J. Anat. (1978), **127**, 2, pp. 425–441
With 8 figures
Printed in Great Britain

Topographical organization of the striatonigral pathway revealed by anterograde and retrograde neuroanatomical tracing techniques

IAN F. TULLOCH*, GORDON W. ARBUTHNOTT AND ANN K. WRIGHT

MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh

(*Accepted* 6 *December* 1977)

INTRODUCTION

Since its first description by Edinger in 1911 the existence of the striatonigral pathway has been demonstrated by almost every known anatomical technique (Grofova & Rinvik, 1970; Grofova, 1975; Hattori, McGeer, Fibiger & McGeer, 1973; Nauta & Mehler, 1966; Niimi, Ikeda, Kawamura & Inoshita, 1970; Rosegay, 1944; Szabo, 1962, 1967, 1970, 1972; Voneida, 1960). The dearth of studies on this pathway in the rat is no doubt due to the difficulty of making lesions in the striatum without simultaneous damage to the corticofugal axons which pass through this nucleus. In the striatum of the rat these axons are in many separate bundles and are not collected into a clearly identifiable internal capsule. In cats and monkeys the corticofugal fibres form a single band which separates the striatum into caudate nucleus and putamen.

In his comprehensive work on the rat brain fibre connexions, Knook (1965) reported the presence of degeneration in the substantia nigra (SN) following lesions in the striatum, and concluded that it was likely that the pathway had a topographical distribution, with the anterior striatum projecting to medial SN, and posterior areas of the striatum projecting more laterally. Such a topographical representation has been reported for the cat (Niimi *et al.* 1970) and monkey (Szabo, 1962, 1967, 1970, 1972).

The problem of the involvement of fibres of passage in neuroanatomical investigations can be overcome by employing the autoradiographic technique of Cowan *et al.* (1972). Hattori, Fibiger & McGeer (1975), using such a technique, demonstrated that there are neurons which project from the rat striatum to the zona reticulata of the SN. The present experiments use a similar technique in order to demonstrate the entire course of the projection of striatal neurons. They also provide evidence that the pathway in the rat has a similar topographical distribution to that which has been described for other species.

Recently the striatonigral projection has been demonstrated by the horseradish peroxidase (HRP) technique (Bunney & Aghajanian, 1976*b*; Grofova, 1975; Kanazawa, Marshall & Kelly, 1976). Further, to support the autoradiographic results, we have also studied the distribution of neurons in the striatum retrogradely labelled by localized injections of HRP into different areas of the substantia nigra.

* These experiments were done while I. F. Tulloch was an MRC Scholar: his present address is: Reckitt & Colman, Dansom Lane, Kingston-upon-Hull.

Bunney & Aghajanian (1976*b*) earlier reported that the distribution of cells labelled with HRP after discrete injections in the SN follows a topographical arrangement. We can now confirm that this topographical organization is demonstrable by both anterograde and retrograde tracing techniques.

METHODS

Autoradiographic tracing methods

L-[4,5-^{3}H]leucine (Radiochemical Centre, Amersham) was injected into the corpus striatum of 15 male Wistar rats of 200–220 g. Each animal was anaesthetized by a Halothane/air mixture and secured in a David Kopf stereotaxic instrument. The labelled solution was delivered through a 30-gauge needle attached to a 1 μl Hamilton syringe and placed stereotaxically into the appropriate area of the corpus striatum. At dorsal and ventral sites 0·5 μl of the labelled solution (25 μCi/μl) was injected over a period of 40 minutes, and the needle was held in position for a further 20 minutes. At posterior placements labelled leucine was delivered in $2 \times 0{\cdot}2$ μl injections, each lasting 10 minutes. The needle was retracted 1 mm between injections and held at the upper position for 30 minutes after the second injection.

The co-ordinates used were as follows:

	Dorsal striatum	Ventral striatum	Tail of striatum
Anterior (mm)	+0·5	+0·5	−2·1
Lateral (mm)	3·0	3·7	4·2
Vertical (mm)	4·0	7·3	5·2–4·2
No. of rats	4	4	7

Bregma was used throughout as the stereotaxic reference point for the anterior and lateral co-ordinates, and the vertical readings were taken from the cortical surface overlying the injection site.

Three to four days after injection, the animals were anaesthetized with chloral hydrate and perfused through the ascending aorta with 80 ml of 0·1 M phosphate buffer (pH 7·2) followed by 200 ml of 4% paraformaldehyde in 0·1 M phosphate buffer (pH 7·2). The animals were kept on ice throughout the perfusion.

The brains were then carefully removed from the skull and placed in fresh fixative for 1 hour. Thereafter the tissue was post-fixed in Bouin's fluid, dehydrated, cleared in xylene, and vacuum-embedded in paraffin wax (M.P. 54 °C). The brains were subsequently sectioned at 6 μm and the sections were floated on to microscope slides coated with chrome alum-gelatin. Before autoradiographic procedures the sections were deparaffinized in xylene, rehydrated and finally washed for several hours in running water.

For light microscope autoradiography, sections were coated with Kodak AR 10 stripping film and exposed for 30 days at −20 °C.

The emulsion was then developed for 5 minutes in undiluted Kodak D-19 developer at 14–18 °C, fixed in Kodak 'Metafix', and washed in gently running tap water for at least 30 minutes.

All developed slides were stained through the photographic emulsion with haematoxylin and eosin. The sections were examined under both light- and dark-field illumination, and the distribution of silver grains was plotted on drawings of appropriate planes from the Konig & Klippel (1963) atlas of the rat brain. In all

cases a second independent observer verified the grain distribution. Control sections were obtained from non-injected brains which had been processed in an identical manner in order to assess background activity.

Experimental procedures for the horseradish peroxidase technique

The preparation of the animals and the method of injection were identical to those described for the autoradiographic experiments.

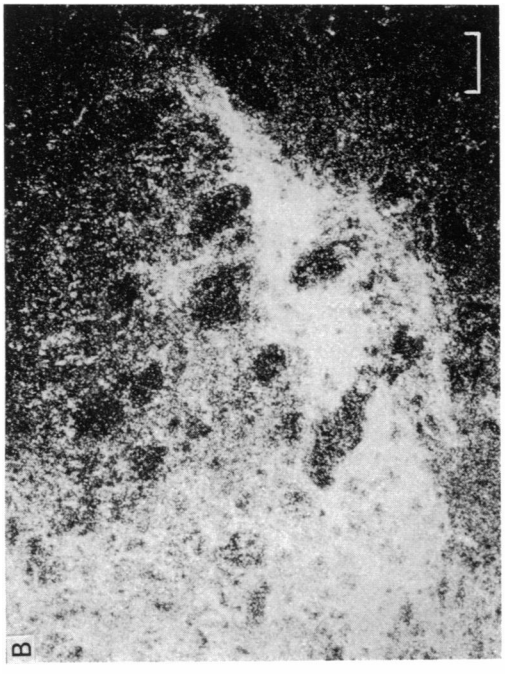
Sixteen rats were injected with HRP in the region of the SN. The delivery of 0·1–0·15 μl of a 10 % HRP solution, over a period of 5 minutes, resulted in adequate localized staining of the SN. The injection needle was inserted into the brain at an angle of 46° and the stereotaxic co-ordinates used were as follows:

	mm
Posterior	4·7–5
Lateral oblique	6·5–7
Depth	6·8–7·2

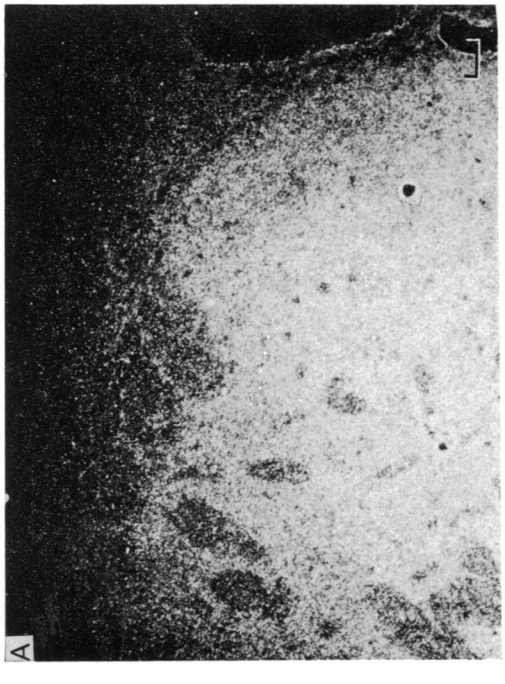
Bregma was used as the stereotaxic reference point, except in the case of the 'depth' co-ordinate, where the reading was taken from the overlying cortical surface. During those injections which were aimed at the zona compacta region (lateral co-ordinate 6·5 mm) the needle was retracted 0·7 mm from the site of most ventral penetration (7·2 mm) in an attempt to confine the HRP to a narrow strip lying along this brain area. After each injection the needle was left in the target area for a further 30 minutes.

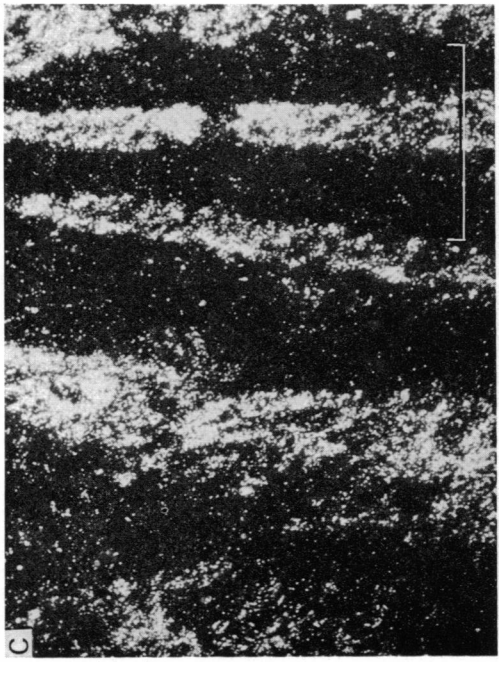
All the operated rats had a post-injection survival time of 24 hours, and they were then re-anaesthetized with chloral hydrate (400 mg/kg) and perfused transcardially with 80 ml of ice cold 0·1 M phosphate buffer (pH 7·4) followed by 150 ml of the same 0·1 M phosphate buffer containing 2 % paraformaldehyde and 2·5 % glutaraldehyde. The entire rat brain was removed immediately after the perfusion and placed in the same fixative for a further 24 hours. Finally the fixed brain was transferred to 0·1 M phosphate buffer (pH 7·4) containing 5 % sucrose and kept there for a further 24 hours.

The brains were cut coronally into two approximately equal parts and cryostat sections 40 μm thick were collected serially in different compartments of 27 compartment plastic ice-boxes containing ice-cold 0·1 N Tris-HCl buffer (pH 7·6). All the sections were rinsed in this buffer for at least 2 hours, but no more than 4 hours. The sections were then pre-soaked for 30 minutes at room temperature in a freshly prepared solution containing 50 mg 3,3-diaminobenzidine tetrahydrochloride (DAB – Sigma) in every 100 ml of 0·1 M Tris-HCl buffer (pH 7·6). The compartmentalized sections were then removed from the DAB-containing vessel and 20 μl of 15 % H_2O_2 per 50 ml of the DAB solution was added and mixed. All pre-soaked sections were then immediately placed in this solution (20–24 °C), with gentle agitation, for a further 30 minutes and then transferred through several rinsing solutions of 0·1 M phosphate buffer (pH 7·4) before being individually mounted on chrome alum-gelatin coated slides, in a water-soluble mountant (Univert-Gurr). This precluded the staining of these sections for Nissl substance, but the tissue quality achieved by this procedure was good. After drying, each section was methodically scanned microscopically for the presence of cell bodies containing HRP reaction product, using both bright and dark-field illumination. The criteria outlined


B


D


A


C

by Nauta, Pritz & Lasek (1974) were used to identify neurons which were retrogradely labelled with HRP. For each injected brain, labelled cells were plotted by two independent observers and projected onto the appropriate plane of section taken from the stereotaxic atlas (Konig & Klippel, 1963).

RESULTS

Autoradiographic studies

Dorsal injection sites in the striatum

All four animals in this group had injection sites well localized to the dorsal area of the head of the striatum, with no involvement of overlying cortical tissue, the corpus callosum seeming to act as an effective barrier to diffusion of the injected [^{3}H]amino acid (Fig. 1A). The extent of the injection site, and the corresponding distribution of label in the target nuclei, were virtually identical in these animals.

Heavily labelled perikarya were present in the dorsal striatum, with only minimal encroachment of label into the extreme dorsal region of the globus pallidus. The small number of silver grains overlying the perikarya in the dorsal globus pallidus did not exceed that over the surrounding neuropil, and it is unlikely that the uptake of label by these cells contributed significantly to the observed distribution of labelled fibres and terminals.

Labelled fibres (Fig. 2) arising from these cells in the dorsal striatum entered the internal capsule dorsally and coursed ventrally before gathering into a relatively compact fibre bundle occupying the medioventral region of the internal capsule. Densely labelled fibres left the internal capsule laterally (Fig. 1B) and appeared to terminate throughout the rostrocaudal extent of the medial part of the globus pallidus. As the labelled fibre bundle in the internal capsule continued caudally and entered the ventral tip of the crus cerebri, fibres were observed to turn dorsally and medially in the comb system of Edinger, (Faull & Carman, 1968; Nauta & Mehler, 1966) (Fig. 1C) before collecting into discrete fascicles. At the level of the anterior substantia nigra, fibres coursed dorsally and left the crus to terminate predominantly in the medioventral region of the zona reticulata (Fig. 1D), and to a much lesser extent in the medial zona compacta area. At planes of section caudal to the oculomotor nerve radioactivity was present in the same relative position within the substantia nigra, although the degree of labelling was much less. No label was found in the most caudal region of the substantia nigra.

Fig. 1. Photomicrographs of the autoradiographs from the 'dorsal injection sites' in the striatum, under dark-field illumination (radiographic grains show as white dots). Calibration bars represent 100 μm. (A) The dorsal region of the injection site is shown at low magnification. The absence of grains over the white matter of the corpus callosum and internal capsule is clear at the top of the picture. The arrowhead marks the border of the ventricle. (B) The fibres en route to the globus pallidus are shown as they leave the bulk of the labelled fibres in the internal capsule to the left of the picture. (C) At higher power the activity in the internal capsule at the level of the mamillary bodies can be seen to be distributed in a pattern reminiscent of the comb system of Edinger (Faull & Carman, 1968; Nauta & Mehler, 1966). (D) The dotted line marks the dorsal border of the crus cerebri in this photograph at the level of the anterior substantia nigra. The autoradiographic grains are concentrated just dorsal to the edge of the white matter, and are only present over the zona compacta area (top right in the picture) at very low concentration.

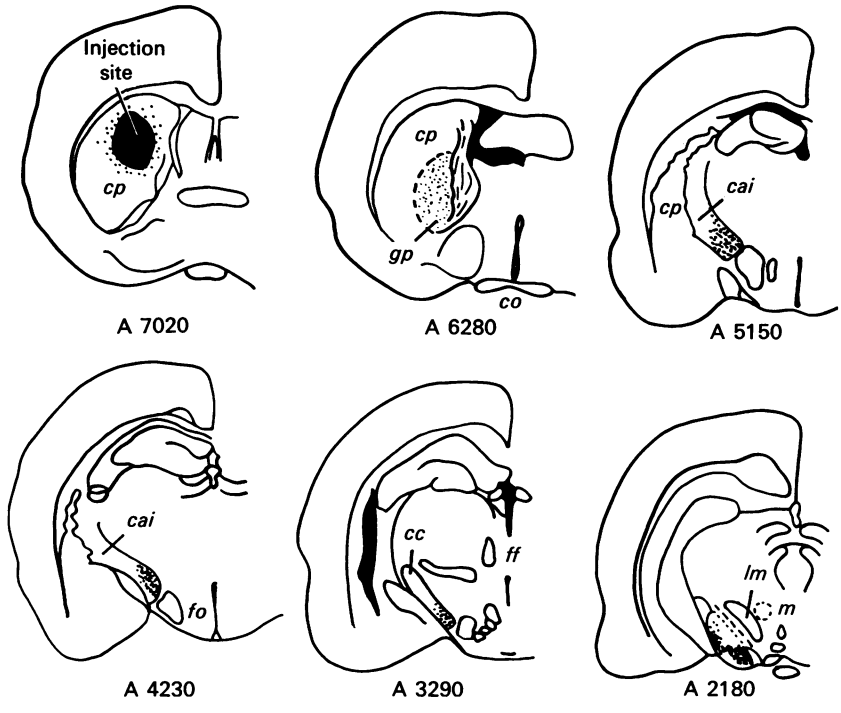


Fig. 2. Diagrammatic representation of the autoradiographic grain distribution in drawings from the rat brain atlas (Konig & Klippel, 1963). The dots represent the summary of the distribution of radioactivity in all four animals with dorsal injection sites as illustrated. The following key applies to all the summary diagrams: *ac*, anterior commissure; *acc*, nucleus accumbens; *cai*, internal capsule; *cc*, crus cerebri; *co*, optic chiasma; *cp*, nucleus caudatus putamen; *dr*, dorsal raphe; *f*, fornix; *fmp*, medial forebrain bundle; *for*, reticular formation; *fr*, fasciculus retroflexus; *gcc*, genu of the corpus callosum; *gp*, nucleus globus pallidus; *hi*, hippocampus; *ip*, interpeduncular nucleus; *lh*, lateral habenula; *lm*, medial lemniscus; *rn*, red nucleus; *snc*, zona compacta of substantia nigra; *snr*, zona reticulata of substantia nigra; *st*, stria terminalis; *sut*, subthalamic nucleus.

The ventral injection sites in the striatum

To avoid spread of label to the globus pallidus this group of animals was injected in the ventrorostral striatum at the level of the anterior commissure, and just rostral to it. All the injections were well confined to this region, with only minimal ventral spread of label out from the striatum. The silver grain distribution following such an injection site is shown in Figure 3.

It proved impossible to distinguish labelled fibres of passage in the globus pallidus from those which might be terminating in this nucleus, thus making plotting of a possible pathway from ventral striatum to globus pallidus difficult. However, it did appear that all the labelled striatonigral fibres arising from the ventral striatum coursed through the ventral region of the globus pallidus before entering the internal capsule laterally. The fibres collected into a distinct bundle in the ventrolateral region of the internal capsule. The course of this pathway within the crus cerebri was slightly lateral to that observed for the fibres from the dorsal injection sites. In this case the distribution of the radioactivity within substantia nigra was topographically distinct from that observed after injection of leucine into the dorsal area of striatum. The label was confined to a lateral position in the anterior zona

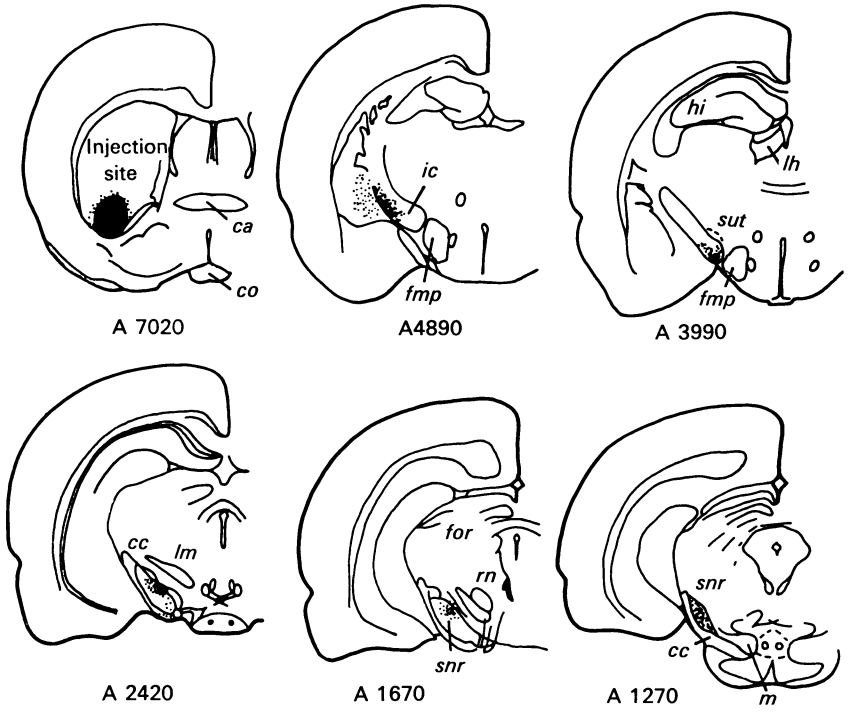


Fig. 3. Diagrammatic representations of the autoradiographic grain distribution in the brains of animals with 'ventral injection sites'.

reticulata, with a small but detectable spread into the lateral parts of the zona compacta. The entire zona reticulata was labelled in planes of section caudal to the exit of the fibres of the third cranial nerve. The activity was limited to SN, and again no label was found caudal to this structure.

The injections in the tail of the striatum

These injections were more variable than the others, and only in two of the seven experimental rats was the label well localized over the tail of the striatum. In four of the animals some of the leucine had clearly entered the striatal cells in the region of the injection site, and the distribution of the grains over the SN was similar in these four animals. In this series there were no animals in which the injection site did not encroach to some extent on the lateral region of the posterior globus pallidus, and so the grain distribution observed may have been contaminated by uptake and transport within the pallidonigral pathway. Figure 4 summarizes the distribution of radioactivity in these cases.

The entry of the labelled fibres into the internal capsule in these animals was very similar to that seen after the ventral injections, but the pathway was localized more dorsolaterally in the peduncle as it descended. The fibres left the crus cerebri by traversing it ventrodorsally. The major innervation area was clearly the lateral edge of the zona reticulata region and the pars lateralis of the substantia nigra. In all the brains there were also some grains over the cells in the lateral zona compacta.

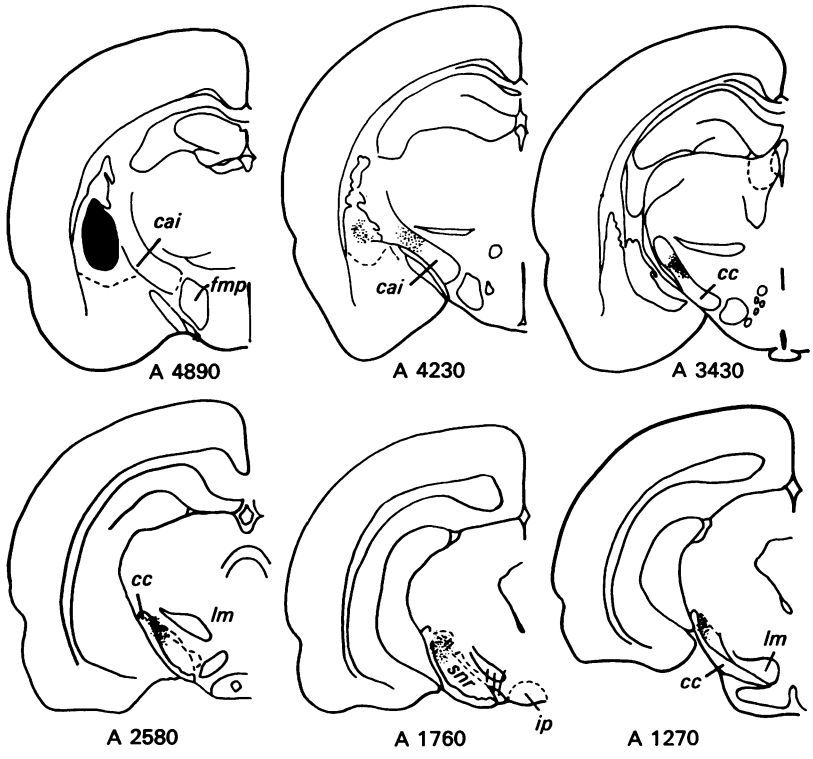


Fig. 4. Diagrammatic summary of the autoradiographic grain distribution in the two most successful injections into the tail of the striatum. In this case the pathway remains lateral in the internal capsule, and the terminal distribution is mainly to the lateral SN.

The studies with horseradish peroxidase

Ventral SN injections

In two of the animals studied the extent of HRP labelling at the injection site was virtually identical. The needle tracks passed ventrally through the zona reticulata of the substantia nigra and the reaction product was visible within all the anterior portion of the nucleus back to the level of the exit of the oculomotor nerve. The distribution of HRP-labelled cells observed following such an injection site is shown in Figure 5.

In these preparations a spur of HRP reaction product extended into the subthalamic nucleus, which under high magnification (Fig. 6A) was observed to be present in both fibres and cells. These HRP-labelled cells had the typical appearance of retrogradely labelled cells.

The majority of HRP-labelled cells found in these brains in planes of section rostral to the SN were found in the striatum (Fig. 6B), mainly in the sections rostral to the optic chiasma. Only a few cells were present in the tail of the striatum, and only a very few cells in the anterior and dorsal portion of the head. The globus pallidus contained many stained cells, also located mainly in the anterior portions of the nucleus.

In these brains a few HRP-labelled cells were present in the cerebral cortex, and a clear accumulation of HRP reaction product was also found in cells in the dorsal raphe nucleus.

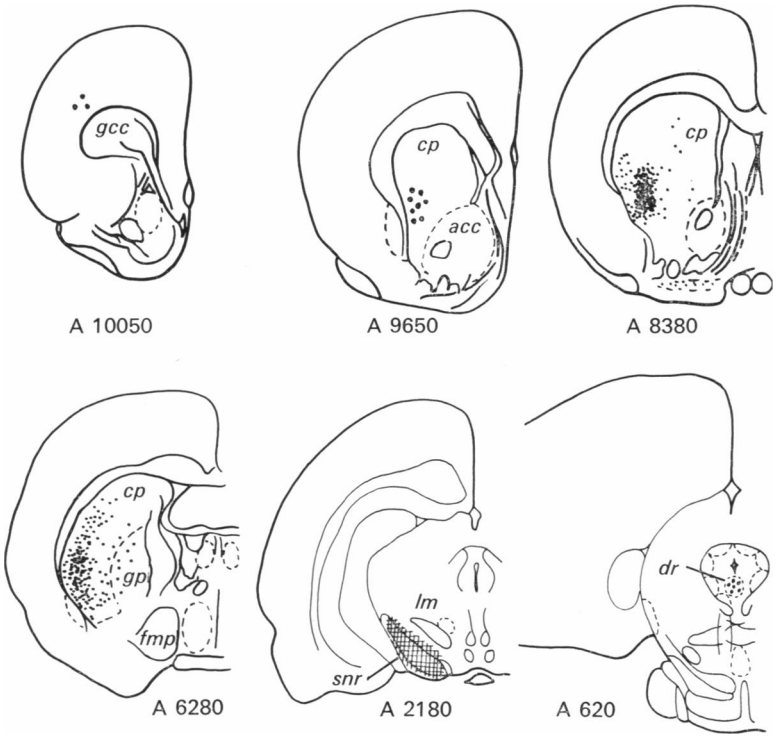


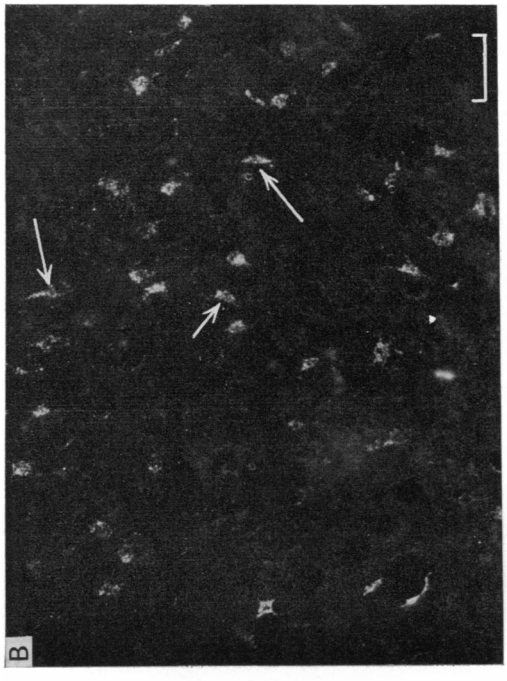
Fig. 5. The diagrams are a summary of the distribution of HRP in one of the brains injected in the anterior zona reticulata (marked by the cross-hatching). In the other drawings each dot represents a cell. The other animal in this group had more cells in the cortex.

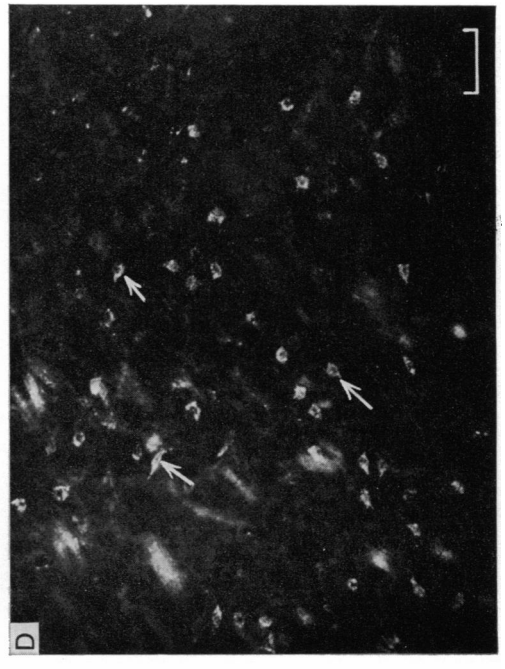
There was only one animal with an injection site in the posterior region of the zona reticulata, and which was therefore comparable to the sites employed by Kanazawa *et al.* (1976). In this animal there was no doubt about the presence of labelled cells in the subthalamic nucleus since there was much less non-specific staining in this region, although there were still HRP-stained fibres coursing through the nucleus. In this animal an even more impressive collection of the cells in the globus pallidus was stained. Stained cells were distributed (Fig. 7) throughout the striatum with the majority being present in planes of section caudal to the level of the optic chiasma.

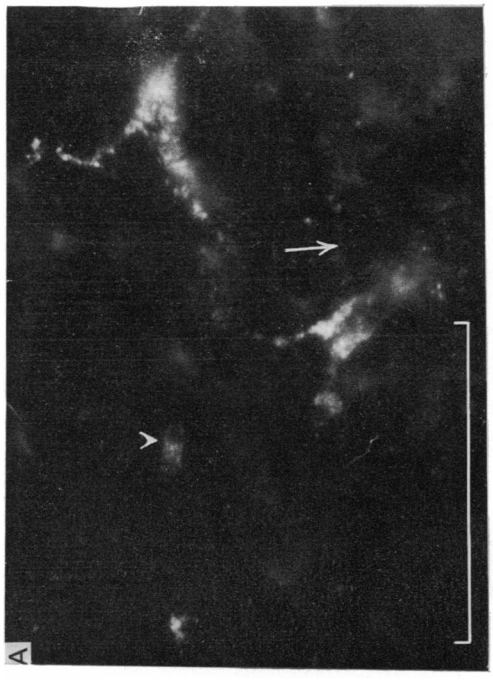
Dorsal SN injections

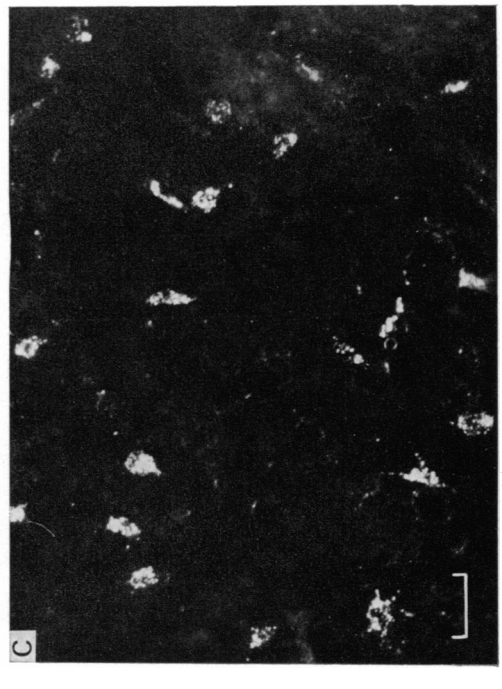
In four animals the injection sites were located in the dorsal substantia nigra. The needle track in these animals followed the ventral edge of the medial lemniscus, with the result that the zona compacta region was densely stained along its length and only a slight staining was seen overlying the zona reticulata and medial lemniscus.

In these animals there were very few stained cells anterior to the optic chiasma (Fig. 8), although a very heavy labelling occurred in the tail of the striatum, especially in the most ventral parts of the nucleus (Fig. 6D). The globus pallidus contained stained cells in its most caudal aspect, and in more anterior planes stained cells were found in the nucleus of the stria terminalis.


B


D


A


C

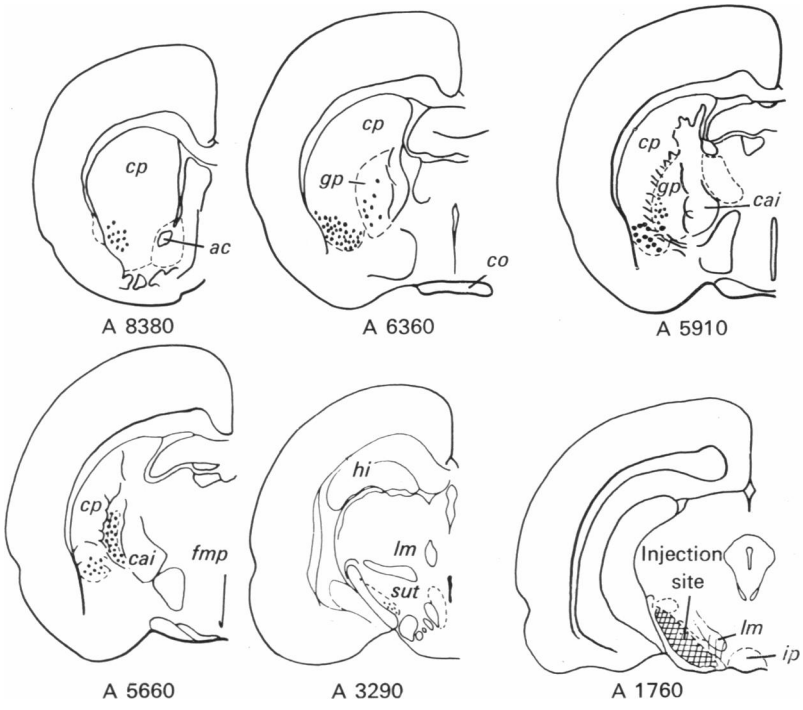


Fig. 7. When the injection site is more posterior in zona reticulata the cell distribution in the basal ganglia is also more posterior, as can be seen in this diagram.

Since all these animals had injection sites in the anterior SN it was very difficult to confirm the presence of specifically stained cells in the subthalamic nucleus because of the relatively large accumulation of HRP in this structure. No HRP-labelled cells were found in the cortices of these animals, but stained cells were found in the dorsal raphe nucleus in each animal.

In two animals injections of HRP were made in the ventral tegmental area of Tsai (A 10) dorsolateral to the interpeduncular nucleus. The dopamine (DA) cells in this region appear to form a medial extension of the group in the SN. In contrast to the SN injections, no striatal cells were labelled in these two brains, although HRP-positive cells were present in the hypothalamus and dorsal raphe as previously described following injections in the dorsal SN. Labelled cells were found in the habenular nuclei of these brains.

Fig. 6. Photomicrographs of sections from the brains of rats previously injected with HRP. Dark-field illumination is used throughout and the calibration bars are 100 μm. (A) Two large cell bodies dominate this picture taken from the subthalamic nucleus of the brain from which Fig. 5 was drawn. The small labelled structures ($\downarrow$) and varicose fibres ($\downarrow$), which also contain reaction product, are typical of this area. (B) A cluster of cells (some of them marked $\downarrow$) in the anterior part of the head of the striatum in tissue from which Fig. 7 (A 6360) is drawn. The lighter grey shading in the background marks the fibre bundles which are a feature of the rat striatum. (C) The larger cells and greater amount of white matter shown in this picture are typical for the globus pallidus in all the animals injected with HRP in SN. (D) The area of the tail of the striatum abutting on the internal capsule shown here contains many cells (some marked $\downarrow$) in the brain used to draw Fig. 8 (A 5340). The bundles of white matter to the top left of the photograph are the lateral and ventral borders of the internal capsule.

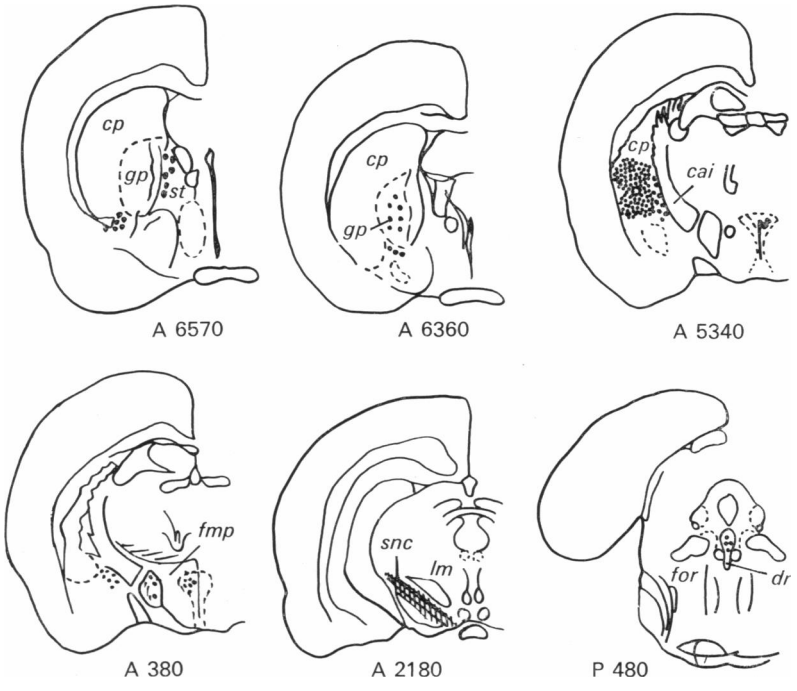


Fig. 8. The diagram shows that the injection of HRP into the dorsal edge of the substantia nigra led to intense labelling in the tail of the striatum, with very few cells in dorsal and anterior areas of the nucleus.

DISCUSSION

Recent interest in the neurochemical topography of the corpus striatum has revealed a differential distribution of the neurotransmitter substances within it (Bockaert *et al.* 1976; Koslow, Racagni & Costa, 1974). Dopamine is distributed predominantly in the head of the striatum, while the concentration of 5-hydroxytryptamine (serotonin or 5HT) is high in the tail region (Tassin *et al.* 1976; Ternaux *et al.* 1977). A 'substance P'-containing pathway originates in the head, while a γ-amino butyric acid (GABA)-containing one may be localized to the posterior part (Hong *et al.* 1977). In the cat and the monkey it has been possible to show that the nigrostriatal system is also topographically arranged, with specific regions of the substantia nigra projecting to localized areas in the striatum (Bedard *et al.* 1969; Carpenter & Peter, 1972). A similar organization is reported for the rat, as determined by fluorescence microscopy (Lindvall & Bjorklund, 1974; Ungerstedt, 1971). Thus different regions of the SN dopamine system would appear to project to regions of the corpus striatum which predominantly contain different transmitter substances. In this respect it is also interesting that our results show that the output pathways from the striatum, projecting to the ipsilateral SN, are also topographically arranged. These results agree well with the earlier anatomical literature on the striatonigral projections, although this in general is concerned with larger animals. For instance, the course and projection of fibres from the tail of the striatum, which is one of the objectives of the present study, correspond closely with the description of the output of the same area in the monkey (Szabo, 1970).

Fonnum *et al.* (1974) studied the distribution of glutamic acid decarboxylase (GAD) in the substantia nigra of the cat and were able to show a topographical

loss of enzyme after small lesions in different regions of the striatum. There is considerable evidence that this pathway from striatum to SN is γ-amino butyric acid (GABA)-containing. Hemisections of the brain at the level of the subthalamic nucleus lead to a loss of GAD and GABA from the substantia nigra (Hattori *et al.* 1973). Stimulation in the striatum results in an evoked response and an inhibition of cell firing in the substantia nigra, which are both blocked by drugs thought to inhibit GABA receptors (Dray, Gonye & Oakley, 1976; McNair, Sutin & Tsubokawa, 1972; Precht & Yoshida, 1971). The inhibitory effect of iontophoretic GABA on the nigral cells is blocked by similar doses of inhibitors (Dray *et al.* 1976). Our own studies with small lesions aimed for the fibres of the striatonigral pathway in the crus cerebri caused a loss of up to 60% of the GABA in the ipsilateral SN (Garcia-Munoz *et al.* 1977; Wright *et al.* 1977).

However, there have been several recent reports that there is also a 'substance P'-containing pathway which has a distribution similar to that outlined in our results (Hong *et al.* 1977; Kanazawa, Emson & Cuello, 1977; Nilsson, Hokfelt & Pernow, 1974). It is not clear how separate these two pathways are in functional terms, but there is evidence that GABA influences 'substance P' release (Jessell, 1977). As far as is known, the autoradiographic technique which we have used would be expected to delineate both projection systems.

In a recent paper, Hattori *et al.* (1973) reported, from electron microscope studies, that the striatonigral pathway in the rat projected mainly to the zona reticulata region. The relative grain density was five times greater for boutons synapsing with dendrites of zona reticulata cells than for those synapsing with dopamine-containing dendrites. However, these results were obtained from leucine injection sites that were largely confined to the dorsal region of the striatum. From the present results it does appear that the ventral and posterior regions may have a more pronounced projection to the zona compacta region, but it cannot be established in our material whether or not these fibres actually synapse with DA-containing neurons.

Hattori *et al.* (1975) have clearly shown a pathway from globus pallidus to substantia nigra which follows an almost identical route to the one seen in our material, and which projects predominantly to the dorsal part of the substantia nigra, and synapses mainly with DA cell dendrites. Since there was some involvement of the pallidum in all our injections into the tail of the striatum, it is not clear whether there was an involvement of the pallidal projection to the zona compacta, such as has been described previously (Hattori *et al.* 1975), or whether there was a greater innervation of the zona compacta region from the tail of the striatum than from the head. The HRP study demonstrated that there was a heavy projection from the tail of the striatum to the region of the SN containing the DA cell bodies. The globus pallidus was found to contain HRP-labelled cells following injections into either the dorsal or the ventral SN. There is evidence that HRP uptake is localized to the immediate vicinity of the injection site (La Vail, 1975) and thus may be greatest in fibres damaged by the needle track. Since the pallidonigral fibres must course through the zona reticulata to reach the zona compacta, it may be that the pallidonigral system takes up HRP from wherever we make the injection in the nigra.

The autoradiographic results, apart from showing a general topographical distribution of striatal efferents within the SN, also demonstrate the topographical distribution of the fibres of the striatonigral pathway coursing caudally in the crus cerebri.

The results from the retrograde labelling studies with HRP are in agreement with the findings obtained from the autoradiographic study, although they do not provide as fine anatomical detail. The recent elegant study by Bunney & Aghajanian (1976*b*), using iontophoretic application of horseradish peroxidase in the SN, achieved a similar definition of the topographical projections to that obtained with the autoradiographic technique. HRP injections in the region of zona compacta of the substantia nigra consistently labelled the most ventral cells in the tail of the striatum, but in contrast to Bunney & Aghajanian's (1976*b*) study, no HRP-labelled cells were found in the central nucleus of the amygdala. On the other hand, we also observed staining in neurons in the subthalamic nucleus, as reported by Kanazawa *et al.* (1976), although such labelling was not reported in the Bunney & Aghajanian (1976*b*) study. It may be that our more extensive injection sites have led to a greater uptake, or an uptake into damaged axons, and that the presence of staining in these cells is only seen after larger injections. In agreement with the previous studies (Bunney & Aghajanian, 1976*b*; Kanazawa *et al.* 1976), the presence of HRP-labelled cells was confirmed in the dorsal raphe nucleus following both dorsal and ventral injections in the SN.

The horseradish peroxidase experiments permitted the visualization of the morphological characteristics of the striatal efferent cells projecting to the SN. In agreement with the earlier studies using similar techniques the cells in the striatum which accumulate HRP are relatively small (Bunney & Aghajanian, 1976*b*; Grofova, 1975; Kanazawa *et al.* 1976). They presumably correspond to output cells described by Di Figlia, Pasik & Pasik (1976) in the Golgi study of the striatum. Preliminary estimates of the number of cells stained with HRP in the most densely labelled areas, and a comparison with the number of cells in a similar area stained with cresyl violet, shows that approximately 30 % of the total number of cells were labelled with HRP. Since the estimates had to be made in different animals (because of our mounting technique) they can only be regarded as giving a rough guide to the proportion of the cells in a given area of the striatum which project to the substantia nigra. Even allowing for the inevitable inaccuracy of our counting method, we cannot agree with the earlier suggestion that only very few cells in the striatum have axons which leave the striatum (Kemp & Powell, 1971). Nor is it true that only the largest cells are output cells (Kemp & Powell, 1971), because, as is obvious from Figure 6, the cells which stain for HRP are in the size range 15–20 μm.

Since we did not find any labelled cells in the striatum after HRP injections in A 10, and since leucine injections in the striatum do not lead to labelling of terminals in A 10, it seems likely that there is no striatal input to A 10. On the other hand, care is needed in the interpretation of negative results with the HRP technique, since the well-documented pathway from cortex to striatum is not labelled after HRP injections in the striatum (Nauta *et al.* 1974).

As for a functional significance of the topographical organization of the striato-nigral projection, it is unlikely that the pathway is part of the feedback control system which modulates striatal DA turnover (Bunney & Aghajanian, 1976*a*) since we have recently shown that lesions of at least the major part of the projection have no effect either on normal striatal DA turnover or on the response of the nigro-striatal DA system to neuroleptics or apomorphine (Garcia-Munoz *et al.* 1977; Wright *et al.* 1977). Although it is still possible that the projection from the tail of the striatum which seems to project preferentially to the DA cells in SN may be the feedback pathway, and function independently of the major part of the striato-

nigral system, it seems more likely that the projection which we have mapped here is part of an output system from the striatum via the zona reticulata to the thalamic and reticular parts of the motor system (Carpenter & Peter, 1972; Deniau, Feger & Le Guyader, 1976; Faull & Carman, 1968; Rinvik, 1975; Rinvik, Grofova & Ottersen, 1976). It is within this framework that we expect to be able to understand how the topography of the system is related to its function.

SUMMARY

L-[4,5-^{3}H]leucine was injected stereotaxically into various regions of the rat neostriatum. Light microscopic autoradiographic techniques were used to plot the entire efferent pathways of the neostriatum, and in particular, the projections to the substantia nigra (SN).

The terminal distribution of the pathways projecting to the ipsilateral SN was predominantly restricted to the zona reticulata region. The dorsal part of the head of the striatum was found to innervate the anterior and medial portions of the zona reticulata, while the ventral area of the head projected to more posterior regions of the SN. A pathway from the tail of the striatum to the lateral and dorsal parts of SN was also demonstrated. Horseradish peroxidase injections, restricted to different areas of the SN, led to retrograde labelling of neurons in the striatum whose distribution confirmed the topographic organization of the striatonigral pathway demonstrated in the autoradiographic studies.

REFERENCES

Bedard, P., Larochelle, L., Parent, A. & Poirer, L. J. (1969). The nigrostriatal pathway: A correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey. *Experimental Neurology* **25**, 365–377.

Bockaert, J., Premont, J., Glowinski, J., Thierry, A. M. & Tassin, J. P. (1976). Topographic distribution of dopaminergic innervation and of dopaminergic receptors in rat striatum. II. Distribution and characteristics of dopamine adenylate cyclase: interaction of D-LSD with dopamine receptors. *Brain Research* **107**, 303–316.

Bunney, B. S. & Aghajanian, G. K. (1976*a*). d-Amphetamine-induced inhibition of central dopaminergic neurons: Mediation by a striato-nigral feedback pathway. *Science* **192**, 391–393.

Bunney, B. S. & Aghajanian, G. K. (1976*b*). The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. *Brain Research* **117**, 423–435.

Carpenter, M. B. & Peter, P. (1972). Nigrostriatal and nigrothalamic fibres in the rhesus monkey. *Journal of Comparative Neurology* **144**, 93–116.

Cowan, W. M., Gottlieb, D. I., Hendrickson, A. E., Price, J. L. & Woolsey, T. A. (1972). The autoradiographic demonstration of axonal connections in the central nervous system. *Brain Research* **37**, 21–51.

Deniau, J. H., Feger, J. & Le Guyader, C. (1976). Striatal evoked inhibition of identified nigrothalamic neurons. *Brain Research* **104**, 152–156.

Di Figlia, M., Pak, P.si & Pasik, T. (1976). A Golgi study of neuronal types in the neostriatum of monkeys. *Brain Research* **114**, 245–256.

Dray, A., Gonye, T. J. & Oakley, N. (1976). Caudate stimulation and substantia nigra activity in the rat. *Journal of Physiology* **259**, 825–849.

Edinger, L. (1911). *Vorlesungen über den Bau der nervozen Zentralorgane*, vol. 1, 8th ed. Leipzig: Vogel.

Faull, R. L. & Carman, J. B. (1968). Ascending projections of the substantia nigra in the rat. *Journal of Comparative Neurology* **132**, 73–92.

Fonnum, F., Grofova, I., Rinvik, E., Storm-Mathisen, J. & Walberg, F. (1974). Origin and distribution of glutamate decarboxylase in substantia nigra of the cat. *Brain Research* **71**, 77–92.

Garcia-Munoz, M., Nicolaou, N. M., Tulloch, I. F., Wright, A. K. & Arbuthnott, G. W. (1977). Striato-nigral fibres – feedback loop or output pathway. *Nature* **265**, 363–365.

Grofova, I. & Rinvik, E. (1970). An experimental electron microscopical study on the striatonigral projection in the cat. *Experimental Brain Research* **11**, 249–262.

GROFOVA, I. (1975). The identification of striatal and pallidal neurons projecting to substantia nigra. An experimental study by means of retrograde axonal transport of horseradish peroxidase. *Brain Research* **91**, 286–291.

HATTORI, T., FIBIGER, H. C. & MCGEER, P. L. (1975). Demonstration of a pallido-nigral projection innervating dopaminergic neurons. *Journal of Comparative Neurology* **162**, 487–504.

HATTORI, T., MCGEER, D. L., FIBIGER, H. C. & MCGEER, E. G. (1973). On the source of GABA-containing terminals in the substantia nigra. Electron microscopic, autoradiographic and biochemical studies. *Brain Research* **54**, 103-114.

HONG, J. S., YANG, H.-Y, T., RACAGNI, G. & COSTA, E. (1977). Projections of substance P containing neurons from neostriatum to substantia nigra. *Brain Research* **122**, 541–544.

JESSELL, T. M. (1977). Inhibition of substance P release from the isolated rat substantia nigra by GABA. *British Journal of Pharmacology* **59**, 486P.

KANAZAWA, I., MARSHALL, G. R. & KELLY, J. S. (1976). Afferents to the rat substantia nigra studied with horseradish peroxidase, with special reference to fibres from the subthalamic nucleus. *Brain Research* **115**, 484–491.

KANAZAWA, I., EMSON, P. & CUELLO, A. C. (1977). Evidence for the existence of substance P containing fibres in striato-nigral and pallido-nigral pathways in rat brain. *Brain Research* **119**, 447–453.

KEMP, J. M. & POWELL, T. P. S. (1971). The structure of the caudate nucleus of the cat: light and electron microscopy. *Philosophical Transactions of the Royal Society* B **262**, 383–401.

KNOOK, H. L. (1965). *The Fibre Connections of the Forebrain.* Assen: Royal Van Gorcum.

KONIG, J. F. R. & KLIPPEL, R. A. (1963). *The Rat Brain.* Baltimore: Williams and Wilkins Co.

KOSLOW, S. H., RACAGNI, G. & COSTA, E. (1974). Mass fragmentographic measurement of norepinephrine, dopamine, serotonin and acetylcholine in seven discrete nuclei of the rat tel-diencephalon. *Neuropharmacology* **13**, 1123–1130.

LA VAIL, J. H. (1975). Retrograde cell degeneration and retrograde transport techniques. In *The Use of Axonal Transport for Studies of Neuronal Connectivity*, (ed. W. M. Cowan & M. Cuenod), pp. 219–248. Amsterdam: Elsevier.

LINDVALL, O. & BJORKLUND, A. (1974). The organisation of the ascending catecholamine neuron systems in the rat brain: as revealed by the glyoxylic acid fluorescence method. *Acta physiologica scandinavica Suppl.* **412**, 1–48.

MCNAIR, J. L., SUTIN, J. & TSUBOKAWA, T. (1972). Suppression of cell firing in the substantia nigra by caudate nucleus stimulation. *Experimental Neurology* **37**, 395–411.

NAUTA, W. J. H. & MEHLER, W. R. (1966). Projections of the lentiform nucleus in the monkey. *Brain Research* **1**, 3–42.

NAUTA, W. J. H., PRITZ, M. B. & LASEK, R. J. (1974). Afferents to the rat striatum studied with horseradish peroxidase. An evaluation of a retrograde neuro-anatomical research method. *Brain Research* **67**, 219–238.

NIIMI, K., IKEDA, T., KAWAMURA, S. & INOSHITA, H. (1970). Efferent projections of the head of the caudate nucleus in the cat. *Brain Research* **21**, 327–343.

NILSSON, G., HOKFELT, T. & PERNOW, B. (1974). Distribution of substance P-like immunoreactivity in the rat central nervous system as revealed by immunohistochemistry. *Medical Biology* **52**, 424–427.

PRECHT, W. & YOSHIDA, M. (1971). Blockage of caudate-evoked inhibition of neurons in the substantia nigra by picrotoxin. *Brain Research* **32**, 229–233.

RINVIK, E. (1975). Demonstration of nigrothalamic connections in the cat by retrograde axonal transport of horseradish peroxidase. *Brain Research* **90**, 313–318.

RINVIK, E., GROFOVA, I. & OTTERSEN, O. P. (1976). Demonstration of nigrotectal and nigroreticular projections in the cat by axonal transport of proteins. *Brain Research* **112**, 388–394.

ROSEGAY, H. (1944). An experimental investigation of the connections between the corpus striatum and the substantia nigra in the cat. *Journal of Comparative Neurology* **80**, 293–310.

SZABO, J. (1962). Topical distribution of the striatal efferents in the monkey. *Experimental Neurology* **5**, 21–36.

SZABO, J. (1967). The efferent projections of the putamen in the monkey. *Experimental Neurology* **19**, 463–476.

SZABO, J. (1970). Projections from the body of the caudate nucleus in the rhesus monkey. *Experimental Neurology* **27**, 1–15.

SZABO, J. (1972). The course and distribution of efferents from the tail of the caudate nucleus in the monkey. *Experimental Neurology* **37**, 562–572.

TASSIN, J. P., CHERAMY, A., BLANC, G., THIERRY, A. M. & GLOWINSKI, J. (1976). Topographic distribution of dopaminergic innervation and of dopaminergic receptors in the rat striatum. I. Micro estimation of (H3) dopamine uptake and dopamine content in microdiscs. *Brain Research* **107**, 291–302.

TERNAUX, J. P., HERY, F., BOUGOIN, S., ADRIEN, J., GLOWINSKI, J. & HAMON, J. (1977). The topographical distribution of serotonergic terminals in the neostriatum of the rat and the caudate nucleus of the cat. *Brain Research* **121**, 311–326.

UNGERSTEDT, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta physiologica scandinavica Suppl.* **367**, 1–48.

VONEIDA, T. J. (1960). An experimental study of the course and destination of fibres, arising in the head of the caudate nucleus in the cat and monkey. *Journal of Comparative Neurology* **115**, 75–87.

WRIGHT, A. K., ARBUTHNOTT, G. W., TULLOCH, I. F., GARCIA-MUNOZ, M. & NICOLAOU, N. M. (1977). Are the striatonigral fibres the feedback pathway? In *Psychobiology of the Striatum* (ed. A. R. Cools, A. H. M. Lohman & J. H. L. van der Berken), pp. 31–50. Amsterdam: North-Holland.