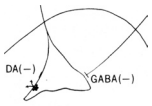

Monoaminergic Synapses, Including Dendro-Dendritic Synapses in the Rat Substantia Nigra

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ABSTRACT

Intraventricular administration of 1 or 2 mg of the osmiophilic "false transmitter" 5-hydroxydopamine (5-OHDA) was used to label monoamine storage and release sites in the rat substantia nigra. Vesicles containing unusually dense cores indicative of the presence of the marker were seen forming from the Golgi apparatus in the cell bodies of medium-sized neurons of the substantia nigra, pars compacta, and from smooth endoplasmic reticulum in the dendrites of those neurons and in small unmyelinated axons of unknown origin. In serial sections, both axons and dendrites containing synaptic vesicles marked with 5-OHDA were seen to form synapses "en passage" in pars compacta, and some presynaptic dendrites containing vesicles filled by the marker were also observed to form contacts with dendrites in pars reticulata. The only identified postsynaptic elements engaging in monoaminergic synapses in the substantia nigra were dendrites of medium-sized pars compacta neurons.

Key words: Substantia nigra - 5-OHDA - Dendro-dendritic synapses - Self-inhibition - Monoamine storage sites

INTRODUCTION

Among the earliest observations made in the central nervous system with the catecholamine histofluorescence method of Falck and Hillarp (Dahlstrom and Fuxe, 1964; Anden et al., 1964) was that of a small group of catecholamine-containing neurons in the substantia nigra, pars compacta, whose axons form the dopaminergic nigro-striatal pathway. Although interest in these neurons has generally centered around their role as a source of dopaminergic axons innervating the forebrain (e.g. Usunoff et al., 1976), recent improvements in the histological demonstration of catecholamines have allowed the description of a dense net of dopamine-containing neuron processes throughout the neuropil of the substantia nigra, pars compacta, and extending into pars reticulata (Lindvall and Bjorklund, 1974; Bjorklund and Lindvall, 1975). These processes, which appear to be dendrites of the dopaminergic neurons, have been suggested to represent a possible substrate for dendro-dendritic interactions within the substantia nigra (Bjorklund and Lindvall, 1975; Groves et al., 1975, 1976).

A role for dopamine as a neurotransmitter within the substantia nigra has been suggested from experiments examining the electrophysiological effects of local microinfusion of drugs which affect dopaminergic transmission (Groves et al., 1975, 1976), and is supported by the demonstration of dopamine release in the substantia nigra in vitro (Geffen et al., 1976; Paden et al., 1976) and in vivo (Korf et al., 1976; Cheramy et al., in press). At least some of the dopamine release observed in neurochemical studies is probably dendritic, since Geffen et al. (1976) were able to obtain measurable potassium-induced release from samples of tissue taken from pars reticulata, which contains dendrites, but not axons or cell bodies of dopaminergic neurons (Bjorklund and Lindvall, 1975; Juraska et al., 1977). Any more complete description of dopamine release sites within the substantia nigra, however, requires their identification at the ultrastructural level.

The existence of dendro-dendritic synapses in the substantia nigra of the rat was suggested by the ultrastructural evidence of Hajdu et al. (1973), but in the absence of a marker for dopaminergic synapses, the identity of both the pre- and postsynaptic elements involved in these synapses is uncertain. The ultrastructural identification of monoaminergic synapses has been greatly facilitated by the introduction of 5-hydroxy-dopamine (5-OHDA) as a synaptic marker (Tranzer and Thoenen, 1967; Richards and Tranzer, 1970). Whether administered intraventricularly or by incubation in vitro, this substance apparently acts as a "false transmitter", being specifically accumulated and released by monoaminergic neurons. Due to its precipitation by glutaraldehyde and ability to subsequently reduce osmium tetroxide, it allows direct visualization of its presence by forming an electron-dense core in the small (30-50 nm) electron-lucent synaptic vesicles usually seen in central monoaminergic neurons after aldehyde-osmium fixation. It has been extensively tested in the central nervous system (e.g. Richards and Tranzer, 1970; Ibata et al., 1974; Tennyson et al., 1974). One report of the ultrastructural appearance of the substantia nigra after 5-OHDA administration is presently available (Ibata et al., 1974) in which the presence of monoaminergic synapses is demonstrated, but no description of the nature or source of the elements involved in such synapses has yet been presented.

The experiments reported here were designed to identify the source of monoaminergic release sites within the substantia nigra by electron microscopic examination of serial sections taken from animals pretreated with 5-OHDA.

MATERIALS AND METHODS

Nine male Sprague-Dawley rats weighing 250-300 grams were used in these experiments. Subjects were anesthetized with sodium pentobarbital supplemented with ether, and a 32 gauge stainless steel injection cannula connected by means of a teflon tube to a Hamilton

microsyringe was placed stereotaxically into the left lateral ventricle. Isotonic saline (3 rats) in a volume of 10 ul, or 5-hydroxydopamine hydrochloride (Aldrich) dissolved in 5 or 10 ul of isotonic saline at a dose of 1 mg (4 rats) or 2 mg (2 rats) respectively was injected over a period of 15 min. The cannula was then removed, the wound closed, and the animal was returned to its cage. In order to avoid the severe and in some cases fatal convulsions seen in initial experiments to occur from 30-60 min after administration of 5-OHDA, later animals were maintained at a light stage of anesthesia with supplemental doses of sodium pentobarbital for the remainder of the survival period.

After a survival period of from 30 min to 3 hours, animals were again deeply anesthetized with sodium pentobarbital and perfused intracardially, first briefly with Krebs-Ringer solution, followed for 5 min by a solution of 0.5% glutaraldehyde and 2% paraformaldehyde in 0.16M cacodylate buffer (pH 7.4), and finally for 20 min with 1% glutaraldehyde and 4% paraformaldehyde in the same buffer. The brain was then removed and stored overnight in the latter fixative at 4° C. Tissue blocks from the midbrain were prepared to include a fairly large portion of the substantia nigra and surrounding tissue to facilitate orientation, washed briefly in buffer and postfixed for 60 min in 1% OsO4 in 0.16M cacodylate buffer. They were then stained "en bloc" by immersion for 16 hours in 0.5% aqueous uranyl acetate, dehydrated with a graded acetone series and embedded in Epon-Araldite.

Semi-thin sections were cut from blocks oriented in the coronal or saggital plane, stained with toluidine blue and examined in the light microscope to determine how each block should be further trimmed. Ribbons of from 20 to 100 consecutive sections were cut with a Sorvall MT-2b ultramicrotome from blocks trimmed to only include tissue ffom either pars compacta or pars reticulata of the substantia nigra. They were mounted on Formvar-coated slotted grids, stained with lead citrate, and examined with a JEM-1OOB electron microscope at 80 KV. The placement of the injection cannula was verified in every case from 60 um frozen sections stained with cresyl violet.

OBSERVATIONS

Saline-Treated Rats:

The essential features of the normal substantia nigra of the rat have been described by other investigators (e.g. Gulley and Wood, 1971; Hajdu et al., 1973), and the observations made on the saline-treated animals in the present material are consistent with those earlier reports, as well as those made on the cat (Rinvik and Grofova, 1970) and monkey (Schwyn and Fox, 1974). In pars reticulata, two neuron types may be distinguished on the basis of their cytological features. The principal neuron is characterized by its relatively large volume of cytoplasm which is richly endowed with organelles. The cell body tapers gradually into the large dendritic trunks, which even in short series of coronal or saggital sections can often be followed for considerable distances. The small pale neuron, which almost certainly corresponds to the nigral interneurons seen in Golgi-stained preparations (Cajal, 1955; Gulley and Wood, 1971; Juraska et al., 1977) generally has only a narrow ring of relatively empty cytoplasm surrounding its highly indented nucleus, and is conspicuous for its paucity of rough endoplasmic reticulum. The thin dendrites of this cell usually form abruptly from the round cell body, and take an irregular course making them more difficult to follow even in relatively long series of consecutive sections. Axo-somatic synapses are not plentiful on either cell type, but dendrites, and even very large dendritic trunks, are frequently covered with a latticework of presynaptic boutons. This characteristic arrangement of synaptic contacts in the pars reticulata, which has been reported previously (e.g. Gulley and Smithberg, 1971; Rinvik and Grofova, 1970; Schwyn and Fox, 1974), gives that area of the nucleus an appearance of organization not shared by pars compacta.

Although large dendritic trunks of pars compacta are, like the dendrites of pars reticulata, sometimes covered with synaptic boutons, these are much less frequently seen, and most synaptic contacts are between single boutons and small dendrites of similar size. Despite this difference in the organization of synaptic contacts, all of the major bouton types that have been described in pars reticulata (Gulley and Smithberg, 1971; Hajdu et al., 1973; Rinvik and Grofova, 1970) can be observed in pars compacta. The most common type of bouton is shown in Figure 1. It is characterized by its densely packed, highly pleomorphic synaptic vesicles, and forms contacts with symmetrical membrane "thickenings". This bouton, which corresponds to the boutons of type I described by Rinvik and Grofova (1970) in the cat substantia nigra and by Hajdu et al. (1973) in the rat, is of the kind reported to be formed by strio-nigral fibers (Hattori et al., 1975; Hajdu et al., 1973; Grofova and Rinvik, 1970; Kim et al., 1972). A second type of bouton, which differs in appearance from these mainly in the density of packing of synaptic vesicles, is shown in Figure 2. Boutons of this type are more common in pars compacta than in pars reticulata, and correspond to the type III boutons of Rinvik and Grofova (1970) and the boutons of type V of Hajdu et al. (1973). Two types of boutons containing round vesicles are seen in pars compacta, and differ mainly in the size of their vesicles. The type containing larger vesicles is shown in Figure 3. The bouton in that figure contains predominantly round synaptic vesicles and forms a contact with a small dendrite with a prominent postsynaptic density. As in many synapses of this type, several dense bodies like those described in the habenula and interpeduncular nucleus by Milhaud and Pappas (1966) are associated with the postsynaptic membrane.

In saline-treated animals, all synaptic boutons contain small electronlucent synaptic vesicles of the kind usually seen in the central nervous system after aldehyde-osmium fixation. No small dense core vesicles of the type often seen in adrenergic synapses in the peripheral nervous

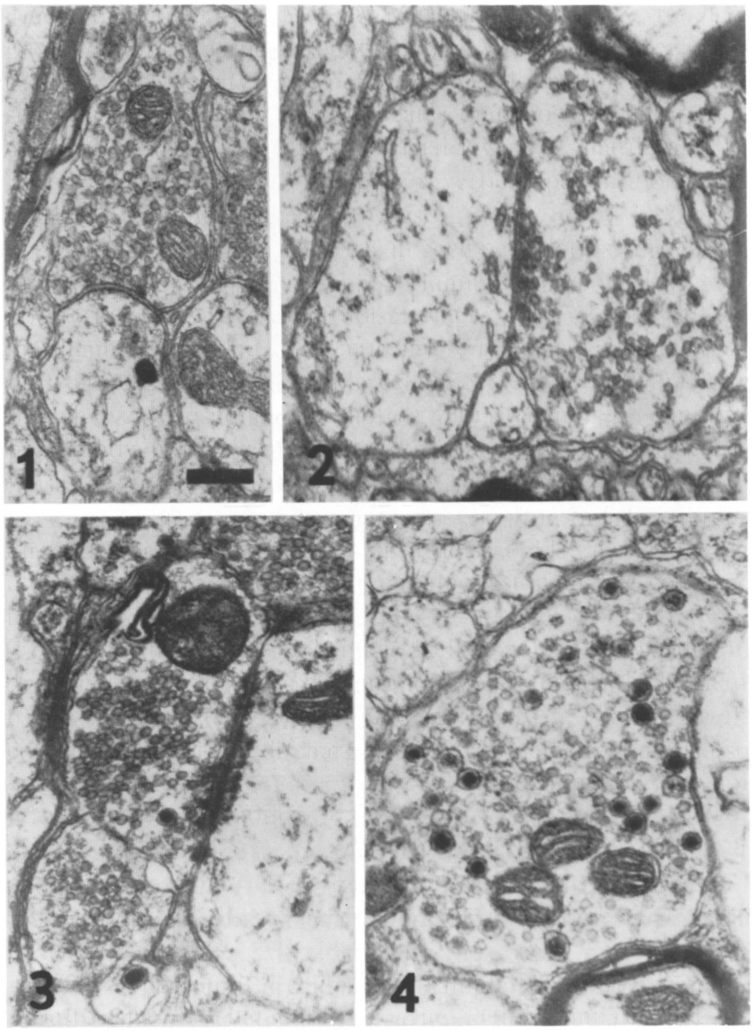

Fig. 1. A bouton containing densely packed pleomorphic vesicles forms a contact with a small dendrite in the substantia nigra, pars compacta. Saline-treated rat. bar: 0.25 um

Fig. 2. A bouton containing loosely packed pleomorphic vesicles forms a contact with a dendrite of similar size in the substantia nigra, pars compacta. Saline-treated rat. Magnification as in Figure 1

Fig. 3. A synapse made by a bouton containing predominantly large round vesicles. Note the prominent pre- and postsynaptic densities, and the presence of a single large dense core vesicle. Substantia nigra, pars compacta. Saline-treated rat. Magnification as in Figure 1

Fig. 4. A bouton containing many large dense core vesicles in the substantia nigra of a saline-treated rat. Small synaptic vesicles, which are also present, are electron-lucent. Magnification as in Figure 1

system after this fixation (e.g. Bloom, 1970) are seen. Larger vesicles (60-130 nm) with central dense cores that do not fill the vesicle are commonly seen in boutons of all types as well as in the somata of all neurons. The number of these varies greatly across various types of boutons however, and one bouton is characterized by the presence of large numbers of large dense core vesicles. A bouton of this type is shown in Figure 4. They usually appear in clusters of from two to five, and may oppose cell bodies or dendrites, but do not appear to make clearly distinguishable synaptic contacts. They correspond to boutons of type VI described by Hajdu et al. (1973).

Small neurons, identical to those described in pars reticulata are also seen in pars compacta, but are much less common there, and most of the postsynaptic elements in pars compacta synapses arise from the medium-sized neurons of that area. These cells, which are almost certainly dopaminergic neurons, resemble in many ways the principle neuron of pars reticulata. The cell body is usually elongated, and in most sections one or more large dendritic trunks are seen to gradually emerge from it. The largest of these is directed toward pars reticulata and

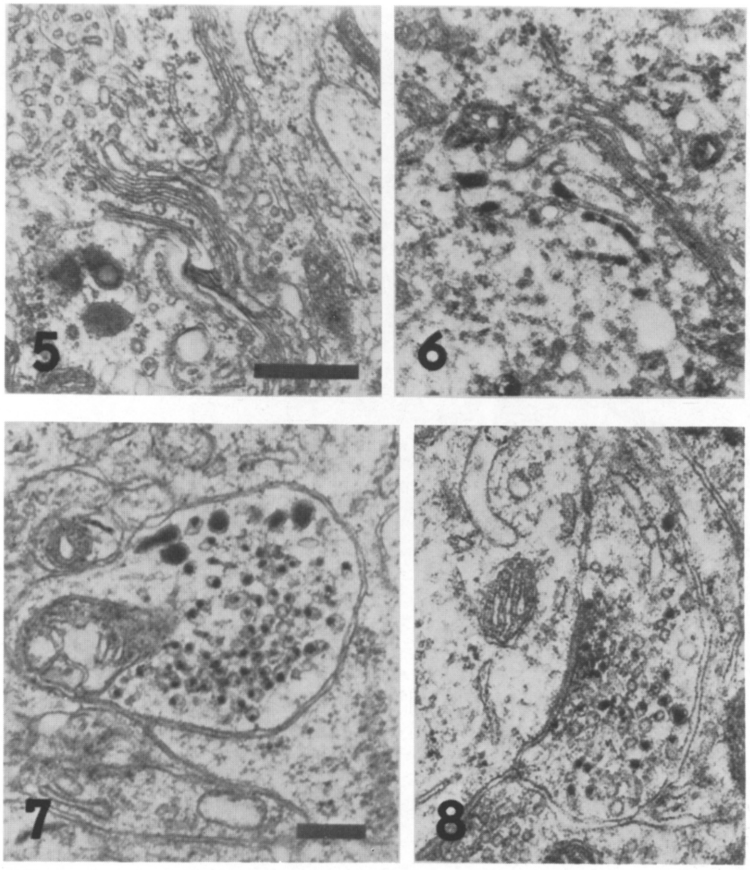

Fig. 5. The typical appearance of the Golgi apparatus in a dendritic trunk of a medium-sized pars compacta neuron. bar: 0.5 um

Fig. 6. A Golgi apparatus from a medium-sized pars compacta neuron after 5-OHDA administration. Osmiophilic material is contained in the saccules of the concave face. Magnification as in Figure 5

Fig. 7. A bouton containing small and large dense core vesicles in the substantia nigra of a rat treated with 2 mg 5-OHDA 60 min before fixation. Small pleomorphic vesicles contain an eccentrically placed dense dot, and filling of the large vesicles with the marker is indicated by the increased size and electron density of the dense core. bar: 0.25 um

Fig. 8. A bouton containing small dense core vesicles forms a "symmetrical" contact with a large dendrite in the substantia nigra. Rat pretreated with 1 mg 5-OHDA. Magnification as in Figure 7

maintains its large caliber for a great distance. The other dendrites are smaller, taper more rapidly and branch relatively soon after leaving the cell body. Dendritic trunks contain stacks of cisternae of rough endoplasmic reticulum and usually have a centrally located, well developed Golgi apparatus around which are many small electron lucent and a few large dense core vesicles. The Golgi apparatus of one such dendritic trunk is shown in Figure 5.

5-OHDA-Treated Rats

Within 30 min after intraventricular administration of 1 mg of 5-OHDA, an increase in the size and electron density of the osmiophilic cores of large vesicles, and the formation of dense cores has begun in some small vesicles observed in the cytoplasm of medium-sized pars compacta neurons. In animals given 2 mg of the marker, the increased density of cytoplasmic vesicles in these cells is more pronounced, and is accompanied by a substantial increase in the number of small dense core vesicles. In all cases, vesicles acquiring dense cores appear most concentrated in the vicinity of the Golgi apparatus. In some cases, the saccules of the concave face of the Golgi apparatus in these neurons exhibit accumulations of osmiophilic material similar to that seen in the cores of cytoplasmic vesicles as shown in Figure 6, taken from a rat given 2 mg of 5-OHDA and surviving for 30 min. Note the absence of dense material in Figure 5, which is typical of the Golgi apparatus of medium-sized pars compacta neurons in animals treated with saline. These effects of 5-OHDA were not seen in the small interneurons of pars compacta, or in cells of pars reticulata. Also after 30 min, vesicles in scattered synaptic boutons have begun to acquire dense cores. These are present in both pars compacta and pars reticulata, although they comprise a small proportion of synapses in either area. They are much more common in pars compacta, where from 5 to 20 of them are seen in a given 200-500 um square section, and are more plentiful in material taken from animals treated with the higher dose of 5-OHDA or surviving for more than one hour. In Figure 7, the typical appearance of the 5-OHDA-marked vesicles is shown. Small, pleomorphic vesicles are characteristically labelled in which the osmiophilic core is seen as a dense spot of variable size eccentrically placed within the vesicle. Some larger vesicles are also filled, as indicated by an increase in the electron density of the osmiophilic core, and its enlargement so that it nearly fills the vesicle. Boutons marked with 5-OHDA in the substantia nigra form contacts with symmetrically membrane densities primarily onto small dendrites, although contacts onto larger dendrites, as shown in Figure 8, are occasionally seen.

Identification of neuronal processes as axonal or dendritic is difficult in the substantia nigra, especially in pars compacta, where most synapses are between small unmyelinated axons and dendrites of small caliber which contain few identifying organelles, and which may course for some distance between synaptic contacts. Axons usually require fewer sections for identification than dendrites,

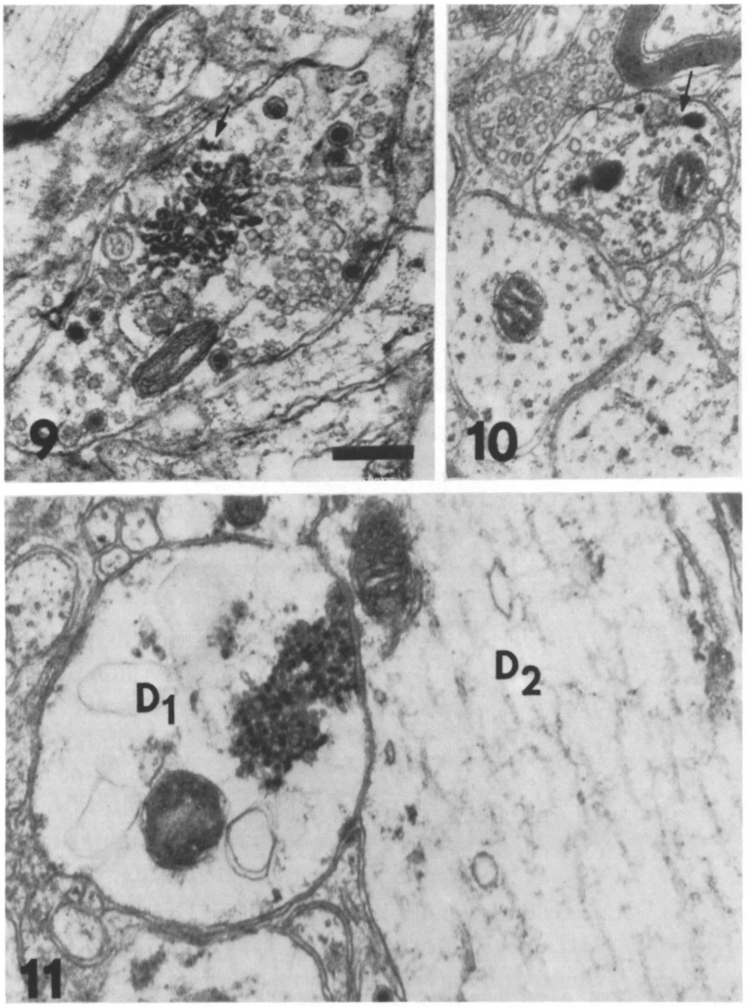

Fig. 9. A section from a series through a presynaptic dendrite in which vesicles appear to be forming from a tubular system of smooth endoplasmic reticulum that contains 5-OHDA. This less common pattern of labelling with 5-OHDA was seen only after short periods of exposure to the marker. Rat pretreated with 1 mg 5-OHDA, 30 min before fixation. Ribosomes are seen at arrow. bar: 0.25 um

Fig. 10. A dendritic shaft containing large and small filled vesicles. One dense core vesicle appears to be continuous with a cistern of smooth endoplasmic reticulum (arrow). Magnification as in Figure 9

Fig. 11. A section from a series through a dendrodendritic synapse in the substantia nigra, pars compacta. The presynaptic dendrite (D1) contains vesicles uniformly filled by 5-OHDA and makes a "symmetrical" contact with a dendritic shaft (D2). Pretreatment with 1 mg 5-OHDA, 3 hours before fixation. Magnification as in Figure 9

since they can often be followed to bundles of small profiles with uniform diameter characteristic of unmyelinated fibers. Dendrites are identified by the presence of polyribosomes, or more commonly, by tracing them to their cell body of origin or to a larger dendritic process containing polyribosomes and uniform arrays of microtubules.

Sixteen presynaptic processes containing small dense core vesicles were identified from serial sections in the above manner, ten of which were dendrites, and six of which were axons. Both types of presynaptic elements were occasionally encountered in tissue taken from animals surviving for 30-60 min, in which the vesicles appeared to be in an intermediate stage of filling with S-OHDA. The presynaptic dendrite shown in Figure 9 is typical of these. It contains a group of electron-lucent vesicles, several apparently unfilled large dense core vesicles, and a centrally located group of tubular elements of smooth endoplasmic reticulum which contains the marker and from which filled vesicles appear to be forming. In the small dendritic shaft shown in Figure 10, small and large filled vesicles can be seen, and a vesicle of intermediate size, which contains the marker, appears to be in continuity with a cisternal element of smooth endoplasmic reticulum. Other 5-OHDA-labelled elements in this material, and all of those observed after a survival time of 3 hours, exhibit a more uniformly filled appearance, although the size of the dense cores seen in the vesicles of these synapses varies from a small dot within most of the vesicles, as in Figures 7 and 8, to a uniform and complete filling of all the vesicles, as in the presynaptic dendrite shown in Figure 11. While the presynaptic elements exhibiting small dense core vesicles often also contain at least one large dense core vesicle which also appears to be altered by the treatment, the large dense core vesicles in other synapses which lack small filled vesicles are indistinguishable from those seen in saline-treated animals.

Presynaptic dendrites are generally thin, distal dendrites of the dopaminergic neurons, or fine branches arising from larger more proximal dendrites. In only a few cases, such as the one shown in Figure 11, were relatively large dendrites seen to make synaptic contacts. Presynaptic dendrites were not usually contacted by axon terminals in the vicinity of the release site. Indeed, only one such contact was seen, and it is shown in Figure 12. The presynaptic bouton contains small flattened electron-lucent synaptic vesicles and one large dense core vesicle which appears unaffected by the 5-OHDA treatment, and forms a symmetrical contact onto a dendrite containing a few small dense core vesicles.

Axons identified in serial sections and containing filled

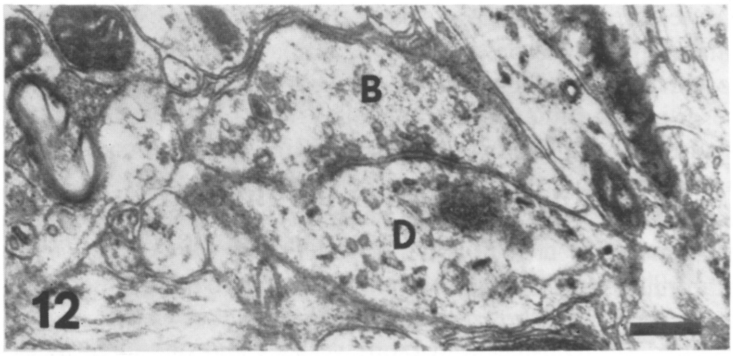

Fig. 12. A dendrite containing small dense core vesicles (D) is contacted by a bouton containing pleomorphic electron-lucent vesicles (B). Not all of the dendritic vesicles contain visible amounts of the marker. The dendritic release site is out of the plane of the section. bar: 0.25 um

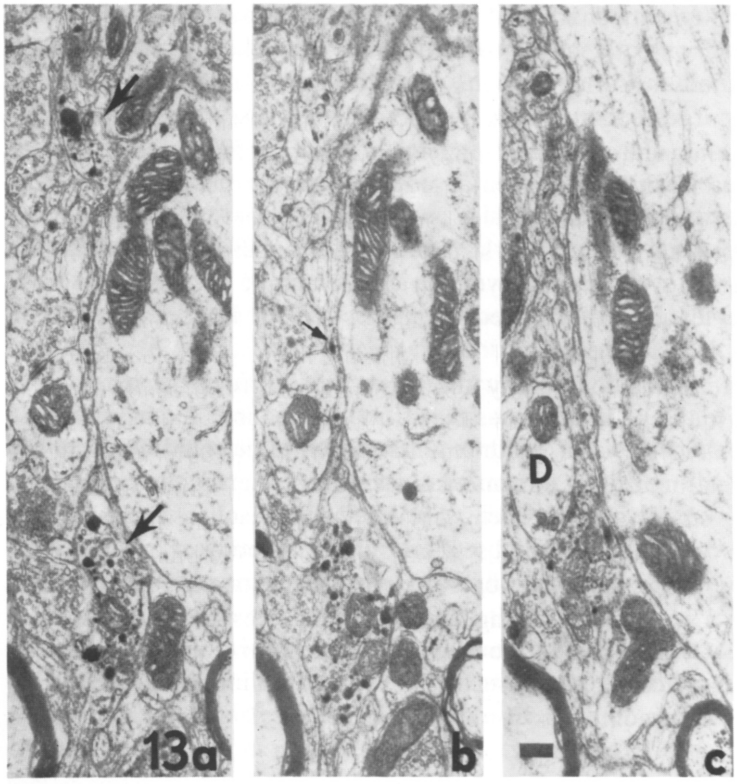

Fig. 13. Sections selected from a series through an axon containing vesicles filled by 5-OHDA. Two vesicle-filled varicosities (large arrows in a), are connected by a narrow strand of axon (arrow in b). One of the varicosities forms a "symmetrical" synapse with a small dendrite (D) in c. Pretreatment with 1 mg 5-OHDA, 60 min before fixation. bar: 0.25 um

vesicles after 5-OHDA treatment were seen only in pars compacta. These were of very small caliber (0.1-0.5 um), and remained unmyelinated for as far as they could be followed. Small varicosities were seen at regular intervals along the axon, and contained vesicles of heterogeneous size and shape and some tubular elements of smooth endoplasmic reticulum which sometimes appeared to contain the marker. They formed synapses with symmetrical membrane densities onto small dendrites. These features of labelled axons are shown in the examples in Figure 13.

Attempts were made to identify the postsynaptic processes involved in monoaminergic synapses. In numerous cases this was possible and in every case the postsynaptic element was a dendrite. In some cases, it could be positively identified as originating from a dopaminergic neuron, either by the presence of scattered large or small vesicles which were filled as a result of the 5-OHDA treatment, or by following it in serial sections to the cell body of origin. In one case, the postsynaptic dendrite was observed in serial sections to contact a third dendrite, forming a synapse containing small dense core vesicles.

Vesicles in that class of nigral boutons which are characterized by a large number of large dense core vesicles, such as the one shown in Figure 4, appeared to be unaffected by the administration of 5-OHDA.

DISCUSSION

The existence of presynaptic dendrites in the substantia nigra was first demonstrated by Hajdu et al. (1973), and although they suspected that the presynaptic dendrite might derive from the nigral interneuron, the synaptic profiles described by those authors are similar to those observed in our material between dendrites of dopaminergic neurons. Both the presynaptic dendrites and the axon varicosities which are labelled by the 5-OHDA method also resemble the boutons of type III described by Rinvik and Grofova (1970) in the cat substantia nigra. Also consistent with the present findings is their observation that boutons of this type are sometimes postsynaptic to other boutons, of their type I, which contain flattened vesicles and which may degenerate after lesions of the striatum (Grofova and

Rinvik, 1970). If it is the case that their type III boutons correspond to the dendritic processes identified here in serial sections and labelled by 5-OHDA, nigral synapses previously described as axo-axonic may actually represent axo-dendritic or dendro-dendritic synapses. An exception to this would be the synapses made onto the initial segments of nigral axons described by Gulley and Smithberg (1971).

Although the postsynaptic process involved in a dendro-dendritic synapse could not always be identified, in all cases in which this was possible, it proved to be a dendrite of a dopaminergic neuron. The possibility that dopaminergic dendrites make contact with neurons of other types in the substantia nigra can not be excluded, but in view of the specificity of responsiveness to dopamine suggested by recent neuropharmacological evidence (Aghajanian and Bunney, 1973; Dray and Straughn, 1976; Groves et al., in press), this would seem unlikely. Also absent in our observations were dendro-axonic synapses. Recent reports concerning the localization of dopamine-sensitive adenylate cyclase, which is believed to be associated with a receptor for dopamine, have indicated that this enzyme might be associated with membranes of strio-nigral fibers (Gale et al., 1977; Premont et al., 1976). Terminals similar to those formed by strio-nigral fibers were occasionally observed in the vicinity of dendritic dopamine release sites, but no consistent relationship between these elements was discernable.

The origin of the axons which were seen in the 5-OHDA-treated animals to contain small dense core vesicles can not be determined with certainty. They may represent axons of serotonergic neurons of the raphe nuclei, which are known to project to the substantia nigra (e.g. Dray et al., 1976). Serotonergic terminals have been shown to accumulate 5-OHDA (Richards et al., 1973), although somewhat higher concentrations are required (Bloom, 1973). The resemblance of the vesicle population in the labelled axons to that seen in presynaptic nigral dendrites suggests the possibility that they may be the axons of dopaminergic neurons. The failure to identify any of these in pars reticulata is also consistent with this possibility, and their small caliber and evenly spaced varicosities are consistent with the light microscopic appearance of these axons (Bjorklund and Lindvall, 1975). Thus, while axons of dopaminergic neurons probably do not possess axon collaterals (Juraska et al., 1977), perhaps they contact nigral elements "en passage" as they exit the nucleus. If so, the boutons containing large numbers of large dense core vesicles and which are not labelled by 5-OHDA in the doses used here may represent the terminals of serotonergic fibers. Boutons of this type have been reported to degenerate after lesions of the raphe nuclei (Bak et al., 1975).

The formation of dense cores in cytoplasmic vesicles of monoamine neurons after fixation with potassium permanganate (e.g. Hokfelt, 1967) or glutaral-dehyde-dichromate (Tranzer and Richards, 1976) has been reported to take a form similar to that observed here, and is consistent with other evidence for the existence of a vesicular, although not readily releasable store of monoamine in the cell body (e.g. Dahlstrom, 1969). In the present observations, all regions of the dopaminergic neurons except the nucleus were seen to contain dense core vesicles, but they were most common in the perinuclear area, around the extensive Golgi system there and in the dendritic trunks. In animals treated with high doses of 5-OHDA the dense core vesicles were present in sufficient quantities to allow speculation that, if normally filled with dopamine, they might account for the fluorescence of these neurons. Since the monoamine localized using this method is exogenous in origin, however, and since the dense core vesicles appeared to be forming from the Golgi apparatus, there must be within the treated cells a pool of 5-OHDA which either is not sufficiently bound or sufficiently concentrated to be visible, but from which the Golgi apparatus, and the vesicles are filled. Similarly, while some dense core vesicles seen in dendrites may arrive by somatofugal transport, many are almost certainly filled with the marker locally in the dendrites. Thus, in material taken from animals exposed to 5-OHDA for only 30 min elements of dendritic endoplasmic reticulum were seen to contain the marker, from which both large and small dense core vesicles appeared to form. After longer survival times the vesicles were more consistently filled, and filling of endoplasmic reticulum was less commonly seen. These obsenations suggest a sequence of events similar to that proposed for the formation of synaptic vesicles in axons (Droz et al., 1975; Holtzman et al., 1973; Sotelo and Taxi, 1973; Tranzer, 1972). Visualization of the events which lead to the concentration of monoamines within these membrane-bound compartments will require the development of a more sensitive method.

Our observations provide evidence that dendrites of dopaminergic neurons contain transmitter release sites that would be required for a process of self-inhibition by dopaminergic neurons (Groves et al., 1975,1976). Inhibitory interactions between dopaminergic neurons located near each other in the substantia nigra have also been revealed by our recent neurophysiological observations (Wilson et al., in press). The dendro-dendritic contacts between dopaminergic neurons observed in these experiments could provide a structural basis for such inhibition. Such processes would involve diffusion of dopamine a short distance away from the release site where it could affect autoreceptors located on or near the presynaptic and postsynaptic dendritic membranes. Since noradrenergic and serotonergic as well as dopaminergic neurons are inhibited by local application of their own transmitters (e.g. Svensson et al., 1975; Aghajanian et al., 1972; Mosko and Jacobs, 1977; Bunney et al., 1973) dendro-dendritic interactions may be a consistent feature of populations of monoaminergic neurons in the central and peripheral nervous system.

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