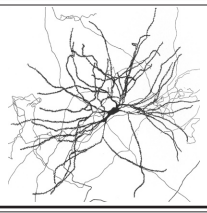

Fine Structure and Synaptic Connections of the Common Spiny Neuron of the Rat Neostriatum: A Study Employing Intracellular Injection of Horseradish Peroxidase

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ABSTRACT

Medium-sized spiny neurons of the rat neostriatum, identified by intracellular injection of horseradish peroxidase, were examined at both light and electron microscopic levels. These neurons were characterized by their heavy investment of dendritic spines, beginning about 20 um from the soma and continuing to the tips of the dendrites. Their axons arose from the soma or from a large dendritic trunk very near the soma, and tapered rapidly to form a main axonal branch from which arose several smaller initial collaterals. These arborized extensively throughout an area of about the same size as, and highly overlapping with, the dendritic field of the cell, while the main axon could be followed for distances of up to 1 mm in the direction of the globus pallidus. Three major synaptic types were seen in contact with spiny neurons.

Boutons containing small round synaptic vesicles formed synapses exclusively with spiny regions of the dendrites, and most of these were axo-spinous. Small, very pleomorphic synaptic vesicles characterized a second bouton type of unknown origin, which made contacts with somata, initial segments, and dendrites, but not dendritic spines. Boutons containing large pleomorphic synaptic vesicles had the most widespread distribution, contacting all regions including dendritic spines. Spines receiving these contacts also were postsynaptic to boutons containing small round vesicles. Axon collaterals of spiny cells formed synapses with large pleomorphic vesicles and made synapses with somata, initial segments of axons, dendrites, and dendritic spines of striatal neurons, including other spiny cells.

INTRODUCTION

The caudate nucleus and putamen, which together constitute the striatum of mammals, have in recent years been the subject of renewed interest and intensive anatomical, electrophysiological, and pharmacological and investigation (for recent reviews see Kitai et al., '79; Pasik et al., '79). Careful analysis of striatal neurons prepared using the methods of Golgi have extended the observations of early neuromorphologists, and have led to major revisions of the traditional scheme of neuronal organization in this large forebrain structure (e.g., Kemp and Powell, '71a; Leontovich, '54; Fox et al., '71/'72; DiFiglia et al., '76; Somogyi and Smith, '79). In parallel with these discoveries, the application of extracellular and intracellular recording techniques, electron microscopy, and advanced neurochemical and cytochemical procedures has shed new light on the synaptic events and neurochemical mechanisms that underlie the integration of neuronal activity in striatum and closely related structures of the basal ganglia. Taken together, these new data indicate the existence of complex local interactions among neurons of several different morphological and functional varieties in striatum, and emphasize the need for direct analysis of the synaptic interconnections and patterns of innervation for striatal cells of known somatodendritic morphology.

The development of intracellular staining techniques compatible with electron microscopy has provided a new means for unambiguous identification of synaptic connections made by individually labelled single neurons and their processes. Neurons filled by intracellular application of horseradish peroxidase and processed for identification of this enzyme become both light opaque and electron dense, so that both dendritic and axonal processes may be traced over long distances and identified in electron micrographs (e.g., Jankowska et al., '76; Cullheim and Kellerth, '76; Christensen and Ebner, '78).

In the present report, we describe the application of this technique to the common spiny neuron ofthe rat striatum. Cells ofthis type are the most numerous neuronal elements in the mammalian striatum, and are easily recognized by the appearance of their dendrites, which exhibit a heavy investiture of dendritic spines, starting about 20 um from their origin at the soma and extending to the tips of their finest branches. They have been described in Golgi-impregnated striatal of humans (Cajal, '11), as well as in monkeys (Fox et al., '71/'72: DiFiglia et al., '76), cats (Kemp and Powell, '71a), and rats (Mensah and Deadwyler, '74; Lu and Brown, '77; Somogyi and Smith, '79). Electrophysiological studies of striatal spiny neurons identified at the light microscopic level by intracellular injection of horseradish peroxidase suggest that these cells play a major role in the integration of afferent activity and that they may give rise to powerful intrinsic influences within the nucleus (e.g., Kocsis et al.,'77; Park, et al., '80). Their light microscopic appearance after intracellular injection of horseradish peroxidase has been described in detail by Preston et al. ('80). Observations on the fine structure of their somata and dendrites have been reported from electron microscopic studies of Golgi-impregnated material by DiFiglia, et al. ('78) and Somogyi and Smith ('79). Smith ('79).

METHODS

A sample of 33 intracellularly injected medium spiny neurons from the caudate-putamen of male Sprague-

Dawley rats was collected in the course of a study of the spontaneous firing patterns of these neurons. Details of the surgical procedures employed will be presented elsewhere in conjunction with the results of that study. Neurons were impaled using bevelled microelectrodes with tip diameters of 0.1-0.5 um as judged by scanning electron microscopy, and filled with a solution of 20% horseradish peroxidase (HRP) (Sigma, Type II) in 0.05 M Tris buffer (pH 8.6) and 0.5 M potassium chloride. Peroxidase was ejected from the microelectrode by application of from 4 to 12 nA positive current pulses of 100-300 msec duration at rates of 3-6/second for 1-5 minutes. After injection of from two to three neurons in each striatum, animals were deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused intracardially, first with oxygenated Krebs-Ringer's solution, and then briefly with 2% formaldehyde and 0.5%glutaraldehyde in 0.15 M phosphate buffer (pH 7.4). This fixative was followed by a longer perfusion with 4% formaldehyde, 1% glutaraldehyde in the same buffer. The brain was removed and stored in the stronger fixative at 4°C for a period ranging from 12 hours to 3 days. Serial 40-60 um coronal or saggital sections throughout the neostriatum were cut on a vibratome in 0.15 M phosphate buffer and reacted for peroxidase in 3',3'-diaminobenzidine tetrahydrochloride (0.05%) and hydrogen peroxide (0.03%) for 15 minutes at room temperature.

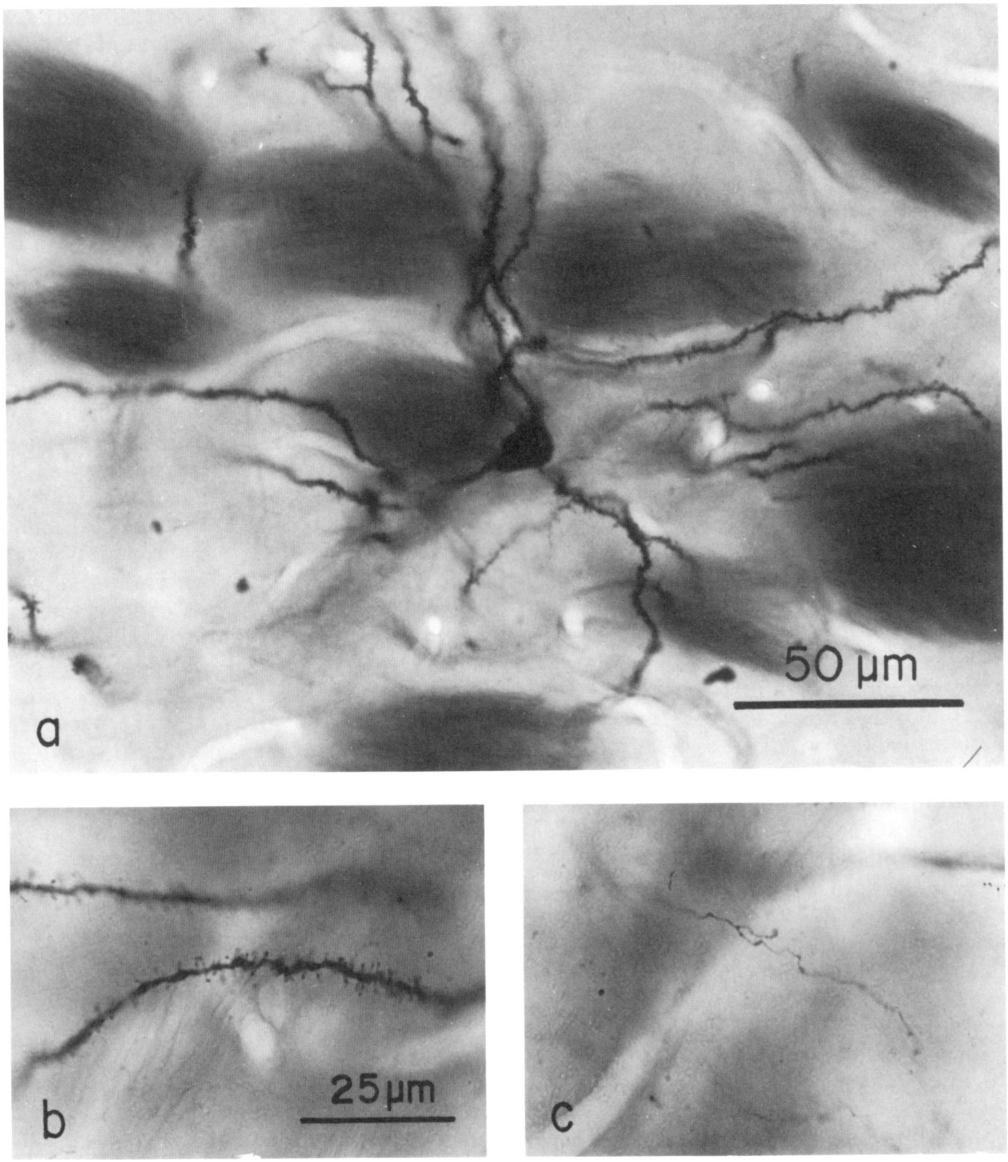


Fig. 1. Light micrographs of a neostriatal spiny neuron injected with HRP, as it appears in a 60 um section after osmium postfixation and section embedding. a. Low-magnification view of the soma and dendrites of the spiny neuron. In this coronal section, the myelinated fibers of the capsular bundles appear in oblique section. The intracellularly stained neuron has a smooth soma and lacks spines on its proximal dendrites. b. Higher-magnification reveals the high density of dendritic spines on a portion of a distal dendrite of the same neuron. c. A small portion of an axon collateral of the same neuron is shown at the same magnification used in b.

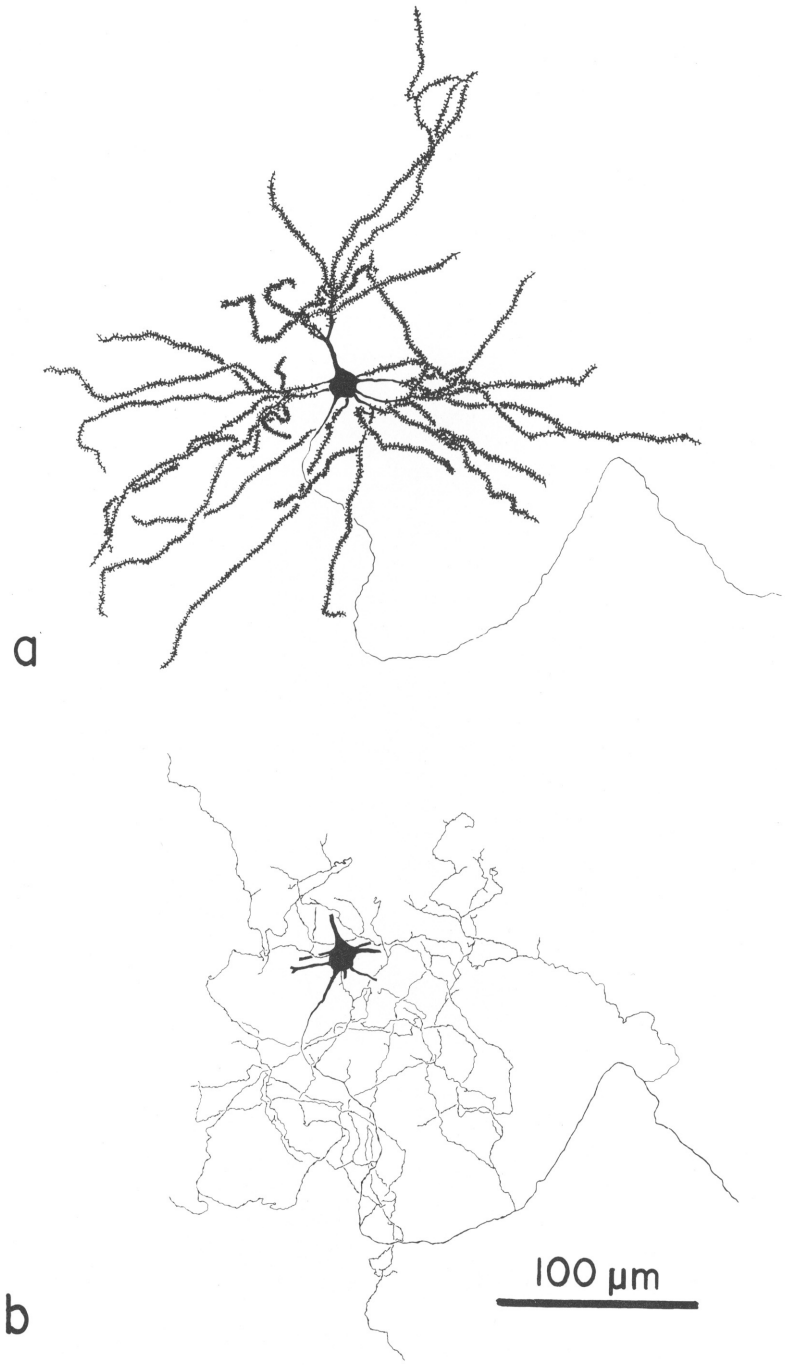


Fig. 2. a. A camera lucida drawing of the dendritic field of a neostriatal spiny neuron reconstructed from serial thick sections. The axon is shown without its collaterals. b. The collateral field of the axon of the same cell. Six collaterals arising at right angles to the main axon give rise to a dense arborization which is largely coextensive with the dendritic field.

After thorough washing in buffer to remove all traces of the reaction mixture, sections were immediately postfixed in 0.5% osmium tetroxide in 0.15 M phosphate buffer (pH 7.4). Sections were then dehydrated, stained "en block" with uranyl acetate, and embedded in serial order in Spurr's embedding medium between teflon-coated glass slides and coverslips using the section-embedding procedure described elsewhere (Wilson and Groves, '79). Section-embedded serial sections were examined under bright-field illumination in a Leitz Ortholux II microscope, and drawn at a magnification of 500 x. A smaller sample of intracellularly stained medium spiny neurons was selected for subsequent electron microscopic study. For these, the coverslip and slide were removed and the area containing the stained neuron was cut out and prepared for thin sectioning. Short ribbons of ten to 50 serial thin sections were cut throughout a selected area of the stained neuron, mounted on formvar-coated slotted grids, stained with lead citrate, and examined in a JEM 100C electron microscope at 80 kV. Serial reconstructions of some portions of injected neurons were performed on a CDC 6400 computer at the computing facility at the University of Colorado.

RESULTS

Light microscopic appearance of spiny neurons

Twenty HRP-injected neurons, all possessing the morphological characteristics of medium spiny neurons were selected for detailed light microscopic study on the basis of relatively complete staining of dendritic and axonal processes and morphological integrity. Photomicrographs from a 60 um section containing the cell body and proximal dendrites of one of these are shown in Figure 1. Bundles of myelinated capsular fibers are seen as large dense bodies due to their staining by osmium tetroxide. Camera lucida drawings made from seven consecutive thick sections through another spiny neuron are shown in Figure 2.

HRP-injected medium spiny cells had rounded or polygonal cell bodies from which arose four to eight spine-free primary dendrites. These usually branched within 30 um of the soma, giving rise to secondary dendrites of more variable length. Dendritic spines appeared at distances in excess of 20 um from the cell body, and maintained a high density throughout the remainder of the dendritic length. Third- and fourth-order dendrites were traced to their terminations at distances of up to 280 um from the cell body. The dendritic fields were generally spherical except where distorted by bundles of capsular fibers. The main axons of both neurons shown in Figures 1 and 2 were followed for over a millimeter in serial thick sections. Each HRP-injected medium spiny neuron in the present sample exhibited a clearly distinguishable main axonal branch from which from five to ten smaller collaterals could be seen to arise. In most, this main branch of the axon could be followed for a distance of over 500 um from the cell body of origin. In many, but not all cases, the axon was seen to join a capsular bundle and in a few it was followed to the border of the globus pallidus with no sign of a terminal arborization.

Collateral axon branches arose from the early portion of the axon, often from varicosities which are common in that region. Once outside the area of the dendritic field however, the axons assumed a relatively uniform caliber and collaterals were rarely seen. At distances of over one millimeter, most axons became less intensely stained and more difficult to follow. Presumably, this was due to inadequate staining of these very distal cell processes, rather than to any morphological feature of the axons themselves. Axon collaterals were best seen in the most heavily stained neurons. Due to the small caliber of the finest branches, the entire local axonal arborization was difficult to reconstruct. Possibly, the smallest axonal branches, even when uniformly stained, are too small to be resolved in the light microscope. The appearance of a larger collateral branch is shown in Figure 1. As shown in Figure 2, axonal processes branch profusely to form an extensive plexus of varicose fibers which extends throughout an area overlapping with, and about the same size as, the dendritic field.

Not counted in the sample described above were a few attempted intracellular injections in which from two to five neurons and/or glia were found to be HRP-positive. Usually, the impaled cell intended for labelling could be clearly made out, since it was stained more darkly than the others. In addition, the stained cells in these cases usually did not have adjacent cell bodies, but were separated by 50-200 um. In the neuropil between them, unstained somata of neurons and glia could often be detected. Probably, most of the cells inadvertently filled with HRP were injected by way of their dendrites, which passed near the soma of the most darkly stained neuron. This pattern of labelling suggests that it may be possible to inadvertently stain afferent or intrinsic axons by the same course of events which leads to multiple stained cells. Despite repeated attempts to do so no light microscopic sign could be found of stained free axons not arising from nearby HRP-positive neurons.

Often present in the vicinity of stained neurons were perivascular cells containing granules of peroxidase-positive material. These cells were similar to those reported present in the area around large extracellular injections of the enzyme (e.g., Nauta et al.,'74). They were more numerous in cases of multiple stained cells or stained neurons undergoing degenerative changes in response to the injection.

In addition to those described above, five neurons were excluded from the sample due to their extreme state of degeneration. HRP-injected neurons often showed some indication of degenerative changes, such as shrunken somatic contours or large somatic indentations, which did not interfere with their identification. In these five, however, the process was so advanced that little could be ascertained about their neuronal identity.

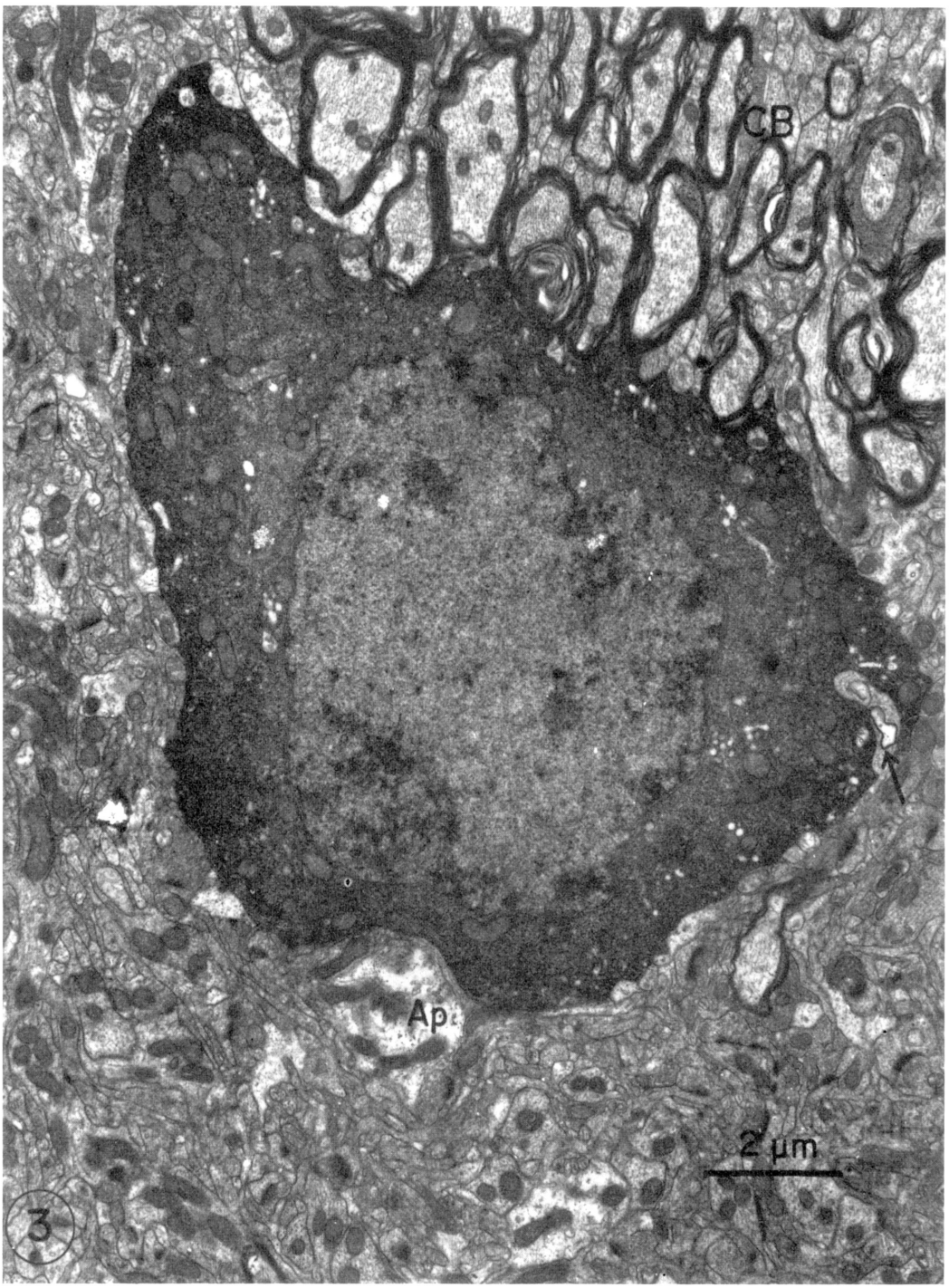


Fig. 3. A low-magnification electron micrograph of the neuron shown in Figure 1. HRP reaction product is visible in the nucleus as well as the cytoplasm. A portion of a capsular fiber bundle (CB) is visible at the upper right. The swollen process of a nearby astrocyte (Ap) is seen adjacent to the injected neuron, and another process is seen indenting the cytoplasm (arrow). Aside from these indications of damage caused by the intracellular electrode, the cytoplasm and the surrounding neuropil have a normal appearance.

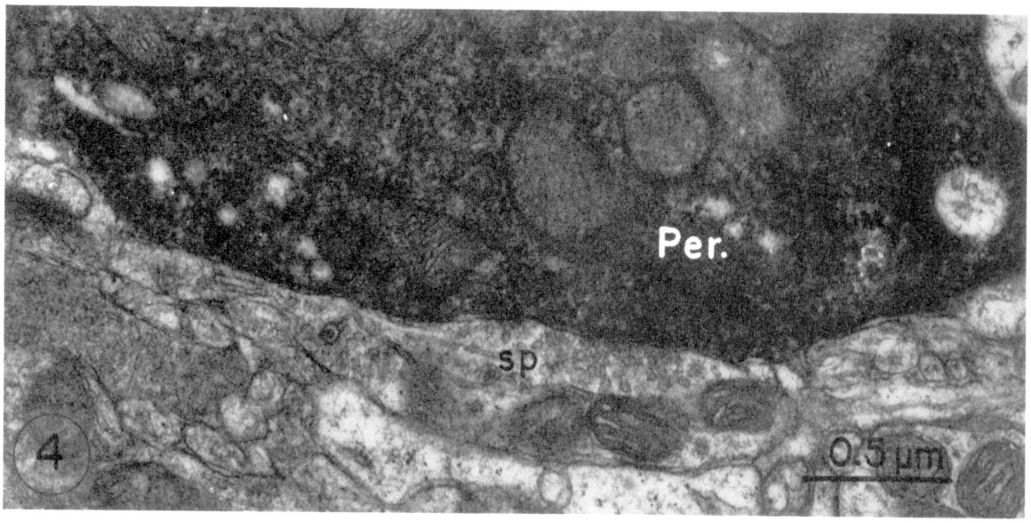
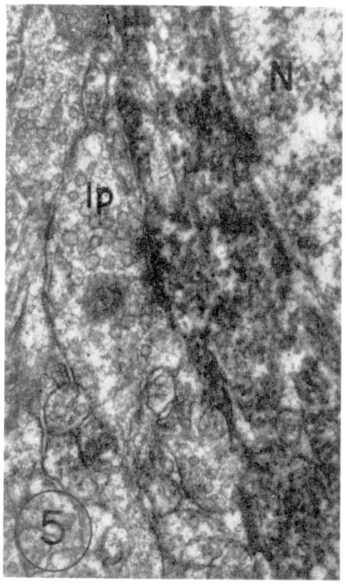


Fig. 4. An axo-somatic synapse formed on an HRP-injected spiny neuron (Per.) by a small unidentified axonal process containing small highly pleomorphic vesicles (sp.).



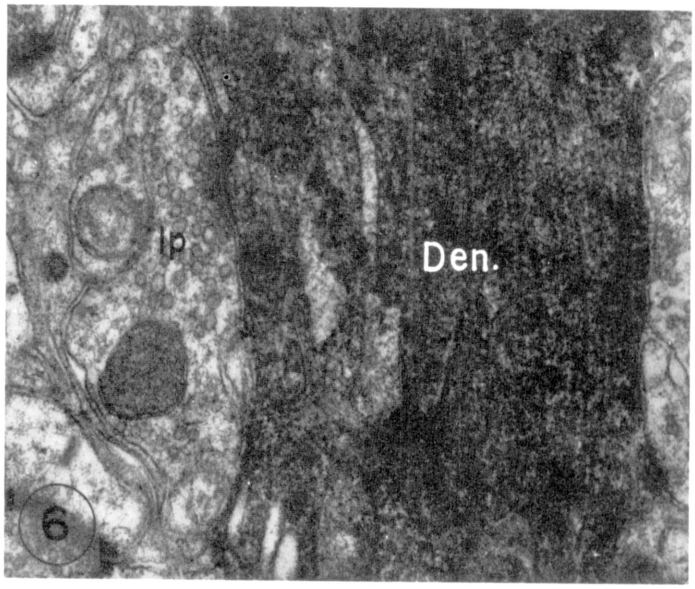


Fig. 5. A second type of synapse seen on the somata of injected spiny neurons, made by a bouton containing large pleomorphic vesicles (lp). Magnification as in Figure 4. A portion of the nucleus of the injected neuron is visible (N).

Fig. 6. A synapse formed by a bouton with large pleomorphic vesicles (lp), on the proximal spine-free portion of an identified spiny neuron dendrite. Magnification as in Figure 4.

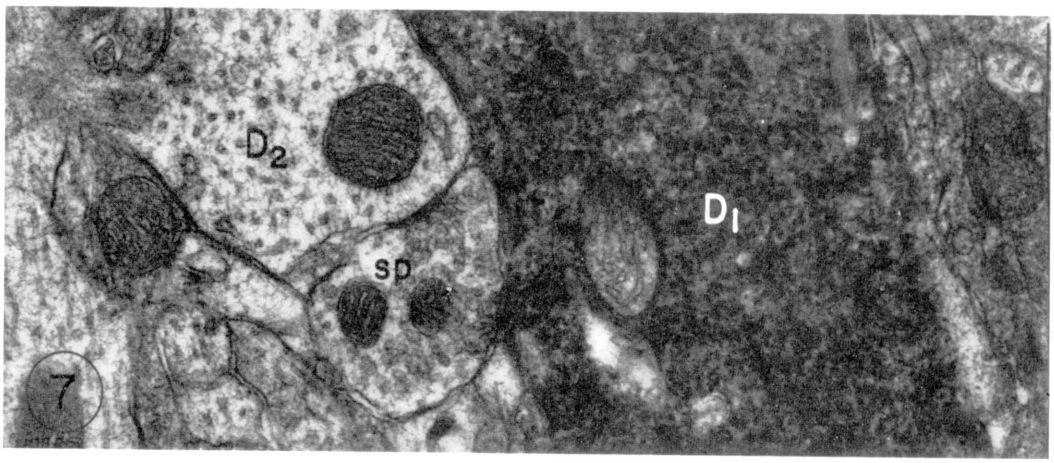


Fig. 7. A bouton containing small pleomorphic vesicles (sp) forming synaptic contacts with the proximal portion of a spiny cell dendrite (D1) and an unidentified dendrite of smaller diameter (D2). Magnification as in Figure 4.

Electron microscopy

Eight identified medium spiny neurons were selected for examination at the ultrastructural level. All of these exhibited features similar to those of the neuron shown in Figure 3. Peroxidase reaction product in the cell body and dendrites appeared as a uniform coarse granularity filling the cytoplasm. Some neurons, like the one shown in Figure 3, had nuclei containing deposits of the reaction product. In others, the nuclei were not stained, presumably due to failure of the microelectrode to penetrate the nuclear envelope. The interiors of membrane-bound cytoplasmic compartments such as the nuclear envelope, Golgi apparatus, endoplasmic reticulum, and mitochondria were free of reaction product. No extracellular reaction product could be recognized. The density of reaction product varied widely across neurons, but was relatively uniform within a stained cell.

Most HRP-injected neurons, including some of those showing no evidence of degenerative changes when examined by light microscopy, exhibited some indication of damage at the electron microscopic level. The neuron shown in Figure 3 is typical of these. Swollen glial processes can be seen in that figure occupying identations of the soma of the injected neuron. In other sections through this cell, these glial processes were traced to an astrocyte lying adjacent to the neuronal cell body. Despite such abnormalities all of the identified neurons examined were clearly of the common cytological type of neostriatal neuron described by numerous other authors (e.g., Mori, '66) and recently identified with the spiny type I neuron of monkey striatum (DiFiglia, et al., '78). All had round unindented nuclei which nearly filled the somata, and lacked heavy localized accumulations of microtubules, lysosomes, or rough endoplasmic reticulum.

Axo-somatic synapses

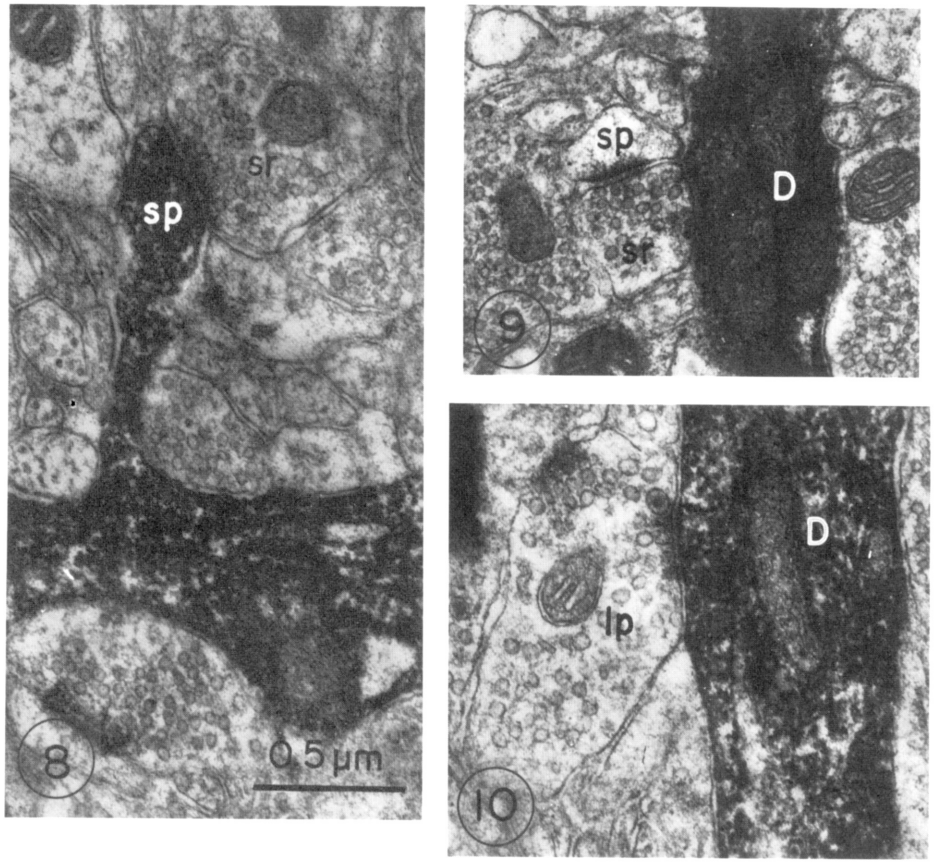


Fig. 8. A dendritic spine (sp) arising from the distal region of a spiny cell dendrite heavily filled with HRP reaction product. The most distal expanded portion of the dendritic spine receives a synaptic contact from a bouton containing small round synaptic vesicles (sr).

Fig. 9. A synapse involving the shaft of a spiny, distal dendrite of an identified spiny neuron (D) and a bouton containing small round vesicles (sr). Another synapse made by this bouton on a nearby dendritic spine of an unidentified neuron (sp) exhibits a pronounced postsynaptic specialization. Magnification as in Figure 8.

Fig. 10. A synaptic contact involving a bouton containing large pleomorphic vesicles (lp) and the shaft of a distal dendrite of a neostriatal spiny neuron (D). Magnification as in Figure 8.

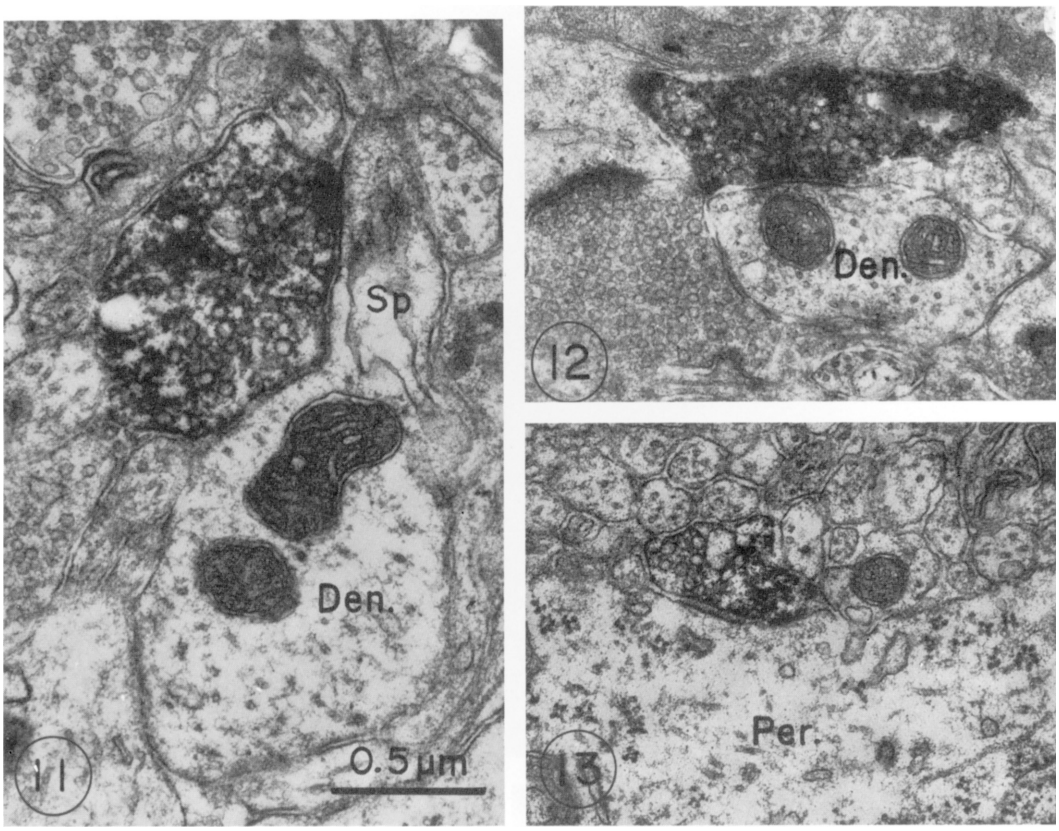


Fig. 11. A varicosity arising from the axon collateral of a HRP-injected spiny neuron forming a synaptic contact with the neck of a dendritic spine (sp) arising from the dendrite of an unidentified striatal neuron.

Fig. 12. A synapse made by the axon collateral of an identified spiny neuron on the shaft of an unstained neostriatal dendrite (Den). Magnification as in Figure 11.

Fig. 13. A small varicosity formed by an axon collateral of an identified spiny neuron contacting the perikaryon of an unstained neostriatal neuron (Per). The postsynaptic cell body exhibits cytological features similar to those of identified spiny neurons. Magnification as in Figure 11.

All HRP-injected neurons were examined for axosomatic synapses and synapses on initial axonal segments using several 10-50 consecutive section series taken throughout the region of the cell body. Two types of synapses were present on both somata and initial segments of identified spiny neurons. Examples of these synapses are shown in Figure 4-7. One of them (Figs. 4 and 7) is formed by a bouton containing small, highly pleomorphic synaptic vesicles. Axons forming synapses of this type were generally of very small caliber and formed their contacts "en passant." Synapses of the other type contained large moderately pleomorphic synaptic vesicles (Figs. 5 and 6). The accumulation of HRP reaction product usually obscured the postsynaptic density to some degree, making difficult fine distinction between symmetrical and asymmetrical junctional specializations. When observed on unstained neurons, synapses of these types usually exhibited symmetrical junctional densities. Synapses of each type were present in approximately equal numbers on all portions of the somata and the initial part of the axons of identified spiny neurons. Neither was plentiful, however, and many sections through these regions of the neurons showed no synapses at all.

Axo-dendritic and Axo-spinous synapses

Density of synapses on dendrites of identified medium spiny neurons increased only slightly with distance from the soma over the initial spine-free portion of the primary and secondary dendrites. Like somata, these dendritic regions receive contacts almost exclusively from axons containing small or large pleomorphic synaptic vesicles, and forming mostly symmetrical junctional specializations.

Synapses of all types were more common along the spiny regions of the dendrites. Most numerous were those formed by boutons containing small round vesicles and contacting the heads of HRP-filled dendritic spines. An example of this type of synapse is shown in Figure 8.

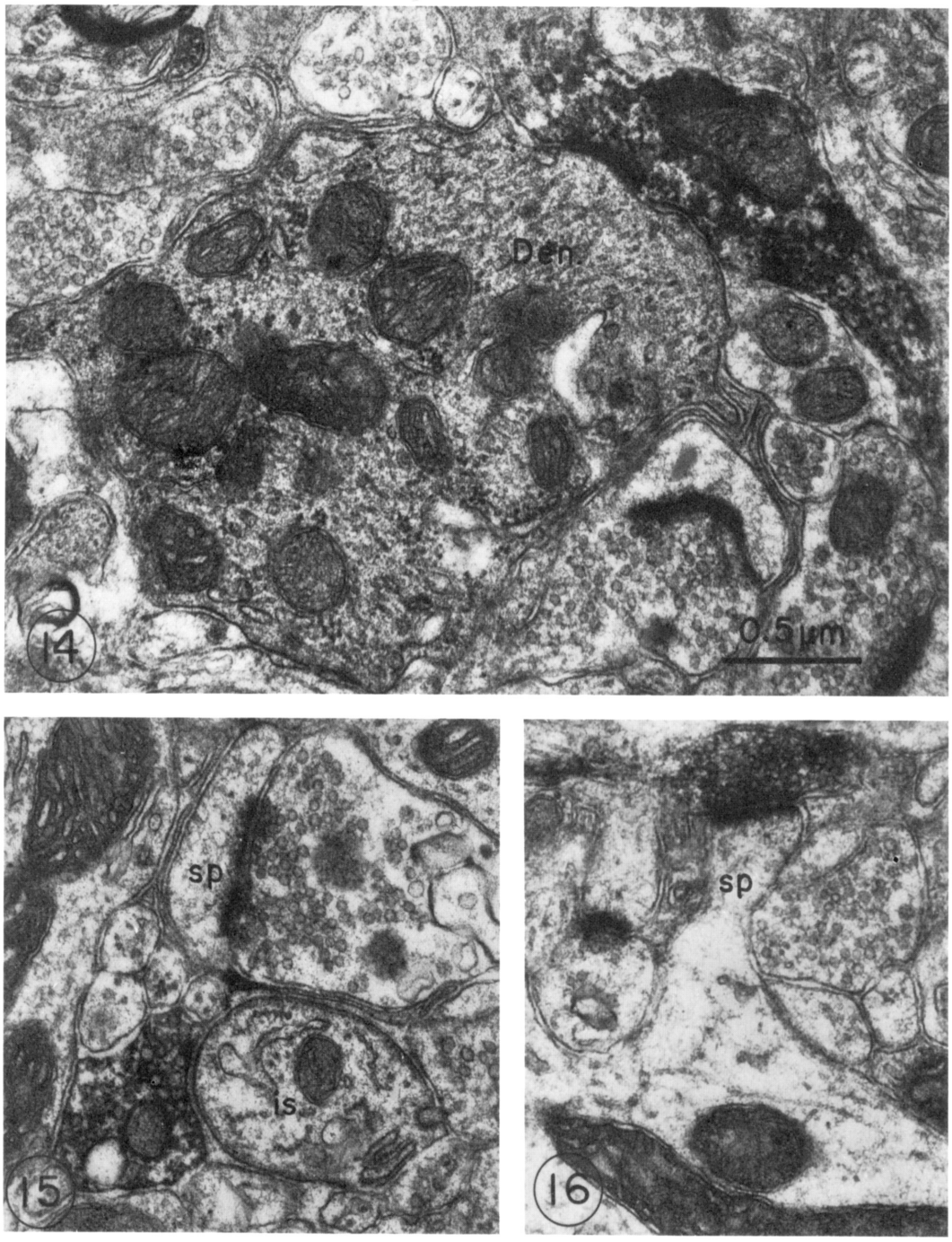


Fig. 14. A synapse involving the axon collateral of an identified spiny neuron and a large dendritic trunk of an unidentified neostriatal neuron.

Fig. 15. An axo-axonal synapse involving the axon collateral of a HRP-injected spiny neuron and the initial axonal segment (i.g.) of an unlabelled neostriatal neuron. The postsynaptic element is identified by its prominent axolemmal undercoating and microtubule fascicles. The synaptic specialization appears highly symmetric when compared with the unlabelled axo-spinous contact seen immediately above (sp). Magnification as in Figure 14.

Fig. 16. A synapse by a HRP-labelled process which could not be located in light micrographs taken before thin sectioning, and so could not be identified. Note the unusual features of the labelled element, its small synaptic vesicles, asymmetric junctional specialization, and synaptic location on the head of a dendritic spine. This may represent an axon stained inadvertently during peroxidase injection. Magnification as in Figure 14.

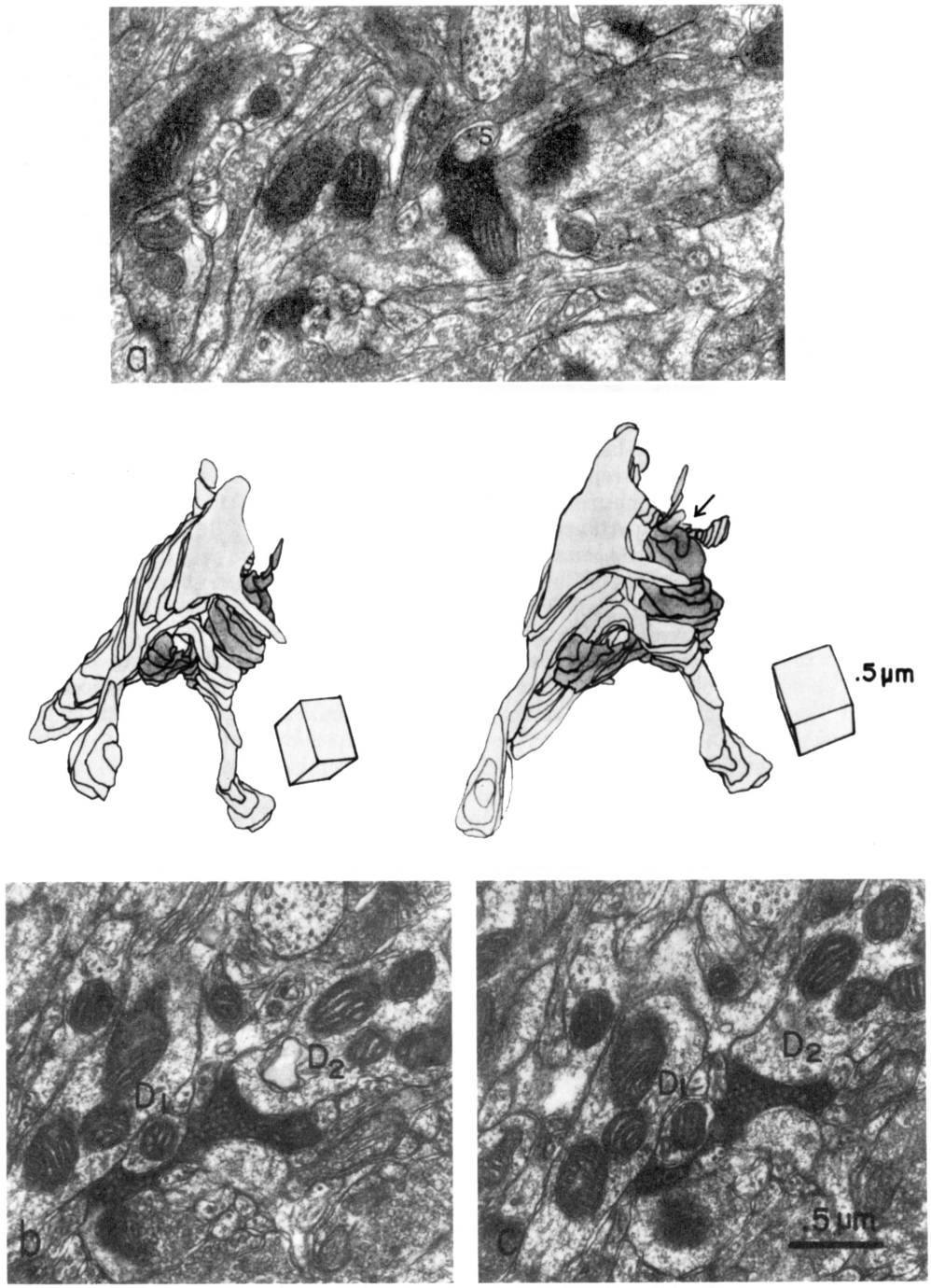


Fig. 17. Three micrographs (a, b, and c) from a set of consecutive sections through an HRP-labelled axonal varicosity along the rotations of a computer-assisted three-dimensional reconstruction of the axon varicosity (shaded) and the spiny dendrite marked as D1 (in band c). The sense of the rotations is indicated by the orientation of the .5 um cube shown to the right of each drawing. A synapse made onto the neck of a spine arising from D1 is shown in the lefthand drawing, and the location of that synapse is indicated by an arrow in the drawing on the right. Dendrite D2 is not included in the reconstruction.

Boutons of similar appearance were occasionally seen to form contacts with the dendritic shafts of identified medium spiny neurons, but only along the spiny regions of these dendrites (Fig. 9). More commonly, synapses on dendritic shafts, even in the most distal regions of the dendritic field, were of the types present on somata and proximal regions of the dendritic surface (Fig. 10). One ofthese latter, containing large pleomorphic vesicles, was also sometimes seen contacting dendritic spines. In these cases, the synapse formed was placed proximally on the spine, usually not upon the expanded "head" region, but rather on the "neck," and usually near its junction with the dendritic shaft. An appreciation of this synaptic arrangement was usually not possible except by examination of serial sections through the whole of the dendritic spine. In all cases so examined, spines contacted by boutons containing large vesicles also received a more distally placed contact from a bouton of the more common type containing small round synaptic vesicles.

Axon collaterals of identified neurons

In axons of peroxidase-injected neostriatal neurons, reaction product was usually more finely granular than in large dendrites and somata. In heavily stained cells, stained axons usually appeared uniformly black with unstained synaptic vesicles and endoplasmic reticulum standing out as if negatively stained. Often these axonal processes were difficult to section and became torn or distorted. Less heavily stained axons showed fine grains of reaction product condensed primarily onto intracellular membranes and microtubules. Attempts were made in all cases to identify HRP filled boutons observed in this section on corresponding light micrographs and drawings of the same area as it appeared in thick sections. In most cases, this was successful, and allowed positive identification of labelled boutons as axonal processes of identified spiny neurons.

Examples of synapses made by axon collaterals identified in this way are shown in Figures 11-15. As evident in those examples, boutons arising from the axon collaterals of spiny neurons resembled those with large moderately pleomorphic vesicles described earlier as ending on the somata, initial segment, dendritic-shafts, and necks of dendritic spines of cells of the same type. In addition to the similarity between their vesicle populations, these synapses were seen to have identical distributions of postsynaptic sites. Most commonly, they contacted small dendrites, or "necks" of dendritic spines, as shown in Figures 11 and 12. Occasionally, however, they could be seen to make synaptic contacts with somata, initial segments of axons, and large dendritic trunks of unidentified neostriatal neurons, as shown in Figures 13-15.

In order to obtain a quantitative estimate of the relative distribution of postsynaptic elements involved in synaptic contacts with collaterals of medium spiny neurons, 50 HRP-labelled synapses found in the vicinity of injected neurons have been examined in series of consecutive thin sections. In many, but not all of these cases, the labelled presynaptic element was identified in light micrographs from the same material prior to thin sectioning and traced to the injected neuron. All but three of these boutons, and all of those which could be traced directly to axon collaterals of identified neurons, contained large pleomorphic vesicles as described earlier. Of these 47 synapses, 19 (40%) were formed with dendritic spines. These all occupied spines which also received another more distally placed synaptic contact from a bouton containing small round vesicles. Twenty-two other HRP-labelled boutons (48%) contacted dendritic shafts in the neostriatum, while six (12%) made synaptic contacts with somata or the initial axonal segments of neostriatal neurons. Three neostriatal boutons containing HRP reaction product had a strikingly different appearance. None of these could be traced to the collateral field of an injected neuron, but they were found within the dendritic fields of stained neurons. These rare boutons contained small round vesicles and formed asymmetric synaptic junctions with dendrites and dendritic spines. They may represent axons of other cells stained inadverently during injection of HRP. An example of one of these is shown in Figure 16.

The finding that axons of medium spiny neurons make synaptic junctions with dendritic spines, and that they have morphological characteristics similar to boutons ending on identified medium spiny neurons suggests that such cells form synaptic contacts among themselves. This was confirmed by examination of serial sections in which the identity of both presynaptic and postsynaptic elements involved in synapses formed by collaterals of HRP-injected neurons could be identified. A reconstruction of one such set of serial sections is shown in Figure 17. Two rotations of the reconstructed profiles, drawn with the aid of the CDC 6400 computer at the University of Colorado, are shown in that figure along with representative micrographs from the series. The peroxidase-filled axon collateral, located approximately 100 um from its cell body of origin, was of extremely fine caliber (< 0.1 um), but expanded to form a varicosity filled with vesicles and which made three synaptic contacts with unlabelled neuronal processes within the series of 28 sections used in the reconstruction. One of these was a contact with the unidentified dendrite (marked D2 in Fig. 17b and c), and is not included in the reconstruction. The other dendrite (marked D1 in Fig. 17b and c) received two synaptic contacts from the axon varicosity. One of these was onto the spine neck, (marked s in Fig. 17a), and one was onto the dendritic shaft near the point at which the spine arises. This dendrite, which is shown in the drawing, was seen to give off four other dendritic spines in slightly more than 2 um of its length. This high density of dendritic spines makes it unlikely that it could be a dendrite of any neuron type other than the medium spiny cell.

DISCUSSION

Although only one of its several neuronal constituents, the heavily spine-laden neuronal type described here

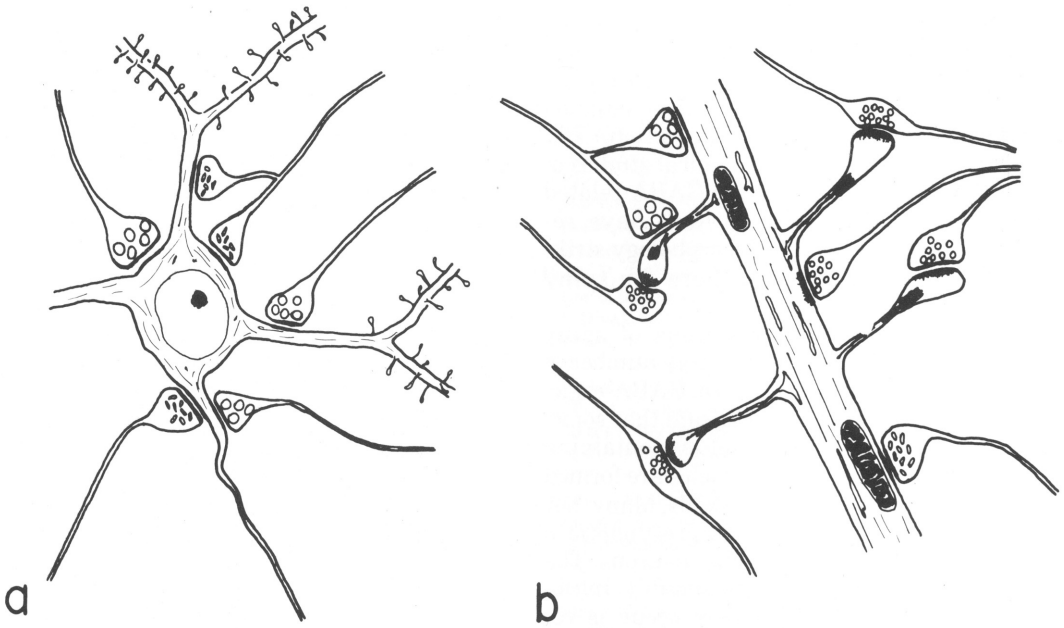


Fig. 18. A diagram summarizing the synaptic contacts on the spiny neurons. a. On somata, proximal spine-free dendritic segments, and initial axonal segments, boutons containing small, very pleomorphic synaptic vesicles and boutons containing large synaptic vesicles sensitive to flattening in some fixatives are present. Both types form mostly symmetric junctions, and seem to be present in relatively small numbers on all of these postsynaptic regions. b. On the more distal, spiny regions of the dendritic field, numerous dendritic spines are contacted by boutons containing small round vesicles. Occasionally, this type of contact is seen on the dendritic shaft. Most of these junctions appear to be asymmetric. The shaft of the spiny dendrite more commonly forms junctions with large and small pleomorphic vesicle boutons similar to those seen contacting the proximal surfaces of the spiny neuron. The boutons containing large pleomorphic vesicles also make contacts with the necks of dendritic spines. Some or all of the boutons containing large synaptic vesicles arise from the axon collaterals of spiny neurons within the neostriatum.

probably represents the overwhelming majority of neurons in the mammalian striatum. The importance of these cells in the local circuitry of the striatum has been recognized since the studies of Cajal ('11), which demonstrated their local axonal arborizations, and has been stressed repeatedly by subsequent investigators (e.g., Fox et al., '71/'72 Kemp and Powell, '71a; DiFiglia et al., '76). Their long-suspected role as the major target of neostriatal afferents has in recent years been confirmed by electron microscopic observations demonstrating that most synapses formed by all three major afferents to neostriatum are axo-spinous in nature (e.g., Kemp and Powell, '71c; Bak et al., '75; Chung et al., '77; Hattori et al., '73). Electrophysiological evidence for convergence of monosynaptic input onto individual identified spiny neurons (Kocsis et al., '77) suggests that these neurons may play a dominant role in the receipt and integration of information impinging on basal ganglia from cerebral cortex, thalamus, and substantia nigra. Finally, evidence has now accumulated to indicate that some and perhaps all of these neurons possess long axons and together constitute a major source of neostriatal efferents to substantia nigra and paleostriatum (Somogyi and Smith, '79; Preston et al., '80). Thus, the medium spiny neuron may represent the predominant direct input-output pathway for that structure, as well as a common pathway for the less direct routes through the complex network of its neuropil.

In view of their role in the integration of activity from neostriatal afferents, the rigid segregation of synaptic contacts on these neurons may be of some functional significance. This segregation according to morphological synapse types as seen in HRP-injected spiny neurons is summarized in Figure 18. Boutons containing small round vesicles and forming clearly asymmetric synapses have been repeatedly associated with the termination of neostriatal afferents from cortex, thalamus, and substantia nigra (e.g., Kemp and Powell, '71c; Hattori et al., '73; Hokfelt, '68; Chung et al., '77; Hattori et al., '79). In unlabelled material, contacts of this type can be observed on somata and large proximal dendrites of unidentified neostriatal neurons (Kemp and Powell, '71b). The present observations on identified spiny neurons suggest, however, that these cells rarely, if ever, receive axosomatic contacts of this type. On these neurons, junctions of the kind associated with striatal afferents appear to be restricted to the spiny regions of the dendritic field. The great majority of them form their contacts onto dendritic spines. Intracellular recordings of synaptic potentials evoked in identified spiny neurons indicate that all three major afferents to these cells may be excitatory in nature

(e.g., Kitai et al., '79). The exclusion of these synapses from proximal sites on the spiny neurons, and their placement on dendritic spines, would seem to insure that no one afferent synapse or small group of synapses could become dominant in the control of activity in these cells. The great number of afferent synapses formed on each spiny neuron (Kemp and Powell, '71d), and the "cruciformaxodendritic" organization of these contacts (Fox et al., '71/'72) indeed suggest that the activation of these cells may normally be dependent upon the coordinated effort of a great number of afferent fibers, probably from more than one source. Some evidence is available to suggest the possibility of an intrinsic source of some striatal boutons of this type (Kemp and Powell, '71c; Tennyson and Marco, '73; Hattori et al., '76), perhaps from an excitatory striatal interneuron. If so, it seems likely that those synapses are subject to the same limitations as afferent synapses in the degree to which they may influence the behavior of spiny neurons.

The origin of presynaptic boutons containing small highly pleomorphic synaptic vesicles is unknown. These boutons, which correspond to the small flat vesicle boutons of Kemp and Powell ('71b) and to the type VI boutons of Hassler and Chung ('76), are most likely of intrinsic origin, although the striatal neuron from which they arise has not yet been identified. In contrast to the report of Kemp and Powell ('71b), they have not been seen in the present material to make synaptic contacts with dendritic spines, but occupy sites on the somata, initial axonal segment, and dendritic shafts of striatal spiny neurons. Since some spines do arise from dendrites of other striatal neurons (e.g., DiFiglia et al., '76), it is possible that the synapses seen by Kemp and Powell were on spines of these less spiny neurons.

Boutons containing large pleomorphic synaptic vesicles have been described repeatedly in striatum (Mori, '66; Rafols and Fox, '71/'72; Kemp and Powell, '71b; Bak et al., '75; Pasik et al., '76). The synaptic vesicles within these boutons are highly sensitive to flattening in cacodylate buffer (e.g., Kemp and Powell, '71b), and can appear in different degrees of flattening with relatively minor differences in the conditions of fixation. With the phosphate buffer used in the present experiments (310-320 mosm), the vesicles often appeared quite rounded and uniform, although a few elliptical or flattened vesicles could usually be seen. These synapses enjoy the widest distribution on the surface of the medium spiny neuron, contacting both proximal regions such as spinefree dendritic segments, somata and initial axonal segments, and distal dendritic regions including dendritic spines. The similarity between striatal boutons containing large pleomorphic synaptic vesicles and those formed by striato-nigral fibers has led some investigators to suggest that they may represent axon collaterals of striato-nigral cells (Fox et al., '71/'72; Kempt, '70; Hassler and Chung, '76).

The present finding that axon collaterals of identified spiny neurons form synapses of the same morphology and distribution as the large pleomorphic vesicle boutons suggests that these cells may indeed be the source of the extensive gamma-amino butyric acid (GABA) containing striato-nigral projection. That spiny neurons do send axons to the substantia nigra has been demonstrated electrophysiologically (Kitai et al., '79). Studies of striato-nigral neurons identified by retrograde axonal transport have also indicated that these represent too large a proportion of striatal neurons to be accounted for entirely by the other neurons types, and include cells having somatic morphology suggestive of the spiny neurons (Grofova, '75; Bunney and Aghajanian, '76). Very recently, immunocytochemical studies of striatal neurons containing the GABA-related enzyme glutamate decarboxylase have revealed cells and synapses of morphology strikingly similar to that seen here for spiny neurons (Ribak et al., '79).

The local axonal arborizations of spiny striatal neurons give rise to a large number of synapses, which are probably also GABA-ergic. Indeed, the present results indicate that some, perhaps all, of the striatal boutons containing large pleomorphic synaptic vesicles are formed by the axon collaterals of these cells. Many, but certainly not all such profiles are presynaptic to processes of other, nearby, spiny neurons. The range over which these (presumably inhibitory) lateral interactions may occur is restricted by the size and shape of the local axonal arborization. Because the axon collaterals of HRP-injected spiny neurons appear to remain within a volume roughly corresponding to the dendritic fields of these cells, it seems likely that direct lateral interactions would occur only between spiny neurons with overlapping dendritic fields (i.e., cells located within about 0.5 mm of each other). The contacts made by local axon collaterals may occupy either proximal or distal regions of the postsynaptic neuron, suggesting as well that the strength of the interaction between two nearby spiny neurons might be highly dependent upon the distance between them. Thus, cells less than about 250 um apart might exercise a very powerful influence upon each other by way of axosomatic contacts, synapses onto proximal dendritic trunks, or contacts onto initial axonal segments. Neurons separated by a greater distance may interact in a more restricted manner, making contacts only within those portions of their dendritic fields which overlap. This spatially organized system of lateral interconnections could play a major role in the processing of information converging upon the neostriatum via afferent fibers now believed to themselves possess a highly structured spatial topography.

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