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A SECOND TYPE OF STRIATONIGRAL NEURON: A COMPARISON BETWEEN RETROGRADELY LABELLED AND GOLGI-STAINED NEURONS AT THE LIGHT AND ELECTRON MICROSCOPIC LEVELS

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Abstract—In a light and electron microscopic examination of the neostriata of rats that had received injections of horseradish peroxidase into the ipsilateral substantia nigra, two morphologically distinct types of horseradish peroxidase-labelled neurons were observed. In confirmation of previous findings, one type was of medium-size and was characterized by Golgi-staining and gold-toning as the densely spinous type. The second type of neuron was in contrast, larger, had an indented nucleus and numerous cytoplasmic organelles. The synaptic input to the perikarya of the latter neurons consisted of numerous boutons containing large round and oval vesicles. The boutons formed symmetrical synaptic contacts and were similar to those of the local axon collaterals of medium-size densely spiny striatonigral neurons.

In an attempt to establish what type of Golgi-impregnated neuron the second type of horseradish peroxidase-labelled neuron was, seventeen Golgi-stained or gold-toned neurons were examined in the electron microscope. Three of them were very similar in their ultrastructural features and synaptic input to the horseradish peroxidase-labelled neurons. All three were of a similar morphological appearance in the light-microscope and characteristically had long (up to 700 μm), essentially smooth dendrites. Both the large horseradish peroxidase-labelled neurons and the Golgi-impregnated neurons with long dendrites have so far only been found in the most ventral regions of the neostriatum.

It is concluded that there are at least two morphologically distinct types of striatonigral neurons.

FROM the numerous Golgi studies that have been performed on the mammalian neostriatum, there is general agreement that there are up to six morphologically distinct types of neuron. These neurons have been classified into two major groups, medium-size and large, and further subdivided according to the presence or absence of dendritic spines and the arrangement of dendrites. The most frequently impregnated neuron is the medium-size densely spinous type, the remainder comprising medium-size and large neurons with sparsely spiny or smooth dendrites and a population of small neurons (for review see PASIK, PASIK & DI FIGLIA, 1979; see also DANNER & PFISTER, 1979; DIMOVA, VUILLET & SEITE, 1980).

The major projection areas of neostriatal efferents are the globus pallidus and the substantia nigra; attempts to correlate the type of projection neurons with Golgi-stained neurons have led to conflicting opinions. Thus, LEONTOVICH (1954) considered that the medium-size neurons with densely spinous dendrites are efferent cells while the classic views of the Vogts (VOGT & VOGT, 1920), supported by KEMP & POWELL (1971*a*) and FOX, ANDRADE, HILLMAN & SCHWYN (1971), were that these neurons are local circuit neurons and that the large neurons are the efferent neurons of the neostriatum. Any conclusions drawn from Golgi material must, however, be tentative since, unless a Golgi-stained axon is traced outside the striatum, it cannot be stated with certainty that the neuron projects. Even if the axon is traced outside the neostriatum, the target area of the neuron cannot be determined unless an axonal aborization is observed.

A different approach to the study of efferent neurons is the labelling of cell somata following the retrograde transport of substances taken up at the axon terminals. In a study of the striatal and pallidal neurons projecting to the substantia nigra using the retrograde transport of horseradish peroxidase (HRP), GROFOVÁ (1975) established that at least one type of striatonigral neuron is of medium-size. This observation has been confirmed by other workers using the retrograde transport of horseradish peroxidase (HRP) (BUNNEY & AGHAJANIAN, 1976; SZABÓ, 1979) or Herpes simplex virus (BAK, MARHAM, COOK & STEVENS, 1978). However, since retrogradely-transported markers only label the somata, and sometimes the proximal dendrites of efferent neurons, information concerning the morphology of the efferent neuron is limited.

The morphology and ultrastructural features of one type of striatonigral neuron have been described by SOMOGYI & SMITH (1979) using a combination of the retrograde transport of HRP and Golgi-staining in one animal. It was demonstrated that the medium-size densely spinous neuron of the neostriatum can be retrogradely-labelled by injection of HRP in the sub-

Abbreviations: GABA, γ-aminobutyrate, HRP, horseradish peroxidase, STN, striatonigral neuron.

stantia nigra. These observations have been confirmed (SOMOGYI, HODGSON & SMITH, 1979; SOMOGYI, BOLAM & SMITH, 1981*a*) and are supported by the results of PRESTON, BISHOP & KITAI (1980), who showed that medium-size densely spinous neurons, identified by intracellular injection of HRP, may occasionally be antidromically stimulated from the substantia nigra.

However, these findings do not exclude the occurrence of a second type of striatonigral neuron. This is made likely by the presence of two putative transmitters, γ-aminobutyrate (GABA) and substance P, in the striatonigral pathway (BROWNSTEIN, MROZ, TAPPAZ & LEEMAN, 1977; GALE, HONG & GUIDOTTI, 1977; KANAZAWA, EMSON & CUELLO, 1977; JESSELL, EMSON, PAXINOS & CUELLO, 1978; STAINES, NAGY, VINCENT & FIBIGER, 1980). More direct evidence of a second type of striatal efferent neuron has come from retrograde tracing studies. In addition to medium-size striatal neurons labelled with Herpes virus following injection in the substantia nigra, BAK *et al.* (1978) also observed a small proportion of large labelled neurons. SZABÓ (1979) detected occasional large striatal neurons in the cat and monkey that were retrogradely-labelled with HRP following injection into the substantia nigra and in a report by GROFOVÁ (1979) some large striatal neurons, also in the cat, were labelled following HRP injection into the retrorubral nucleus. This area has been suggested to be a dorsal extension of the zona compacta of the nigra (SZABÓ, 1979).

We have looked for a second type of striatonigral neuron in rats that received injections of HRP in the substantia nigra. The ipsilateral neostriatum from each of these animals was processed to reveal HRP activity and was then impregnated by the Golgi method, sometimes followed by gold-toning. Many striatonigral neurons, that were labelled with HRP, were also Golgi-stained but these were all of the medium-size densely spiny type and studies on these have been described elsewhere (BOLAM, SOMOGYI & SMITH, 1980; SOMOGYI *et al.*, 1981*a*). In the same material, some larger striatal neurons were also retrogradely-labelled with HRP. We have now studied these neurons in the electron microscope and compared their cytological characteristics and afferent synaptic boutons with those of Golgi-impregnated neurons.

EXPERIMENTAL PROCEDURES

All experiments were performed on female albino Wistar rats (150–170 g) using the stereotaxic atlas of KÖNIG & KLIPPEL (1963). The rats were anaesthetized with chloral hydrate (350 mg/kg i.p. in 0.9% w/v NaCl) and injected into the substantia nigra with 6% HRP conjugated with wheat-germ agglutinin (WGA), prepared according to the method of GONATAS, HARPER, MIZUTANI & GONATAS (1979). The stereotaxic co-ordinates were: A, 2.2; L, 6.2; V, 6.4 using an oblique approach at an angle of 45°. The results of studies on HRP-labelled neurons were obtained from three rats. Rat 1 received 20 nl of the HRP-WGA conjugate, rat 2 35 nl and rat 3 30 nl. All injections were made using glass micro-pipettes (tip diameter 20–30 μm) and a controlled gas pressure system (SOMOGYI *et al.*, 1979). Rat 1 and some of the animals from which Golgi-stained neurons were studied had received multiple electrolytic lesions in the cortex, as described previously (SOMOGYI *et al.*, 1981*a*). Twenty hours following the injection of HRP the animals were re-anaesthetized and perfused through the heart with approximately 20 ml of 0.9% (w/v) NaCl followed by approximately 200 ml of fixative consisting of 2.5% glutaraldehyde, 0.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The area of HRP injection, i.e. the mesencephalon, was removed and washed in buffer followed by 15% sucrose in buffer. Forty μm cryostat sections were then taken and stained for HRP histochemistry using *o*-tolidine (dimethylbenzidine) as a substrate, or were Nissl stained. The neostriatum was then sliced in 1 mm thick blocks (necessary for the demonstration of HRP endproduct and Golgi impregnation in the same material), processed to reveal retrogradely-transported HRP and subsequently Golgi-stained, sectioned at 80 μm with a tissue chopper and the majority of the sections were then gold-toned. The combined HRP histochemistry Golgi-staining procedure has been described in detail elsewhere (SOMOGYI *et al.*, 1979; SOMOGYI *et al.*, 1981a).

The Golgi-sections were examined in the light-microscope; cells of interest (i.e. HRP-labelled perikarya) were photographed, their position within the striatum noted and they were re-embedded for examination in the electron microscope. Golgi-stained and gold-toned neurons were also drawn using a Leitz Camera Lucida and a X 100 oil immersion objective. Serial ultrathin sections (silver/grey) were mounted in single slot formvar-coated grids and examined using a Philips 201C electron microscope with 20–30 μm objective apertures. In order to improve contrast the Golgi sections were stained *en bloc* with uranyl acetate and the ultrathin sections with lead citrate (SOMOGYI, 1978). All the measurements given in the paper were made on Golgi sections and have not been corrected for shrinkage.

RESULTS

The location of horseradish peroxidase reaction endproduct at injection sites

The extent of spread of the injected HRP is shown in Fig. 1. It is important to note that when *o*-tolidine is used as a substrate a much greater area of reaction end-production is observed than when diaminobenzidine is used (SOMOGYI *et al.*, 1979). In all three animals, the heaviest area of reaction endproduct was confined to the substantia nigra, including parts of both the zona compacta and zona reticulata. In animal number 3, there was a spread of the most marginal zone to the red nucleus, parts of the reticular formation and the ventral tegmental area. In animals 1 and 2, there was a slight spread beyond the substantia nigra but this was only in the most marginal zones. However, as has been previously reported (SOMOGYI *et al.*, 1981*a*), even with the largest injection, which included most of the substantia nigra, retrograde labelling occurred only in restricted parts of the neostriatum indicating that the area of HRP uptake into terminals is smaller than that revealed by histochemical examination 20 h after injection of the enzyme.

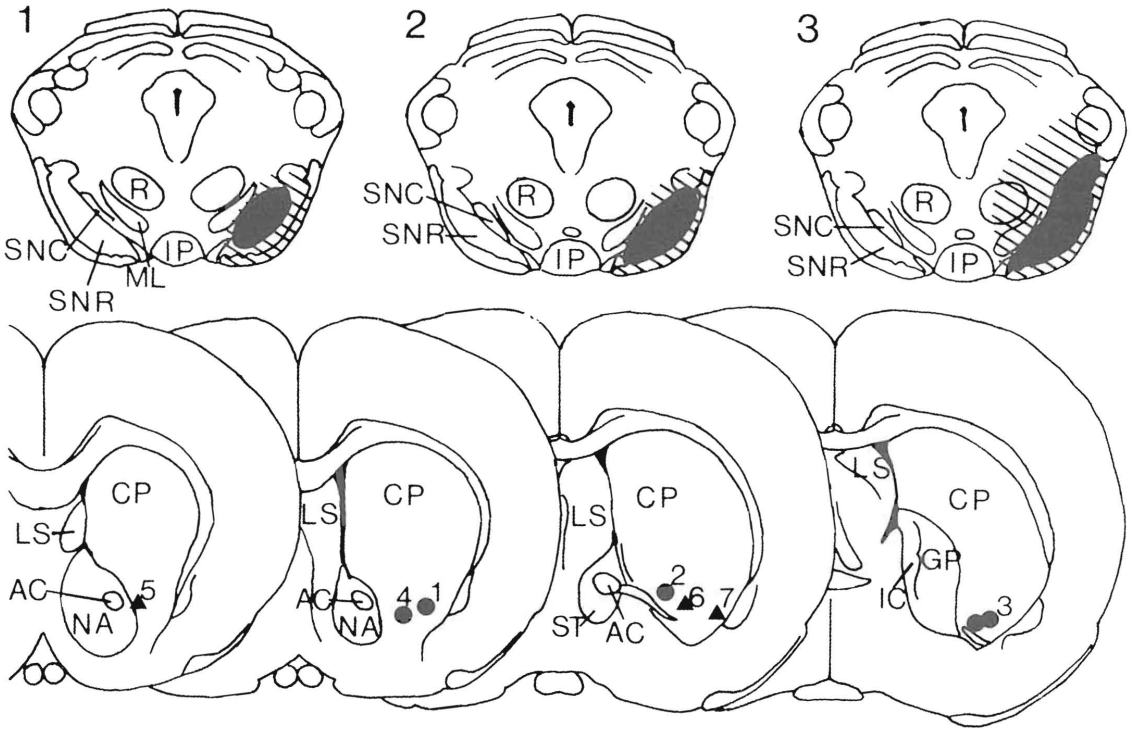

FIG. 1. The upper part illustrates the site of injection of horseradish peroxidase into the substantia nigra. The black area indicates the most heavily stained area at the centre of the injection; the hatched area indicates the maximum extent and spread of the HRP or reaction end-product with *o*-tolidine as substrate. The lower half illustrates the location within the neostriatum of the HRP-labelled striatonigral type 2 neurons (black circles) and the similar Golgi-stained neurons (black triangles). Neurons 1, 3, 4, 6 and unlabelled black circle came from rat 1; neuron 2 from rat 2. Neurons 5 and 7 were from different animals. The location of the neuron from rat 3 was not recorded. *Abbreviations*: AC, anterior commissure; CP, caudate-putamen (neostriatum); IC, internal capsule; IP, interpeduncular nucleus; LS, lateral septal nucleus; ML, medial lemniscus; SNC, substantia nigra zona compacta; SNR, substantia nigra zona reticulata; ST, stria terminalis; NA, nucleus accumbens; R, red nucleus.

Appearance of horseradish peroxidase reaction endproduct in retrogradely-labelled neurons in the neostriatum

The use of horseradish peroxidase conjugated with wheat-germ agglutinin resulted in the labelling of a greater proportion of neurons in the neostriatum than with HRP alone. The reaction end-product was mainly of the homogenous type, varying from a light orange colour in lightly labelled neurons (Fig. 2A) to a deep red colour in heavily labelled neurons. The appearance of the reaction end-product in the electron microscope varied according to the intensity of labelling. Thus, in lightly labelled neurons the reaction end-product was not visible in the electron microscope (Figs 2B, 3, 5); in moderately labelled neurons there were electron-dense areas in the cytoplasm and, occasionally a rim of electron-dense material along the cell membrane (Fig. 7A), while in heavily labelled neurons the cytoplasm was filled with an electron-dense material, which obscured most of the ultrastructural details of the cytoplasm, with some reaction end-product in the nucleus (Fig. 6). On some occasions (results not presented) the whole of the nucleus was obscured by reaction end-product. The presence of large amounts of reaction end-product made cutting ultrathin sections for the electron microscope difficult; the sections were often torn and the reaction end-product had a ruffled appearance (Fig. 6).

The amount of HRP reaction end-product in the retrogradely-labelled neurons is presumably related to two factors. First, the amount of HRP retrogradely transported; second, the position of the neuron within the 1 mm slice when incubated with the reaction mixture; in those cells that are located close to the surface of the slice, i.e. in a position where the substrate does not have to penetrate far, a greater amount of reaction product will be formed than in those cells located in or close to the centre of the block.

It does not matter that the reaction end-product is not visible in the electron microscope in lightly labelled neurons since they were first of all identified as HRP-labelled, and thus striatonigral neurons, in the light-microscope and were then examined in the electron microscope by direct correlation (Fig. 2).

Medium-size striatonigral neurons

The majority of HRP-labelled neurons were medium-sized with a slightly oval shape (10–14 μm in

diameter) with a round or oval nucleus that filled most of the cytoplasm. All the neurons that were both retrogradely-labelled with HRP and Golgi-stained were of the medium-size densely spinous type (Fig. 2A, C) (see also SOMOGYI & SMITH, 1979; SOMOGYI *et al.*, 1979; SOMOGYI *et al.*, 1981*a*). In the electron microscope these neurons have a centrally located smooth, round or oval nucleus with a relatively thin rim of cytoplasm which is poor in organelles except mitochondria (Fig. 2C) (see also SOMOGYI & SMITH, 1979; SOMOGYI *et al.*, 1979; SOMOGYI *et al.*, 1981*a*). The ultrastructural appearance agrees well with that observed by others for medium-size densely spiny neurons that were simply Golgi-stained (DIMOVA *et al.*, 1980; DI FIGLIA, PASIK & PASIK, 1980*b*). We propose to call this type of striatonigral neuron striatonigral neuron type 1 (STN type 1). The synaptic input to these neurons has been described previously (SOMOGYI & SMITH, 1979); the axosomatic synapses consist of a sparse input of boutons containing flattened or pleomorphic vesicles and making symmetrical contact with the soma.

A second type of striatonigral neuron

In addition to the medium-size neurons, six HRP-labelled neurons were observed in the light-microscope, and subsequently in the electron microscope, that were markedly dissimilar from the medium-size HRP-labelled cell somata and the identified STN type 1. Four were found in rat 1, and one each in rats 2 and 3, and all were located (excluding the neuron in rat 3, the position of which is not indicated) in the most ventral regions of the neostriatum (Fig. 1). We propose to call these neurons striatonigral neuron type 2 (STN type 2). In the light-microscope, they had an oval shape, larger than the medium-size neurons (20–30 μm long axis; 11–18 μm short axis), with at least one thick primary dendrite visible (2.5–5.6 μm in width). The nucleus was fairly large and there was a proportionally larger area of cytoplasm. The nucleus occasionally had a slightly irregular form and contained a large prominent nucleolus (diameter 2.8–4.2 μm) (Fig. 2A).

In the electron microscope the size and shape of the neurons was confirmed: they were round or oval depending on the plane of section (Figs 2B, 3A, 5, 6, 7A). The relatively large area of cytoplasm was rich in organelles including numerous mitochondria, ribosomes (mainly arranged as polysomes), several regions of Golgi apparatus, well developed granular endoplasmic reticulum, often arranged as Nissl bodies, and a moderate number of dense bodies (Figs 2B, 3A, 5). One of the most characteristic features of these neurons were the prominent nuclear indentations (Figs 2A, 3A, 5, 6, 7A).

The synaptic input to the perikarya of these neurons was also characteristic and markedly different from that to STN type 1 neurons. The input was extremely heavy, as many as 24 vesicle-containing boutons were seen in close apposition to the perikaryal membrane in a single section, with as many as 12 in synaptic contact (Figs 3A, B, 7A). The boutons were fairly large (up to 1 μm in width) and contained large round and oval vesicles with occasional dense-core vesicles. Presynaptic dense projections were conspicuously well developed (Fig. 3B). Some of the boutons contained fewer vesicles congregated at the synaptic site and generally contained at least one mitochondrion (Fig. 3B) while others contained numerous tightly packed vesicles without any mitochondria (Fig. 3C), but the morphology of the vesicles and structure of the synaptic specialisation was the same. All the axosomatic synaptic boutons observed so far have had symmetrical membrane specialisations (Figs 3B, C).

The initial parts of the proximal dendrites were also rich in cytoplasmic organelles (Figs 4A, 7A) but these become scarce in the more distal regions. The synaptic input to the proximal dendrites was also very heavy, consisting mainly of boutons in symmetrical membrane contact, very similar to those forming axosomatic synapses (Figs 4B, 7C). Less frequently observed were synaptic boutons forming asymmetrical membrane contacts and containing small round vesicles (Fig. 4B). The rarest type contained very few flattened or pleomorphic vesicles and was in symmetrical membrane contact with the dendrites (Fig. 7B).

Comparison of striatonigral type 2 neurons with Golgi-stained neurons

As we were unable to detect any neurons that were both Golgi-stained and retrogradely-labelled with HRP from the substantia nigra other than the medium-size densely spiny type, a study of the ultrastructural appearance of different types of striatal neurons, that were either Golgi-stained or Golgi-stained and gold-toned, was carried out to see if any of them had morphological features similar to those of the STN type 2 neurons. In all, 17 neurons were examined in the electron microscope; they were selected for study on the following basis: (1) smooth or sparsely spiny dendrites; (2) soma size larger than STN type 1 neurons.

Although most of the neurons studied have some ultrastructural similarities with STN type 2 neurons, most notably the indented nucleus; only one type had both similar ultrastructural features and a similar type of axosomatic synaptic input. Three neurons of this type were found, one of which was in rat 1 (number 6 in Figs 1 and 8), and all three were located in the most ventral regions of the neostriatum (Fig. 1). These neurons had an oval cell body with dimensions of approximately 25 μm in the long axis and 12–16 μm in the short axis with up to five primary dendrites, one of which was very thick (3.5–5.5 μm) and came from the pole of the cell located dorsally or medially. The thick primary dendrites were smooth and fairly straight, and divided up to 3 times. They extended as far as 700 μm from the cell soma and

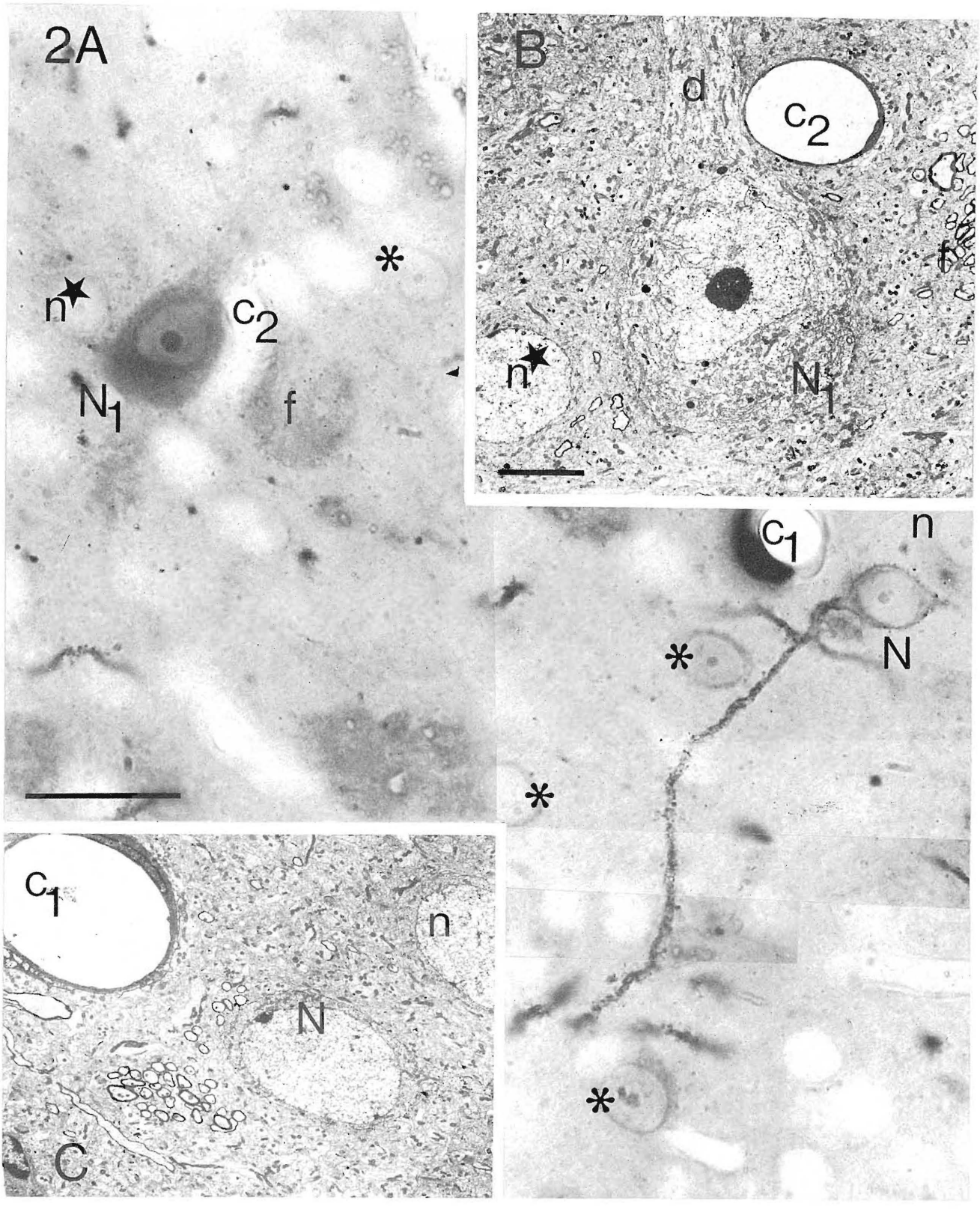

FIG. 2. Correlation between light and electron microscopic levels of investigation. (A) Light microscopic photomontage showing neuron number 1 (N_1) in Fig. 1 and a Golgi-stained gold-toned medium-size densely spiny neuron (N) that was also retrogradely-labelled following injection of HRP into the substantia nigra. Asterisks denote medium-size neurons that were only HRP-labelled. The micrographs were taken from the surface of the resin block in which the cells were re-embedded. (B) Low power electron micrograph of striatonigral type 2 neuron N_1 in A. Additional structures common to both micrographs are the neuron labelled n*, capillary, C_2 and fibre bundle, f. Higher power electron micrographs of this neuron are shown in Figs 3 and 4. (C) Low power electron micrograph of striatonigral type 1 neuron labelled N in A. Additional structures common to both micrographs are capillary, C_1 and neuron, n. Scales: A, 25 μm; B and C, 5 μm.

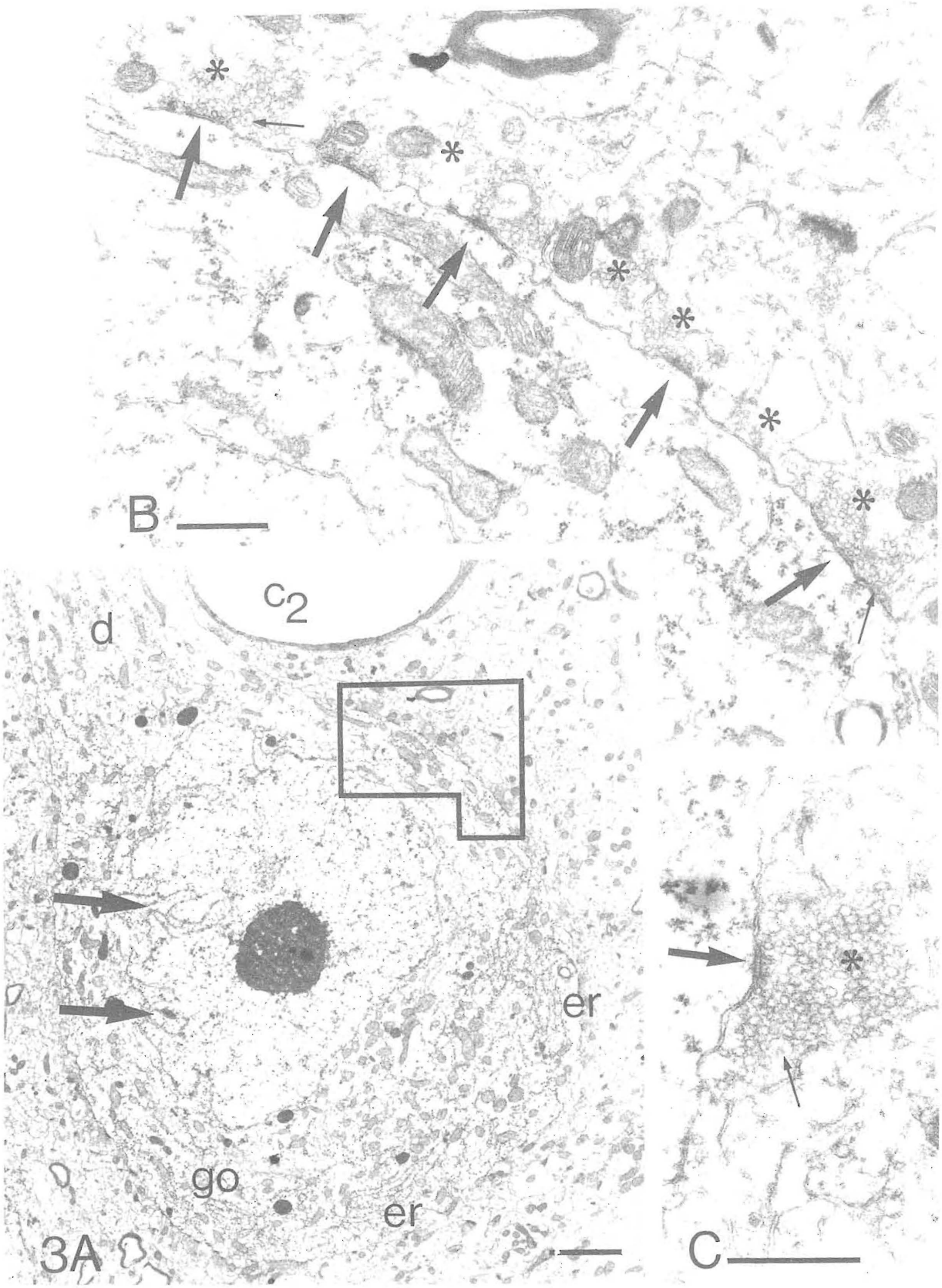

FIG. 3. Medium power electron micrograph of the striatonigral type 2 neuron (N_1 in Figs 1, 2A, B). Capillary (C_2) also appears in Figs 2A, B. This neuron was only lightly labelled with HRP: the reaction end-product is not visible in the electron microscope. Note indented nucleus (arrows) areas of endoplasmic reticulum (er) and Golgi apparatus (go) and the thick primary dendrite (d), the continuation of which appears in Fig. 4A. (B) Higher power electron micrograph of boxed area in A. Six boutons (asterisks) containing large round and oval vesicles are in close apposition to the perikaryal membrane. Four of the boutons are in symmetrical synaptic contact with the neuron (arrows). Small arrows: dense-core vesicles in the boutons. (C) High power electron micrograph of another bouton (asterisk) in symmetrical synaptic contact (arrow) with the perikaryon of neuron N_1. This bouton contains numerous tightly packed vesicles and a dense core vesicle (small arrow). Scales: A, 2 μm; B, 0.5 μm; C, 0.5 μm.

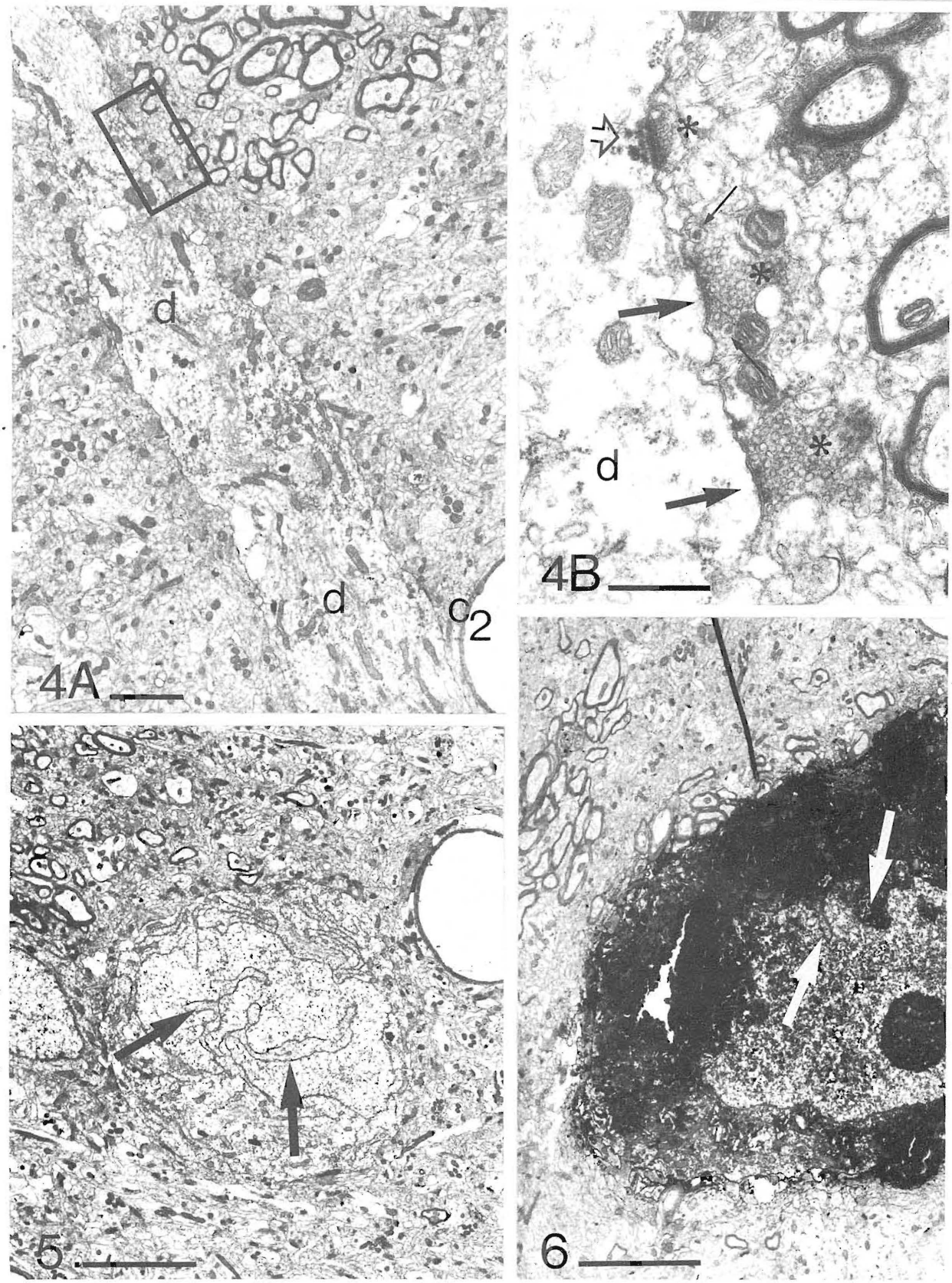

FIG. 4. Medium power electron micrograph of the thick primary dendrite (d) of the neuron (N_1) in Figs 2A, B and 3. The dendrite is a continuation of that shown in Fig. 3A and the capillary (C_2) also appears in those micrographs. The boxed area is shown at higher power in B. (B) High power electron micrograph of boxed area in A but a few sections away. Three boutons (asterisks) are in synaptic contact (arrows) with the dendritic shaft (d). Two of the boutons make symmetrical synaptic contact (filled arrows) and a third makes asymmetrical synaptic contact (open arrow). Small arrows: dense-core vesicles. Scales: A, 2 μm; B, 0.5 μm.

FIG. 5. Electron micrograph of the cell body of the horseradish peroxidase-labelled neuron (striatonigral type 2) from rat 2 (neuron 2 in Fig. 1). Note deeply invaginated nucleus (arrows). Scale: 5 μm.

FIG. 6. Electron micrograph of a striatonigral neuron (neuron 3 in Fig. 1) heavily labelled with horseradish peroxidase (STN type 2). The electron-dense reaction end-product has obscured ultrastructural details of the cytoplasm. Note indented nucleus (arrows). Scale: 5 μm.

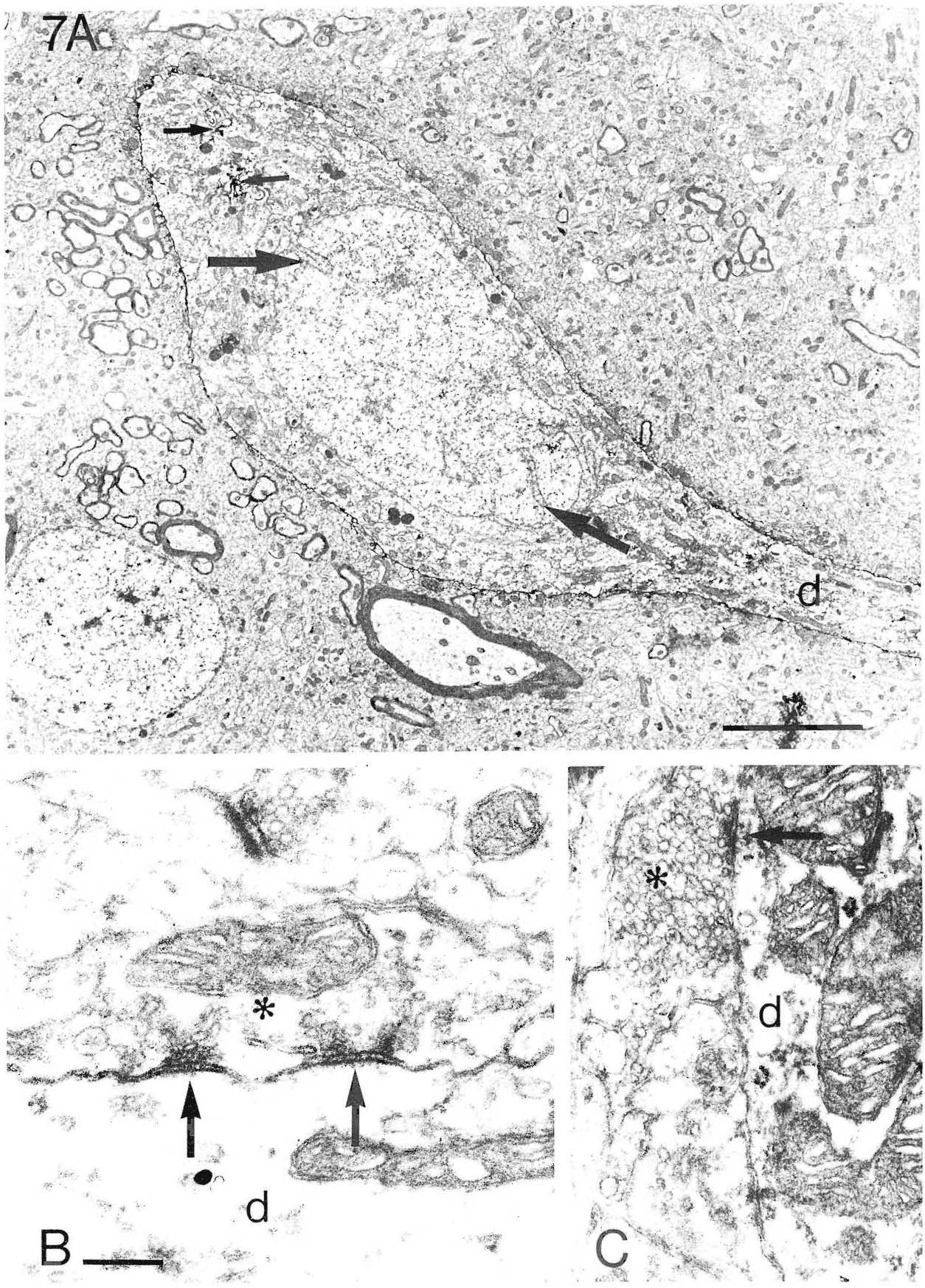

FIG. 7. (A). Low power electron micrograph of striatonigral type 2 neuron, number 4 in Fig. 1. The HRP reaction end-product can be seen as a thin rim of electron-dense material along the cell membrane and occasional electron-dense particles in the cytoplasm (small arrows). Note the indented nucleus (large arrows) and thick primary dendrite (d). (B and C) Axonal boutons (asterisks) in symmetrical membrane contact (arrows) with the primary dendrite (d) of the neuron illustrated in A. The bouton in C contains large round and oval vesicles; the bouton in B contains pleomorphic vesicles. Scales: A, 5 μm; B and C, 0.2 μm.

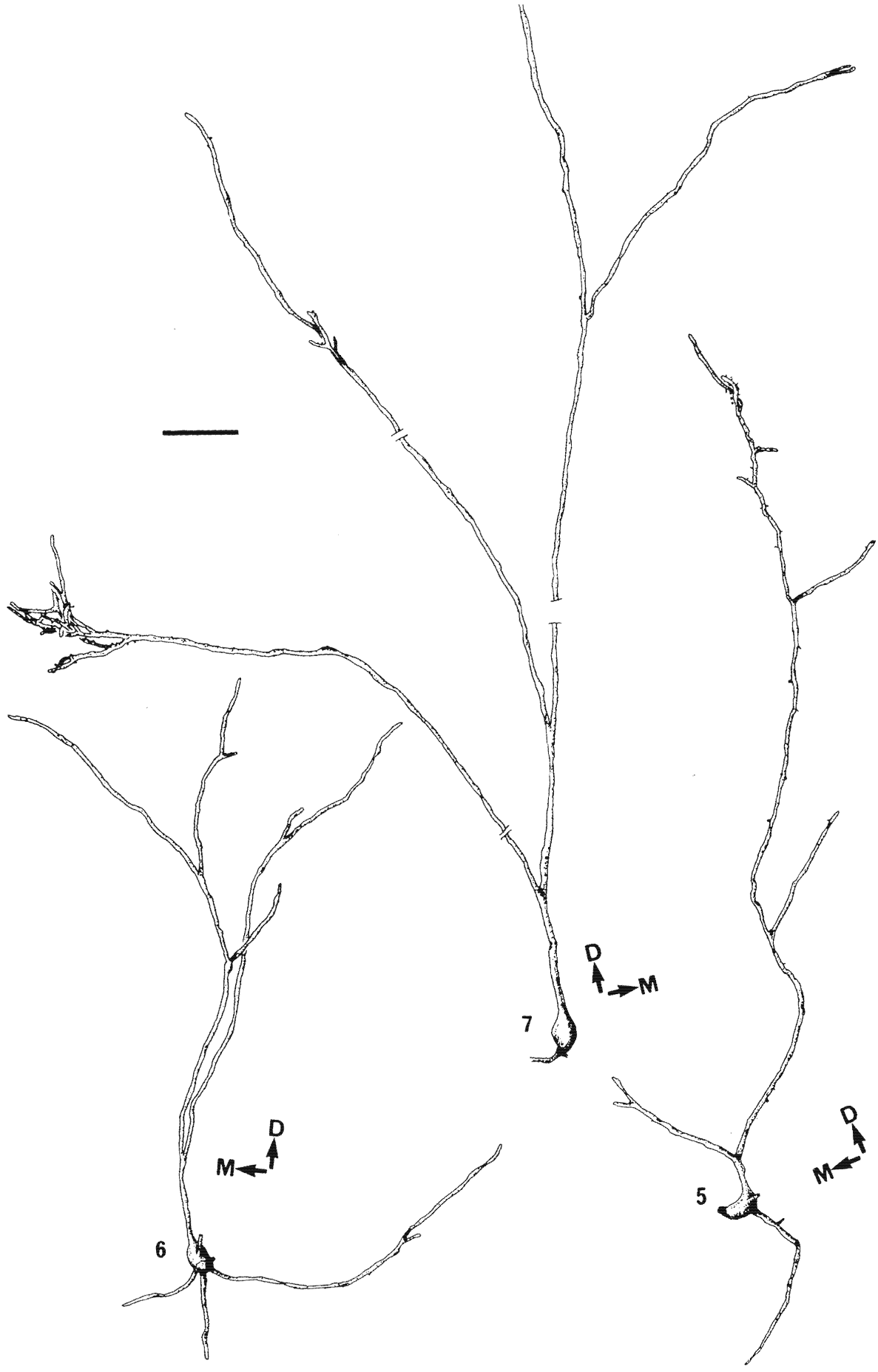

FIG. 8. Camera lucida drawing of 3 Golgi-stained neurons from the neostriatum that were ultrastructurally similar and had a similar synaptic input to striatonigral type 2 neurons. Numbers refer to the numbers in Fig. 1, where the location of these neurons within the striatum is shown. Neuron 5 was only Golgi-stained while 6 and 7 were also gold-toned. D, dorsal; M, medial. Scale: 50 μm.

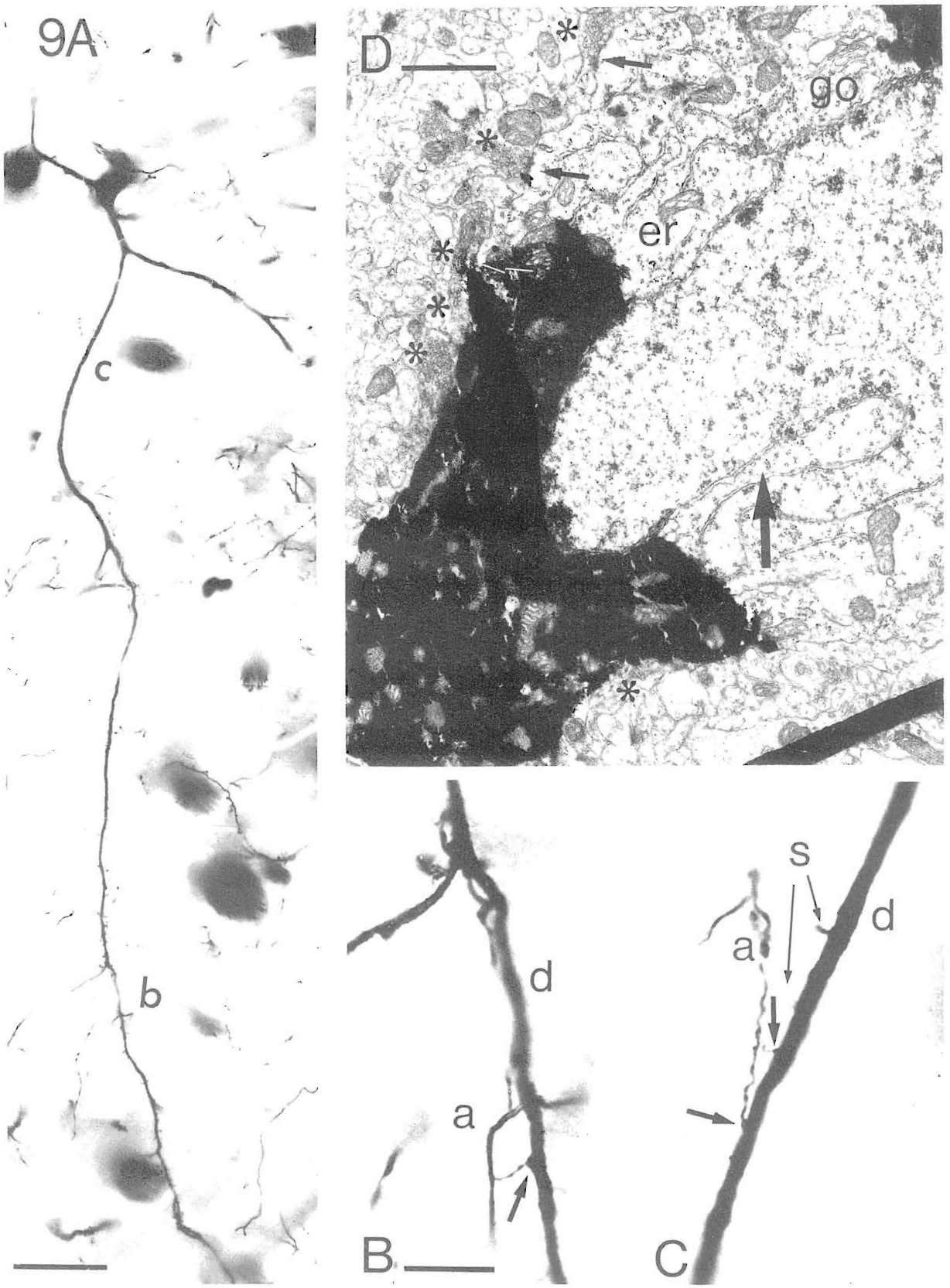

Fig. 9. Photomontage of Golgi-stained neuron, number 5 in Figs 1 and 8. Areas labelled b and c are shown at high power in B and C respectively. (B) The essentially smooth dendritic shaft (d) has Golgi-stained axons (a) in close apposition, one of which (arrow) appears to form a bouton and make contact with the shaft. This axon was seen to join a bundle of five fibres. (C) A different axon (a) which also appears to make contact (arrows) with the dendritic shaft (d). Note presence of occasional dendritic spines (s). (D) Medium power electron micrograph of the same neuron. The perikaryon was only partially impregnated with the electron-dense Golgi deposit. Note the deep nuclear indentation (large arrow), endoplasmic reticulum (er) and Golgi apparatus (go). Six vesicle-containing boutons (asterisks) are in close apposition with the perikaryal membrane, three of which are in symmetrical synaptic contact (small arrows). Scales: A, 50 μm; B and C, 10 μm; D, 1 μm.

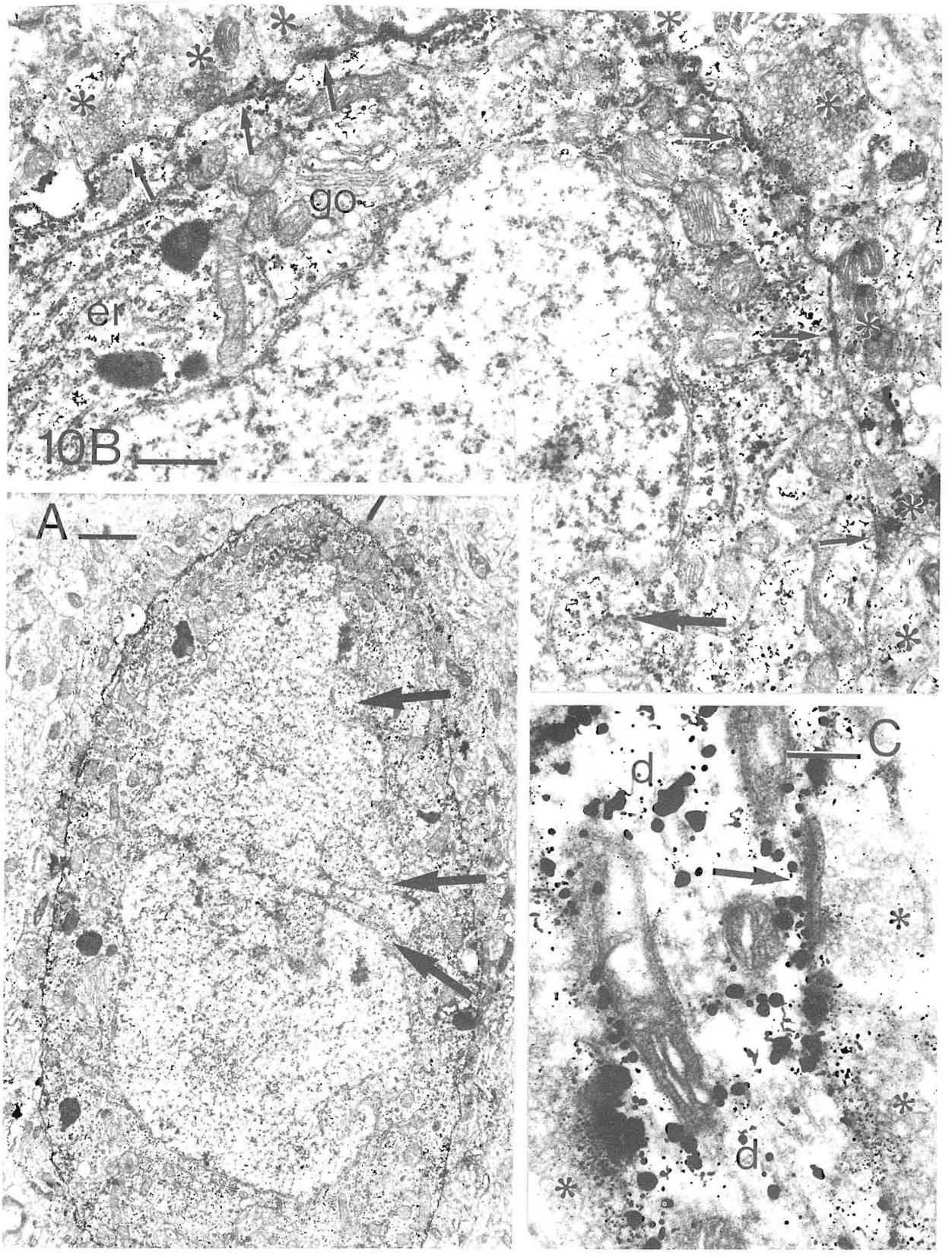

FIG. 10. (A) Low power electron micrograph of the Golgi-stained and gold-toned neuron, number 6 in Figs 1 and 8. The nucleus has several indentations (arrows) and the cytoplasm is rich in organelles. (B) Higher power electron micrograph of upper part of the neuron in A. Eight vesicle-containing boutons (asterisks) are in close contact with the cell membrane, 6 of which are in symmetrical synaptic contact (small arrows). Large arrow, nuclear indentation; er, endoplasmic reticulum; go, Golgi apparatus. (C) High power electron micrograph of a distal dendrite (d) of the same neuron. Three boutons (asterisks) are in close apposition to the dendrite; the lower two contain large round vesicles and are similar to the axosomatic boutons and those making synaptic contact with the striatonigral type 2 neurons; the upper one, which is in asymmetrical synaptic contact (arrow) contains small round vesicles. Scales: A, 1 μm; B, 0.5 μm; C, 0.2 μm.

projected in either a dorsal or medial direction. Occasional dendritic spines were observed. The three neurons studied were not completely impregnated but in one of them (number 7 in Figs 1 and 8) the dendrites were traced in two adjacent sections; one of the branches of a thick primary dendrite terminated in a dense aborization of thinner dendrites with more numerous spines (Fig. 8).

The perikarya of all 3 of these neurons were examined in the electron microscope (Figs 9D and 10). The ultrastructural features were similar to those of STN type 2 neurons, i.e. indented nucleus, numerous cytoplasmic organelles including mitochondria, ribosomes, Golgi apparatus and well developed granular endoplasmic reticulum. (Compare Figs 9D and 10A, B with 3A, B; 5, 6, 7A). In addition, these neurons had a synaptic input both quantitatively and qualitatively similar to that of the STN type 2 neuron, i.e. numerous axosomatic boutons containing large round and oval vesicles and in symmetrical synaptic contact (Figs 9D, 10B). Although the preservation in the area of neuron number 6 was poor, preliminary observations indicate that both the proximal and distal dendrites also have a heavy synaptic input, consisting of boutons in symmetrical contact similar to those of the axosomatic synapses and boutons containing small round vesicles that were in asymmetrical synaptic contact with the dendrites (Fig. 10C). The number of asymmetrical synapses was greater in the distal regions of the dendrite.

DISCUSSION

The results confirm earlier observations that the major type of striatonigral neuron is of medium-size (Grofová, 1975; Bunney & Aghajanian, 1976; Bak *et al.*, 1978; Szabó, 1979) and that at least some of these cells belong to the medium-size densely spinous class as defined by Golgi-staining (Somogyi & Smith, 1979; Somogyi *et al.*, 1979; Somogyi *et al.*, 1981*a*). In addition, they clearly demonstrate the existence of a second class of striatonigral neuron that is morphologically distinct from the medium-size densely spinous type. These neurons may be similar to the 'rare large neurons' retrogradely-labelled with Herpes simples virus in the rat (Bak *et al.*, 1978) and the 'occasional large cell' retrogradely-labelled with HRP in the cat and monkey (Szabó, 1979). These reports, however, gave no morphological details of the labelled neurons. The large striatal efferent neurons in the cat described by Grofová (1979) may also be of the same type. However, these neurons were labelled following injection of HRP into the retrorubral nucleus and we have been unable to label striatal cells following injections of HRP into this area in the rat (unpublished observations). The ultrastructural features of the neuron type that degenerates following chronic isolation of a column of neostriatum in the cat (Hassler, 1979) are very similar to those in the present study, i.e. large size and indented nucleus. Furthermore, both neuron types have a heavy axosomatic synaptic input. However, the results of this technique must be viewed with caution because although there is evidence that medium-sized striatal neurons project (Grofová, 1975; Bunney & Aghajanian, 1976; Bak *et al.*, 1978; Szabó, 1979; Somogyi & Smith, 1979; Somogyi *et al.*, 1981*a*), the 'chronic isolation' method failed to demonstrate that any medium-size neurons projected from the neostriatum. This may be because the medium-size densely spiny neurons that project from the neostriatum have local axon collaterals (Bolam, Somogyi & Smith, 1980; Preston, Bishop & Kitai, 1980; Somogyi *et al.*, 1981*a*) and damage to only the projecting part of the axon may be insufficient to cause retrograde degeneration.

The question may be asked, why are striatonigral neurons of the second type so infrequently detected? In our material we found only six neurons, from three rats, that were positively identified (i.e. by ultrastructural examination) as a morphologically distinct type of striatonigral neuron. In all, areas of retrograde labelling were examined in 21 rats. Only three Golgi-stained neurons, from a collection of 29 rat brains, have been observed that also positively fit into this category, i.e. have ultrastructural features and synaptic input similar to the HRP-labelled neurons. In all the ultrastructural studies of the neostriatum (for references see review by Pasik *et al.*, 1979; also Di Figlia, Pasik & Pasik, 1980*a*; 1980*b*; Dimova *et al.*, 1980) except possibly that of Hassler (1979), neurons of this type have not been described. Thus, it is likely that these neurons represent only a small proportion of striatal neurons. It is important to note that the HRP-labelled neurons were only observed in those animals in which HRP conjugated with wheatgerm agglutinin was used. This material is a far more sensitive retrograde marker than HRP alone (Gonatas *et al.*, 1979). It is possible, therefore, that the axon terminal aborization of these neurons within the substantia nigra is spread over a large area or volume and only with the more efficient HRP conjugated with wheatgerm agglutinin is sufficient material taken up and retrogradely transported to allow for adequate histochemical demonstration of the HRP.

Comparison of retrogradely-labelled neurons of type 2 with Golgi-stained neurons

It would have been ideal to study the morphological features, in particular the form of the dendritic tree, of the second type of striatonigral neuron by examining neurons that were both retrogradely-labelled and Golgi-impregnated. However, probably due to the scarcity of these neurons, no 'double labelled' cells were observed. The next best approach to this problem is to compare ultrastructural features of the HRP-labelled STN type 2 neurons and Golgi-impregnated neurons. Of the Golgi-stained cells that were studied, only three had a similar ultrastructure and synaptic input to the STN type 2 neurons, and at

the light-microscopic level all three of these cells had a similar morphology and may be classed as one type. Thus, although the evidence is indirect it strongly suggests that the Golgi-stained cells that have long, predominantly smooth dendrites are of the same type as the HRP-labelled cells and therefore probably also project to the substantia nigra.

This type of Golgi-impregnated neuron does not appear to have been described in the literature (see review PASIK *et al.*, 1979; also DANNER & PFISTER, 1979; DIMOVA *et al.*, 1980). There are some similarities to the larger version of the spiny type II described by DI FIGLIA, PASIK & PASIK (1976) in the monkey, namely the size of the soma and dendritic radius. However, these neurons have a higher density of spines (5 spines/10 μm), while only occasional spines were observed in the present material. Additionally, there are differences in the arrangement of the dendrites. This comparison is limited to light-microscopic morphology as there are no published electron micrographs of identified large spiny type 2 neurons and the possibility of species differences cannot be excluded. The type IV neurons of DIMOVA *et al.* (1980) are similar in light-microscopic appearance, i.e. similar shape of soma and arrangement of dendrites, although the soma is slightly smaller and the dendrites shorter. However, the ultrastructural appearance of Dimova's type IV neurons is different from the neurons described here, in that the amount of cytoplasm is less, there is no endoplasmic reticulum arranged as Nissl bodies and examination of the electron micrographs shows only few boutons in close apposition to the perikaryal membrane. It is possible, therefore, that the neurons with long, predominantly smooth dendrites are a class of striatal neurons that has not been described previously.

The classification of neurons on the basis of their light or electron microscopic appearance is useful as long as the categories are sufficiently distinct. In the striatum, however, there is only one category of neuron that is universally recognised: the medium-size densely spiny neuron (some of which have been identified as striatonigral type 1 neurons). The lack of general agreement about the different categories of striatal neurons with smooth or sparsely spiny dendrites emphasizes the importance of using criteria based on connectivity (input-output relationships), as in the present study, when trying to establish what different types of neuron occur in a complex area of the brain.

Functional considerations

All the HRP-labelled and Golgi-impregnated neurons presented in this paper (excluding the neuron from rat 3, the position of which was not noted) lay in the most ventral regions of the neostriatum. It is possible that this does not represent a true distribution because of the small sample, but is does, nevertheless, raise the possibility that the neostriatum is not homogeneous in its cytoarchitecture. Although the majority of cellular studies of the neostriatum (PASIK *et al.*, 1979) suggest that it is homogeneous, there are indications that there are functionally distinct regions (NEILL & HERNDON, 1978; NEILL, PEAY & GOLD, 1978) and it is clear that afferents from different areas project preferentially to different parts of the neostriatum (GRAYBIEL & RAGSDALE, 1979). Histochemically, marked inhomogeneities have been found in the distribution of acetylcholinesterase (GRAYBIEL & RAGSDALE, 1978; 1980) and several putative transmitter substances (GRAYBIEL, RAGSDALE, YONEOKA & ELDE, 1981). One of the major indications of regional variations is the apparent differential distribution of the two putative transmitters in the striatonigral pathway, namely GABA and substance P. GABA has been shown to originate in the dorsal and caudal regions while substance P has been shown to originate mainly in rostral and ventral regions (BROWNSTEIN *et al.*, 1977; JESSELL *et al.*, 1978; CUELLO & KANAZAWA, 1978; LJUNGDAHL, HÖKFELT & NILSSON, 1978; STAINES *et al.*, 1980). One would expect therefore, an anatomical basis for these differences, based either on cellular distribution or connectivity. The apparent localization of the STN type 2 neurons and the long dendrite Golgi cells of the present study in the ventral region, may in part be an anatomical basis for regional variations.

Transmitters. As stated above both GABA and substance P are putative transmitters of the striatonigral pathway. It has been suggested previously that the medium-size densely spinous neurons that project to the substantia nigra are the GABA-containing neurons of this pathway (SOMOGYI & SMITH, 1979). This suggestion is supported by the findings of RIBAK, VAUGHN & ROBERTS (1979) that glutamic acid decarboxylase immunoreactivity is present in medium-size striatal neurons that possess spines. In view of the apparent distribution of neurons in the present study and the distribution of substance P and GABA (see above) it is reasonable to speculate that the STN type 2 neurons might contain substance P. However, the possibility remains that sub-populations of medium-size densely spinous neurons contain different, or more than one, transmitter substance. Support for this proposal comes from the observation of PICKEL, SUMAL, BECKLEY, MILLER & REIS (1980) who showed enkephalin immunoreactivity in medium-size neurons that possess spines.

Neuronal circuits. One of the most striking features of both the HRP-labelled STN type 2 and the similar Golgi-impregnated neurons is the pattern and type of synaptic input. This consists of numerous boutons, of essentially similar morphology, in symmetrical synaptic contact with the perikarya and with the proximal and distal dendrites. This type of input strikingly resembles that received by neurons in the globus pallidus and substantia nigra pars reticulata (KEMP & POWELL, 1971*b*; SOTELO, JAVOY, AGID & GLOWINSKI, 1973; SOMOGYI *et al.*, 1979; SOMOGYI, BOLAM, TOTTERDELL & SMITH, 1981*b*). The boutons that form sym-

metrical contacts with STN type 2 neurons are very similar to the boutons of local axon collaterals of STN type 1 neurons (BOLAM *et al.*, 1980; SOMOGYI *et al.*, 1981*a*; 1981*b*) and the local boutons of the same morphological class of striatal neurons that were identified only by intracellular HRP injections (WILSON & GROVES, 1980). This is the only type of synapse that has unequivocally been shown to originate from neurons within the neostriatum.

The proximal and distal dendrites also receive synaptic input from some boutons containing small round vesicles and making asymmetrical membrane contacts. This type of bouton has been described as the major type of afferent bouton in the neostriatum (KEMP & POWELL, 1971*b*; CHUNG, HASSLER & WAGNER, 1977; HASSLER, CHUNG, RINNE & WAGNER, 1978; SOMOGYI *et al.*, 1981*a*) originating in the cortex, thalamus and substantia nigra. Possible input from extrinsic neurons is suggested by the observation (Fig. 9) that one of the Golgi-impregnated neurons (number 5) had Golgi-impregnated axonal boutons in contact with its dendrites; the axon making these contacts originated in a bundle of five fibres. Thus, STN type 2 neurons may receive an input from the same afferent neurons, located in some or all of the areas that project to the striatum, that also form terminals on the spines of STN type 1 neurons. There would be an important difference, however, between the two neurons. Type 1 neurons could receive a more significant input through their compact dendritic field and numerous spines but in a small area of the neostriatum; type 2 neurons, on the other hand, would receive a sparse input in any striatal area but could cover large areas by means of their long dendrites. Following activation by the same afferents, type 1 neurons could exert a powerful feedback effect on type 2 neurons *via* their local axon collaterals. We may speculate that STN type 2 neurons (possibly containing substance P, see above) receive a massive synaptic input from the local axon collaterals of medium-size densely spiny neurons (possibly containing GABA) located close to the neuron and as far away as approximately 1 mm (the dendritic radius plus the radius of medium spiny cell axons).

Electrophysiological studies have shown that stimulation of the striatum leads to a sequence of excitatory and inhibitory postsynaptic potentials in the substantia nigra (PURPURA, 1976; DRAY, 1979). If we speculate that our type 2 neurons might have larger diameter myelinated axons and that the type 1 neurons might have slow conducting unmyelinated or partially myelinated axons, impulses evoked by afferents of the striatum and mediated by STN type 2 neurons would reach the substantia nigra before the impulses of STN type 1 neurons. Thus, activation of the striatum *via* excitatory afferents could result in the following sequence of events: (i), simultaneous activation of type 1 and type 2 neurons; (ii), substance P-mediated excitation of substantia nigra neurons (DAVIES & DRAY, 1976); (iii), GABA-mediated inhibition of substance P neurons in the neostriatum, followed by inhibition of neurons in the substantia nigra. It should be noted that this sequence of events might happen only if large areas of the striatum are activated, resulting in summation along the long dendrites of type 2 neurons. Small local activation might lead only to the firing of type 1 neurons and evoke inhibitory postsynaptic potentials in the substantia nigra.

Finally, we have previously demonstrated (SOMOGYI *et al.*, 1981*b*) that substantia nigra neurons that project to the main body of the neostriatum (identified by the retrograde transport of HRP) receive a direct synaptic input (identified by anterograde degeneration) from neurons located in the ventral striatum-nucleus accumbens region. In addition, these nigrostriatal neurons received synaptic input from two morphologically distinct types of boutons that were labelled with HRP by anterograde transport from the neostriatum. One of the bouton types (type 1) correlated well with the local synaptic boutons of STN type 1 neurons; it is tempting to speculate therefore, that the second type might be terminals of the STN type 2 neurons described in the present study.

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