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41
An Approach to the Identification of Neurotransmitters in Characterized Synapses of Complex Neuronal Networks: Application to the Basal Ganglia of the Rat

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Introduction

In spite of many experimental studies, we still know little more about the functions of the neostriatum than can be deduced from the symptoms of diseases which affect this part of the brain. Parkinsonism, in which there is a degeneration of afferent fibres containing dopamine, is associated with difficulty in carrying out voluntary motor activity. Huntington's disease, in which some striatal neurons have degenerated, is associated with excessive involuntary motor activity. The neostriatum is, therefore, generally believed to play a key role in the control of motor activity (Divac and Öberg, 1979).

In order to understand how the striatum is involved in the control of motor activity, it is clearly necessary to know what information it receives from other brain areas and how it processes this information before passing it on to other regions concerned in motor control. Such major questions cannot be answered fully without a detailed knowledge of the neuronal 'wiring diagram' of the striatum. For example, although we know that the primary lesion in Parkinson's disease is the degeneration of the dopaminergic nigrostriatal pathway (see Hornykiewicz, Chapter 45 in this volume), we cannot explain why this leads to hypokinesia. This is because we cannot describe how input from the substantia nigra is processed in the striatum;

we do not even know the nature and connections of the neuron that receives the dopaminergic input.

The application of many different experimental methods (e.g. morphological, pharmacological, biochemical and electrophysiological procedures) has provided important clues about the neuron networks involving the striatum (reviewed in Divac and Öberg, 1979; Dray, 1979, 1980). These clues do not, however, add up to a coherent picture. This is partly because the striatum is so complex (Fig. 1). It receives input from four different

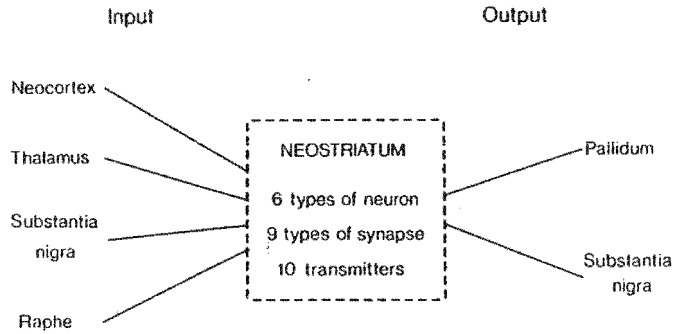


FIG. 1. *Diagram to illustrate the complexity of the neostriatum. The anatomical studies on input and output from the neostriatum have been reviewed by Graybiel and Ragsdale (1979).*

regions of the brain (Nauta and Domesick, 1979; Graybiel and Ragsdale, 1979); it contains at least six different types of neuron (Pasik *et al.*, 1979; Dimova *et al.*, 1980), about nine types of synapse (Hassler, 1978) and 12 or more different putative transmitters (Table 1). However, another reason why it is so difficult to build up a clear picture of the 'wiring diagram' of the striatum may be because the experimental methods in common use cannot, on their own, unequivocally answer the key questions. Such questions include: what types of neuron (local circuit or projection neuron) receive input from each of the four brain regions that send afferents to the striatum and what are the transmitters used by the terminals of each afferent pathway and by their respective postsynaptic target neurons?

The difficulty in answering questions of this type arises because they are concerned with a problem that is at one and the same time both macroscopic and microscopic. On the macroscopic scale the problem is to trace connections between two, three, or more brain regions; while on the other hand the very same connections have to be demonstrated at the synaptic level. The electrophysiologist's approach to this problem is to use microelectrodes to record synaptic events in individual neurons: an input from another

TABLE 1

Putative neurotransmitters found in the striatum

Acetylcholine
Dopamine
5-Hydroxytryptamine
γ-Aminobutyric acid
Glycine
Glutamate
Noradrenaline
Substance P
Enkephalin
Cholecystokinin
Somatostatin
Vasoactive intestinal polypeptide (VIP)

References: Dray (1980) except for cholecystokinin, (Hökfelt *et al.*, 1980; Emson *et al.*, 1980), somatostatin (Graybiel *et al.*, 1981) and VIP (Lorén *et al.*, 1979).

region can be characterized as excitatory or inhibitory and, by antidromic stimulation, it may be possible to identify the projection area of the neuron being studied. Such methods have certain technical limitations (Schlag, 1978) which may be particularly difficult to overcome in studies on the striatum (Preston *et al.*, 1980). Nevertheless, a lot of valuable information about the striatum has been obtained by electrophysiology alone, or in combination with pharmacological or morphological methods (Dray, 1980; Feger *et al.*, 1979).

The morphologist's approach to the problem of studying connections between brain areas at both the macroscopic and microscopic levels has also suffered from serious limitations. Tracing neuronal pathways by light microscopic methods can demonstrate connections between two brain regions, but cannot be used to demonstrate connections at a synaptic level. Thus, we know that the striatum receives input from the neocortex and that the striatum sends projections to the globus pallidus and to the substantia nigra; but, even if a close topographical relationship could be shown between the sites of termination of the corticostriatal pathway and, for example, the cells of origin of the striatonigral pathway, it would not be justifiable to conclude that the two pathways are functionally (i.e. synaptically) connected. Synaptic connections can only be shown in the electron microscope. After placement of a lesion in the neocortex, degenerating terminal boutons from cortical neurons have been shown to be in asymmetric synaptic contact with dendritic spines in the striatum (Kemp and Powell, 1971*b*). However, because only a very tiny part of a

neuron can be studied in the electron microscope, it is not possible to tell from such studies (1) what type of spine-bearing neuron receives the cortical input, (2) whether these neurons are local circuit neurons or project outside the striatum (and, if so, to where they project) or (3) what neurotransmitter is used by the striatal spiny neuron that receives input from the cortex.

These problems about the connections between the neostriatum and two other brain areas are examples of a general problem: if neurons in region A project to region B, how can it be demonstrated that they are in synaptic contact with the neurons in B that project to a third area, C (Fig. 2)? Furthermore, how can we identify the neurotransmitters used by the two neurons that are synaptically connected?

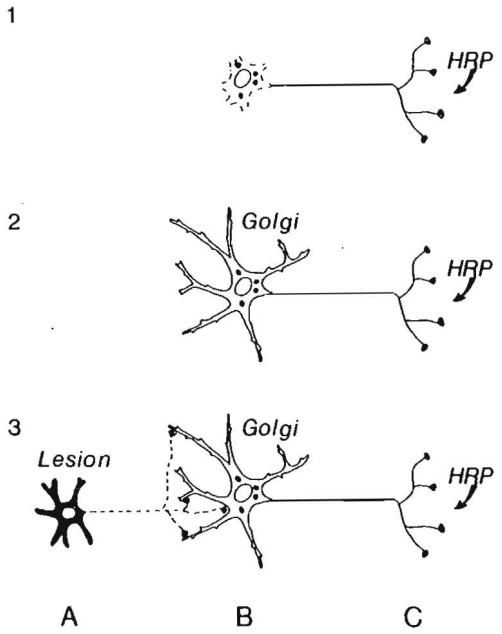


FIG. 2. *Diagram illustrating how a combination of Golgi staining, retrograde transport of horseradish peroxidase (HRP) and anterograde degeneration can be used to show a neuron chain linking three distant regions of the brain. (1) Retrograde labelling by horseradish peroxidase shows a projection from region B to region C; the labelled neurons contain horseradish peroxidase reaction product in granules in the cytoplasm. (2) Combining retrograde labelling with Golgi staining makes it possible to describe the morphological characteristics (in particular the form of the dendritic tree) of the neurons that project from B to C. (3) Adding a third method, the identification of degenerating synaptic boutons in the electron microscope, makes it possible to demonstrate that neurons in region A are in monosynaptic contact with the neurons in region B that project to region C.*

We are approaching this problem in two stages: first, we have developed a morphological procedure that allows us to demonstrate a monosynaptic link (in region B) between regions A and C; second, we are trying to combine this tracing procedure with methods that will identify the neurotransmitters in the characterized synapses and neurons. In this chapter we will summarize the results we have obtained by application of the morphological tracing procedure to connections of the basal ganglia.

The Golgi–Peroxidase Transport–Degeneration Procedure for Demonstrating a Monosynaptic Link between Three Brain Regions

This procedure exploits the power of morphological methods to tell us about macroscopic relations (connections between two different brain regions) and about connections at the synaptic level. Instead of applying the approaches separately, however, we have combined them in the same material (Fig. 2).

The first step involves the use of horseradish peroxidase, which is injected in region C, to identify neurons in region B that project to C. Horseradish peroxidase reaction product can be detected in the perikaryon and, sometimes, in the proximal dendrites, but very rarely in the distal dendrites. This tells us very little about the morphological features of the neurons that project from B to C: we simply get information about the shape and size of the cell body.

The classical way of studying the morphology of neurons is the Golgi method, since this leads to impregnation of the whole dendritic tree. However, although axons may be impregnated, they can rarely be traced over long distances and so the Golgi method cannot be used to demonstrate projections to a distant region of the brain.

We found a way of overcoming the limitations of the horseradish peroxidase-transport method and the Golgi method by combining them in the same tissue (Somogyi *et al.*, 1979). By using a new substrate for horseradish peroxidase (*o*-tolidine) it was possible to observe reaction product of horseradish peroxidase in brain tissue that contained neurons impregnated by the Golgi method; indeed, sometimes neurons could be found that contained retrogradely transported horseradish peroxidase and were also themselves Golgi-stained (Fig. 2). In this way, we could give a complete morphological description (in the light microscope) of the type of neuron that projects from one region to another region. Furthermore, by using an important modification of the Golgi method called 'gold-toning' (Fairén *et al.*, 1977) we were able to study the Golgi-stained, retrogradely labelled neurons in the electron microscope; in this way the nature of their afferent

nerve terminals could be studied. In addition, if they had local axon collaterals that were impregnated by the Golgi stain, details of their own nerve terminals could be described.

When the above procedure was carried out in an animal which had had a lesion placed in an area (area A in Fig. 2) that contained neurons known to project to region B, we could study boutons undergoing anterograde degeneration in region B and identify those that were in synaptic contact with the characterized projecting neuron. This provided unequivocal morphological evidence of a chain of connections between three distant regions of the brain.

Morphological Characterization of Striatonigral Neurons

Striatonigral neurons have been labelled by the retrograde transport of horseradish peroxidase (Bunney and Aghajanian, 1976; Grofová, 1975) or *Herpes simplex* virus (Bak *et al.*, 1978). Such studies have shown that the great majority of the cells labelled are of medium size (13–20 μm in diameter). Studies in the rat with the Golgi method have identified at least four types of striatal neuron in this size range (see Dimova *et al.*, 1980) and so no conclusion can be drawn from retrograde transport studies alone about the type of neuron that projects to the nigra. To overcome this problem we combined the retrograde transport of horseradish peroxidase from the substantia nigra to the striatum with the Golgi method. We found that some medium-sized striatal neurons that are labelled by horseradish peroxidase can be Golgi-stained and gold-toned; so far, they all fall into one morphological class: the neurons with densely spiny distal dendrites (Somogyi and Smith 1979; Somogyi *et al.*, 1981*a*). An example of one of these striatonigral spiny neurons is shown in the photomontage in Fig. 3.

The medium-sized densely spiny neuron is by far the most common type seen in Golgi preparations of the striatum of different species; in the cat, for example, 96% of the neurons impregnated by the Golgi procedure were of this type (Kemp and Powell, 1971*a*). This type of neuron has often been thought to be an interneuron in the striatum, although in the mouse an axon of such a neuron was traced as far as the globus pallidus (Leontovich, 1954). On the grounds of morphological similarity, it could now be argued that since some striatal medium-sized spiny neurons are efferent neurons perhaps all of them are, some projecting to the substantia nigra and some to the globus pallidus (or the same neuron may have long axon collaterals to both areas). Alternatively, there may be subpopulations of medium-sized densely spiny neurons, some of which are just local circuit

neurons. These questions will be difficult to answer by Golgi studies since the impregnation of neurons is so capricious.

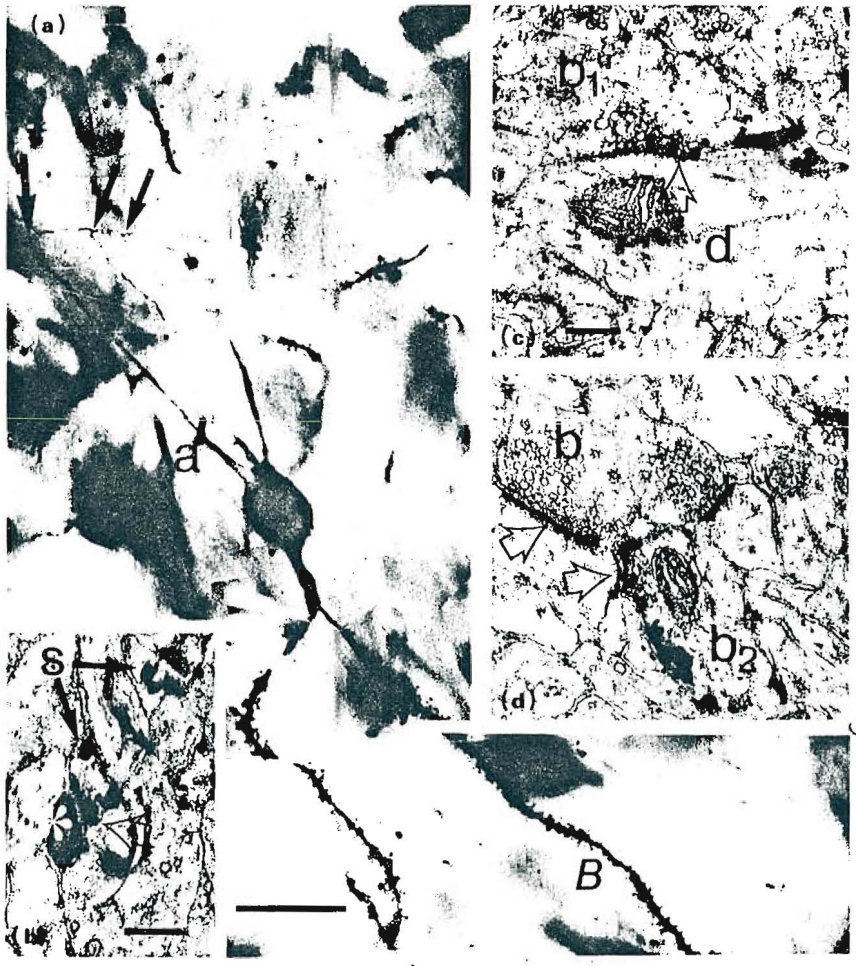
A second type of striatonigral neuron

Because other types of striatal neuron are much less commonly impregnated in the Golgi procedure (and may, indeed, really be in a minority), we pointed out that the medium-sized spiny neuron may not be the only type of striatonigral neuron (Somogyi and Smith, 1979). Subsequent studies (J. P. Bolam, P. Somogyi, S. Totterdell and A. D. Smith, unpublished) have identified a second type of striatonigral neuron in the rat. These are relatively rarely horseradish peroxidase-labelled and, as a result, none has so far been found that is both retrogradely labelled and Golgi-impregnated. However, examination of the cell bodies of horseradish peroxidase-labelled neurons in the electron microscope shows that the second type of striatonigral neuron can be distinguished from the medium-sized densely spiny neuron on the following grounds: it is somewhat larger; it has an indented nucleus, rather than a smooth one; and it has a larger amount of cytoplasm. Indirect correlation of these ultrastructural features with those of Golgi-stained neurons suggests that the second type of striatonigral neuron falls into a class of Golgi neuron that has long predominantly smooth dendrites which extend as far as 700 μm from the cell soma.

Afferents of Identified Striatonigral Medium-sized Spiny Neurons

When a striatonigral spiny neuron is identified in the light microscope, a record is made of its dendritic tree and local axon arborization (if the axon is impregnated) by serial photography (see Fig. 3) and drawing using the camera lucida. (Examples of such drawings and photographs can be seen in Somogyi and Smith (1979) and Somogyi *et al.* (1979, 1981*a*).) The part of the section containing the neuron is then re-embedded in epoxy resin and ultrathin sections are taken for electron microscopy. By examining the surface of the plastic block in which the neuron is embedded at intervals during the cutting of ultrathin sections, it is possible to correlate the electron micrographs with the light microscopy. Identified regions of the soma, dendritic tree or axon initial segment can be examined in the electron microscope for the presence of afferent axonal boutons in synaptic contact with the neuron. The types of boutons we found in synaptic contact with striatonigral spiny neurons are listed in Table 2.

In order to see if any of the afferent boutons originate from neurons outside the striatum, the Golgi–horseradish peroxidase procedure must be combined with one of the methods of anterograde tracing, e.g. anterograde



transport of horseradish peroxidase; autoradiography following axonal transport of labelled protein; or degeneration of boutons following destruction of the cell bodies. We have carried out some studies using the last-mentioned approach (Somogyi *et al.*, 1981*a*). Lesions were placed in parts of the cerebral cortex (motor, frontal and prefrontal) known to send projections to the striatum; these animals also received an injection of horseradish peroxidase into the substantia nigra. The striatum was processed by the combined Golgi–horseradish peroxidase procedure and Golgi-impregnated striatonigral neurons were examined in the electron microscope to see if they were in contact with any degenerating boutons

TABLE 2

Afferent synaptic boutons on identified striatonigral medium-size spiny neurons

Postsynaptic structure	Type of contact	Type of vesicle
Cell body	Symmetrical	Pleomorphic
	Symmetrical	Flattened
Axon initial segment	Symmetrical	Pleomorphic
Dendritic shafts (all)	Symmetrical	Pleomorphic
Dendritic shaft (distal)	Asymmetrical[a]	Spheroidal
Dendritic spines	Asymmetrical[b]	Spheroidal
	Symmetrical[a]	Spheroidal

For further details see Somogyi and Smith (1979); Somogyi *et al.* (1979); Somogyi *et al.* (1981*a*).

[a] This type is less common.

[b] Boutons forming asymmetrical contacts on spines are heterogeneous with regard to their size and the packing of vesicles.

FIG. 3. *A striatonigral neuron that receives input from the cortex and has local axon collaterals in the striatum. (This is neuron 6 from Somogyi* et al. *(1981*a*) where further results are given.)*

(a) Photomontage of part of the Golgi-impregnated and gold-toned neuron that was retrogradely labelled with horseradish peroxidase injected in the substantia nigra. Note the densely spiny secondary dendrites; B indicates the region of the dendrite from which the electron micrograph (in (b)) was taken. The axon (a) emerges from the perikaryon and was found to give rise to two local axon collaterals, one of which appears in this montage (large arrow). This axon collateral branched twice (small arrows) and two of the varicosities from along these collaterals are shown in electron micrographs (c) and (d). Scale: 20 μm.

(b) Three dendritic spines (s) from the part of the neuron labelled B in (a). The spines are identified as belonging to the striatonigral neuron by their content of electron-dense gold particles which are formed when the Golgi precipitate is replaced in the gold-toning process. A degenerating bouton (asterisk) originating from a neuron in the frontal/prefrontal cortex (where a lesion had been placed) is in asymmetrical synaptic contact (open arrow) with one of the spines of the striatonigral neuron. Scale: 0.2 μm.

(c) One of the varicosities of a local axon collateral of the striatonigral neuron shown in (a). The varicosity contains vesicles and is, therefore, a bouton (b_1). The bouton, identified by its content of electron-dense gold particles, is in symmetrical contact (open arrow) with a dendritic shaft (d). In serial sections, a spine was seen to emerge from this dendritic shaft. Scale: 0.2 μm.

(d) A second bouton (b_2) in symmetrical synaptic contact with part of a dendrite. An unlabelled bouton (b) from another neuron is also in synaptic contact with the same dendrite. Scale: 0.2 μm.

of cortical neurons. Asymmetrical synapses between degenerating boutons and the spines of dendrites of striatonigral spiny neurons are the only type so far observed. The degenerating boutons did not form a homogeneous population: some were small (0.1–0.3 μm in diameter), whereas others were much larger (up to 1 or 2 μm).

These studies, described in full in Somogyi *et al.* (1981*a*), establish that some of the boutons that form asymmetrical synaptic contacts with the dendritic spines of striatonigral neurons originate from neurons in the cerebral cortex. Thus, we have unequivocal morphological evidence of a monosynaptic cortico-striatonigral pathway. We have discussed elsewhere the relationship between these findings and electrophysiological studies and have also speculated on the neurotransmitters that may be involved (Somogyi *et al.*, 1981*a*).

Local Axon Collaterals of Identified Striatonigral Neurons

Medium-size spiny neurons in the striatum have been found by several workers (reference in Somogyi *et al.*, 1981*a*) to have extensive local axon collaterals. The presence of such local axon collaterals would, of course, support the view that the spiny neuron is a local circuit neuron, rather than an efferent neuron. Since we now know that some medium-sized spiny neurons project to the substantia nigra, there might be two populations of spiny neurons, only one of which has local axon collaterals and which does not project to another region of the brain. However, this does not turn out to be the case, since we have now found two striatonigral spiny neurons that also have local axon collaterals within the striatum (Bolam *et al.*, 1980; Somogyi *et al.*, 1981*a*); one of these is illustrated in Fig. 3. In Golgi material, the axons of these (and similar) neurons have a varicose appearance. Morphologists often assume that such varicosities are synaptic boutons. By examining the axons of Golgi-stained, gold-toned neurons in the electron microscope we found that the varicosities did indeed contain synaptic vesicles and that sometimes they could be shown to form synapses (see Fig. 3). The synapses were of the symmetrical type and the postsynaptic element was usually a dendritic shaft (in one case a dendritic spine). Examination of serial sections showed that the dendritic shaft receiving a synaptic bouton of an axon collateral had some spines. In view of this, and because the ultrastructural features of the postsynaptic elements were very similar to those of medium-sized spiny neurons, we suggested that the axon collaterals of striatonigral spiny neurons might form synapses with the dendrites of other spiny neurons (Somogyi *et al.*, 1981*a*). The possibility

arises, therefore, of lateral interactions between spiny neurons in the striatum; further work will be necessary to see whether the postsynaptic spiny neuron in such interactions may also be a projection neuron. These morphological findings give support to suggestions from electrophysiological studies (Park *et al.*, 1980) that spiny neurons participate in recurrent inhibition in the striatum.

Input from the cortex

The two striatonigral spiny neurons with local axon collaterals that we have studied so far were in animals that had received lesions in their motor cortex and frontal cortex, respectively. We found that dendritic spines of these two striatonigral neurons were both in contact with degenerating boutons (one is illustrated in Fig. 3), showing that they receive direct input from the cortex (Bolam *et al.*, 1980; Somogyi *et al.*, 1981*a*).

These morphological observations have certain functional implications. The cerebral cortex can simultaneously influence, via the same neuron in the striatum, not only the activity of neurons in the substantia nigra but also the activity of other neurons in the striatum that lie within the axonal field of the striatonigral neuron. It will clearly be important to identify the connections of the spiny neurons that receive input from the local axon collaterals of these striatonigral neurons. Do they also project from the striatum? Do they also receive input from the cortex or does their input come from another area that sends afferents to the striatum?

In summary, the medium-sized densely spiny neurons that project from the striatum to the substantia nigra receive input from several regions of the cortex and some of them also have local axon collaterals within the striatum. The next question to be discussed concerns the nature of the postsynaptic target of striatonigral neurons.

Monosynaptic Targets of Striatonigral Neurons

The substantia nigra sends projections to at least four different regions of the brain and it may also contain some interneurons (for references see Dray, 1979, 1980; Beckstead *et al.*, 1979; Graybiel and Ragsdale, 1979). Even though the neurons that project from the substantia nigra are topographically organized (Faull and Mehler, 1978), it is not possible by conventional morphological methods to identify which of them receives input from the striatum. We have applied our procedure to see whether nigrothalamic and nigrostriatal neurons receive monosynaptic input from striatonigral neurons.

Striato-nigro-thalamic pathway

Horseradish peroxidase was injected into the ventromedial nucleus of the thalamus and the same rats received an injection of kainic acid in the striatum in order to destroy the cell bodies of striatonigral neurons. Some of the nigrothalamic neurons (identified by retrograde horseradish peroxidase transport) situated in the pars reticulata were also Golgi-stained and gold-toned. On examination in the electron microscope, it was found that the long, thick, smooth dendrites of these neurons were ensheathed by numerous large synaptic boutons, most of which established symmetrical synaptic contact with the dendrite. Degenerating boutons of striatonigral neurons were sometimes found in contact with the perikarya of nigrothalamic neurons, but more frequently with the smooth distal dendrites (Somogyi *et al.*, 1979). Thus, some striatonigral fibres terminate on nigrothalamic neurons. This provides a morphological basis for the electrophysiological evidence that striatonigral fibres can inhibit nigrothalamic fibres (Deniau *et al.*, 1976).

Striato-nigro-striatal pathway

Nigrostriatal neurons occur predominantly in the pars compacta, an area of the substantia nigra which receives afferents from the ventral striatum and nucleus accumbens (Nauta *et al.*, 1978). We therefore retrogradely labelled nigrostriatal neurons by injecting horseradish peroxidase into the main body of the striatum and, in the same rats, placed lesions in the ventral striatum accumbens region. In some rats, the substantia nigra was processed by our combined Golgi–horseradish peroxidase procedure, but in others we used diaminobenzidine as a substrate for horseradish peroxidase and did not use the Golgi method. In the latter animals it was possible to study the afferent terminals on the soma and proximal dendrites of the nigrostriatal neurons because the projecting neurons could be identified in the electron microscope by the presence of horseradish peroxidase reaction end-product in characteristic intraneuronal structures. Degenerating boutons of axons from neurons in the ventral striatum accumbens were found in symmetric contact with the cell bodies and dendrites of nigrostriatal neurons. Individual nigrostriatal neurons often received many such degenerating boutons: in one section six were observed in contact with one neuron (Somogyi *et al.*, 1981*b*). These studies provide a morphological basis for neurons in the ventral striatum and nucleus accumbens to influence the activity of neurons in the substantia nigra that project to the main body (i.e. the more dorsomedial part) of the striatum. Thus, this part of the striato-nigro-striatal loop is not closed monosynaptically at the striatal end. It has been speculated that the function of this pathway might be to relay information coming from limbic regions into the ventral striatum and

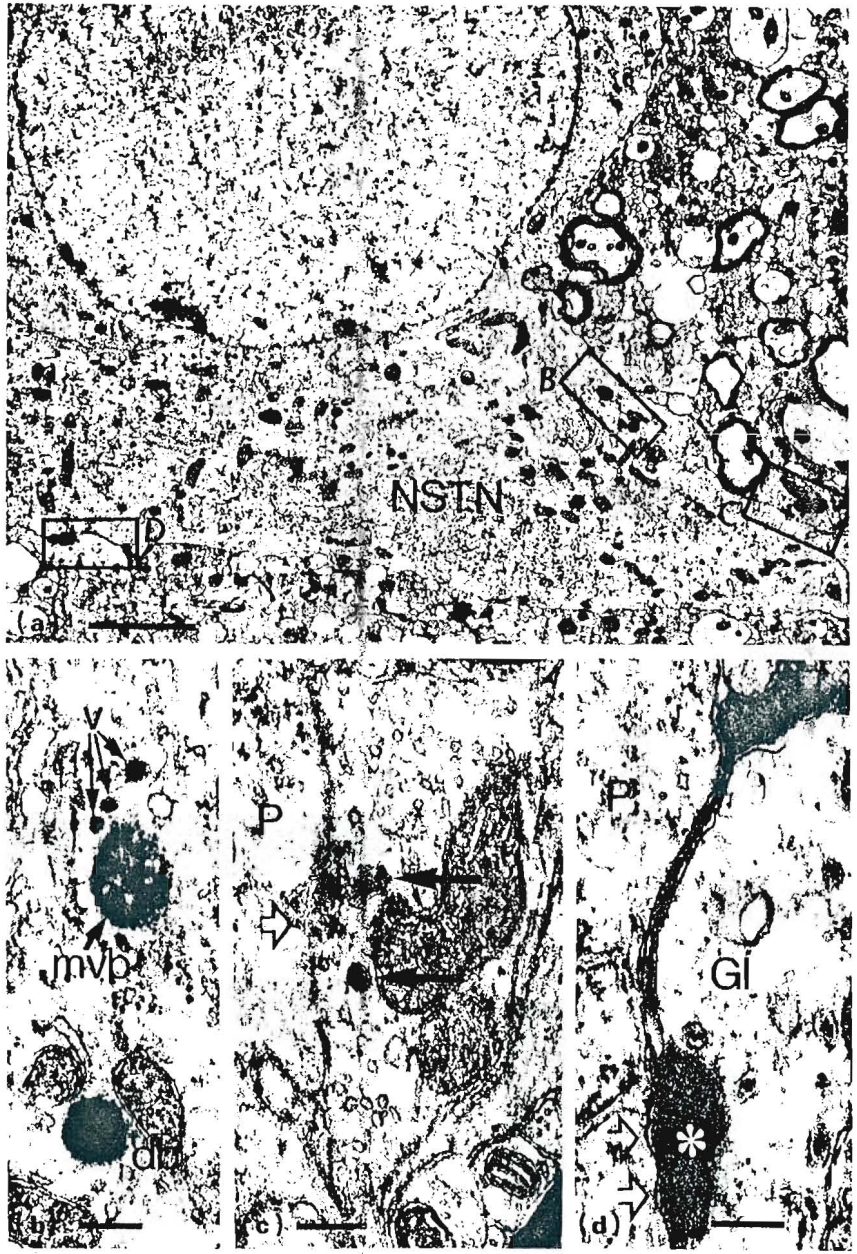
accumbens, through the substantia nigra to the main body of the striatum (Nauta and Domesick, 1978).

When studying the substantia nigra from these animals we were struck by the frequent occurrence of horseradish peroxidase reaction end-product in axons and boutons. Such findings have, in the past, been attributed to the anterograde transport of horseradish peroxidase along axons of striatonigral neurons (Sotelo and Riche, 1974; Nauta *et al.*, 1975). The horseradish peroxidase was present in two morphologically distinct types of bouton (Somogyi *et al.*, 1981*b*), one of which was mainly in synaptic contact with dendrites and the other was in contact with perikarya. The cell bodies of retrogradely labelled nigrostriatal neurons were often found in synaptic contact with horseradish peroxidase-labelled boutons; an example is shown in Fig. 4. Since the horseradish peroxidase that labelled the neurons retrogradely and labelled the boutons anterogradely was taken up following a single injection in the main body of the striatum, this implies a point-to-point (area-to-area) reciprocity in this part of the striato-nigro-striatal circuit. However, although these findings show that the loop is closed monosynaptically in the nigra, they do not allow us to conclude the same about the striatal end. At the best, it can be said that they do not exclude such a closed loop. They provide a morphological basis within the substantia nigra for the feedback loop from the striatum long postulated on pharmacological grounds (Aghajanian, 1978).

A further finding revealed more complexities about the afferents to the nigrostriatal neurons that project to the main body of the striatum. Individual nigrostriatal neurons received synapses (on their dendrites and/or perikaryon), both from degenerating boutons of cells located in the ventral striatum-accumbens and from horseradish peroxidase-labelled boutons presumed to originate from neurons in the main body of the striatum (see Fig. 4). In other words, there was a convergence of input from two different parts of the striatum. Since there are functionally distinct areas within the striatum (Neill and Herndon, 1978; Neill *et al.*, 1978), it is possible that striatal afferents from areas which process different information might converge on to single nigral neurons which, in turn, project the integrated information back to the striatum.

Circuits Involving the Neostriatum

We summarize in Fig. 5 the monosynaptic links which we have so far been able to demonstrate within neuronal networks in and around the striatum. A very great deal remains to be done, but we are just beginning to glimpse some of the details of networks by which the striatum might integrate input



from the sensory side (thalamus and cortex) with that from the parts of the brain concerned more with emotion and motivation (limbic areas, which might relay through the nucleus accumbens).

Neurotransmitters of Identified Synapses

We have refrained from speculating about the possible transmitters and their possible actions at the characterized synapses in the networks (some speculations can be found in Somogyi and Smith, 1979; Somogyi *et al.*, 1979, 1981*a*,*b*). It is essential now to find ways of identifying these transmitters with the same degree of rigour as the networks themselves can be revealed. Two ways of approaching this problem are being tested. First, we are trying to combine immunohistochemical methods for the localization of transmitters or their biosynthetic enzymes with Golgi staining and/or a retrograde tracing method. The combination of immunohistochemistry with retrograde tracing has already been achieved at the light microscopic level (Ljungdahl *et al.*, 1975; Hökfelt *et al.*, 1980; Priestley *et al.*, 1981) but information about neuronal networks will only be obtained when such neurons can also be studied in the electron microscope.

The second approach is to try to combine autoradiographic localization of radiolabelled transmitters, following their uptake, with Golgi staining. Conditions have been found where this is technically possible in the striatum

FIG. 4. *Convergence of input to a nigrostriatal neuron from the main body of the striatum and from the nucleus accumbens.*

(a) Retrogradely labelled nigrostriatal neuron (NSTN) in the dorsal part of the zona reticulata in the substantia nigra. Framed areas are shown in (b), (c) and (d) respectively. Scale: 2 μm.

(b) Horseradish peroxidase reaction end-product is present in vesicles (V), a multivesicular body (mvb) and a dense body (db) in the nigrostriatal neuron, following horseradish peroxidase injection into the main body of the striatum. Scale: 0.2 μm.

(c) Horseradish peroxidase-labelled bouton in synaptic contact (open arrow) with the perikaryon (P) of the nigrostriatal neuron. Horizontal arrows indicate reaction end-product in membrane-limited structures. This bouton was labelled as a result of anterograde transport following injection of horseradish peroxidase in the main body of the striatum. Scale: 0.2 μm.

(d) Degenerating bouton (asterisk) in synaptic contact (open arrows) with the same perikaryon (P). A lesion had been placed in the nucleus accumbens–ventral striatum region. GP, glial process engulfing the degenerating bouton. Scale: 0.2 μm.

(This previously unpublished material comes from rat labelled ST24 in Somogyi et al. *(1981*b*).)*

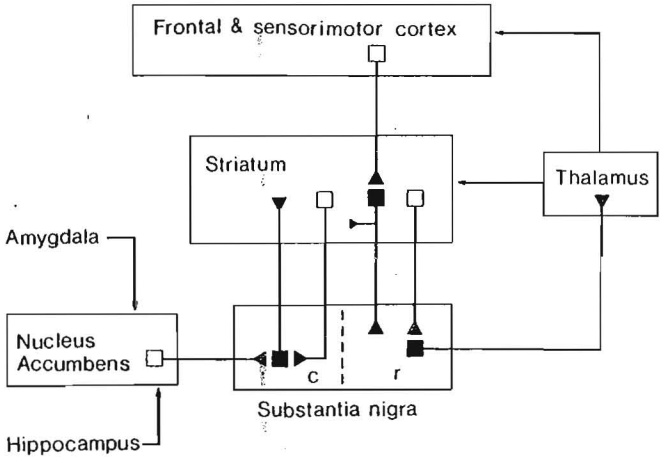


FIG. 5. *Summary of the monosynaptic links found so far in the basal ganglia. The squares indicate cell bodies of neurons and triangles indicate synaptic boutons. The two parts of the substantia nigra are pars compacta (c) and reticulata (r). Connections not yet studied by the new procedure, but known to connect two regions from earlier studies, are shown by arrows. This is a highly simplified diagram: no attempt has been made to indicate, for example, other projection areas of the striatum or substantia nigra reticulata that might be involved in the control of motor activity (see review by Graybiel and Ragsdale, 1979).*

and there is every reason to hope that we shall before long be in a position to identify which putative transmitter substance is taken up into a neuron in the striatum that has been characterized by Golgi staining, the source of some of its afferent synapses and the site to which it sends a projection.

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