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Monosynaptic Cortical Input and Local Axon Collaterals of Identified Striatonigral Neurons. A Light and Electron Microscopic Study Using the Golgi-Peroxidase Transport-Degeneration Procedure

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ABSTRACT Following the injection of horseradish peroxidase into the ipsilateral substantia nigra, 36 retrogradely labelled neurons in the striatum were characterized (in three rats) by Golgi staining and gold toning: each neuron was of the medium-size, densely spinous type. Prior to the injection of horseradish peroxidase, two of the rats had had lesions placed in the ipsilateral motor cortex, the third rat had had a lesion placed in the ipsilateral frontal and prefrontal cortex. In the electron microscope, degenerating boutons of cortical neurons were found in asymmetrical synaptic contact with the spines of proximal and distal dendrites of all six of the identified striatonigral neurons that were studied. Some of the degenerating boutons were small (diameter 0.1–0.3 μm), while others were larger (1–2 μm). An individual dendrite of a striatonigral neuron was in synaptic contact with very few degenerating boutons.

Local axon collaterals in the striatum could be traced from two of the identified striatonigral neurons that received degenerating cortical boutons. These were studied in the electron microscope; their boutons formed symmetrical synapses with spines or dendritic shafts of other striatal neurons. The synaptic boutons contained large, clear, round and pleomorphic vesicles. The postsynaptic targets of these boutons morphologically resemble the dendrites of medium-size spiny neurons.

It is concluded that afferents from the cortex make monosynaptic contact with the dendritic spines of medium-size spiny striatonigral neurons and that such neurons have local axon collaterals in the striatum that form synapses with other spiny neurons.

The mammalian neostriatum receives its main afferents from the cerebral cortex, from the thalamus, and from certain cell groups in the mesencephalon (for references see Carpenter, '76; Grofová, '79; Nauta and Domesick, '79). The cortical projection is topographically organized, and apparently the whole neocortex sends fibres to the neostriatum (Whitlock and Nauta, '56; Webster, '61, '65; Kemp and Powell, '70). However, the projection does not show a simple point-to-point topography, since more than one cortical region may project to the same region of the striatum in a discontinuous fashion, and the area of the projection is not related to the dimensions of the cortical area (Kemp and Powell, '70; Goldman and Nauta, '77; Garcia-Rill et al., '79; Veening et al., '80).

The way in which the neostriatum integrates and transforms information arriving from the cortex (or other nuclei) before passing it on to the pallidum and substantia nigra is poorly understood, largely because we know so little

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about the neuronal circuits involved. We need to know, for example, what type of neuron in the striatum receives the cortical input, how many synapses occur between the terminals of cortical neurons and the striatal efferents, and what type of local synaptic interactions occur in the striatum. Electrophysiological studies combined with the intracellular injection of HRP have indicated that the medium-size spiny neuron receives a monosynaptic excitatory input from the cortex (Kitai et al., '76; Kocsis et al., '77), but attempts to demonstrate that such morphologically identified striatal neurons could be excited antidromically by stimulation in the substantia nigra were not successful (Preston et al., '80). There are, in fact, technical problems that make the electrophysiological study of striatal neurons difficult both to carry out (Preston et al., '80) and to interpret unequivocally (see Moore and Bloom, '78).

Likewise, the application of individual morphological procedures has not led to clear-cut answers to the above questions about neuronal connections in the striatum. Following the placement of lesions in the cortex, most of the degenerating terminals found in the striatum are in synaptic contact with spines and, to a lesser extent, with the shafts of dendrites (Kemp and Powell, '71b; Hassler et al., '78; Pasik et al., '79). Since the most frequently impregnated cell in Golgi material is the medium-size spiny neuron (Kemp and Powell, '71a), it has been suggested that the spines of these neurons are the principal targets of cortical afferents (Kemp and Powell, '71c). However, it is now known that the neostriatum contains more than one type of spine-bearing neuron (DiFiglia et al., '76), and so no firm conclusions can be made from morphological studies alone about which type of neuron receives the cortical input. Morphological studies on the nature of the striatal neurons that project to the substantia nigra have been limited by the fact that retrograde tracing procedures, such as the use of HRP, can only indicate the size of the soma of the efferent neurons, because the HRP does not reach the distal dendrites (Grofová, '75; Bunney and Aghajanian, '76).

In order to overcome these and related problems, a new morphological approach has been developed (Somogyi, '78; Somogyi et al., '79) that provides direct information about the synaptic connections of neurons that have been identified by Golgi staining. Furthermore, by combining the retrograde transport of horseradish peroxidase (HRP) with Golgi staining, it has been possible to show that, in the rat neostriatum, some of the medium-size neurons with densely spinous dendrites project to the substantia nigra (Bolam et al., '79; Somogyi and Smith, '79). Since the Golgi stain impregnated all regions of the HRP-labelled striatonigral neurons, we were able to describe the different types of terminals in synaptic contact with these neurons (Somogyi and Smith, '79; Somogyi et al., '79). The next stage is to identify the source of the boutons that are in synaptic contact with identified striatonigral neurons. In this study we have examined whether some of these boutons are of cortical origin by looking for degenerating terminals in the neostriatum after placement of a lesion in the ipsilateral cerebral cortex.

Since some medium-size spiny neurons in the striatum have been shown to have extensive local axon collaterals (Fox et al., '71; DiFiglia et al., '76; Kocsis et al., '77; Lu and Brown, '77; Preston et al., '80), we have also tried to identify what structures are postsynaptic to the Golgi-stained boutons of these cells, and, furthermore, we have studied synapses established by local axon collaterals of identified striatonigral neurons.

A preliminary report of some of the findings was made to the Physiological Society (Bolam et al., '79).

MATERIALS AND METHODS

Seventeen female albino Wistar rats (150–170 gm) were used. They were deeply anaesthetized with chloral hydrate (350 mg/kg, i.p.), and multiple electrolytic lesions (1 mA, 3 sec, anodal) were placed in the cerebral cortex, 0.8 mm below the pial surface, using a stainless steel electrode (0.3 mm diameter) with an exposed tip of 0.2 mm. In the same animals the ipsilateral substantia nigra was injected with HRP (Sigma type VI) by means of a fine glass capillary (tip diameter 20–30 μm), as described previously (Somogyi et al., '79). Because both the corticostriatal and striatonigral projections are topographically organised, only those animals could be used where there was an overlap between areas of terminal degeneration in the striatum and areas where retrograde labelling of cells with HRP had occurred. Cells for the present study were selected from three animals (Fig. 1). One rat (R4) received a series of lesions in the right hemisphere at coordinates A, −1, 1, 2.5, 4.2 Bregma zero; L,1,2.5 mm, and 24 hr later 80 nl of 20% (W/V) HRP was injected at coordinates A (earbar zero), 2.2; L, 6.2; V, 6.4 (König and Klippel, '63) by an oblique lateral

approach (Somogyi et al., '79). A second animal (R10) received electrolytic lesions at coordinates A, −1, 1, 2.5, 3.5; L, 1, 2.5 mm, and 24 hr later 50 nl of 20% HRP was injected into the substantia nigra by an oblique lateral approach. The third animal (R16) received electrolytic lesions in the prefrontal cortex at coordinates A, 4.5; L, 1.5, and A, 5.4; L, 1.3, 2.4 mm, followed 24 hr later by an injection of 20 nl of HRP into the substantia nigra as above (Fig. 8E). For the latter animal we used a conjugate of HRP with wheatgerm agglutinin (final concentration approximately 6% in HRP) prepared by the procedure of Gonatas et al. ('79).

Twenty hours following injection of the HRP, the animals were anaesthetized with chloral hydrate and perfused through the heart with 0.9% NaCl (approximately 20 ml) followed by fixative (2.5% glutaraldehyde, 0.5% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4; approximately 200 ml). The brains were then processed according to the combined HRP-transport-Golgi-degeneration procedure (Somogyi et al., '79). Briefly, the mesencephalon, containing the HRP injection site, and representative 0.8 mm -thick slices from the forebrain, including the area of the cerebral cortex with the lesion and the striatum, were processed in the form of cryostat sections for light microscopic HRP histochemistry (*o*-tolidine as substrate) and/or were Nissl-stained. From the remaining slices of the forebrain the striatum was dissected, cut into blocks, and processed for HRP histochemistry followed by Golgi staining. For both the cryostat sections and Golgi blocks, *o*-tolidine was used as substrate to reveal HRP activity. From the blocks, 70–100 μm 'Golgi sections' were cut using Sorvall TC-2 tissue chopper. Most of the sections were gold-toned to convert some of the original Golgi precipitate into metallic gold, using a slightly modified procedure (Somogyi et al., '79) to that developed by Fairén et al. ('77).

Areas tested for degeneration and containing retrogradely labelled cells were examined in the light microscope. Double-labelled cells, i.e., neurons containing both HRP reaction endproduct and secondary Golgi deposit, were photographed and drawn using a Leitz camera lucida and a × 100 oil immersion objective. They were subsequently re-embedded for electron microscopy. To confirm HRP labelling, 1-μm sections were cut at the level of the perikaryon, when required. Serial ultrathin sections were mounted on single slot formvar-coated grids. To improve contrast for electron microscopy, Golgi sections were stained en bloc with uranyl acetate and ultrathin sections with lead citrate as described previously (Somogyi, '78). Electron micrographs were taken on a Philips 201C electron microscope using 20–30 μm objective apertures.

Terminology

We shall use the same terms as defined in a previous paper (Somogyi et al., '79). Thus, the term "Golgi stain" (or deposit) will refer to the substance(s) formed during the original Golgi staining; the substance(s) formed as a result of "gold toning" will be called "secondary gold precipitate" or "gold particles."

The term "Golgi section" refers to sections 70–100 μm thick cut with the tissue chopper from the Golgi impregnated slice of brain tissue.

RESULTS

The localization of cortical lesions (Fig. 1)

The lesion in animal R4 included the cytoarchitectonic areas 4, 6, and the medial part of 3 in the pariatal cortex, and the dorsal part of area 10 in the frontal cortex as delineated by Kreig ('46). The cingulate cortex was only slightly damaged. In animal R10 the lesion was similar but included only the very posteromedial part of area 10. These two lesions were thus largely localised to motor areas as identified by electrical stimulation (Hall and Lindholm, '74). In the third animal (R16) the lesion was confined to the most anterior part of the frontal and prefrontal cortex, including part of area 10 of Kreig ('46) and the anteromedial cingulate or mediodorsal projection cortex (Beckstead, '79). In all animals care was taken to avoid damage to the corpus callosum, and the lesions affected only the gray matter.

The extent of HRP spread at the injection site

It has been pointed out that when *o*-tolidine is used as a substrate, the HRP endproduct can be seen over a greater area than when diaminobenzidine is used (Somogyi et al., '79). In addition to the fibres around the injection track, the walls of blood vessels are also stained due to HRP taken up by phagocytotic elements (Fig. 8E). In all three animals the HRP injection area included parts of both the substantia nigra pars compacta and the reticulata. In animal R4 there was spread to the ventral tegmental area and nucleus ruber, and in animal R10 a slight spread to the ventral tegmental area. However, even with the largest injection

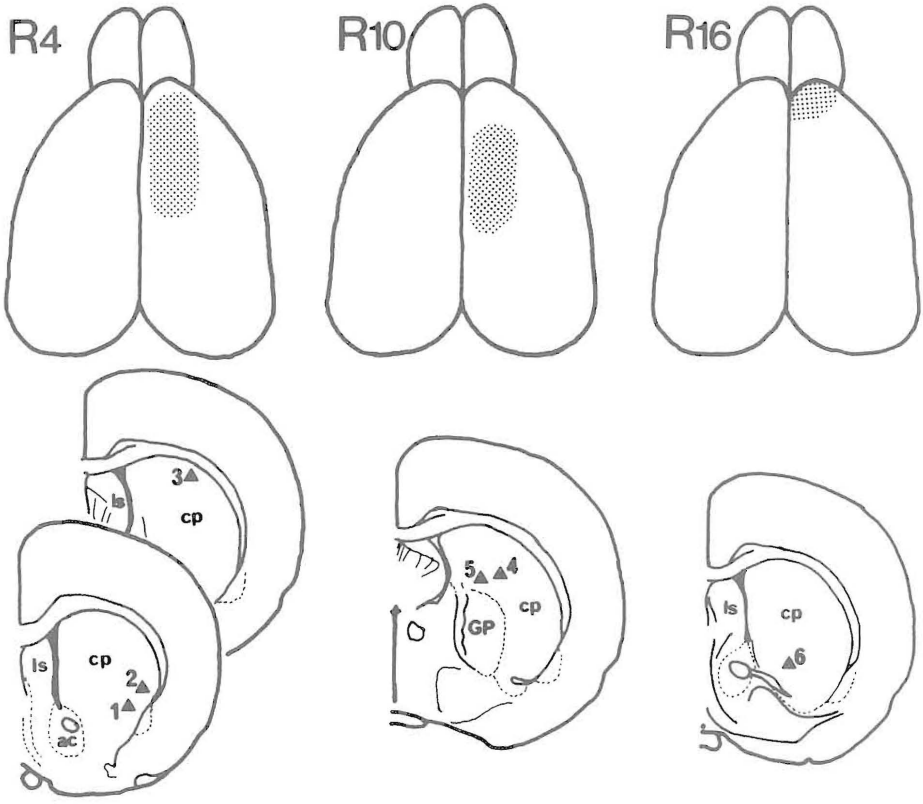


Fig. 1. The upper part illustrates the approximate extent of the electrolytic lesions in the cortex in the three rats. In the lower part, the triangles indicate approximate positions of Golgi-stained neurons in the striatum that were also retrogradely labelled following injection of HRP into the substantia nigra. Each neuron is given a number (see Fig. 2). ac, nucleus accumbens; cp, caudate-putamen; GP, globus pallidus; ls, lateral septal nucleus.

in rat R4, where most of the substantia nigra was stained for HRP activity, only restricted parts of the neostriatum contained retrogradely labelled cells, indicating that the area of HRP uptake into terminals is smaller than that revealed by histochemical examination 20 hr after the injection of the enzyme.

Retrograde HRP labelling in the neostriatum

Labelled neurons were found in all parts of the neostriatum according to the topography described in previous studies (Bunney and Aghajanian, '76, see also Nauta and Domesick, '79). In frozen sections the neurons are stained blue with the *o*-tolidine procedure, while in the Golgi sections the reaction endproduct is brown. In both kinds of section both granular and homogenous types of reaction endproduct were observed in perikarya and proximal dendrites. In the Golgi sections medium-size neurons that contained brown reaction endproduct granules were found side by side with homogenously filled cells. In this material the most commonly Golgi-stained cell type was the medium-size spiny neuron. It is easy to recognise HRP reaction endproduct within Golgi-stained neurons when the cell is heavily labelled, because the perikaryon is filled with the brown reaction endproduct (Fig. 3A), while unlabelled Golgi-stained cells are transparent, delineated only by the greyish gold deposit. In the case of lightly labelled neurons, the HRP reaction endproduct granules are also visible in the Golgi section (Fig. 5A), but they became more apparent in the 1-μm plastic sections (Fig. 5B). Finally the HRP reaction endproduct is also recognisable in both the granules and the cytoplasm at the electron microscopic level (Fig. 5C) (see also Somogyi and Smith, '79; Somogyi et al., '79).

Golgi staining and HRP labelling of the same neuron

In the present study 36 neurons have been found, in the three animals, which were both Golgi-stained and retrogradely labelled after injection of HRP into the substantia nigra. They were all of the medium-size, spiny type (Figs. 2, 3A, 5A, 6A) described previously to project to the substantia nigra (Somogyi and Smith, '79). Three to six smooth, primary dendrites emerge from the perikaryon, which is 12–18 μm in diameter. The dendrites usually branch at distances from 10–30 μm from their origin. The secondary dendrites are initially also smooth but become densely laden with spines, as are the tertiary dendrites. The number of spines slightly decreases toward the most distal regions of the dendrites. The axon emerges from the perikaryon. In gold-toned material it is difficult to follow the axon of stained neurons. Nevertheless, in two cases we have been able to observe local axon collaterals of gold-toned neurons retrogradely labelled by HRP from the substantia nigra (Fig. 2, nos. 1, 6).

Local axon collaterals of Golgi-stained spiny neurons

In material not processed for gold toning, between 2 and 5 axon collaterals could frequently be observed leaving the main axon and having bulbous enlargements along their whole length (Fig. 9). These collaterals branch profusely, but the axon arborisation as seen in the 80–100 μm thick sections is only 200–300 μm in diameter and does not extend far beyond the area occupied by the dendrites.

Electron microscopy of striatonigral neurons and terminal degeneration of corticostriatal fibres

Six striatonigral neurons (Fig. 2), including two with axon collaterals, were re-embedded and sectioned for electron microscopy. The gold deposit appears as small, electron-dense granules within the neurons and allows the identification of the profiles within the microscope. It has been extensively demonstrated previously (Somogyi, '78; Somogyi et al., '79) that with our method any Golgi-stained process can be correctly identified in the electron microscope. Therefore, the correlation between the light and electron microscopic levels is shown only in one example (Figs. 6A–D).

All parts of the identified striatonigral neurons were examined to assess the synapses they receive. Only symmetrical synapses were found on the perikaryon (Fig. 3C), axon initial segment, and the primary smooth dendrites. Symmetrical synapses were also found on the secondary and tertiary dendritic shafts, but in two cases asymmetrical junctions were also observed. No degenerating terminals were seen in synaptic contact with these parts of the neuron. The dendritic spines receive predominantly asymmetrical synapses from boutons containing spheroidal vesicles. Some of these boutons are very small—only about 0.1–0.3 μm in diameter; others are much larger—up to 1–2 μm in diameter. Rarely, two or three (Fig. 3D) different boutons may converge onto the same spine. In this example only one forms an asymmetrical contact; the others establish symmetrical contacts.

The six neurons were in areas where terminal degeneration could also be observed. However, except in the area of neuron no. 4 in Figure 2, the degeneration was sparse. The late stage of electron-dense degeneration was the most frequently encountered type. (Figs. 4A–C, 6A, D, 7A–F, 8C, D). All degenerating boutons that were seen formed asymmetrical synapses. Degenerating boutons of corticostriatal fibres were observed among the axo-spinous asymmetrical synapses of the six striatonigral neurons. The degenerating boutons had strongly electron-dense cytoplasm (Figs. 4A–C, 6C, D, 7A–F, 8C, D), shrunken, dark mitochondria (Figs. 7A–D), and occasionally clusters of aggregated vesicles (Figs. 7A, B). The synapses could be identified on the basis of the postsynaptic membrane specialization, the synaptic cleft material, and often an electron-dense line within the cleft (Figs. 7E–F). We found degenerating boutons of both the small (Figs. 4A–C, 6B–D, 8C, D) and the large type (Fig. 7), and they were encountered on both proximal and distal parts of the dendrites. Any individual dendrite received only one or two degenerating boutons, and other dendrites of the same neuron could have none at all. This may reflect the sparsity of degeneration in the examined areas.

Synapses formed by axon collaterals of striatonigral spiny neurons

Two of the identified striatonigral neurons, which received degenerating cortical boutons, had local axon collaterals, and these were studied at the electron microscopic level. Two boutons of neuron no. 1 in Figure 2 were identified; they formed symmetrical synapses with a spine (Fig. 4D) and a small dendritic shaft, respectively. Two boutons of neuron no. 6 in

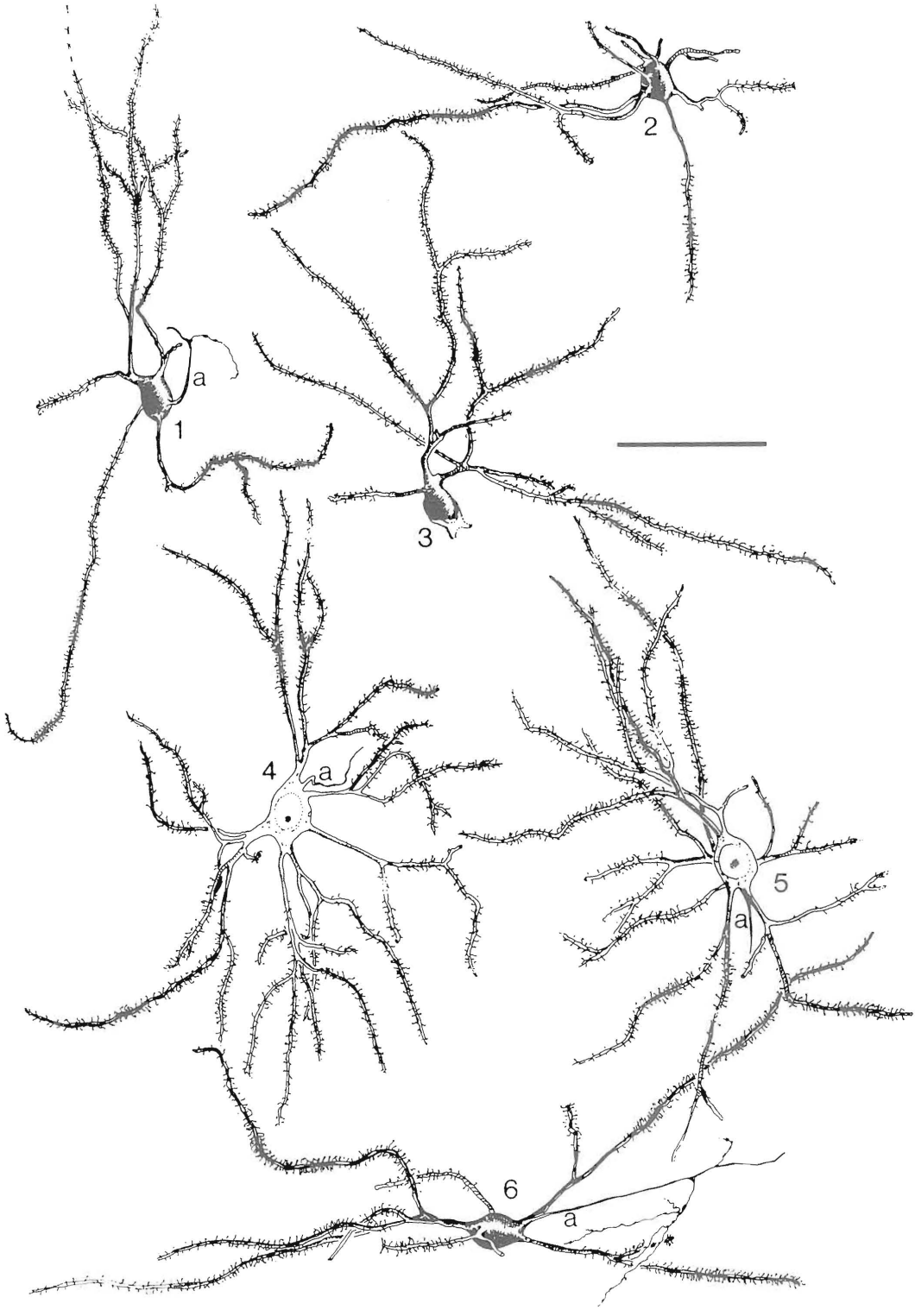
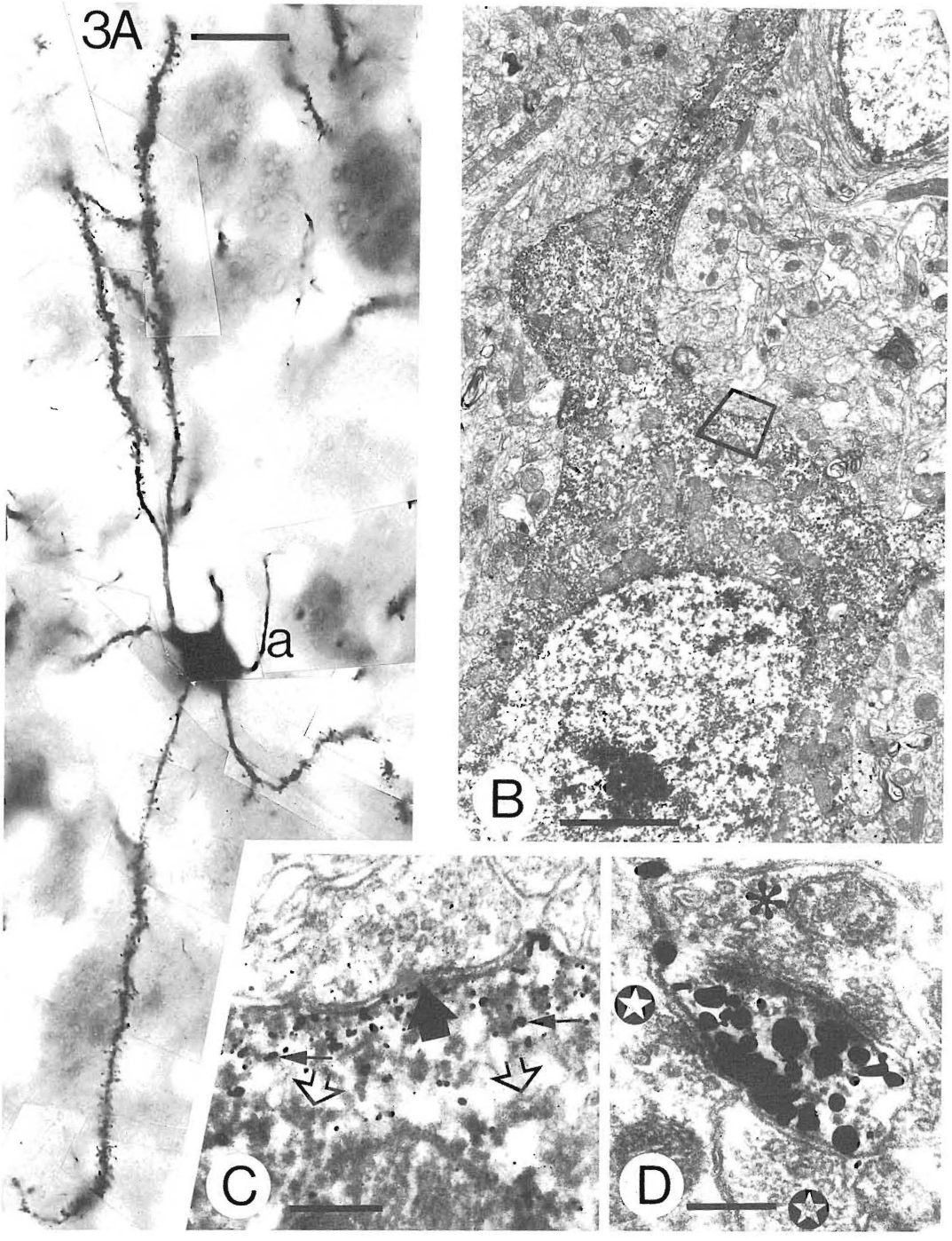


Fig. 2. Camera lucida drawings of Golgi-stained, gold-toned striatal neurons shown to project to the substantia nigra by the retrograde transport of HRP. The same neurons were found to receive synapses from degenerating boutons following the cortical lesions illustrated in Figure 1. All the cells belong to the medium-size spiny type, and two of them (nos. 1 and 6) have local axon collaterals. The origin of the axon is indicated by "a." Scale, 50 μm.



Figs. 3, 4. Illustrations of our observations on the striatonigral neuron labelled no. 1 in Figure 2.

Fig. 3. A) Photomontage of the neuron. Note the densely spinous secondary dendrites and the axon (a) originating from the perikaryon, which is homogeneously filled with HRP reaction endproduct. B) Low-power electron micrograph of the perikaryon of the same neuron. The cytoplasm is filled with amorphous HRP reaction endproduct. The framed area is shown at higher magnification in C. C) An axo-somatic symmetrical synapse is established by a bouton containing flattened synaptic vesicles. At this magnification the gold particles (thin solid arrows) and the HRP endproduct (open arrows) in the perikaryon can easily be differentiated. D) Three boutons are in synaptic contact with a dendritic spine of this striatonigral neuron. One of them (asterisk) forms an asymmetrical synapse; the other two (stars) form symmetrical synapses. Scales: A, 20 μm; B, 2 μm; C and D, 0.2 μm.

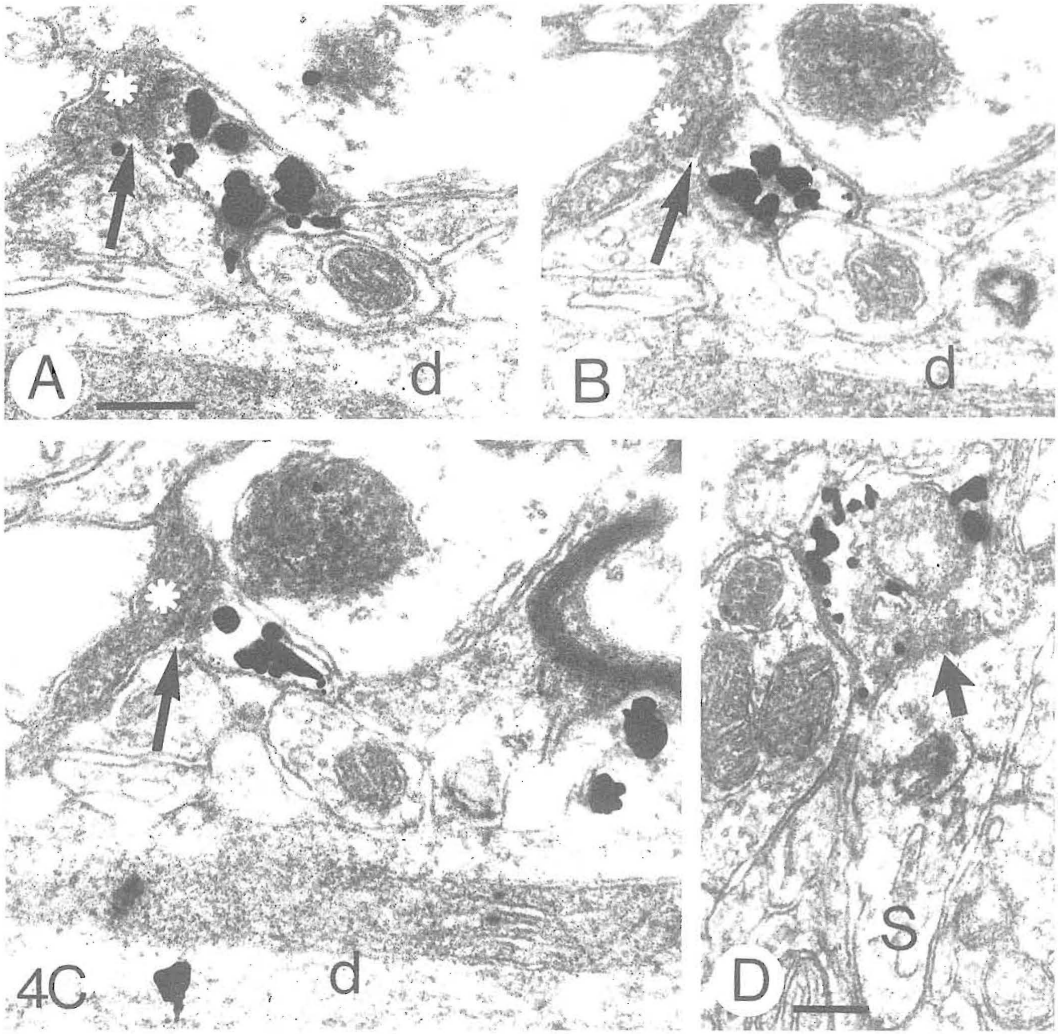
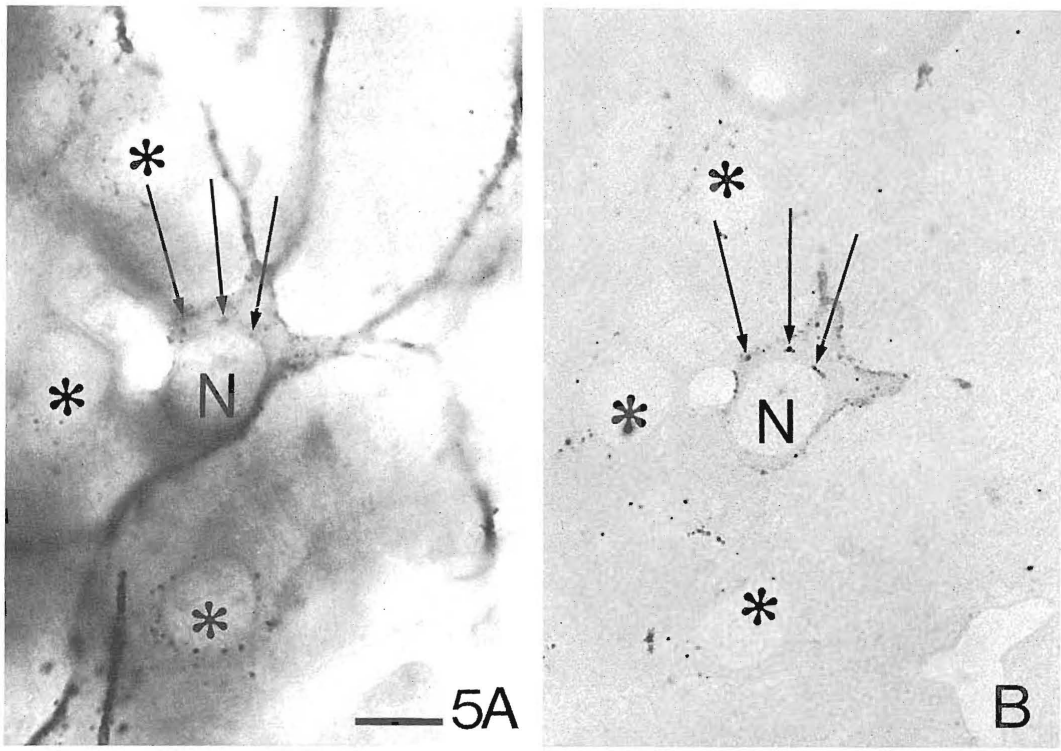


Fig. 4. A–C) Serial sections showing a spine of the striatonigral neuron to be in synaptic contact (arrow) with a degenerating cortical en passant terminal (white asterisk). In serial sections, the spine was shown to originate from the dendrite (d) D) A bouton from the local axon collateral of the same neuron is in synaptic contact (arrow) with a spine (s). Scales: A–C, 0.2 μm; D, 0.2 μm.

Figure 2 were studied (Figs. 8A, B) and found to establish symmetrical synapses with three dendritic shafts. These dendrites contained large numbers of microtubules, numerous mitochondira, but were poor in endoplasmic reticulum (Figs. 8A, B). A spine could be traced to one of the dendrites in serial sections. The boutons contained large, clear, round and pleomorphic vesicles. The presynaptic dense projections were specially prominent (Figs. 8A, B).

Since it was difficult to follow the axon collaterals in the gold-toned material, we also sampled the axonal arborization of Golgi-stained, medium-size, spiny neurons that had not been gold-toned (Fig. 9). Eight boutons of one cell (Fig. 9) and one bouton of the other were found to make symmetrical synapses with dendritic shafts, one of which is shown in Figures 10A and B. These dendrites contained numerous mitochondria (Figs. 10A, B) and mi-



Figs. 5, 6. Illustrations of our observations on the striatonigral neuron labelled no. 4 in Fig. 2.

Fig. 5. A) Light micrograph of a Golgi section showing the HRP-labelled, Golgi-stained and gold-toned neuron (N). Other HRP-labelled striatonigral neurons that were not impregnated with the Golgi stain are indicated by asterisks. Note the numerous cytoplasmic granules containing the HRP reaction endproduct, some of which are indicated by arrows in the gold-toned neuron. B) A plastic section (1-μm thick) of the same area at the same magnification as shown in A; the same labelling is used. The granules of HRP reaction endproduct (arrows) can be seen more clearly in this section. Scale: 10 μm.

crotubules. In serial sections, spines were found to emerge from two of the dendrites postsynaptic to Golgi-stained boutons (Fig. 10B). In general, they were very similar to dendrites contacted by boutons of striatonigral neurons.

DISCUSSION

Identification of striatonigral neurons

All 36 Golgi-stained, gold-toned neurons that were shown to project to the substantia nigra by the retrograde transport of HRP belonged to the medium-size, densely spinous type. This confirms and extends our earlier report (Somogyi and Smith, '79) in which eight such striatonigral neurons were described. However, these findings do not necessarily mean that no other type of striatal neuron projects to the substantia nigra or that all medium-size spiny neurons are efferents.

Medium-size spiny neurons have been found to have extensive local axon collaterals, both in Golgi material (Kemp, '68; Fox et al., '71; Kemp and Powell, '71a; DiFiglia et al., '76) and following the intracellular injection of HRP (Preston et al., '80). Our finding that two medium-size neurons that project to the substantia nigra have axon collaterals within the striatum means that the presence of such axon collaterals can no longer be taken as evidence that a medium-size spiny neuron is a striatal interneuron. A similar conclusion was reached by Preston et al. ('80), who observed axons leaving the striatum from medium-size spiny neurons that had been injected with HRP.

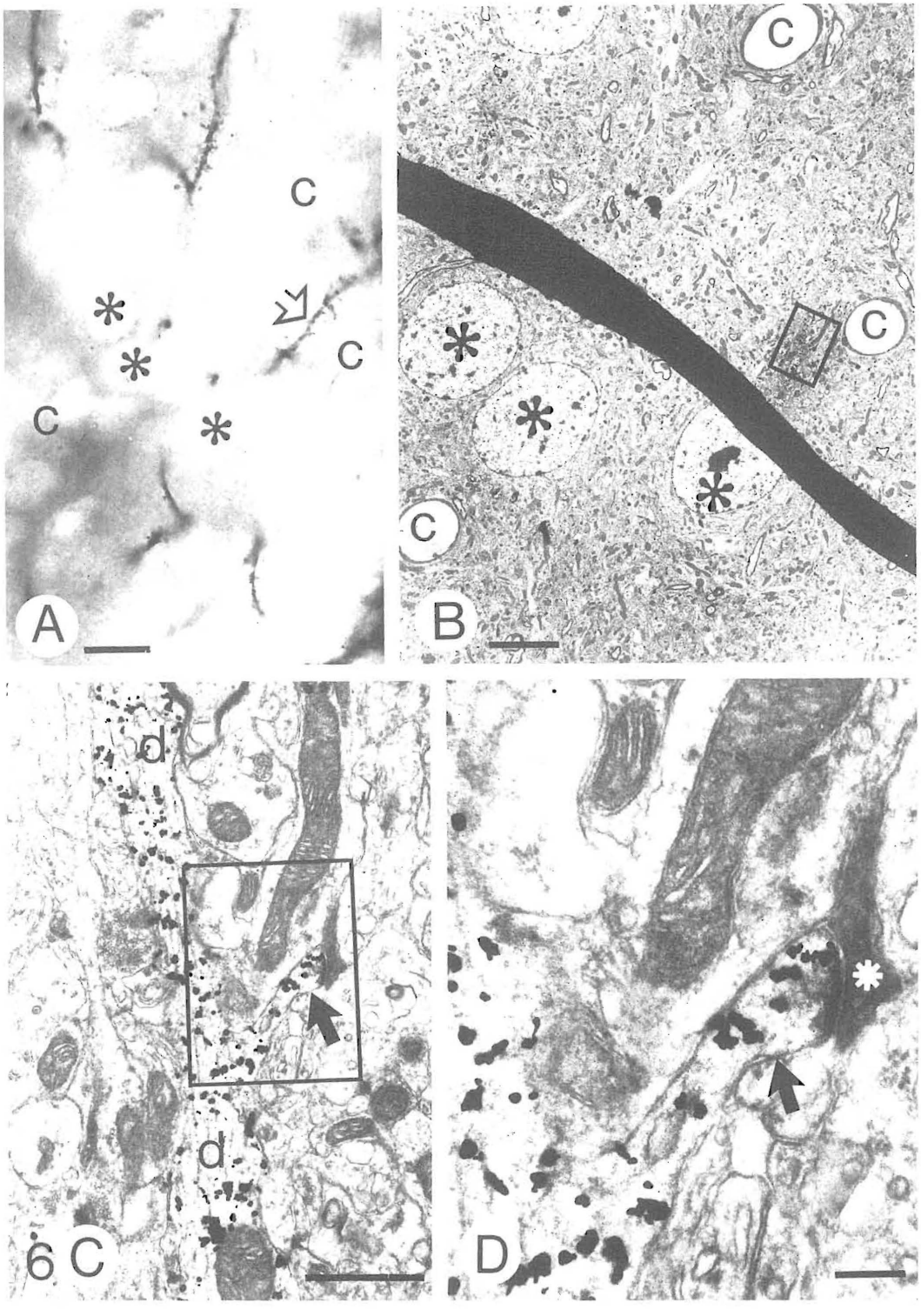


Fig. 6. Correlation of the light and electron microscopic levels of investigation. A) Light micrograph showing part of the dendritic field of the striatonigral neuron. Several Golgi-stained, gold-toned spiny dendrites can be seen, one of which is indicated by an open arrow. The latter dendrite occurs within the rectangle in the electron micrograph in B. Structures common to A and B are three capillaries (C) and three medium-size neurons (asterisks). The black line in B is a fold in the section. C) The framed area in B at higher magnification. The gold-toned dendrite (d) emits a spine (arrow). The dendrite contains a large number of microtubules and mitochondria but is poor in other structures. D) The framed area in C at higher magnification. The spine (arrow) of the striatonigral neuron is in asymmetrical synaptic contact with a small degenerating cortical axon terminal (white asterisk). Scales: A, 10 μm; B, 5 μm; C, 1 μm; D, 0.2 μm.

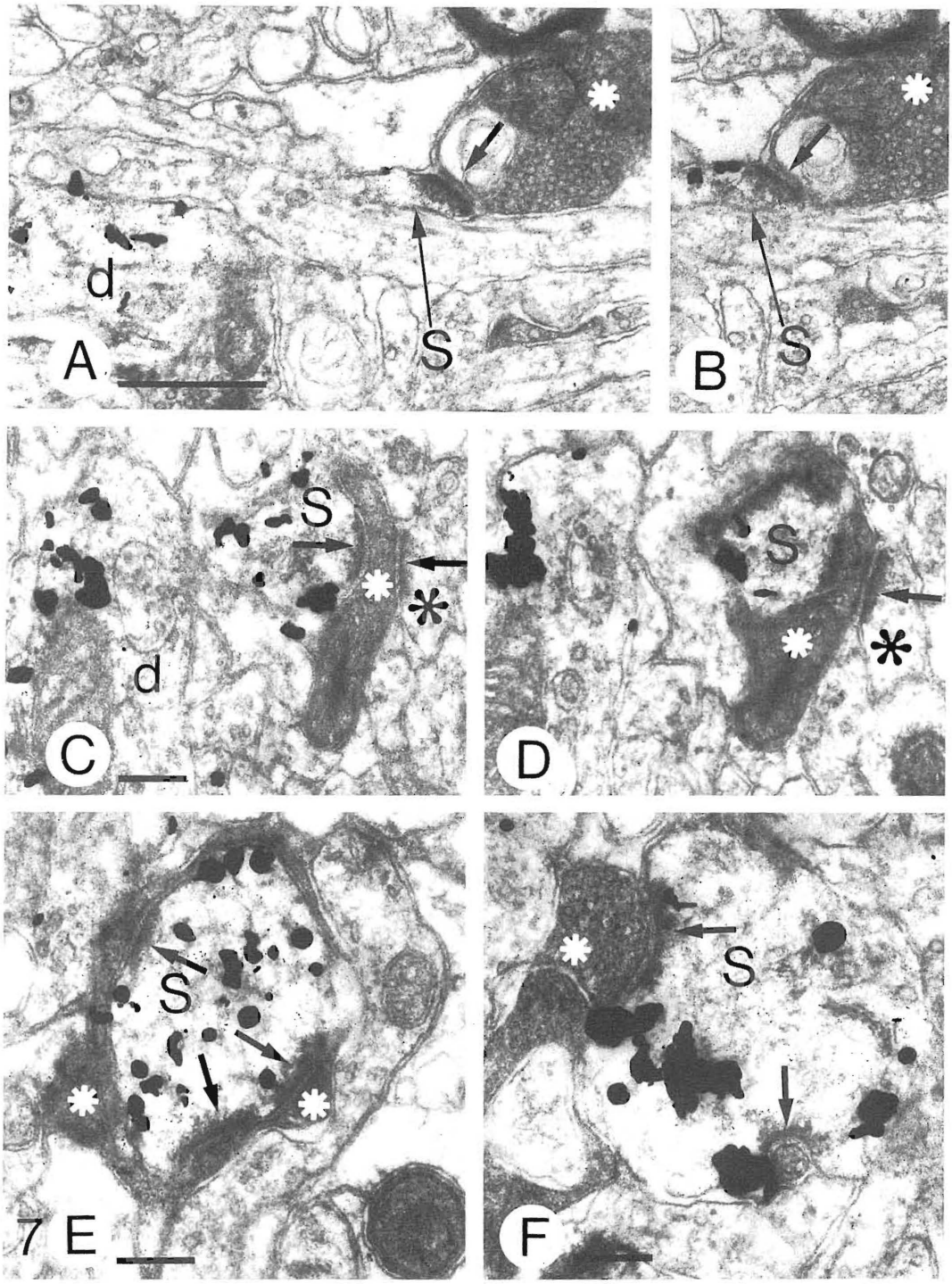


Fig. 7. Degenerating cortical axon terminals (white asterisks) are shown in synaptic contact (short arrows) with spines (s) of identified striatonigral neurons. A, B) Serial sections through a dendrite (d) of neuron 5 (Fig. 2), which gives off a spine (s). The degenerating bouton contains aggregated synaptic vesicles. C, D) Serial sections of a dendrite (d) of neuron 2 (Fig. 2). The degenerating bouton is not only in contact with the gold-toned spine (s) of the striatonigral neuron, but is also in contact with a spine (black asterisk), which belongs to an unidentified neuron. E, F) Two degenerating cortical boutons in contact with two different spines of the same dendrite of neuron 3 (Fig. 2). Scales: A and B, 0.5 μm; C and D, 0.2 μm; E and F, 0.2 μm.

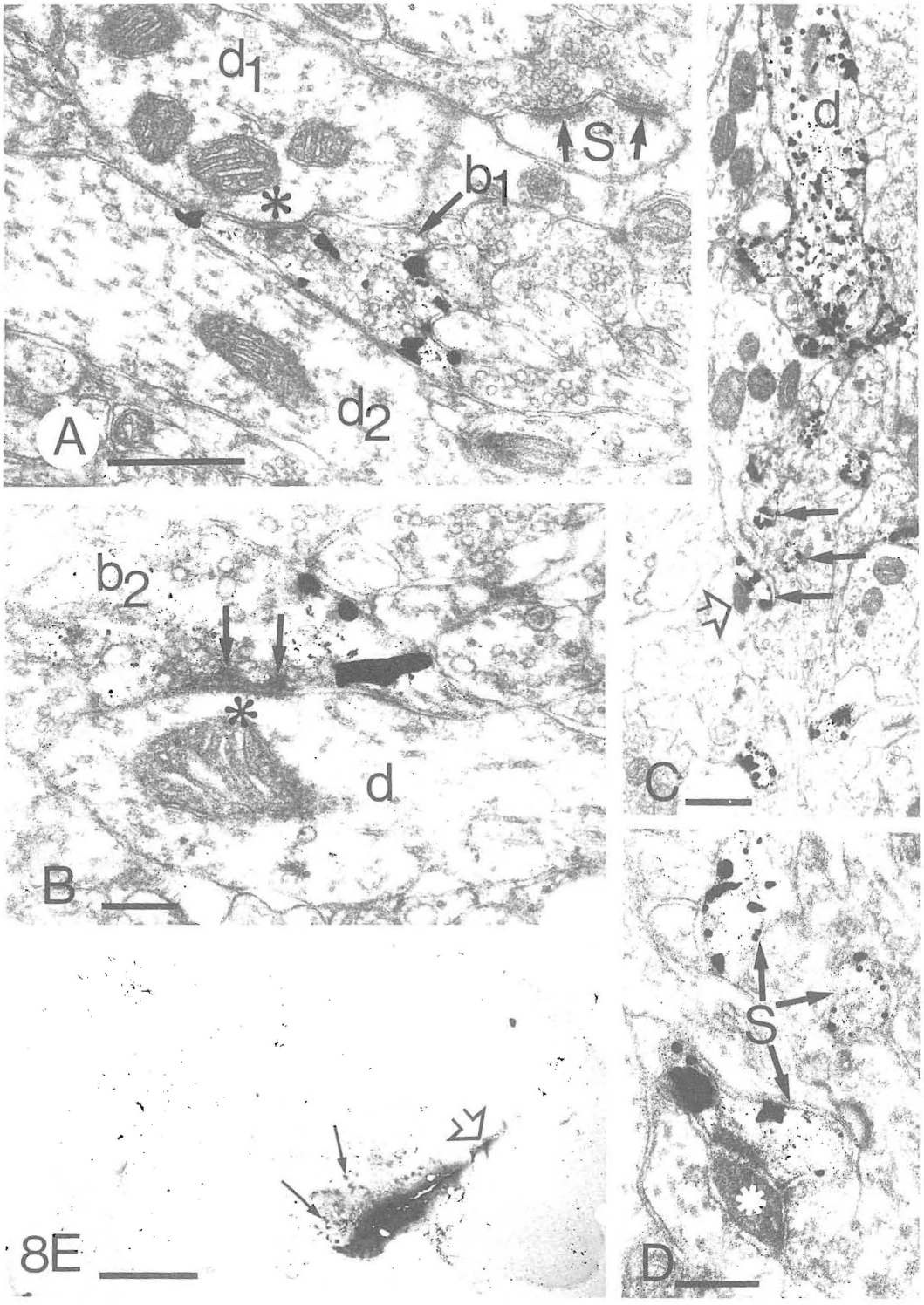


Figure 8

Fig. 9. Camera lucida drawing of a Golgi-stained medium-size spiny neuron in the striatum, which has an extensive axon collateral network. Arrows indicate the origin of the collaterals. The main axon (a) was seen to enter a fibre bundle. The asterisk indicates the bouton, which is shown in the electron micrographs in Figure 10. Scale, 10 μm.

Fig. 8. Input and output of an identified striatonigral neuron (no. 6 in Fig. 2). A, B) Two boutons (b_1 and b_2) of an axon collateral of a gold-toned striatonigral neuron make symmetrical synaptic contacts (asterisk) with dendritic shafts (d, d_1, and d_2). In serial sections it was shown that bouton b_1 was in synaptic contact with both dendrites d_1 and d_2. The boutons contain large, clear, round and pleomorphic vesicles. Note the prominent presynaptic dense projections (arrows) in bouton b_2. The spine(s) in A receives an asymmetrical synapse (arrows) from an unlabelled bouton containing round vesicles; these vesicles are smaller than those in the boutons of the striatonigral neuron. C) The gold-toned dendrite (d) of the same neuron. Numerous spines containing secondary gold precipitate can be seen along the dendrite; three of them are labelled by arrows. An open arrow indicates a degenerating cortical bouton in synaptic contact with one of the spines. The area including this bouton is shown at higher magnification in D, in a subsequent section. D) A degenerating bouton (white asterisk) of a cortical axon in asymmetrical synaptic contact with a gold-toned spine (s) of the striatonigral neuron. E) Light micrograph of a frozen section illustrating the HRP reaction endproduct around the site of injection in the substantia nigra (animal R16). Open arrow, injection track; small arrows, blood vessels. This injection of HRP led to the retrograde labelling of the neuron in the striatum (no. 6 of Fig. 2) that has been illustrated in A–D. Scales: A and C, 0.5 μm; B and D, 0.2 μm; E, 2 mm.

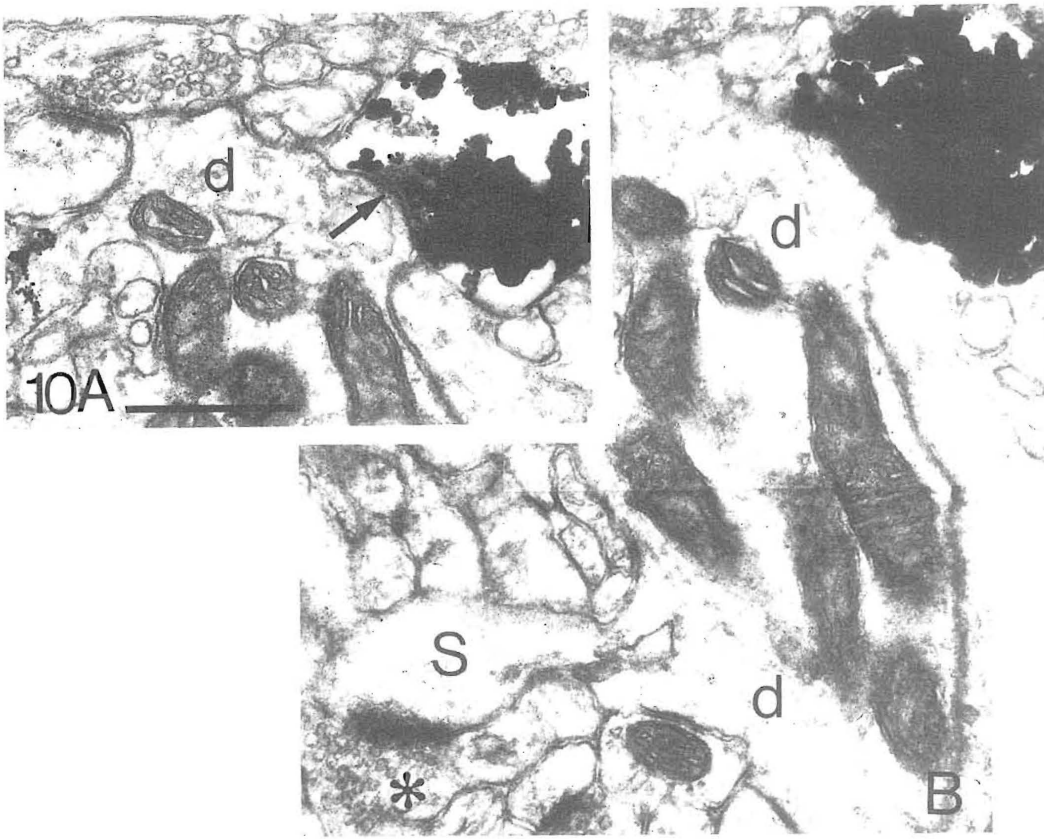


Fig. 10. A, B) Serial sections through a Golgi-stained bouton of a local axon collateral of the neuron illustrated in Figure 9. The electron-dense primary Golgi deposit fills most of the bouton. The bouton is in symmetrical synaptic contact (arrow) with a dendrite (d) that has numerous mitochondria and microtubules and that emits a spine (s). A bouton, containing round synaptic vesicles, from an unstained neuron is in synaptic contact (asterisk) with the same spine. Scale: 0.5 μm.

The monosynaptic target of corticostriatal fibres

One of the major inputs to the striatum, the cortical projection, has now been shown to terminate directly on efferent striatonigral neurons. This finding provides a morphological basis for earlier electrophysiological studies in the cat. After cortical stimulation, monosynaptic excitatory postsynaptic potentials could be recorded in morphologically unidentified striatal neurons, and a very small proportion of these neurons could also be antidromically activated from the substantia nigra (Fuller et al., '75; Kitai et al., '75; Kocsis et al., '77).

Some of the striatal neurons that receive excitatory cortical input have been shown to be of the medium-size spiny type by the intracellular injection of HRP (Kocsis et al., '77). The procedure we have applied provides morphological evidence not only that cortical fibres terminate on medium-size spiny neurons in the striatum, but also that the same neurons project to the substantia nigra.

Previous morphological studies in the cat (Kemp and Powell, '71b; Hassler et al., '78) and monkey (Pasik et al., '79) have shown that, following a cortical lesion, degenerating boutons in the striatum are found in asymmetric synaptic contact with dendritic spines and, sometimes, with dendritic shafts. However, in these studies the type of recipient neuron could not be directly determined. In another study, Kemp and Powell ('71c) showed that there was a reduction in the number of Golgi-impregnated spines of medium-size spiny neurons

chronically after placement of a cortical lesion; they suggested that this was a consequence of the loss of a direct cortical input to this type of neuron. In the rat, it has been shown by autoradiography at the electron microscopic level that, following injection of [^{3}H]proline into the frontal cortex, labelled boutons can be found in the striatum in synaptic contact with dendritic spines (Hattori et al., '79). Our results are in agreement with the termination of cortical boutons on spines, since all degenerating synaptic boutons formed axo-spinous asymmetrical contacts, and, in addition, we have also shown that the recipient neurons are of the medium-size, densely spinous type and project to the substantia nigra.

Convergence and divergence in the termination of corticostriatal fibres

It is difficult to make quantitative estimates of the number of boutons of cortical origin at one particular survival time, because degeneration is a continuous process. Nevertheless, the survival period we chose (44 hr) is likely to give a good indication of the number of cortical terminals originating from neurons in the area of the lesion, because we found very few boutons at an early stage of degeneration and no spines that had become separated from their afferent bouton—which would indicate a very late stage. Thus, our finding of very few degenerating boutons in contact with an individual dendrite is likely to reflect the density and distribution of the terminals of cortical fibres from the area of the lesion. It implies a relatively high degree of both convergence and divergence in afferents from the cortex.

We can assume that when a cortical fibre degenerates, all its boutons within an arborization will show the signs of degeneration at the same time. Thus, the small number of degenerating boutons received by any one striatonigral neuron must mean that one cortical pyramidal cell can have little influence on an individual neuron in the striatum. It is likely, therefore, that there is convergence of a number of cortical neurons from different areas on to each striatonigral spiny neuron. This is quite consistent with the topographical arrangement of the cortico-striatal projection, which shows a considerable degree of overlap in the termination of fibres from different cortical areas (see Grofová, '79), while from the same area many pyramidal cells project to the striatum (Hedreen, '77; Jones et al., '77; Wise and Jones, '77; Veening et al., '80), probably also in an overlapping fashion.

Evidence of divergence in the termination of fibres from an individual cortical neuron is also provided by the observation of very few degenerating terminals per striatal neuron, since it is unlikely that one cortical axon would give rise to so few synapses. More direct evidence is provided by our finding that the same degenerating bouton can be in contact with both Golgi-stained and unstained spines (see Figs. 7C, D); these spines belong to different neurons.

Although the cortical lesions included several cytoarchitectonic areas in two of the animals, they were largely localized to motor regions. Whether neurons from other areas of the cortex terminate in the striatum in the same way and on the same type of neuron remains to be examined. The fact that a spiny striatonigral neuron received a degenerating bouton in an animal (R16) where the lesion was localized to the frontal and prefrontal cortex does, however, suggest that there might be a monosynaptic channel from all cortical areas through the striatum to the substantia nigra.

It has been suggested (Nauta, '79) that information is funnelled from the cortex to the striatum and then to the pallidum or substantia nigra in a way such that the number of neurons is fewer at each stage. This is certainly true if we consider the total number of neurons in each structure. However, only a subpopulation of layer V pyramidal cells in the cortex project to the striatum (Hedreen, '77; Jones et al., '77; Wise and Jones, '77), while the medium-size spiny neurons that project outside the striatum probably comprise the majority of the cells in this structure. It is thus possible that the numbers of cells that are interconnected in the two structures are of the same order of magnitude.

Connections of striatonigral neurons within the striatum

As mentioned above, several studies show that the medium-size spiny neuron has extensive local axon collaterals within the striatum. Varicosities occur along these axon collaterals, and they have been assumed to be synaptic boutons. Our studies of Golgi-stained, gold-toned spiny neurons have directly demonstrated such boutons and have also shown that these local synaptic boutons occur along the local axon collaterals of efferent neurons that project to the substantia nigra. We were only able to observe local axon collaterals in two of the 36 striatonigral cells, but this is probably a reflection of the technical problem of tracing fine axons in Golgi material that has been

gold-toned by our procedure. For this reason we also studied, in the electron microscope, the axon collaterals of Golgi-stained spiny neurons that were not gold-toned and that were not, therefore, shown to be projecting neurons.

The varicosities of the gold-toned axon collaterals of the two identified striatonigral neurons made symmetrical synaptic contacts with dendritic shafts and, in one case, a spine; the varicosities of the axons of the Golgi-stained neurons, likewise, were in synaptic contact with dendritic shafts. These boutons are very similar to the synapses that survive after surgical isolation of part of the striatum (type IX of Hassler et al., '77; Hassler, '79). It is also noteworthy that Kemp and Powell ('71b) described degenerating boutons that formed symmetrical contacts in one part of the cat striatum following placement of a small lesion in another part of the striatum; they suggested that such terminals might arise from intrinsic neurons. Although a similar type of bouton might well derive from local circuit neurons, it is clear from our findings that such boutons can also come from the local axon collaterals of striatonigral neurons.

In our studies the dendritic elements that were postsynaptic to the boutons of axon collaterals were quite similar to the corresponding structures in striatonigral medium-size spiny neurons; indeed, when serial sections were made, spines were seen to be continuous with the dendritic shafts. These findings raise the possibility that striatonigral spiny neurons are in synaptic contact with other medium-size spiny neurons. Although there is no direct evidence that these local target neurons also project to the substantia nigra, the fact that all Golgi-stained efferent neurons so far observed are of a similar type, together with the high proportion of projecting neurons, would suggest that different projecting neurons might be in synaptic contact with each other via their local axon collaterals.

Possible functional implications

The present findings have certain implications for the functioning of the striatum, and these will be discussed briefly in relation to the possible neurotransmitters in the corticostriatonigral pathway and to some of the neuronal circuits that involve the striatum.

Neurotransmitters. Biochemical studies (Divac et al., '77; Kim et al., '77; McGeer et al., '77; Reubi and Cuenod, '79), experiments with push-pull cannulae (Godukhin et al., '80), and electrophysiological evidence (Spencer, '76) are all consistent with the view that L-glutamate is a transmitter in the corticostriatal pathway. Stimulation of the cortex leads to excitation of neurons in the striatum (Kocsis et al., '77), and the effect of L-glutamate is similar (Spencer, '76). The facts that only boutons forming asymmetrical synapses degenerate in the striatum following cortical lesions (see above for references) and that similar boutons are labelled by the autoradiographic tracing method (Hattori et al., '79) are consistent with the excitatory action of this pathway. Thus, we can speculate that the asymmetrical synapses that cortical neurons form on the dendritic spines of striatonigral neurons use glutamate as a transmitter and are excitatory. Since this synapse provides a monosynaptic link between the cortex and the substantia nigra, it is important to know what transmitter is used by the medium-size spiny striatonigral neurons. It was suggested (Somogyi and Smith, '79) that γ-aminobutyrate (GABA) might be the transmitter in this type of striatonigral neuron. More direct evidence on this question has since been published by Ribak et al. ('79), who studied the localization of glutamate decarboxylase by immunocytochemistry. It was found that the enzyme is localized in spines, dendrites, and perikarya of what were interpreted as medium-size spiny neurons. Furthermore, it was suggested that these neurons project to the pallidum and substantia nigra and that the immunoreactive axon terminals in the neostriatum correspond to local axon collaterals of these neurons. However, the neuron illustrated (Fig. 18 of Ribak et al., '79) is clearly very different from the medium-size spiny neurons found in our experiments to project to the substantia nigra. It has invaginations of the nuclear membrane and an intranuclear rod never seen in the neurons studied in the present and previous (Somogyi et al., '79; Somogyi and Smith, '79) work. In addition, the glutamate decarboxylase-containing neuron of Ribak et al. ('79) has a large volume of cytoplasm and a type of large dense body not characteristic of medium-size spiny neurons identified by Golgi staining and examined in the electron microscope (see also Pasik et al., '79). Some of the immunoreactive boutons form symmetrical synapses similar to those we have described for the axon collaterals of striatonigral neurons, but other boutons form asymmetrical synaptic contacts (Ribak et al., '79). A further difference between the glutamate decarboxylase-contain-

ing nerve terminals and local boutons of identified striatonigral neurons is the nature of their postsynaptic targets. Some of the former were found in contact with neuronal perikarya, while none of the latter have so far been observed to form axosomatic synapses. A possible explanation of these findings is that there is more than one type of GABA-containing neuron in the striatum; perhaps one is purely a local circuit neuron and the other is an efferent neuron. Since it is well-established that there is a GABA-containing pathway from the striatum to the substantia nigra (see Dray, '79), we suggest that the GABA-containing efferent neuron is the medium-size spiny neuron that projects to the substantia nigra and that has local axon collaterals that form symmetrical synapses in the striatum.

It is noteworthy that a similar arrangement of GABA-containing neurons exists in the cerebellar cortex, where the densely spinous Purkinje cell, which has local axon collaterals, is a projecting GABA-containing neuron, and where at least two types of GABA-containing local circuit neurons are also found (see Roberts, '74).

The functional significance of these tentative conclusions is that stimulation of the cortex will excite the striatonigral neuron, leading to inhibition not only in the substantia nigra, but also in the neurons that are targets of its local axon collateral field. These predictions could, perhaps, be tested electrophysiologically.

Neuronal circuits. If, as we suggest, the transmitter used by the medium-size spiny striatonigral neuron is GABA, then the axon collaterals could provide a negative feedback loop, since they appear to terminate on other spiny neurons. Furthermore, because the local axon collaterals terminate near the parent neuron, their main role might be to influence a relatively localized group of cells, which could form a functional unit in the neostriatum.

In relation to the output from the striatum, we would like to point out that the corticostriatonigral pathway revealed in this study might well involve the same striatonigral neurons that occur in the striatonigrothalamic pathway demonstrated previously (Somogyi et al., '79). Since the nigrothalamic pathway probably uses GABA as a transmitter (DiChiara et al., '79), we can predict that stimulation of the cortex would lead, via the corticostriatonigrothalamic pathway, to disinhibition in the ventromedial nucleus of the thalamus.

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