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MONOSYNAPTIC INPUT FROM THE NUCLEUS ACCUMBENS–VENTRAL STRIATUM REGION TO RETROGRADELY LABELLED NIGROSTRIATAL NEURONES

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SUMMARY

After placement of lesions (either electrolytic or by injection of kainic acid) in an area including the nucleus accumbens and part of the ventral striatum in the rat, the ipsilateral substantia nigra was studied in the electron microscope. Degenerating axons and nerve terminals were found mainly in the zona reticulata and in the ventral layer of the zona compacta. Degenerating synaptic boutons were found in contact with cell bodies (symmetric synapses) and dendrites (mainly symmetric, but a few asymmetric).

The postsynaptic target of some of the afferent fibres from the accumbens–ventral striatum was established by demonstrating degenerating synaptic boutons of the above types in contact with nigrostriatal neurones which had been identified by the retrograde transport of horseradish peroxidase (HRP) from the main body of the striatum. Some of the HRP-labelled cells were also impregnated by the Golgi stain and degenerating boutons were found in contact with their distal dendrites. We also observed two types of HRP-containing boutons (presumably labelled anterogradely) in the substantia nigra after injection of HRP into the main body of the striatum: type 1 boutons contained large spherical vesicles, and formed symmetrical synapses mainly on dendritic shafts in the zona reticulata and in one case the dendrite was from a nigrostriatal neurone; type 2 boutons had pleomorphic and flattened vesicles and formed symmetrical synapses with perikarya and proximal dendrites, especially in the zona compacta. The latter type of HRP-labelled bouton was frequently found in synaptic contact with the cell bodies of nigrostriatal neurones and the same neurones

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sometimes also received degenerating boutons originating from neurones in the nucleus accumbens–ventral striatum.

It is concluded that part of the striato-nigro-striatal circuit includes a monosynaptic link between neurones in the ventral striatum–accumbens and some nigrostriatal neurones. The possible convergence of input from different regions of the striatum on to single nigrostriatal neurones is also suggested.

INTRODUCTION

The involvement of the basal ganglia in certain neurological disorders has evoked a large volume of research on the reciprocal connections between the substantia nigra and the neostriatum[6,10]. There is evidence that the substantia nigra receives a topographically organized projection from the neostriatum with γ-aminobutyrate (GABA) and substance P involved as transmitters. In turn the substantia nigra sends a topographically organized projection to the neostriatum using dopamine and possibly some other unknown transmitter.

While the details of the complicated topographic projections between the two structures are fairly well understood[1,4,14,23,35] very little is known about the connections at the synaptic level. Since both structures are composed of several types of neurones, as defined by their connectivity, it is difficult to establish with conventional morphological methods which neurone type or types are the monosynaptic targets of the nigrostriatal and striatonigral pathways. Recently we have developed a method in which the projection area of a neurone is determined by retrograde labelling with horseradish peroxidase (HRP), its morphological characteristics by Golgi staining and its input by anterograde degeneration of afferent synaptic boutons[31]. Application of this method has revealed that nigrothalamic neurones receive monosynaptic input from striatonigral fibres[31]. In the same series of experiments, however, we failed to find monosynaptic input to nigrostriatal neurones from the dorsal neostriatum in the rat.

Using the anterograde transport of radiolabelled proteins it has been demonstrated that the nucleus accumbens–ventral striatal region sends fibres to the dorsal substantia nigra into, or close to, the area where the cell bodies of nigrostriatal neurones are located[5,23,26,34]. To establish if this projection from the ventral part of the striatum terminates monosynaptically on nigrostriatal neurones, lesions were placed in the nucleus accumbens–ventral striatum region prior to injection of HRP into the main body of the striatum. Electron microscopic analysis of the substantia nigra was then carried out to see whether retrogradely HRP-labelled nigrostriatal neurones receive monosynaptic input from the degenerating terminals of fibres originating from the area affected by the lesions. In the same animals, we have also studied the synapses formed in the substantia nigra by boutons that contain HRP.

MATERIALS AND METHODS

Ten male Wistar rats (160–180 g) were used. All operations were performed

under deep chloral hydrate anaesthesia (0.4 g/kg i.p.). Three of the rats received unilateral electrolytic lesions (2 mA, 5 sec, anodal) in the ventral striatum–nucleus accumbens region at coordinates A, 8.9; L, 1.8; ventral 5.2 mm from the pial surface according to König and Klippel[17]; 0.2 mm of the electrode tip was free of insulation. Four other rats received an injection of kainic acid (1 %, Sigma, 40 nl delivered during 10–15 min) in the same area. One or two days later all rats, except one of the kainic acid treated animals, received an injection of 40–50 nl HRP (20 % in water, Sigma type VI) into the striatum ipsilateral to the lesion at coordinates A, 7.5; L, 2.5; ventral 4.5 mm from the pial surface. All injections were delivered as described previously[31] using glass capillaries with tip diameters of about 50 μm. After the appropriate survival time the animals were perfused through the heart with a fixative of 2.5 % glutaraldehyde, 0.5 % paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4[31].

The brain was removed from the skull and immersed in the same fixative for a further 3 h before blocks were dissected. From the forebrain 40 μm sections were cut on a crysostat and one series was processed for Nissl staining and another to localize the HRP injection site using *o*-tolidine (3,3'-*dimethylbenzidine)* as substrate[31]. Some blocks containing the substantia nigra were processed by the Golgi–HRP transport-degeneration procedure described previously[31]. Some other blocks from two HRP injected animals and blocks from the kainic acid injected animal which did not receive HRP injection were sectioned at 50 μm on a Sorvall TC-2 tissue chopper. These sections were washed overnight in 0.1 M sodium phosphate buffer (pH 7.4) and incubated for 30 min, at room temperature, to reveal HRP activity using 3,3'-diaminobenzidine (DAB) as substrate in a procedure slightly modified from that of Graham and Karnovsky[9]. The incubation media contained 0.05 % DAB (tetrahydrochloride, Sigma), 0.01 % H_2O_2 in 0.1 M sodium phosphate buffer at pH 7.4. Some sections were incubated in a medium from which either the DAB or the H_2O_2 was omitted. After incubation the sections were washed in two changes of buffer for 30 min, postfixed in 2 % OsO_4 in 0.1 M sodium phosphate buffer (pH 7.4) for 2 h, dehydrated and embedded in Araldite (Durcupan ACM Fluka).

After evaluation of the injection site, the retrograde transport of HRP, the extent of the lesion caused in the ventral striatum–nucleus accumbens region and the anterograde degeneration detectable in the substantia nigra, 3 animals were selected for the present report (Fig. 1). The animal labelled ST24 received kainic acid and, 49 h later, HRP; it was killed after a further 17 h. The animal labelled ST27 received an electrolytic lesion and HRP at the same intervals before death as ST24. The animal labelled ST28 received kainic acid and, 18 h later, HRP; it was killed after a further 22 h.

Semithin sections (1 μm) were cut from the substantia nigra to detect retrograde labelling and ultrathin sections from the area of retrograde labelling were examined for degenerating boutons in the electron microscope. Areas with both retrograde HRP-labelling and anterograde degeneration were then sectioned serially for electron microscopy. The following number of areas were studied: case ST24, 5 areas (out of 6 examined), case ST27, 2 areas (5 examined), case ST28, 2 areas (3 examined). In addition one block was studied from the contralateral substantia nigra from animals

ST24, ST27; one block from both animals when either DAB or H_2O_2 was omitted from the incubation media and two blocks from an animal which had not been injected with HRP.

One further animal was selected from another series of rats. This rat received an injection of HRP conjugated with wheat germ-agglutinin into the substantia nigra (20 nl, approximately 6% HRP) as described earlier[30]. Twenty-four hours later the animal was perfused with fixative as above and the ipsilateral striatum was processed by the Golgi–HRP transport procedure as described previously[31].

For electron microscopy all material was stained en bloc with uranyl acetate (1% in 70% ethanol). Serial ultrathin sections were mounted on formvar coated single slot grids and stained with lead citrate[37]. Electron micrographs were taken on Philips 201C and JEOL 100B microscopes at 80KV using 20–30 μm objective apertures.

RESULTS

Control experiments

No enzyme reaction end-product was found in the substantia nigra if either the substrates DAB and *o*-toluidine, or H_2O_2 were omitted from the incubation media. In the substantia nigra of an animal with a kainic acid-induced lesion in the ventral striatum-nucleus accumbens region, but which was not injected with HRP, reaction end-product was observed in a few dense bodies in some astroglial cells. No reaction end-product was found in neurones. It is concluded that under our conditions only exogenous peroxidase activity is demonstrated in neurones.

In blocks derived from the substantia nigra contralateral to the ventral striatal lesion there were no degenerating axons or nerve terminals, indicating that the degeneration observed in the ipsilateral substantia nigra was caused by the lesions. In two rats that only received an injection of HRP in the striatum 17 and 23 h before death, a search was made in the ipsilateral substantia nigra for the presence of degenerating axons or nerve terminals; however, none was found. Thus, any degeneration due to the lesion caused by the glass capillary used to inject the HRP must have been quantitatively insignificant compared with the degeneration that was readily found after the placement of lesions, electrolytically or with kainic acid, in the ventral striatum–accumbens region.

The spread of injected HRP and the extent of lesions (Fig. 1)

In the case ST24 the spread of HRP was largely confined to the neostriatum with a slight spread to the nucleus accumbens proper and to the bed nucleus of the stria terminalis. In the other two cases in addition there was some spread to the globus pallidus, but in all three cases the spread to structures other than the striatum was in the most marginal zone. It is important to note that the use of *o*-tolidine reveals a larger area of spread of HRP than does DAB[31].

The cell loss as revealed by Nissl staining in all 3 cases involved part of the nucleus accumbens and also the anteroventral striatum. The claustrum, the anterior olfactory nucleus and the piriform cortex were very slightly affected with the kainic

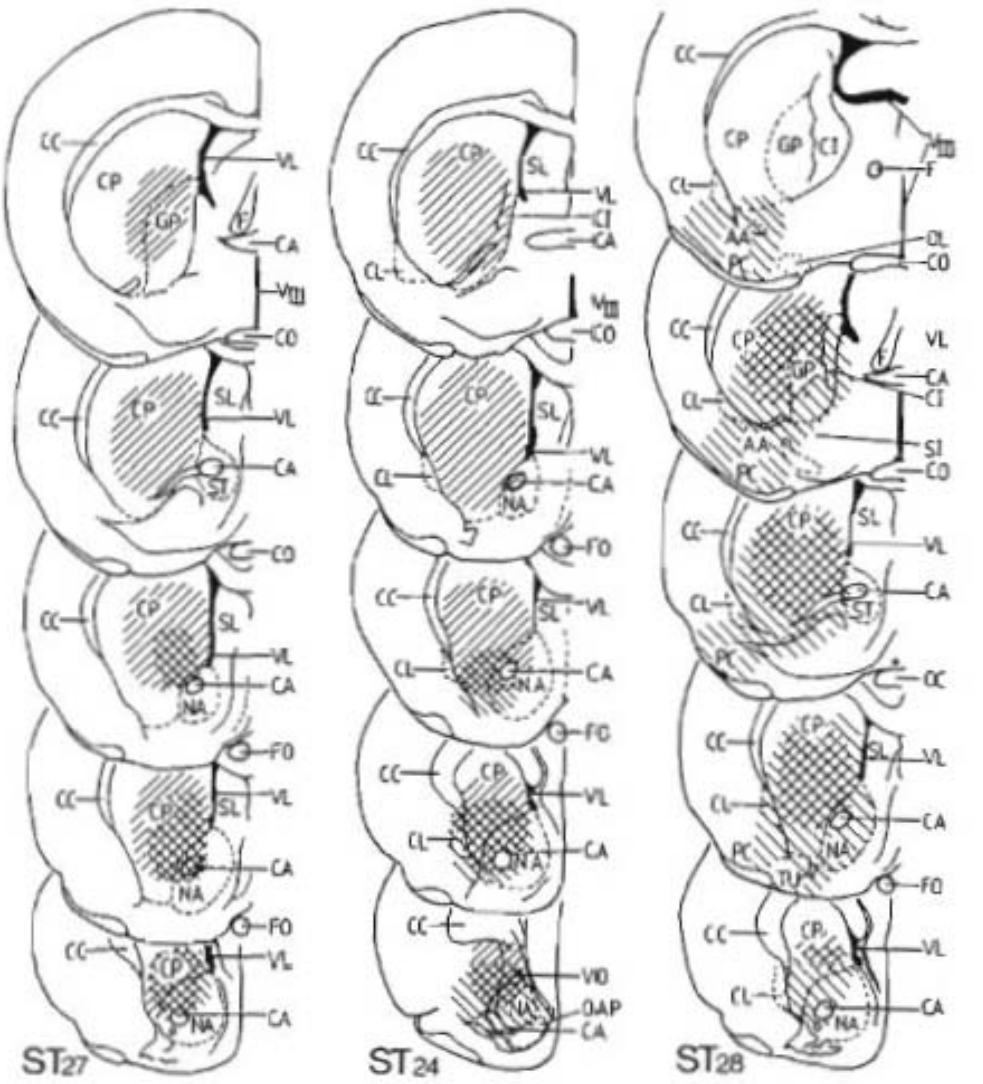


Fig. 1. Charting of the area showing HRP reaction product (*o*-tolidine as substrate) (▨) and the area affected by kainic acid (ST24, ST28) or electrolytic (ST27) lesion (▩). Coronal sections after König and Klippel[17]. AA, anterior amygdaloid area; CA, comissura anterior; CC, corpus callosum; CI, capsula interna; CL, claustrum; CO, chiasma opticum; CP, caudato-putamen; F, fornix; FO, fasciculus opticus; GP, globus pallidus; NA, nucleus accumbens; OAP, nucleus olfactorius anterior, pars posterior; OL, nucleus olfactorius lateralis; PC, cortex piriformis; SL, septum lateralis; SI, substantia innominata; ST, stria terminalis; TU, tuber olfactorium; VL, ventriculus lateralis; VO, ventriculus olfactorius; VIII, ventriculus tertius.

acid lesion in ST24. In case ST28 cell loss was observed in the anterior amygdaloid area, the piriform cortex, the globus pallidus, the bed nucleus of the stria terminalis, the claustrum and the olfactory tubercle as a result of kainic acid injection. Therefore, none of the findings we have illustrated come from the latter animal, although in general the results were the same as in the other two animals.

Retrograde HRP labelling in the substantia nigra

In all 3 animals a dense layer of HRP-labelled cells was found in the pars compacta of the substantia nigra. Scattered cells were also observed in the pars reticulata, especially at more caudal levels, and also in the ventral tegmental area. In t

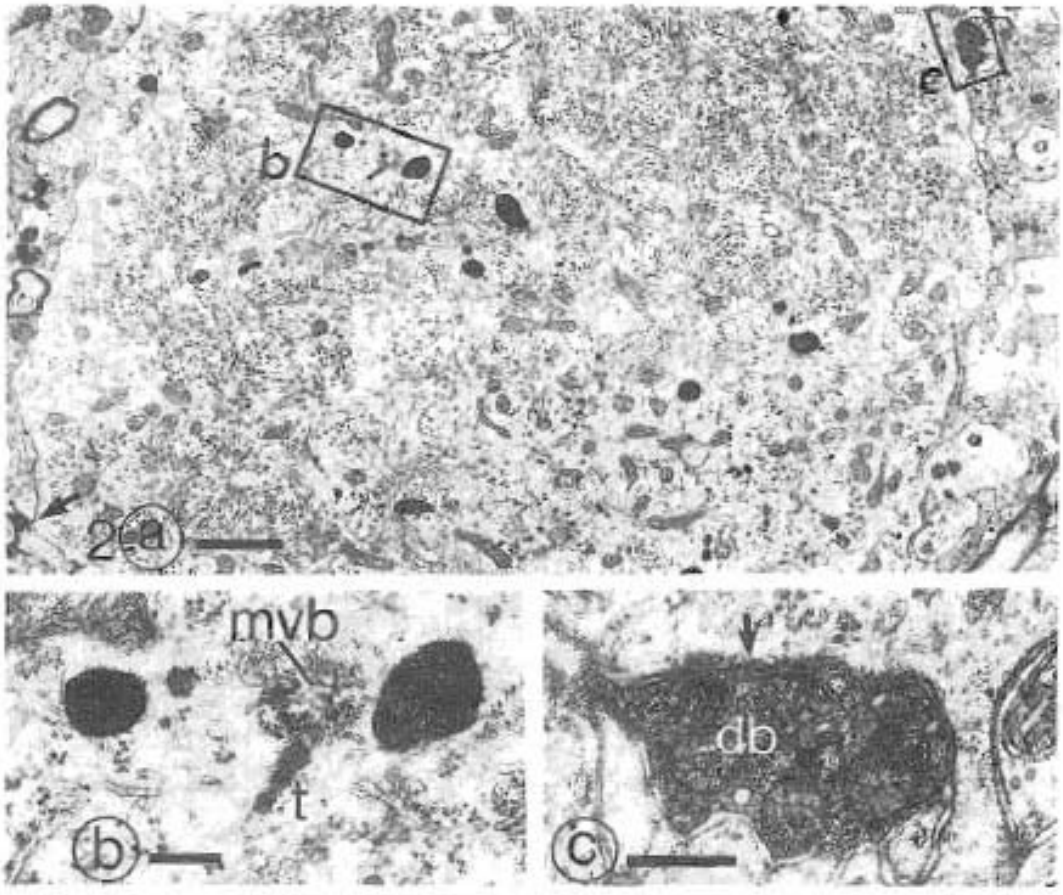
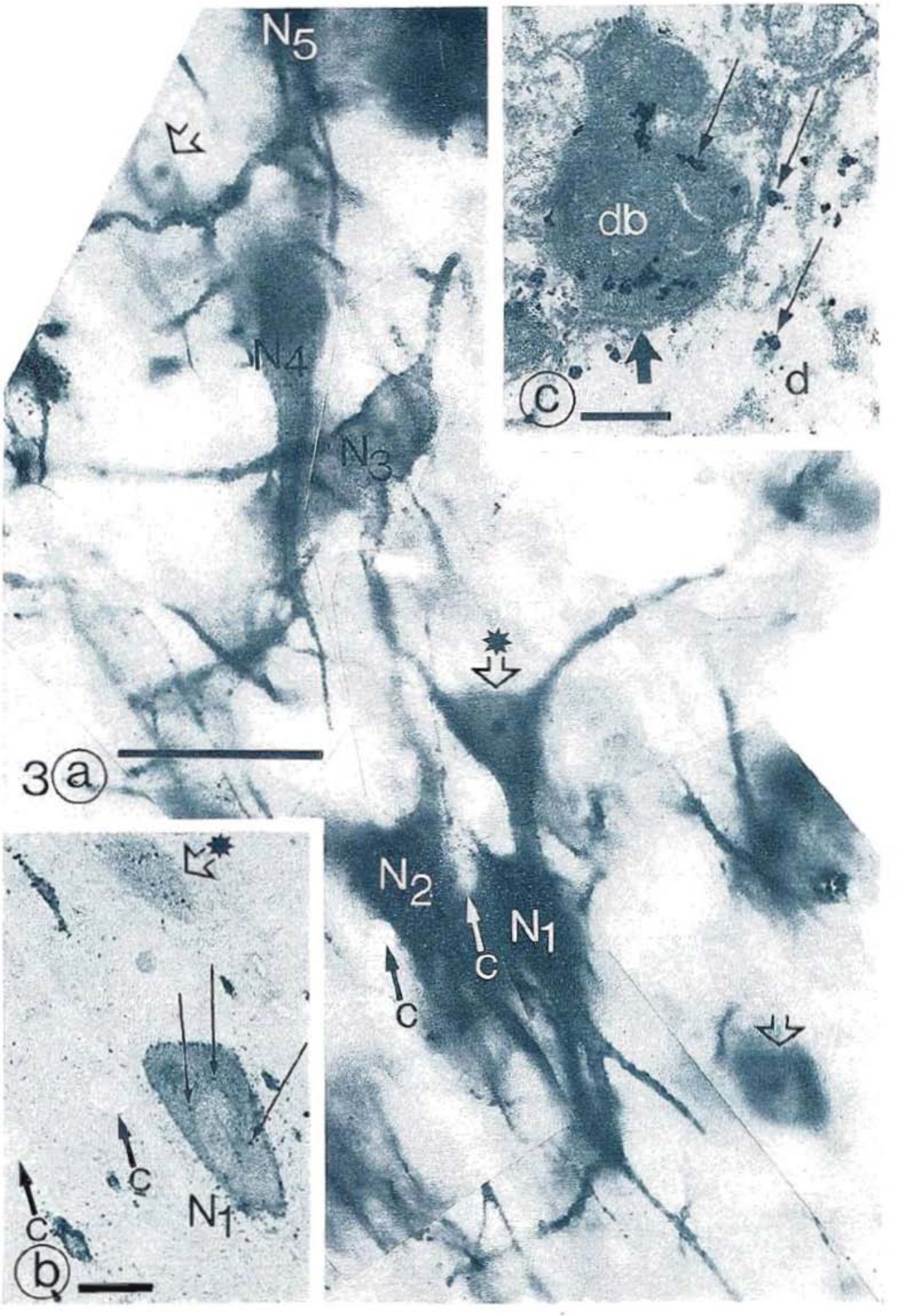


Fig. 2. a: lower power electron micrograph of a retrogradely HRP-labelled nigrostriatal neurone receiving two degenerating boutons (c: framed, and arrow) in the substantia nigra zona compacta. b: framed area in (a) showing HRP reaction end-product in dense bodies, multivesicular body (mvb) and a tubule (t). c: one of the degenerating boutons (db) in (a) is in synaptic contact (arrow) with the perikaryon. Case ST24, DAB as substrate. Scales: a, 1 μm; b and c, 0.2 μm.

Fig. 3. a: photomontage of Golgi-stained, gold toned HRP-labelled nigrostriatal neurons in the zona compacta (N_3–N_4). Two neurones (N_1,N_2) are heavily labelled with HRP. Other neurones (open arrows) are HRP-labelled but not Golgi stained. The zona reticulata is to the right. b: semithin section (1 μm) through the perikaryon of one of the Golgi stained (N_1) and another (open arrow, star) HRP-labelled neurone; both contain HRP reaction end-product in granules (long arrows), as well as in the cytoplasm. Capillaries (C) are indicated in both sections. c: electron micrograph of a degenerating bouton (db) in synaptic contact (thick arrow) with the dendrite (d) of the neurone marked N_1. Long arrows indicate gold particles in and around the Golgi stained dendrite; the particles outside the dendrite result from displacement during the gold toning. Case ST24, o-tolidine as substrate. Scales: a, 50 μm; b, 10 μm; c, 0.2 μm.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N3
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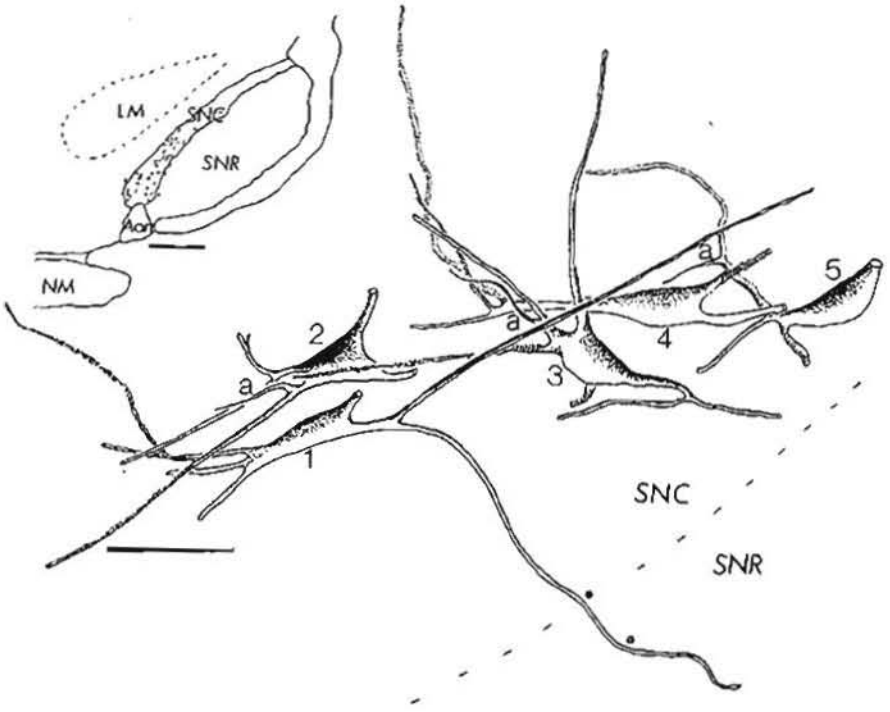


Fig. 4: Camera lucida drawing of the 5 HRP-labelled, Golgi stained nigrostriatal neurones in the zona compacta (SNC) of the substantia nigra shown in Fig. 3. The positions of HRP-labelled neurones (dots) and of Golgi stained HRP-labelled neurons (squares) are shown in the inset. Neurone number one was found to receive two degenerating boutons from the nucleus accumbens-ventral striatum region (stars) on its dendrite. a, axon initial segment; Aon, accessory optic nucleus; LM, medial lemniscus; NM, nucleus mamillaris; SNC, substantia nigra zona compacta; SNR, substantia nigra–zona reticulata. Scales: drawing, 50 μm; inset, 0.5 mm.

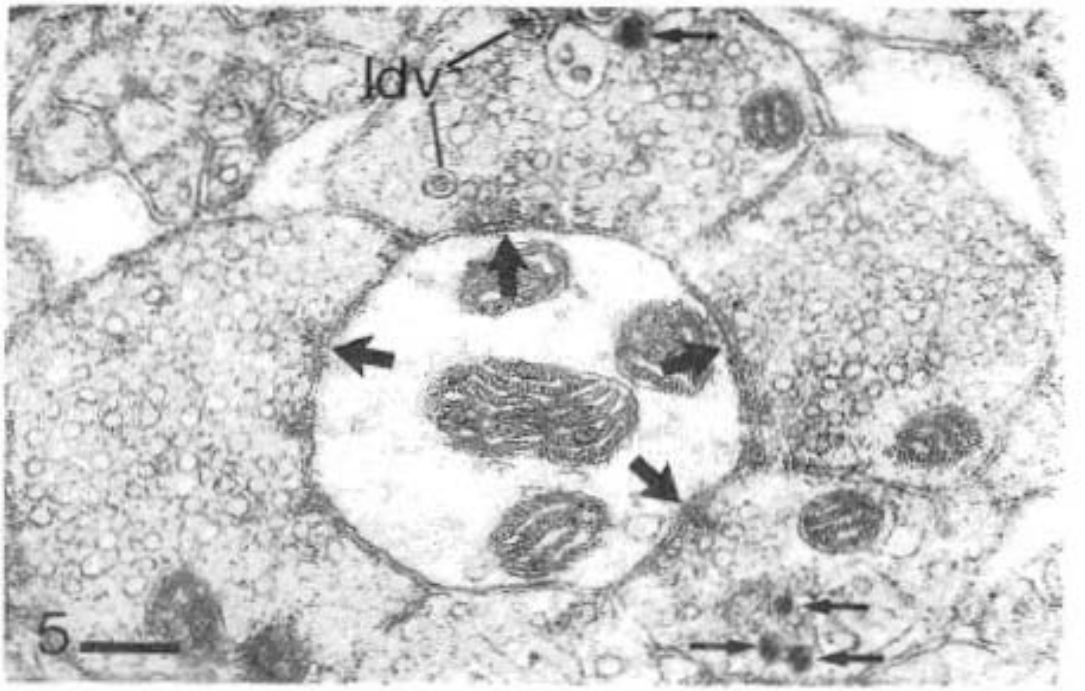
μm sections the DAB reaction end-product appeared as black granules in the neurones while the reaction end-product formed from tolidine was brown and was found both in granules and diffusely in the cytoplasm (Fig. 3a and b). At the electron microscopic level the reaction end-product formed from DAB was highly electron dense. It was

Fig. 5. Several boutons containing large round clear synaptic vesicles make synaptic contact (large arrows) with a dendrite in the zona reticulata of the substantia nigra. Two of the boutons (type 1) contain HRP reaction end-product (horizontal arrows). Note prominent presynaptic dense projections, long synaptic active zones and slight postsynaptic membrane specialization. ldv, large dense core vesicles. Case ST24, DAB as substrate. Scale for figs. 5–8, 0.2 μm.

Fig. 6. Degenerating bouton (db) in the zona reticulata is seen to make synaptic contact (large arrow) with a dendritic shaft (d). The bouton, as well as another normal bouton (star), also contains HRP reaction end-product in membrane limited structures (horizontal arrows). Case ST24, DAB as substrate.

Fig. 7. Golgi-stained, gold toned bouton of a local axon collateral in the striatum originating from a striatonigral neuron. The bouton containing large clear vesicles makes a synapse (large arrow) with a dendritic shaft (d). Long arrows indicate gold particles.

Fig. 8. Synaptic contact (large arrow) between an HRP-labelled (horizontal arrows) type 2 bouton and the perikaryon (P) of a zona compacta neurone in the substantia nigra which was also HRP-labelled retrogradely from the striatum. Case ST27, DAB as substrate.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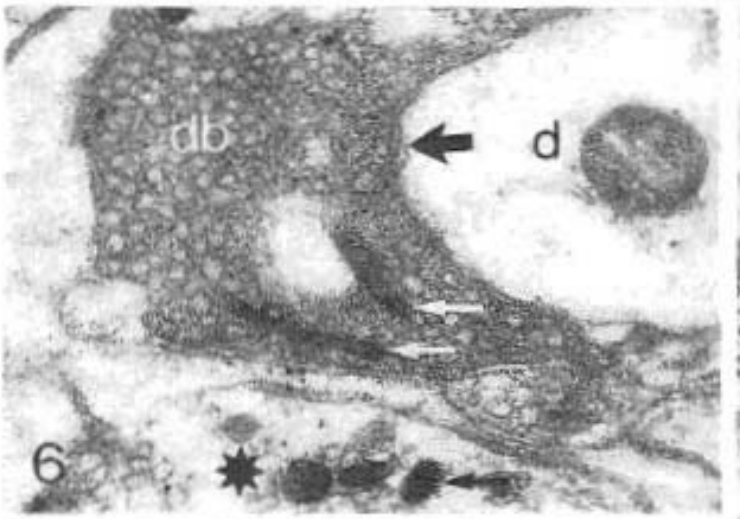
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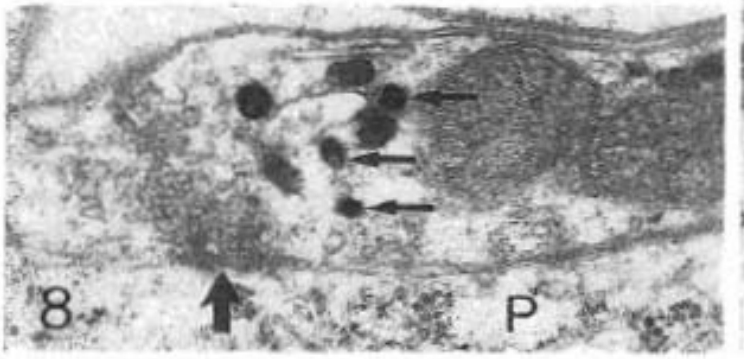
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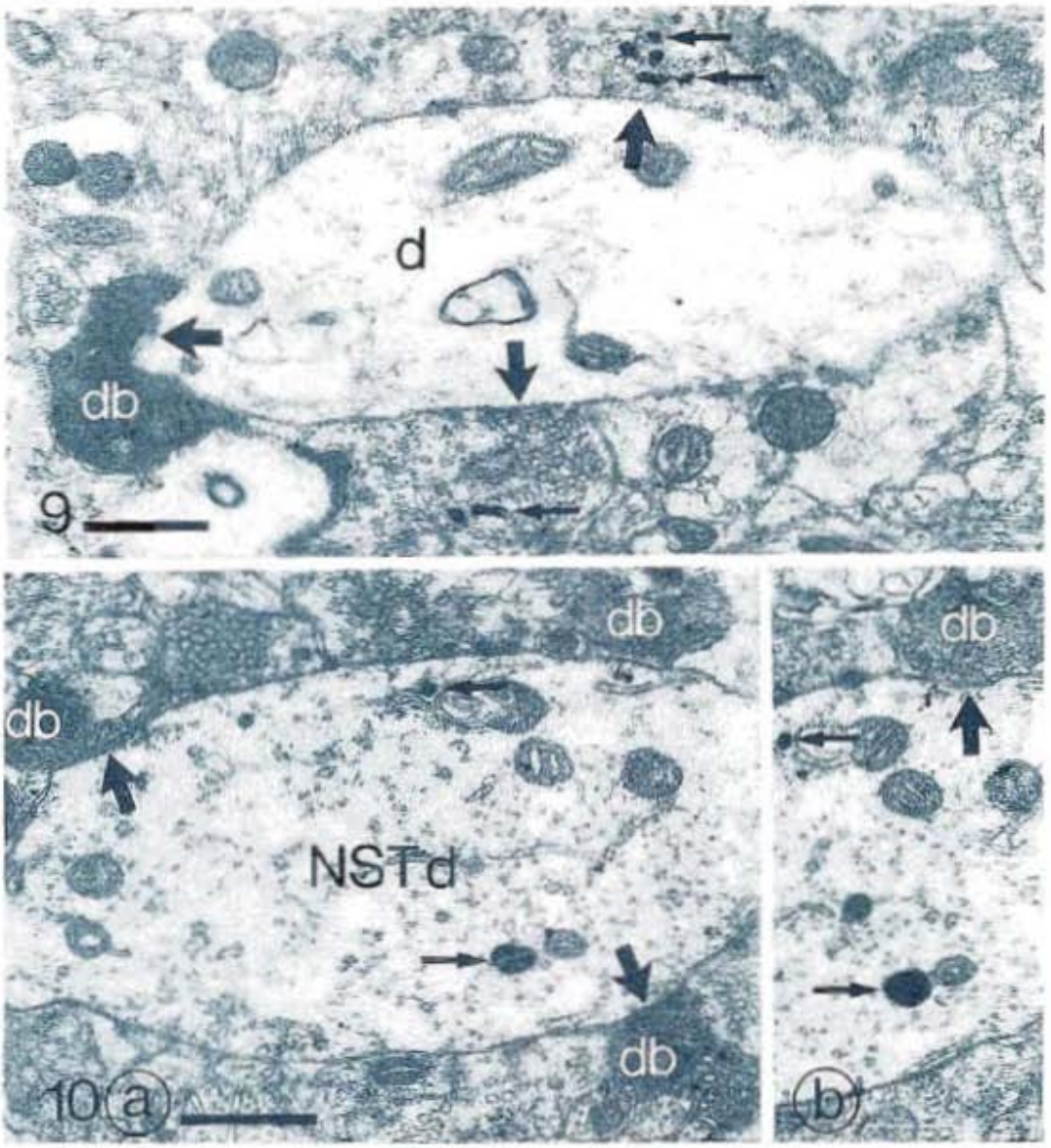


Fig. 9. Convergence of degenerating (db) and HRP-labelled (horizontal arrows) type I synaptic boutons onto the same dendrite (d) in the zona reticulata (large arrows). Case ST27, DAB as substrate. Scale, 0.5 μm.

Fig. 10. a and b: serial sections showing convergence of 3 degenerating boutons (db) onto the same HRP-labelled (horizontal arrows) dendrite of a nigrostriatal neuron (NSTd). Zona reticulata. Case ST27, DAB as substrate. Scale, 0.5 μm.

deposited in large and small dense bodies, vesicles, multivesicular bodies and in tubules of the smooth endoplasmic reticulum (Figs. 2a, b, 10 and 11a). In the perikaryon there was a large number of these structures (Figs. 2a and 11a) but their number gradually decreased in the dendrites more distally. Nevertheless, occasionally

even relatively distal dendrites in the pars reticulata, but more frequently in the pars compacta, contained HRP reaction end-product indicating that they belong to nigrostriatal neurones (Fig. 10a and b).

The HRP reaction end-product formed from *o*-tolidine is not osmiophilic and so is not as electron dense as the product formed from DAB[31]. In heavily labelled neurones it filled the perikaryon and proximal dendrites homogeneously (Fig. 3a and b). Sometimes it formed a crystalline precipitate in nigrostriatal neurones as described previously[31]. Lightly labelled cells could be identified in semithin sections (Fig. 3b).

Golgi staining of retrogradely labelled nigrostriatal neurones

In our adult rat specimens it was difficult to stain the zona compacta neurones with the rapid Golgi method employed in the combined HRP–Golgi-degeneration procedure. Neurones were not only rarely stained but also many partially stained neurones were observed. Nevertheless, from the few Golgi stained retrogradely labelled neurones that were found it was observed that most of the dendrites remained in the zona compacta and had a course parallel with the sheath of the compacta cell layer (Figs. 3a and 4). Some of the dendrites turned ventrally and descended into the zona reticulata (Fig. 4), but the whole course of each dendrite could not be established.

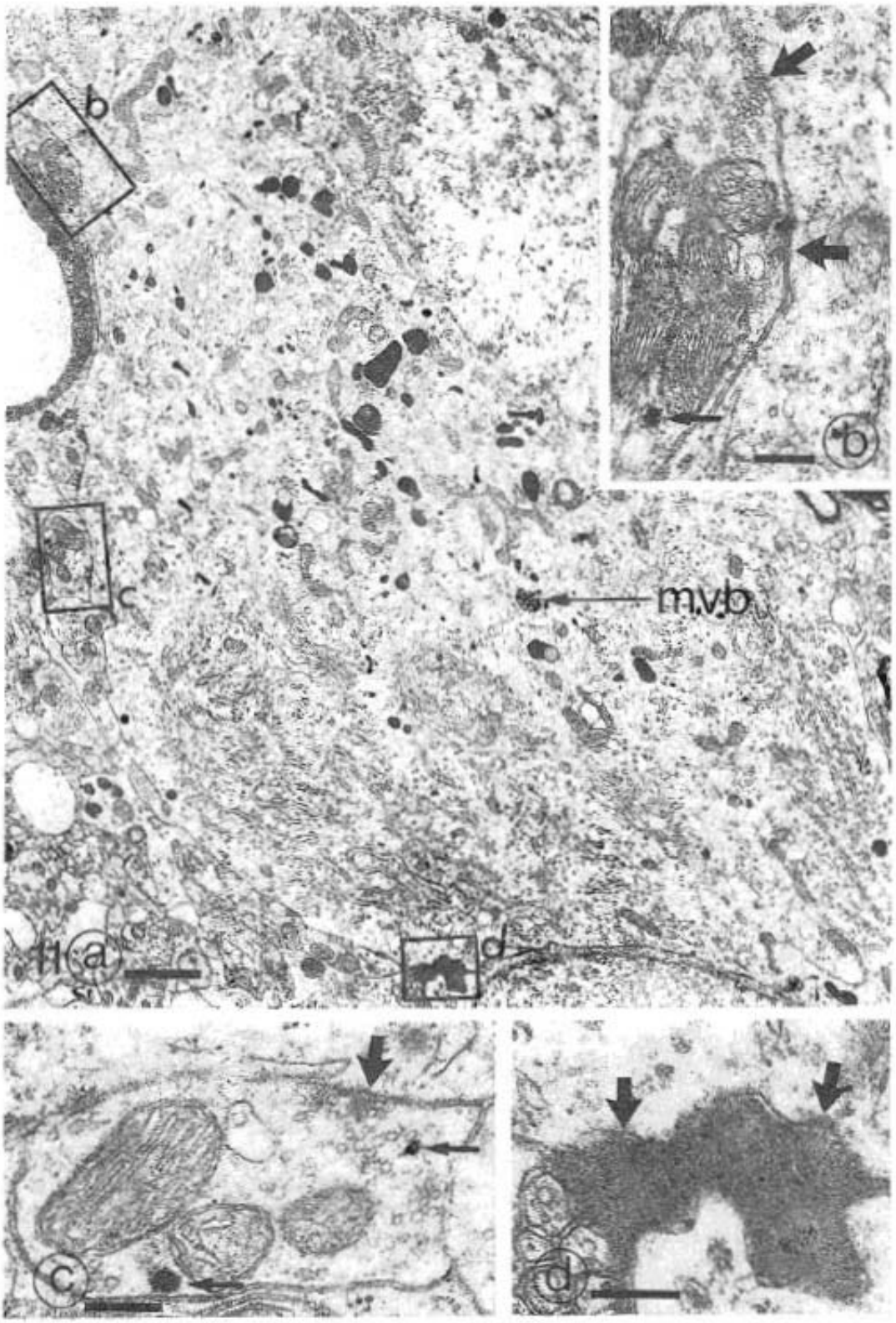
Terminal degeneration

Following lesions in the nucleus accumbens–ventral striatum region, degenerating terminals and axons could be found in the ipsilateral substantia nigra. The electron dense type of degeneration was the most common type observed, (Figs. 2c, 6, 10 and 11a and d). There was no qualitative difference in the appearance of degenerating terminals in the animals whether the lesion was caused by kainic acid or electrolysis, nor was there any difference between the animals in the synaptic relations of retrogradely HRP-labelled structures and degenerating terminals; therefore the results obtained from the 3 animals will be described together.

The heaviest degeneration was found in areas which on the basis of their synaptology correspond to the zona reticulata. However, the border between reticulata and compacta is not sharp and the most dorsal zone with reticulata characteristics contained occasional retrogradely labelled nigrostriatal neurones. The zone of degeneration usually ended at the ventral aspect of the dense layer of retrogradely labelled neurones, although scattered degenerating terminals could be found in the neuropil in the ventral layer of the compacta (Figs. 2a, c and 10).

At all survival times early and late stages of degeneration were observed in both the material processed for histochemistry with DAB (Figs. 2, 6, 9, 10 and 11) and in the Golgi-HRP material (Fig. 3). In the early stages, vesicles could still be recognized in the electron dense cytoplasm (Figs. 2c, 6 and 10a). In the late stages boutons became shrunken and were filled with homogeneous electron dense material (Figs. 9, 10 and 11d).

The synaptic contacts of degenerating terminals could be established on the basis of the synaptic cleft and the postsynaptic membrane specialization. At the contacts between degenerating terminals and perikarya small amounts of punctate


b
c
m.v.b
d
(a)
(b)
(c)
(d)

postsynaptic dense material could be observed (Figs. 2c and 11d). Long apposition of the synaptic membranes with moderate postsynaptic density could be seen at axodendritic synapses (Figs. 3c, 9 and 10). In terminals with this later type of contact, synaptic vesicles resembling those of HRP type 1 boutons (see below) were recognizable (Figs. 6 and 10a) in the early stages of degeneration. In rare cases early stages of degenerating boutons with type 1 characteristics were found to contain HRP-reaction end-product in tubules of smooth endoplasmic reticulum (Fig. 6). In addition to the symmetrical type of degenerating boutons on perikarya and dendrites, a few axodendritic degenerating boutons were found, with extensive asymmetric type postsynaptic density.

Synaptic contacts between degenerating terminals and retrogradely labelled structures

In those cases where the zone of degeneration overlapped areas where retrogradely labelled neurones occurred, numerous symmetrical synaptic contacts between the perikarya of nigrostriatal neurons and degenerating boutons were found (Figs. 2 and 11). In one section up to 6 such synaptic contacts were observed on a single neurone. Dendrites containing HRP-reaction end-product also received degenerating terminals and occasionally several degenerating boutons converged onto one HRP-labelled dendrite (Fig. 10).

One area from animal ST24 which contained 5 HRP-labelled cells that were also Golgi-stained was studied under the electron microscope (Figs. 3 and 4). Degeneration in this region was sparse and the zone of degeneration stopped short of the perikarya. Only two degenerating boutons were found in synaptic contact with the Golgi stained, gold toned dendrite of one of the HRP-labelled nigrostriatal neurones (N_1 in Figs. 3 and 4), although most of the length of this dendrite, as well as the others, was studied in serial sections.

HRP-labelled axons and nerve terminals

Enzyme reaction end-product was not only found in perikarya and dendrites but was also present in myelinated axons and, more frequently, in thin unmyelinated axons. It could usually be found within membrane limited tubules and vesicles, occasionally in dense bodies and multivesicular bodies. There were at least two types of nerve terminals containing HRP reaction end-product in such structures.

Type 1 boutons. These made axodendritic synapses with both thick and thin dendritic shafts as well as with occasional spines of these dendrites (Figs. 5 and 9). They contained large, clear, round vesicles which were tightly packed filling most of the bouton (Fig. 5). A few dense core and granular vesicles, larger in size than the clear ones, could also be observed in these boutons. The boutons had prominent triangular

Fig. 11. a: retrogradely HRP-labelled nigrostriatal neurone in zona reticulata. Framed areas (b) and (c) are shown at higher magnification in (b) and (c). Synaptic contacts (large arrows) between HRP-labelled (horizontal arrows) type 2 boutons and the prikaryon are seen. d: a degenerating bouton framed in (a) is also in synaptic contact (large arrows) with the same perikaryon. mvb, multivesicular body. Case ST27, DAB as substrate. Scales: a, 1 μm; b, c, and d, 0.2 μm.

presynaptic dense projections. The synaptic contact zone was extensive and there was a moderate postsynaptic membrane specialization (Figs. 5 and 9). Type 1 boutons may completely ensheath dendrites in the zona reticulata (Fig. 5).

Type 2 boutons. These HRP-labelled boutons made synapses with proximal dendrites but mainly with perikarya (Figs. 8, 11b and 11c). They were most frequently found in the zona compacta and contained small pleomorphic and flattened vesicles. The vesicles accumulated in small clusters at the synaptic active zones, which were small compared to those of type 1 boutons. The pre- and postsynaptic membrane specializations were small and symmetric.

There were differences in the mitochondria in the two types of boutons; type 1 boutons had much smaller and fewer mitochondria than type 2 boutons (compare Figs. 5 and 8).

Synaptic relationships of HRP-labelled pre- and postsynaptic structures

HRP-labelled perikarya and proximal dendrites of nigrostriatal neurones frequently received synapses from type 2 HRP-labelled terminals (Fig. 11). In one section up to 7 labelled boutons could be observed in contact with one neurone. On the other hand, type 1 labelled boutons formed synapses mainly with unlabelled dendrites, which probably reflects the scarcity of HRP-labelled dendrites in the area of the zona reticulata where most labelled type 1 boutons were found.

Convergence of HRP-labelled and degenerating boutons was found on to both retrogradely HRP-labelled and unlabelled dendrites (Fig. 9) and perikarya (Fig. 11). On the dendrites the HRP-labelled terminals were of type 1, while on the perikarya they were type 2.

Comparison of local axon collateral terminals of striatonigral neurones with type 1 HRP-labelled boutons (Figs. 5 and 7)

The boutons of the local axon collaterals of an HRP-labelled, Golgi stained striatonigral neurone were studied within the neostriatum. This neurone was a medium size cell with densely spiny dendrites and has been described in another report[30]. Gold toning of the Golgi stained striatonigral neurone and its local axon collaterals revealed the internal detail of its structure. The boutons contained large, clear vesicles similar to those in type 1 boutons (Fig. 7). The presynaptic dense projections were well developed and the boutons formed symmetrical synaptic contacts, similar to those of the type 1 HRP-labelled boutons that were found in the substantia nigra in the present study.

DISCUSSION

Monosynaptic targets of striatonigral fibres

Our main finding is that neurones projecting to the neostriatum from the substantia nigra receive monosynaptic input from neurones in the nucleus accumbens-ventral striatum region. That these afferent terminals comprise an important part of the input of nigrostriatal neurones is suggested by the numerous degenerating boutons

on the perikarya as well as on the proximal and distal dendrites. Earlier studies in the rat[13] and in the cat[11,16] showed that following large lesions in the striatum degenerating terminals could be found in synaptic contact with both the dendrites and perikarya of nigral neurones. However, the nigra contains neurones that project to many parts of the brain and local interneurones may also be present[1,6,7]. Thus, in the earlier studies it was not possible to determine with morphological methods which type(s) of neurone receive the monosynaptic afferents from the striatum. Using the retrograde HRP-labelling or its combination with Golgi staining it is now evident that both nigro-thalamic[31] and nigrostriatal neurones receive a monosynaptic input from the striatum, although this input probably originates in different parts of the striatum. In the earlier study on the striato-nigro-thalamic pathway, the lesions were placed in the main body of the striatum[31] whereas in the present study the lesions were located in the ventral striatum and nucleus accumbens.

The origin of HRP-labelled boutons

Our results are in agreement with earlier studies[21,38] on HRP in striatonigral system, demonstrating that the neuronal smooth endoplasmic reticulum and the lysosomal system contain the reaction end-product. Two main types of boutons were found to contain reaction end-product in the substantia nigra, following injection of HRP into the striatum.

Type 1 boutons in the present study are identical with type 1 boutons of Rinvik and Grofová[28] in the cat, with type C boutons in the rat[12] and with endings with large synaptic vesicles in the primate[29]. It is more difficult to correlate the HRP-labelled type 2 boutons with any described previously. We may have selected a subpopulation of boutons hitherto unrecognized, because we studied the synaptic connections of labelled structures. In any case there is a clear difference between the two populations of HRP-labelled boutons not only in morphology but more importantly in termination. Type 2 boutons were mainly found on perikarya and proximal dendrites while type 1 boutons occurred mainly on more distal dendrites and not on the perikarya of nigrostriatal neurones. This difference in termination indicates a differential input onto the various recipient parts of nigrostriatal and other nigral output neurones.

The presence of a large number of HRP-labelled boutons raises the question: are these boutons the terminals of striatonigral neurones? Following injection of HRP into the striatum, labelled boutons could appear in the substantia nigra in 3 ways: (1) through anterograde transport of HRP from the striatum; (2) they could be local axon collaterals labelled anterogradely following retrograde labelling of nigrostriatal neurones; and (3) they may represent the anterogradely labelled boutons of neurones in a third nucleus that was labelled retrogradely from the striatum and which sends long axon collaterals to the substantia nigra.

It is likely that at least some of the type 1 boutons are labelled by the first mechanism. Thus, following placement of lesions in the accumbens–ventral striatum region, the degenerating boutons in the nigra have, at an early stage, the same morphological features as the type 1 HRP-labelled boutons. In rare cases HRP-labelled boutons of type 1 themselves showed the early stage of degeneration (Fig. 6),

indicating that they originate from that part of the striatum where the spread of HRP overlaps with the region of the lesion induced by kainic acid. Finally, the boutons formed by local axon collaterals of identified striatonigral neurons[2,30] have very similar morphological features to type 1 HRP-labelled boutons in the substantia nigra. This suggests not only that type 1 boutons are of striatal origin, but that they are boutons of the medium size neurones with densely spiny dendrites that are known to project to the nigra[30–32].

No HRP-labelled boutons of type 2 were found that also showed signs of degeneration and so their origin is uncertain. It is noteworthy that they had the same postsynaptic target (the perikarya of nigrostriatal neurones) and a similar symmetrical synaptic specialization as some of the boutons that degenerated after the placement of a lesion in the accumbens–ventral striatum. Although the origin of type 2 boutons from neurones in the striatum requires confirmation by more direct methods, it is significant that there is a strong afferent system that can be labelled by HRP injected into the striatum and which terminates on the perikarya and proximal dendrites of nigrostriatal neurones.

Possible correlations with neurotransmitters

In order to understand the functional role of the monosynaptic pathway from neurones in the accumbens–ventral striatum to nigrostriatal neurones, we need to identify the neurotransmitters of both the afferent terminals and the recipient nigrostriatal neurones. Although there is, at present, no direct evidence we can draw some tentative conclusions from histochemical and neurochemical studies. The striatonigral substance P containing projection originates mainly from the anterior striatum in the rat[3,8,14,15] and the caudal nucleus accumbens-ventral striatum region (part of the area affected by our lesions) contains substance P immunoreactive cell bodies[10]. Substance P immunoreactive fibres can be seen to pass among, and ventral to, zona compacta neurones forming ultrastructurally identified boutons on their way (P. Somogyi, J. Priestley, A. C. Cuello, A. D. Smith and J. P. Bolam, unpublished observations). However, it is possible that nigral substance P does not originate from neurones in the ventral striatal region because there was no decrease in the amount of substance P in the substantia nigra following a lesion in the nucleus accumbens[15], although microdissection of the different parts of the substantia nigra was not performed. The importance of subdividing the nigra was shown by Walaas and Fonnum[38], who found a selective decrease in the level of glutamate decarboxylase activity in the rostromedial substantia nigra (including part of the pars compacta) following placement of lesions in the nucleus accumbens. Thus, the evidence at present favours γ-aminobutyrate as a transmitter in the neurones that project from the accumbens to the nigra.

The majority of nigrostriatal neurones are dopaminergic, but some have been found to lack tyrosine hydroxylase[16] or dopamine[37]. Although we cannot exclude the possibility that some afferents from the ventral striatal region terminate on non-dopaminergic nigrostriatal neurones, the finding that degenerating boutons can readily be found on most of the retrogradely labelled neurones when they are within an

area of degeneration implies that the dopaminergic nigrostriatal neurones receive a monosynaptic input from the accumbens–ventral striatum.

Possible functional implications

There seem to be functionally distinct areas within the neostriatum[34,35] and so our findings about the input of nigrostriatal neurones may have functional implications. The degenerating boutons we found in contact with nigrostriatal neurones must originate from a different part of the striatum (the ventral region, including the accumbens) from the great majority of the HRP-labelled boutons because the latter only rarely showed signs of degeneration. It is likely that many of the HRP-labelled boutons originated from neurones in the main body of the striatum. It is noteworthy that we observed convergence of HRP-labelled boutons and degenerating boutons on the same nigrostriatal neurone. It is, thus, conceivable that striatal afferents from wide areas, which process different information, converge on to single nigral neurones which in turn project the integrated information back to the striatum. This nigrostriatal feedback pathway should not be considered only as a simple (monosynaptic) feedback loop since it involves regions of the striatum which feed back on to nigral neurones that project to a different part of the striatum. This can be concluded from our studies on animals with electrolytic lesions in the ventral striatum, since the retrogradely labelled nigrostriatal neurones that received degenerating terminals could not have taken up HRP from the area affected by the lesion because their axons would have undergone electrocoagulation. It is also unlikely that their retrograde labelling occurred via axon collaterals, since the axons of nigrostriatal cells do not branch to large areas of the striatum as indicated by the lack of double labelled cells in experiments involving the use of fluorescent dyes[30].

We tentatively conclude that our finding of HRP-labelled boutons in contact with nigrostriatal neurones is consistent with a point-to-point (i.e. area-to-area) reciprocity involving the nigral projection to the main body of the striatum, but that the circuit involving the nucleus accumbens–ventral striatum region is not exclusively organized in this mode since neurones from this region are in monosynaptic contact with nigral neurones that project elsewhere in the striatum. The latter conclusion is in agreement with the interpretations of Nauta et al.[23].

The ventral striatum–nucleus accumbens region is known to receive limbic afferents (for references see Nauta and Domesick[32]; Graybiel and Ragsdale[10]; Mogenson et al.[20]). On the other hand, the main body of the caudoputamen where most of the HRP was injected participates in somato-motor functions[10]. The monosynaptic connection through the substantia nigra demonstrated in the present study between these two areas thus provides a neuronal basis, at the synaptic level, for an 'interface' between limbic and motor systems[10,23].

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