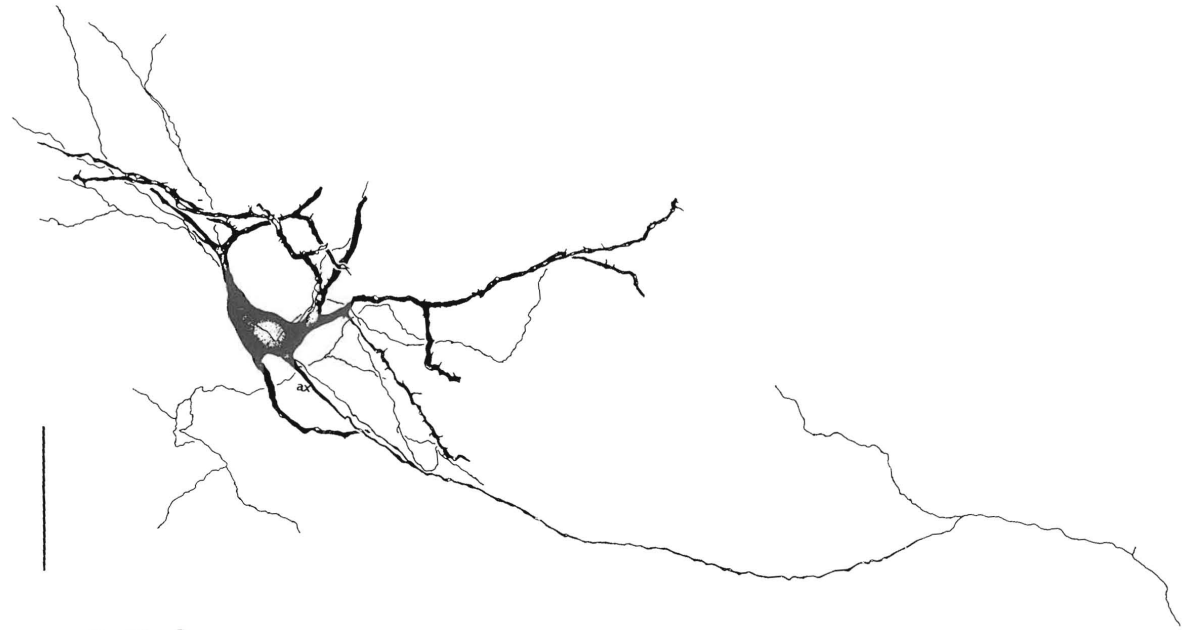

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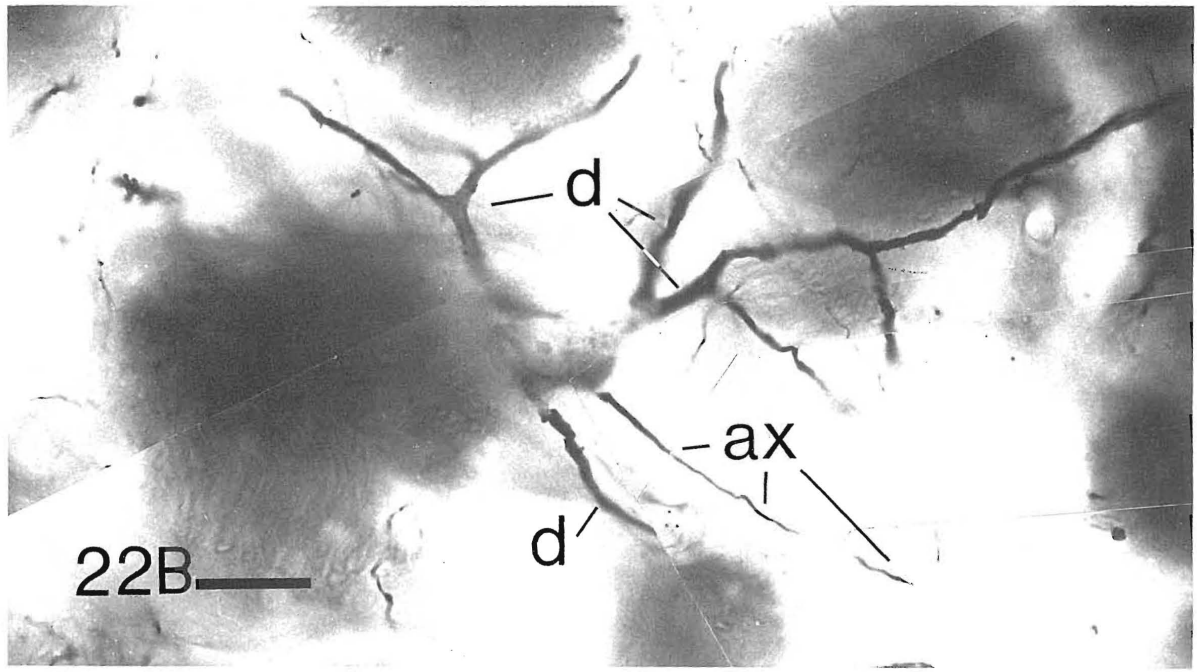

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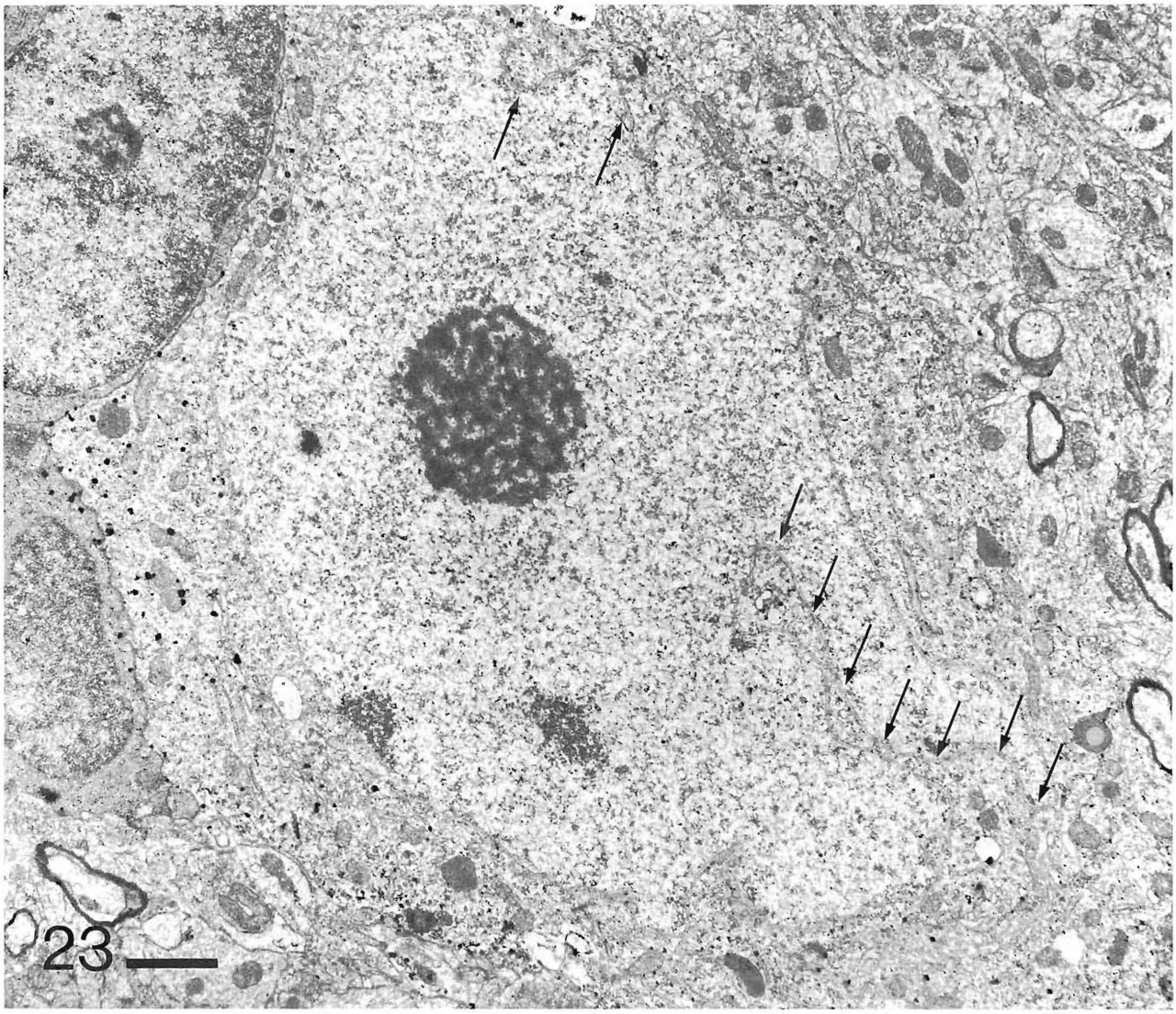
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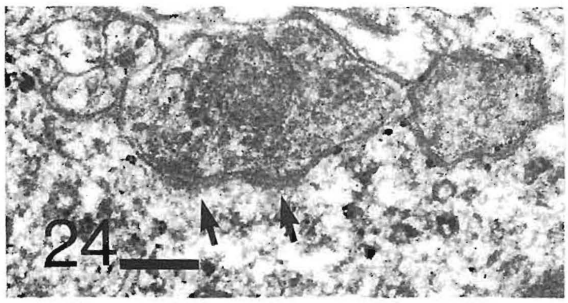
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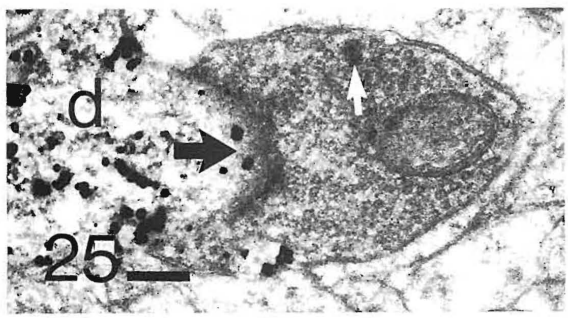
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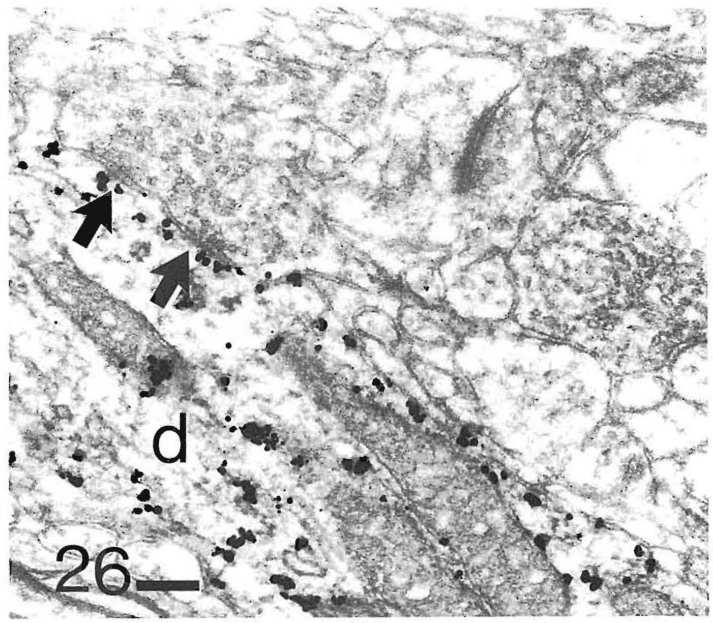
d
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elements were dendritic shafts not giving rise to spines in the immediate vicinity (Fig. 28) and three were dendritic spines (Fig. 27).

It is noteworthy that the impregnated boutons contained a characteristic type of large round or oval electron-lucent vesicle and medium-size mitochondria (Figs. 27C, D, 28); thus, they are clearly very different from the boutons of the MA1 and MA2 neurons.

Discussion

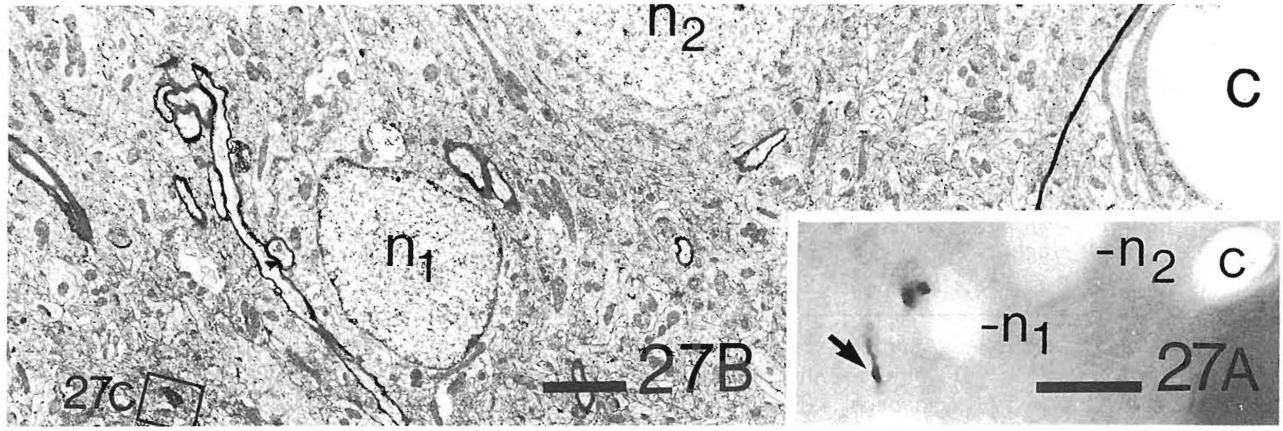
The subclassification of aspiny neurons in the neostriatum based only upon light microscopic studies is not easy and there are many different proposals in the literature. Without more precise methods of analysis, we can only suggest possible correlations between the three types of aspiny neuron we have studied and those described by other workers (Table 3). The examination of Golgi-impregnated neurons in the electron microscope adds further information upon which subclassification can be based and in the present study it has proved particularly useful to study the synaptic input and the ultrastructure of boutons along local axon collaterals.

SPINDLE-SHAPED, MEDIUM-SIZE ASPINY (MA1) NEURON

Although Danner & Pfister (1981) described a very similar spindle-shaped neuron in their Golgi studies on the rat and reported that it was much the most common type of impregnated aspiny neuron, it has only been noticed in very few other Golgi studies. The ultrastructural features of our gold-toned MA1 neurons are similar to those of the neurons Dimova *et al.* (1980) called medium-size type IV, in particular the highly indented nucleus and small volume of cytoplasm, except at the base of the dendrites. A neuron of this type has also been filled with horseradish peroxidase following intracellular recording in the rat striatum (Bishop *et al.*, 1982): this neuron had a similar pattern of dendritic arborization and a similar ultrastructure to our type MA1, but the axon was not filled by the horseradish peroxidase and so no axon collaterals were revealed.

Fig. 27. (A) Light micrograph showing a varicosity along one of the local axon collaterals of the giant aspiny neuron (Fig. 22). (B) Low power electron micrograph of the area shown in 27A; the impregnated varicosity is within the framed area (which is shown at higher magnification in 27C). Structures readily identified in both the light and electron micrographs are a capillary (c) and two unimpregnated neurons (n_1, n_2). (C, D) Serial sections of the area framed in 27B. Filled arrows indicate a symmetrical synaptic contact between the impregnated axonal varicosity of the giant aspiny neuron and a dendritic spine (s). The spine is also in asymmetrical contact (open arrow) with an unimpregnated bouton. Note that the impregnated bouton of the giant aspiny neuron contains large electron-lucent vesicles. Scale bars: (A) 10 μm; (B) 2 μm; (C, D) 0.2 μm.

Fig. 28. (A–C) Serial sections showing a symmetrical synaptic contact (arrows) between an impregnated bouton of a local axon collateral of the giant aspiny neuron (Fig. 22) and an unimpregnated dendritic shaft (d). The bouton contains large round or oval electron-lucent vesicles. Scale bar: 0.2 μm.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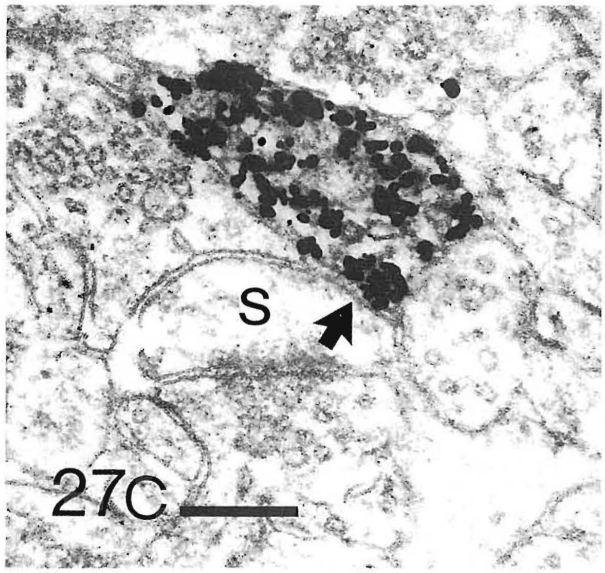
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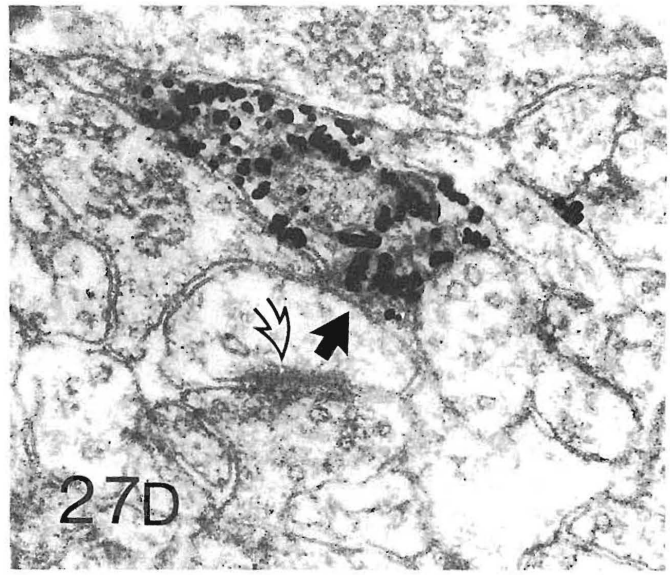
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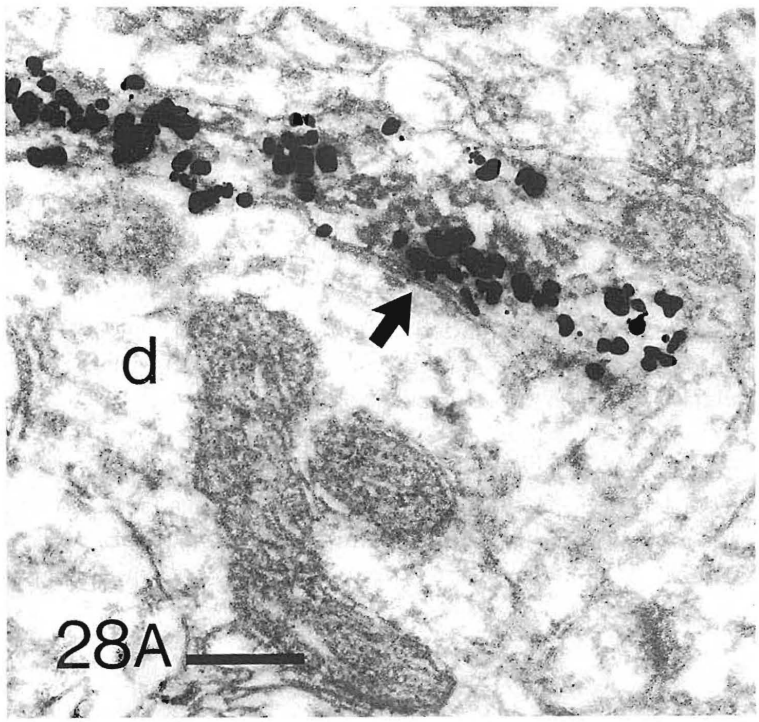
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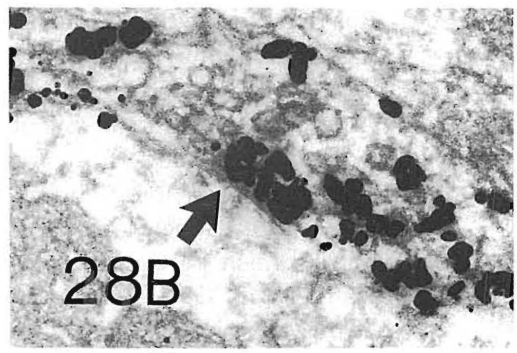
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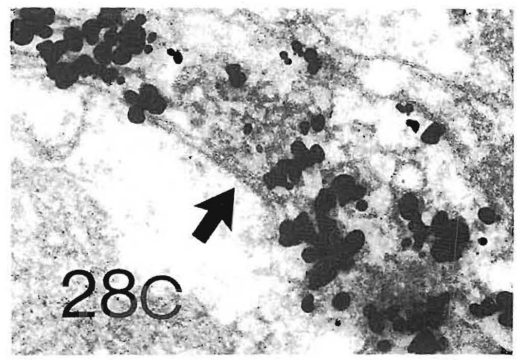
28C

Table 3. Comparison of the three types of aspiny neurons with earlier Golgi studies.

Present study *Rat*	*Dimova et al. (1980)* *Rat*	*Danner & Pfister (1981)* *Rat*	*Chang et al. (1982)* *Rat*	*Kemp & Powell (1971)* *Cat*	*Fox et al. (1971/2)* *Monkey*	*Di Figlia et al. (1976)* *Monkey*	*Tanaka (1980)* *Dog*	*Braak & Braak (1982)* *Human*
MA1	Medium-size type IV	Aspiny spindle-shaped neurons	Small neuron (Figs. 12A–C) or medium neuron, long dendrites (Fig. 13A)	—	—	—	Medium aspiny III	Type V
MA2	Medium-size type II	Aspiny spidery neurons	Type III medium; small neuron (Fig. 7B, C)	Medium-size smooth cell	Spidery neuron	Aspiny type III	Medium aspiny I	Type IV
Giant aspiny	Giant neuron	Aspiny giant neurons	Large type I	Giant cell	Large aspiny neuron	Large spiny type II	Large aspiny II	Type III

It is noteworthy that our MA1 type of neuron resembles in shape, dendritic form and ultrastructure the neurons in rat neostriatum which contain somatostatin-like immunoreactivity (Di Figlia & Aronin, 1982; Takagi *et al.*, 1983).

A remarkable feature of one of the MA1 neurons is that it has two axons that originate from opposite poles of the neuron and which appear to have separate zones of arborization. Local interneurons with two axons have been reported in a few other parts of the C.N.S. (Gobel, 1975; Meyer, 1982). A medium-size spiny projection neuron filled by horseradish peroxidase in the rat neostriatum was considered to have two axons (Preston *et al.*, 1980), but the possibility that the horseradish peroxidase had filled two cells that were in contact at their perikarya cannot be excluded. On the other hand, one of the aspiny neurons in the human neostriatum described by Braak & Braak (1982) had two axons and this neuron was similar to the MA1 type in morphology.

ROUND-SHAPED, MEDIUM-SIZE ASPINY (MA2) NEURON

Neurons similar to this type have been described in many previous Golgi studies (see Table 3). On grounds both of dendritic morphology and ultrastructure our MA2 type of

neuron resembles the medium-size type II neuron of Dimova *et al.* (1980). It also shares several of the features of neurons which have been characterized by Golgi-impregnation and electron microscopy (Bolam *et al.*, 1983a) and shown to take up [^{3}H]γ-aminobutyrate (GABA). However, these [^{3}H]GABA-accumulating cells sometimes have varicose dendrites (Bolam *et al.*, 1983a) and seem to resemble more closely the type III neurons of Dimova *et al.* (1980). The possibility should nevertheless be considered that there may be two types of [^{3}H]GABA-accumulating cell in the neostriatum, distinguishable on the basis of the details of their dendritic morphology.

Aspiny neurons have also been studied in the monkey neostriatum with the electron microscope (Di Figlia *et al.*, 1980). Some of the fine structural details of the neurons were similar to the MA2 type described here, but the dendrites appeared more beaded. Only one of the illustrated neurons from the monkey neostriatum had an impregnated axon collateral and one efferent synapse was shown. The synaptic contact made by the latter was symmetrical and the bouton contained vesicles with a similar morphology to those of our type MA2 neuron. However, a larger sample is required before we can pursue this comparison further.

GIANT ASPINY NEURON

Large, or giant-sized, neurons have frequently been described as occurring in the Golgi-impregnated neostriatum (see Table 3), but in some studies the presence of numerous dendritic spines (Di Figlia *et al.*, 1976), or dendritic and somatic spines (Chang *et al.*, 1982; Chang & Kitai, 1982) has been reported. It was suggested (Di Figilia *et al.*, 1976; Chang *et al.*, 1982) that there are two types of giant neuron in the neostriatum, one with and one without spines on the soma. The giant neuron in this study probably corresponds to the giant neurons of Dimova *et al.* (1980), to the large neuron of Bishop *et al.* (1982) and to the type I neurons of Chang & Kitai (1982). Both our giant cell and those mentioned above displayed a rich granular endoplasmic reticulum, which is also a feature of the large, acetylcholinesterase-positive neurons of the neostriatum (Henderson, 1981). Large acetylcholinesterase-positive neurons in the neostriatum have recently been shown to contain choline acetyltransferase (Levey *et al.*, 1983). It can be speculated, therefore, that our giant neuron might be a cholinergic cell, but clearly more direct evidence is needed such as could be obtained by combining Golgi-impregnation with either immunocytochemistry (Freund & Somogyi, 1983; Somogyi *et al.*, 1983b) or acetylcholinesterase histochemistry (Bolam *et al.*, 1983b, 1984). Further support for this suggestion comes from comparison of the boutons of the axon collaterals of the giant neuron with those that contain immunoreactivity for choline acetyltransferase (see below).

SYNAPTIC INPUT TO ASPINY NEURONS (TABLE 1)

Examination of Golgi-impregnated aspiny neurons with the electron microscope allowed us to study their synaptic input. In general, axosomatic synapses were not very common, but axodendritic synapses were more readily found. The morphological

features of the afferent boutons varied (Table 1) and this indicates some specificity in the input to each type of neuron. It also provides further criteria by which the neuron types can be distinguished, e.g. afferent boutons forming symmetrical contacts with MA1 neurons were nearly all large. This represents another similarity between our type MA1 neuron and the somatostatin-immunoreactive neurons in rat striatum (Takagi *et al.*, 1983). In contrast, the boutons forming symmetrical synapses on MA2 neurons were of medium size. Furthermore, whereas neurons of type MA2 receive both symmetrical and asymmetrical synapses on their cell bodies, we have so far only found symmetrical synaptic contacts on the cell bodies of type MA1.

None of the aspiny neurons in the present study has a similar type of synaptic input to another kind of aspiny neuron in the rat neostriatum (Bolam *et al.*, 1981). The latter type of aspiny neuron was found to project to the substantia nigra from the ventral striatum and was called striatonigral type 2 (Bolam *et al.*, 1981). The size range of the perikarya of the striatonigral type 2 neurons would place them in the large or giant category. However, their synaptic input differs markedly from all other striatal neurons: the perikaryon and dendrites are ensheathed in boutons containing pleomorphic vesicles and forming symmetrical contacts (Bolam *et al.*, 1981). Many boutons of this type contain enkephalin immunoreactivity (Di Figlia *et al.*, 1982; Somogyi *et al.*, 1982).

The Golgi-impregnated giant aspiny neuron described in this study differs from the striatonigral type 2 neuron not only in its synaptic input, but also in its dendritic pattern, which is more branching and covers a smaller area. There is considerable confusion about the naming and comparison of striatal neurons revealed by various methods. Recently, Bolam *et al.* (1984) summarized previous studies on the large neurons of the striatum and concluded that, in addition to the striatonigral type 2 neuron, there are two other types of large neuron (see, for example, Pasik *et al.*, 1979; Dimova *et al.*, 1980; Chang *et al.*, 1982). The efferent connections of these two types remain to be established. The present study shows that one of these types of large neuron has local synaptic output within the neostriatum. Following the classification of Bolam *et al.* (1984), this cell is a type 1 large neuron and is probably cholinergic (for discussion see Satoh *et al.*, 1983; Bolam *et al.*, 1984). It should be noted that a large type of neuron in the cat putamen, which is rich in acetylcholinesterase and so may correspond to one of the giant aspiny types (Bolam *et al.*, 1983b, 1984), has been suggested to project to the neocortex (Parent *et al.*, 1981). The straight course of the partially impregnated axon in the present study could also indicate the efferent nature of this neuron.

SYNAPSES FORMED BY LOCAL AXON COLLATERALS OF ASPINY NEURONS (TABLE 2)

The main purpose of this study was to see whether the local axon collaterals of each of the three types of aspiny neuron examined formed synapses in the neostriatum. It was found that boutons of each type of neuron establish symmetrical synaptic contacts with dendritic profiles, as well as with spines (Table 2). The lack of any axosomatic contacts in our sample of 35 identified synaptic boutons is noteworthy, since these are usually

symmetrical (Somogyi & Smith, 1979; Bolam *et al.*, 1981; present study) and have previously been assumed to derive from intrinsic neurons. This suggests either that there are further local circuit interneurons that innervate the perikarya or that some of the axosomatic contacts may be supplied by extrinsic afferents, as has recently been found (Freund *et al.*, 1984).

Although the ultrastructural features of the gold-toned boutons were not always easy to discern, due partly to poor preservation and partly to heavy deposits of gold, we were able to distinguish between the boutons of the giant aspiny neuron and those of the MA1 and MA2 neurons. The boutons of MA1 and MA2 local axon collaterals were of medium size and contained small round or oval electron-lucent vesicles, while the boutons from the giant aspiny neuron contained substantially larger, electron-lucent vesicles and are probably identical with the boutons in the neostriatum that are immunoreactive for choline acetyltransferase (Wainer *et al.*, 1983).

The postsynaptic targets of all the identified synaptic boutons were dendritic elements (Table 2). Although dendritic shafts (sometimes shown to bear spines) were the principal target, it is noteworthy that dendritic spines were identified as targets of eight of the boutons. In the neostriatum, such symmetrical axospinous contacts are much less common than asymmetrical contacts; thus, in the monkey only 8% of the axospinous contacts have symmetrical specializations (Pasik *et al.*, 1976). We have now shown that the origin of these symmetrical axospinous synaptic boutons is diverse: not only do they come from all three types of aspiny neuron described here, but also from the local axon collaterals of the medium-size densely spiny neurons that receive input from the cortex and project to the substantia nigra (Smith *et al.*, 1981; Somogyi *et al.*, 1981a). The boutons of the latter type of medium-size spiny neuron contain packed large electron-lucent vesicles and can thus be distinguished from boutons of MA1 and MA2 neurons, though they are somewhat similar to those of the giant aspiny neuron.

The diverse origin of boutons forming symmetrical axospinous contacts is consistent with immunohistochemical studies which show that such boutons in the rat neostriatum can show immunoreactivity to either glutamate decarboxylase (Ribak *et al.*, 1979), enkephalin (Di Figlia *et al.*, 1982; Somogyi *et al.*, 1982), substance P (Bolam *et al.*, 1983c), somatostatin (Di Figlia & Aronin, 1982; Takagi *et al.*, 1983), choline acetyltransferase (Wainer *et al.*, 1983) or tyrosine hydroxylase (Pickel *et al.*, 1981; Freund *et al.*, 1984). The vesicles in the enkephalin and substance P-immunoreactive boutons resembled those in boutons of axon collaterals from medium-size spiny neurons, while those in the somatostatin-immunoreactive boutons were similar to those of the MA1 or MA2 neurons and those in the choline acetyltransferase-immunoreactive boutons to the boutons of the giant aspiny neuron.

It is noteworthy that one of the boutons of a local axon collateral of an MA2 neuron formed a symmetrical synapse with a dendritic shaft of its parent neuron: this was established by electron microscopy and is thus an unequivocal example of what has been called an 'autapse' (van der Loos & Glaser, 1972; Calvet & Privat, 1980).

POSSIBLE FUNCTIONAL IMPLICATIONS

Our observations provide morphological evidence of possible chemical neurotransmission between three different types of aspiny neurons and the dendrites of other neurons within the neostriatum. The location of symmetrical synapses on the spines of dendrites could form the morphological basis for a local shunting of presumed excitatory input from the predominant asymmetrical axospinous terminals. This would be consistent with electrophysiological studies on rat striatal slices where it has been shown that shunting of test excitatory postsynaptic potentials in medium-size spiny neurons occurs in the absence of any detectable inhibitory postsynaptic potential (Lighthall *et al.*, 1981).

At least two of the neuron types (MA1 and MA2) would appear to be just local circuit neurons, since no aspiny neurons of this type have been retrogradely labelled so far in studies on striatal efferent neurons (see Bolam *et al.*, 1981; Smith *et al.*, 1981). The nature of the giant aspiny neuron is less certain, because large striatal neurons have been retrogradely labelled from the neocortex (Jayaraman, 1980; Parent *et al.*, 1981; Reinoso-Suarez *et al.*, 1982) and some of these in the cat putamen also contained acetylcholinesterase (Parent *et al.*, 1981). It is possible, therefore, that the giant aspiny neuron subserves both a local circuit function and acts as an efferent neuron, in the same way as the medium-size, densely spiny neuron (Kitai, 1981; Somogyi *et al.*, 1981).

Our finding that both the MA1 and MA2 types of aspiny neuron (see also Bolam *et al.*, 1983a), as well as the medium-size spiny neuron (see Somogyi *et al.*, 1981), each receive input from boutons forming asymmetrical membrane specializations may be a clue to the processing of information in the striatum, since it is likely that most, if not all, boutons forming asymmetrical synapses originate from neurons outside the striatum (Kemp & Powell, 1971; Hassler *et al.*, 1977). This indicates that the processing of extrinsic inputs occurs in a parallel fashion rather than serially.

It can be concluded that local circuit interactions within the neostriatum involve at least the three aspiny types studied here as well as the medium-size spiny neuron. Studies on the neurotransmitters and afferent connections of these identified neurons are now necessary in order to gain insight into their possible functions.

Acknowledgements

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