

ANATOMICAL AND HISTOCHEMICAL STUDIES OF THE GLOBUS PALLIDUS  
AND RELATED BASAL GANGLIA NUCLEI

by

William Alan Staines

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Department of Psychiatry, Division of Neurological Sciences.

The University of British Columbia  
1956 Main Mall  
Vancouver, Canada  
V6T 1Y3

Date August 3, 1983

## ABSTRACT

The anatomical organization of the connections of the major components of the basal ganglia was investigated in detail. A sensitive procedure for the simultaneous study of afferents and efferents was carried out on the striatum (CP), globus pallidus (GP), and substantia nigra (SN). Previously well characterized connections of the CP were confirmed, additional evidence for a projection to the CP from the ventromedial nucleus of the thalamus was obtained and a topographically organized projection to the CP from the GP was discovered. A similar study of the SN revealed a nigral projection to the ipsilateral lateral dorsal nucleus of the thalamus and nigral input from the contralateral posterior lateral hypothalamus. The projection of the GP to the SN was found to be linked topographically to the striatonigral and pallidostriatal pathways. A study of the connections of the GP confirmed a massive projection from the CP and provided further evidence of a reciprocal connection. In addition, pallidal innervations of the entopeduncular nucleus and reticular nucleus of the thalamus were indicated.

Because of the potential importance of a pallidostriatal projection and the significant number of technical difficulties associated with its demonstration, additional experiments were carried out to confirm the presence of this pathway and to determine its anatomical relationship to other basal ganglia connections. Retrograde labelling of pallidostriatal neurons,

studied with electron microscopy and in combination with lesions of the striatum, confirmed that pallidal neurons project either to or through the striatum. Evidence for possibly two groups of pallidal neurons that project to the CP was obtained, and it was observed that both of these cell groups were congruent with the striatopallidal terminal fields. Comparisons of the distribution of cells retrogradely labelled after tracer injections into the cortex and CP in combination with histochemistry for acetylcholinesterase demonstrated that the population of pallidal neurons projecting to the CP was distinct from that of peripallidal cholinergic neurons which may project through the striatum to the cortex. Double retrograde fluorescent tracing experiments indicated that pallidal neurons which project to the CP also have collateral projections to the substantia nigra and perhaps to the subthalamic nucleus. The application of a new technique for studying efferent projections allowed the confirmation and morphological description of the projection of the globus pallidus to the striatum. The characteristic morphology of this projection was shared by pallidal efferents which project to the entopeduncular nucleus, the reticular nucleus of the thalamus, the subthalamic nucleus and the substantia nigra. The fine morphological detail afforded by this method of anterograde tracing was utilized in combination with a histochemical protocol to show that pallidostriatal terminals end in part on somatostatin-containing neurons in the CP.



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## LIST OF ABBREVIATIONS USED IN FIGURES

ab,	basolateral nucleus of the amygdala
ac,	nucleus accumbens
AC,	anterior commissure
BC,	brachium conjunctivum
ce,	central nucleus of the amygdala
cem,	centromedial nucleus of the thalamus
cg,	central gray area
cl,	centrolateral nucleus of the thalamus
cp,	striatum (caudate-putamen)
CP,	cerebral peduncle
ctx,	cortex
dmt,	dorsal midbrain tegmentum
dr,	dorsal raphe nucleus
ep,	entopeduncular nucleus
F,	fornix
FM,	forceps minor
FR,	fasciculus retroflexus
g,	nucleus gelatinosus
gp,	globus pallidus
h,	habenular complex
ic,	inferior colliculus
IC,	internal capsule
ICB,	bundles of internal capsule fibers

ip, interpeduncular nucleus  
ld, laterodorsal nucleus of the thalamus  
lh, lateral habenula  
lhp, posterior lateral hypothalamus  
LV, lateral ventricle  
md, mediodorsal nucleus of the thalamus  
MFB, medial forebrain bundle  
ML, medial lemniscus  
MT, mammillothalamic tract  
na, nucleus accumbens  
nsp, non-specific thalamic afferents  
ntp, nucleus tegmenti pedunculopontis  
pb, peribrachial region  
pc, paracentral nucleus of the thalamus  
pf, parafascicular nucleus  
pfc, prefrontal cortex  
ppn, nucleus tegmenti pedunculopontis  
r, red nucleus  
rr, retrorubral area  
rt, reticular nucleus of the thalamus  
sc, superior colliculus  
snc, substantia nigra pars compacta  
snr, substantia nigra pars reticulata  
sp, specific thalamic afferents  
sum, supramammillary nucleus

sut, subthalamic nucleus  
vl, ventrolateral nucleus of the thalamus  
vm, ventromedial nucleus of the thalamus  
vp, ventral pallidum  
vta, ventral tegmental area of Tsai  
zi, zona incerta

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## GENERAL INTRODUCTION

The two main goals of the study of the neurosciences are to understand the biological principles and mechanisms underlying normal brain function and to gain an understanding of brain pathology that can be directed toward the more immediate needs of the clinical neurosciences. These two ambitions are well met in the study of the basal ganglia. In recent years, much has been learned about the chemistry and physiology of this system and research into the many diseases involving the basal ganglia has benefitted not only the patient but also the researcher.

The basal ganglion is not a circumscribed brain area, but rather a system of identifiable brain regions closely linked by anatomical and functional considerations. The most readily apparent function carried out by this system is the control of motor behavior of the type that has come to be termed "extrapyramidal" (DeLong and Georgopoulos, 1981). In essence, these are the coarse background movements upon which more conscious fine motor behavior is superimposed. Anatomically, the basal ganglia are composed of a number of deep forebrain and bulbar cell clusters including the striatum (formed by the caudate nucleus and putamen in humans), globus pallidus (termed external globus pallidus in humans), entopeduncular nucleus (internal globus pallidus in human), subthalamic nucleus and substantia nigra. Some workers include the nucleus accumbens and olfactory tubercle as well, as anatomical considerations have led them to be considered ventral extensions of the striatum (DeLong and Georgopoulos, 1981).

Historically, the identification of this system with motor behavior was inferred from the observation of motor disturbances arising from pathological states involving components of the basal ganglia. These include Huntington's chorea, Parkinson's disease, Wilson's disease and hemiballismus (see DeLong and Georgopoulos, 1981). Experimental lesion studies in animals have approximated many of these conditions, allowing corroboration of the anatomical and biochemical presentation of the pathology seen in humans. Our appreciation of the anatomy, biochemistry and function of the basal ganglia, therefore, represents a synthesis of clinical and experimental studies.

Neurobiological studies usually focus on a single, spatially defined nucleus rather than on a functionally defined system.

Often such nuclei represent distinct functional units, but modern neuroanatomical techniques have led to a de-emphasis on classical cytoarchitectural boundaries (Nauta, 1979a). The inputs and outputs of this spatially defined nucleus are investigated to determine what other brain structures it is associated with. This information can be obtained by following fiber bundles, the transport of tracer substances in a retrograde or anterograde manner, or from electrophysiological techniques. Once this circuitry has been elucidated, biochemical, immunohistochemical or electropharmacological methodologies may be employed to determine the transmitter(s) used by individual connections (ie. the "biochemical neuroanatomy" of the system). With the data derived by these means the system may be manipulated by the placement of selective lesions or by selective

pharmacological perturbation to determine functional principles. In some instances, these data also contribute significantly to our understanding of neurological disease and suggest therapeutic approaches.

Discussions of the connections of the basal ganglia most often begin with the cortical projection to the caudate nucleus and putamen (CP). The CP receives a divergent input from most cortical areas, both motor and sensory, but the majority of fibers from a given area end in clusters, indicating a modular type of input (Kemp and Powell, 1971b; Oka, 1980; Royce, 1982). Cortical areas linked to one another by association fibers give rise to overlapping terminal fields within the striatum (Yeterian and van Hoesen, 1978; Royce, 1982). Most of the cortical input to the striatum arises from the cortex on the same side, but there is also a significant input from the contralateral cortex. Cortical cells giving rise to this projection are located mainly in layer V and in some cases have been shown to have branched axons that continue caudally to the pyramids after innervating the CP via a collateral (Jinnai and Matsuda, 1979; Donoghue and Kitai, 1981; Royce, 1982). Biochemical and electrophysiological evidence indicates that glutamate is used as a neurotransmitter by at least a portion of the neurons making up this projection (Divac et al., 1977; McGeer et al., 1977; Kocsis, et al., 1977; Spencer, 1976).

In considering the pattern of neuronal activity involved in movement of the extrapyramidal type it is usual to think in terms of a cortical input to the striatum as the first step. However,

it should be kept in mind that many animals have minimal cortical tissue and yet have a well developed striatum. The striatum, therefore, cannot be considered to be a cortically dependant structure but rather may act in concert with the motor regions of the cortex such that fine cortically controlled motor behavior is expressed on a background of striatal motor behavior. In this context the cortical input to the CP may serve more a coordination role than one of initiation.

The CP receives a well characterized input from the pars compacta of the substantia nigra (SNc) which utilizes dopamine as a neurotransmitter (see Dray, 1979). The physiological action of dopamine released in the striatum has been variously characterized as excitatory (Kitai et al., 1976) or inhibitory (Siggins, 1978) and it is possible that its action may depend on the state of the postsynaptic neuron at the time it is addressed. Anatomically, this input has been shown to be very diffuse and to display a patchy distribution which is correlated with the cortical glutamate input and endogenous cholinergic parameters within the CP (Graybiel et al., 1981; Lehmann et al., 1983). On a behavioral level the function of this input is better understood. In Parkinson's disease the loss of DA neurons in the substantia nigra is the main feature and appears to be correlated to the functional aberrations (Marsden, 1982). These data indicate a permissive role of striatal DA activity in the initiation of motor behavior. This impression gains support from microinfusion studies in which direct or indirect DA agonists acting within the striatum increase the level of motor behavior.



In rats, infusion sites in the dorsal striatum elicit orofacial stereotypies and those in the ventral striatum (nucleus accumbens) produce an increase in locomotion (Anden and Johnels, 1977).

Other striatal inputs include those from intralaminar nuclei of the thalamus, a serotonin containing projection from the dorsal raphe nucleus and a noradrenergic innervation originating in the locus coeruleus (see Dray, 1979). Recently, afferents to the striatum from the amygdala, globus pallidus and nondopaminergic neurons within the pars reticulata of the substantia nigra (SNr) have been described (Dafny et al., 1975; Kelley et al., 1982; Staines et al., 1981; van der Kooy et al., 1981b). These last two are of particular interest in that these regions are in receipt of dense termination from striatal neurons and have been suggested to be homologous structures on the basis of cell morphology and development (Nauta, 1979b).

Lesions of the striatum itself are the consistent pathological finding in Huntington's chorea (see Dray, 1979; DeLong and Georgopoulos, 1981). The motor disturbances characteristic of this disorder take the form of a hypermobility with uncontrolled twitches and grimaces. These symptoms respond to treatments which antagonise the permissive influence of the nigrostriatal projection by blockade of DA action. Fiber sparing lesions of the CP of experimental animals mimic the biochemical changes seen in Huntington's chorea (McGeer and McGeer, 1976; Coyle and Schwarcz, 1976).

The efferents of the striatum project to only three

structures; the globus pallidus, entopeduncular nucleus and substantia nigra (see Dray, 1979). Over 65% of striatal neurons have been estimated to project to the substantia nigra alone (Bolam et al., 1981a). As it can be inferred from lesion studies that not all striatal projections are collaterals of those fibers innervating the SN, this figure is undoubtedly an underestimate of the total percentage of cells which are projection neurons (Staines et al., 1980a).

Biochemical data suggest that each of the sites of striatal termination receive both gamma-aminobutyric acid (GABA)- and substance P-containing projections (Staines et al., 1980a) and there are electrophysiological data for dual excitatory and inhibitory inputs (Kanazawa and Yoshida, 1980; Collingridge and Davies, 1982; Mogenson et al., 1983).

In most animals the globus pallidus is immediately adjacent to the CP and is innervated by striatal fibers containing GABA, substance P and enkephalin (Nagy et al., 1978a; Cuello and Paxinos, 1978; Staines et al., 1980a). The globus pallidus also receives a DA input from the SNc (Lindvall and Bjorklund, 1979), a serotonergic projection from the dorsal raphe nucleus (Parent et al., 1981b; Pasik et al., 1981) and a projection from the subthalamic nucleus (Nauta and Cole, 1978; van der Kooy and Hattori, 1980a). The seeming simplicity of the afferents of the GP may be due to the fact that this question has not yet been addressed in a direct manner. The vast majority of efferents from this nucleus project to the subthalamic nucleus (Nauta, 1979a; McBride and Larsen, 1980; Carpenter et al., 1981; van der Kooy et

al., 1981). Attempts to determine the transmitter used by this projection have as yet provided no clear answer (Fonnum et al., 1978; Rouzaire-Dubois et al., 1980; van der Kooy et al., 1981a). The GP may also have projections to the reticular nucleus of the thalamus, substantia nigra, striatum and a minor projection to the mediodorsal nucleus of the thalamus (Carter and Fibiger, 1978; Nauta, 1979a; McBride and Larsen, 1980; Staines et al., 1981).

The globus pallidus is involved in the neuropathology in Wilson's disease which is characterized by tremour, rigidity, hypertonicity and spasmotic contractions (Owen, 1981). However, animal studies in which unilateral or bilateral lesions of the globus pallidus are made reveal, on the whole, no long term disturbance in motor behavior (see DeLong and Georgopoulos, 1981), and it may be that the symptomology of Wilson's disease is related to the degeneration of the putamen which is more prevalent than that seen to occur in the GP. The features of the disease on the other hand correspond better to the picture of nigral degeneration than that normally associated with caudate-putamen damage (Marsden, 1982). The clinical pharmacology sheds little light on this as both the dopamine precursor, l-dihydroxyphenylalanine (L-DOPA), and the dopamine antagonist, haloperidol, have been claimed to improve the symptoms of Wilson's disease - L-DOPA the "extrapyramidal symptoms" (presumably referring to the tremour and rigidity) and haloperidol the involuntary movements (Owen, 1981).

The entopeduncular nucleus receives terminals from the

striatum and the nucleus tegmenti pedunculopontis and projects back to the latter nucleus (van der Kooy and Carter, 1981; Moon-Edley and Graybiel, 1980; Larsen and McBride, 1979). It also innervates the motor divisions of the thalamus, intralaminar cell groups of the thalamus, and the lateral habenular nucleus (Kim et al., 1976; Larsen and McBride, 1979; Nauta, 1979a; DeVito and Anderson, 1982). The EP receives a dense innervation from the subthalamic nucleus (SUT) (Carpenter et al., 1981). Therefore, although the two divisions of the globus pallidus (GP and EP) have relatively minor direct connections (see below), the GP can influence the EP via this relay.

As already mentioned, the SUT receives a massive input from the GP and projects to the GP and EP. Virtually every cell in the SUT appears to project to the EP and each of these seems to also give rise to a collateral innervating the SNr (Deniau et al., 1978a; van der Kooy and Hattori, 1980a). An excitatory input to the SUT arising from both motor and premotor cortices has been observed (Hartmann-von Monakow et al., 1978; Romansky et al., 1979; Kitai and Deniau, 1981).

The substantia nigra receives afferents from each of the other main components of the basal ganglia; the CP, GP and SUT (Gerfen et al., 1982). Aside from the ascending dopaminergic projection arising from the pars compacta, the pars reticulata of the SN projects to motor divisions of the thalamus, the superior colliculus, the midbrain reticular formation and has reciprocal connections with the nucleus tegmenti pedunculopontis. The projections to the tectum and thalamus have been determined to

arise partly as collaterals of the same neurons (Bentivoglio et al., 1979) and to use GABA as a neurotransmitter (Vincent et al., 1978a; DiChiara et al., 1979a).

Much more insight into the function of a nucleus may be obtained by the examination of its outputs than its inputs. The clearest indication of the function of the striatum has emerged from the study of the electrophysiological response characteristics of the EP and SNr (see DeLong and Georgopoulos, 1981). The majority of neurons in these loci show a correlation between firing rate and muscular activity. Of the more interesting findings arising from these correlative studies are the observations that cell firing within the internal globus pallidus of the monkey (ie. EP) does not always precede the onset of movement, indicating that the basal ganglia are not necessarily involved in the initiation of movement, and that correlations were obtained between neuronal activity and fine, distal movements in addition to more gross, slow movements. These data are at odds with more classical impressions of basal ganglia function arising from clinical and lesion studies.

Similar attempts to correlate activity of neurons within the external segment of the globus pallidus (ie. GP) with motor activity have yielded little or no positive data. The same is true of decades of study on the effects of lesions of the GP. Perhaps as a result of this and the observation that the GP appears only to interconnect other basal ganglia components, this nucleus has not been subjected to the extensive research that other parts of the basal ganglia have received. The present

studies attempt to arrive at a more precise understanding of the anatomy of the basal ganglia, with a mind to biochemical heterogeneity underlying this anatomy, and will emphasize the anatomy of the external globus pallidus.

## STATEMENT OF THE PROBLEMS EXAMINED

The groundwork was laid for a more detailed study of the anatomical circuitry of the basal ganglia nuclei by a reexamination of the connections of the striatum, globus pallidus and substantia nigra. The understanding of the anatomy of many brain systems has been expanding due to the increased sensitivity of recently developed techniques. The use of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) as a neuroanatomical tracer allows for small injections to be made and both efferents and afferents of the injected area to be visualized with great sensitivity. These advantages were employed to study the inputs and outputs of the three main components of the basal ganglia.

Findings from Experiments 1 and 3, suggesting a pallidostriatal projection, were examined in greater detail in Experiment 4, to control for the possibility of axosomatic or transynaptic artifacts in the observation of the apparent retrograde labelling of pallidal neurons. The topography of this projection was studied with injections of WGA-HRP into the CP, in some instances in conjunction with the perikaryal neurotoxin, kainic acid, to block the anterograde labelling of the GP. The ultrastructural localization of the peroxidase activity within pallidal neurons labelled by striatal tracer injections was also examined.

In Experiment 5, further control studies addressed the possibility that the apparent labelling of a pallidostriatal projection was in fact due to labelling of peripallidal neurons

projecting through the striatum to the cortex. A comparison was made of the distribution of neurons projecting to the striatum and cortex in conjunction with histochemistry for acetylcholinesterase under conditions which differentiated neurons with cortical projections from the typical pallidal neurons.

Topographical data from Experiments 1 and 3 had suggested a possible collateralization of pallidal projections to the CP and SN. This possibility was evaluated by double retrograde fluorescent transport studies in Experiment 6.

In Experiment 7 an attempt was made to provide further evidence for a pallidostriatal projection by the application of an anterograde transport technique capable of demonstrating axon terminals at the light microscopic level. This was achieved by the injection of a novel lectin into the globus pallidus and then visualizing its transport using immunohistochemistry.

Finally, Experiment 8 was conducted to determine if synaptic contact could be observed between terminals of the pallidostriatal projection and chemically identified cell types within the striatum. The anterograde tracing technique used in Experiment 7 was combined with a histochemical procedure for NADPH-dependent diaphorase activity, a reliable marker for somatostatin-containing neurons in the striatum.



## EXPERIMENT 1: EXAMINATION OF THE EFFERENT AND AFFERENT CONNECTIONS OF THE STRIATUM

### INTRODUCTION

The striatum is the most well-studied component of the basal ganglia. It is in receipt of the majority of the input to the basal ganglia from other brain areas and stands as the first link in the chain of successive nuclei which make up this system (Nauta, 1979a; McGeer et al., 1978). The striatum has been the subject of a great deal of neuroanatomical research aimed at demonstrating both its efferent and afferent connections (see Graybiel and Ragsdale, 1979) but the majority of these reports have dealt with observations on single connections. To date, there has been no systematic study mapping the distribution and relative densities of the efferents and afferents of the striatum of the rat. Furthermore, previous studies have used less sensitive neuroanatomical techniques than are now available (Mesulam, 1978; Gonatas et al., 1979; Staines et al., 1980b) and have not had the advantage of simultaneous examinations of both input and output elements.

In the present experiment, wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was utilized to demonstrate the projections of and inputs to the striatum in the rat. Confirmations of previous findings were obtained and expanded upon, and, in addition, an input to the striatum from the globus pallidus was demonstrated for the first time.

## METHODS

Male Wistar rats, anesthetized with pentobarbital, were given 50 to 200 nl pressure injections of a solution of 1 to 5% WGA-HRP in isotonic saline via a 5 ul Hamilton syringe mounted on a stereotaxic instrument. WGA-HRP was synthesized as described previously (Staines et al., 1980b). The striatal coordinates were chosen to correspond to 8.9/1.0/2.0 (anterior/dorsal/medial) in the stereotaxic atlas of the rat brain by Konig and Klippel (1963). Solution was infused over a ten minute period and the cannula was left in place for a further five minutes to limit diffusion up the cannula tract. After 24-48 hours, rats were reanesthetized and perfused transcardially, after clamping the descending aorta, with an isotonic saline solution containing 0.25% procaine and 0.001% heparin. This was followed by perfusion with a fixative containing 1% paraformaldehyde and 1% glutaraldehyde in 100 mM sodium phosphate buffer (PB), pH 7.4 (250 mls over 30-45 minutes) and finally, an ice cold solution of 10% sucrose in 100 mM PB (200 mls over 30 minutes). Brains were removed and stored for at least four hours in the latter solution. Sections were cut on a freezing microtome at a thickness of 50 um and mounted on chrome-alum coated slides from 50 mM PB. Slide mounted sections were reacted for peroxidase using tetramethylbenzidine (TMB) and hydrogen peroxide as substrates under standard conditions (Mesulam, 1978).

In brief, slides were rinsed three times in distilled water and then transferred to a reaction medium containing 100 mg sodium nitroferricyanide and 5 mg TMB (added as a 2% solution in

absolute ethanol) per 100 ml of sodium acetate buffer (50 mM, pH 3.3). After presoaking for 5 to 10 min, the reaction was begun by the addition of 250  $\mu$ l of 3% hydrogen peroxide and allowed to proceed for 20 minutes in total darkness. The reaction medium was usually replaced at least once during this period. After a number of post-reaction rinses in the reaction buffer and a brief rinse in distilled water, slides were air dried, dehydrated in 100% ethanol for 10-20 seconds, cleared in xylene and coverslipped with permount. Slides were routinely examined with dark field illumination on a Zeiss Universal Research microscope.

In all cases, the criterion used to determine a WGA-HRP-labelled terminal field was the presence of labelled fibers to and within an area in which a moderately dense, punctate distribution of reaction product was evident. The density, distribution, and orientation of the reaction product appeared to allow differential identification of terminal fields and areas containing projection fibers, but it is acknowledged that this was a subjective evaluation.

## RESULTS

The results of a series of injections will be reported through comparisons of one large injection (200  $\mu$ l, 5% WGA-HRP; CP-1; Fig. 1) with numerous smaller injections (CP-2, CP-3, CP-4; Fig. 2) directed at locations within the head of the striatum (that portion rostral to the decussation of the anterior commissure). The distribution of labelling resultant from the large injection is depicted in Figure 1. Unless otherwise stated, all presentations arise from observations of this case.

Figure 1. Line drawings illustrating the WGA-HRP injection site (stippled area) and resultant anterograde and retrograde labelling in case CP-1. Labelled perikarya are represented as filled circles. Fibers containing peroxidase reaction product and areas of terminal labelling are drawn in. Abbreviations in this and following figures: ab, basolateral nucleus of the amygdala; AC, anterior commissure; cem, central medial nucleus of the thalamus; cl, centrolateral nucleus of the thalamus; cp, striatum; CP, cerebral peduncle; dr, dorsal raphe nucleus; ep, entopeduncular nucleus; gp, globus pallidus; IC, internal capsule; pc, paracentral nucleus of the thalamus; pf, parafascicular nucleus; rr, retrorubral area; snc, substantia nigra pars compacta; snr, substantia nigra pars reticulata; vl, ventrolateral nucleus of the thalamus; vm, ventromedial nucleus of the thalamus.

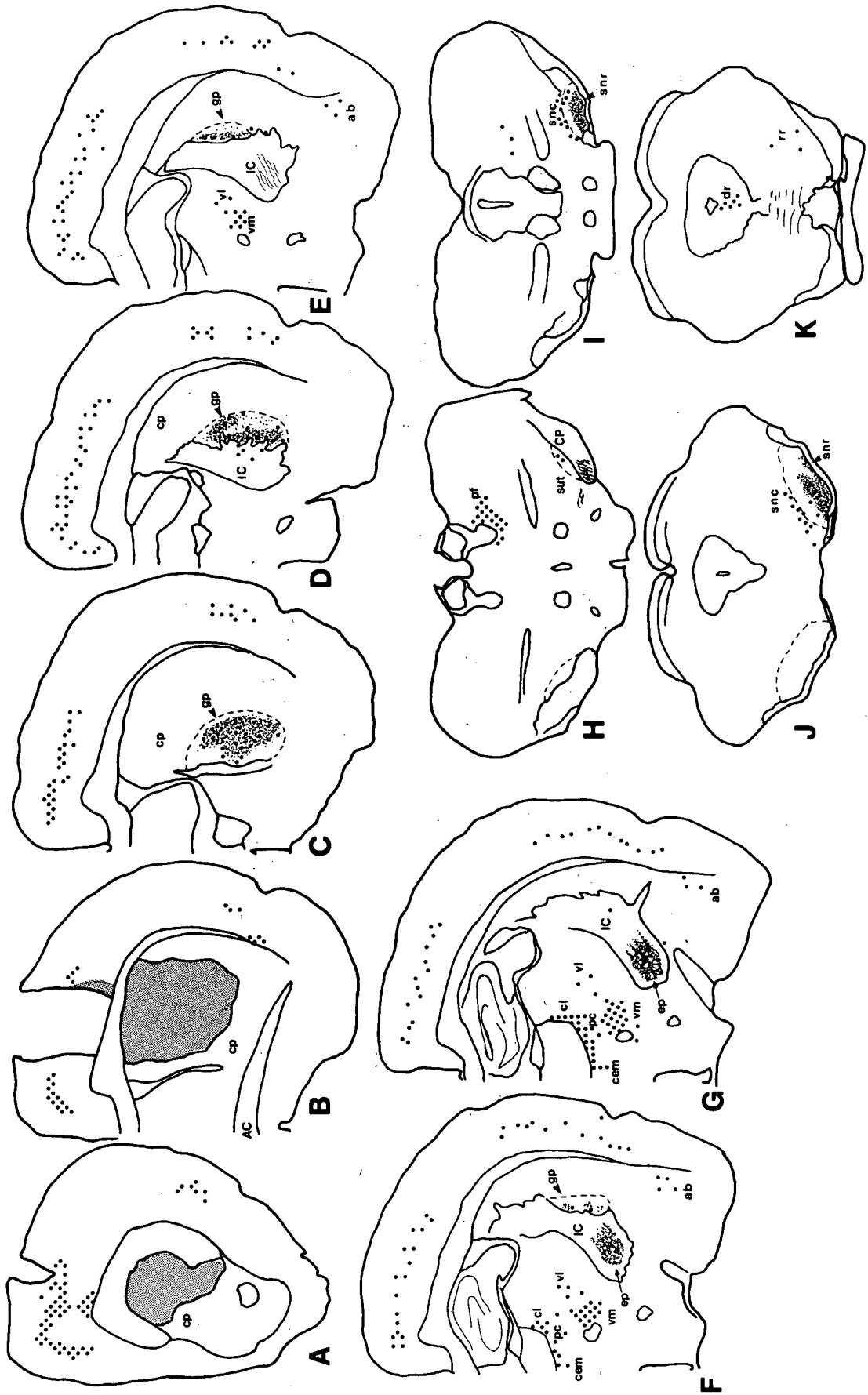
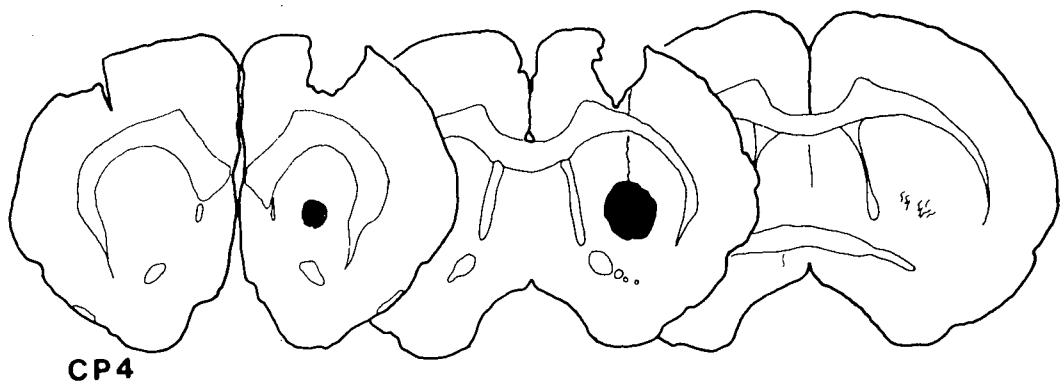
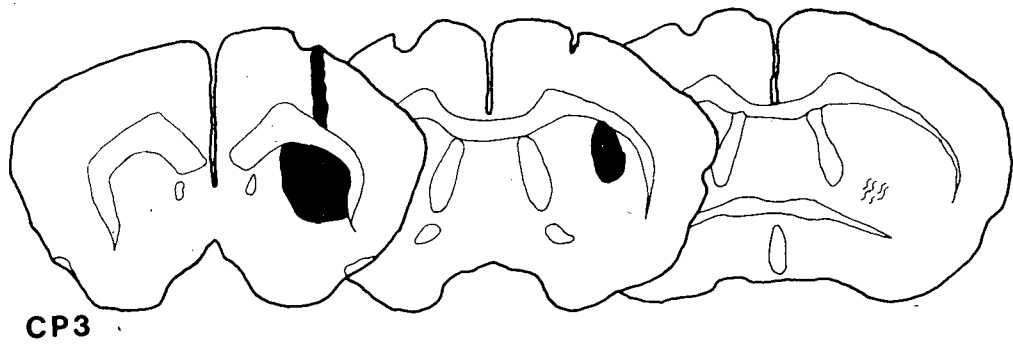


Figure 2. Line drawings illustrating the injection sites (filled area) in cases CP-2, CP-3 and CP-4.



## STRIATUM

As depicted in Figure 1 the large injection site (CP-1) involved most of the head of the striatum. The injection did not spread ventral or caudal to the anterior commissure and therefore did not label the tail of the striatum or accumbens. There was some labelling of the cortex immediately overlying the striatum at the level of the needle tract; however, most of this tissue had been destroyed by thermal damage during trephination. The corpus callosum acted as a lateral boundary to the injection site and medially the injected WGA-HRP did not diffuse as far as the lateral ventricle, leaving a thin medial strip of striatum free from labelling. At the boundaries of the injection site a disorganized punctate deposit (indicative of a featureless distribution) rapidly tapered off into clear striatum. A remarkable feature of the injection site was the absence of locally labelled striatal neurons, around its perimeter or within the tail of the striatum. In case CP-1, only 3 labelled striatal neurons could be seen beyond the bounds of the injection site, and all of these were within 500  $\mu$ m of the edge of the injection site (Fig. 3A). No neurons were seen in the tail of the striatum. The relative lack of retrograde labelling of striatal cells was a consistent feature of all cases examined (including many not documented in the present report). Heavily labelled cells were seen within the injection site but their codistribution with glial elements indicated that they were labelled by direct uptake of peroxidase by the cell body. In cases where WGA-HRP injections were made in caudal regions of the



head of the striatum rostral regions were free of labelling, indicating little or no potential for uptake of WGA-HRP into the projection fibers of the anterior cells which passed through the injection site. Again, the labelled striatal neurons which were found beyond the bounds of the injection site were invariably few in number and never more than 500  $\mu$ m from an edge of the injection site. There were too few of these neurons for detailed study but most appeared to be large ( $> 25 \mu$ m), triangular or spindle shaped cells. There was also no clear evidence for intrastriatal anterograde transport of WGA-HRP. A limited amount of fine, diffuse reaction product was seen in striatal areas beyond the injection site boundaries but this was most likely due to some limited diffusion of peroxidase activity or seeding of the peroxidase reaction. In some cases (CP-2 for example) there was clear evidence that no anterograde transport to either the tail of the striatum or the lateral head of the striatum had occurred. There was, however, sparse anterograde labelling in the rostromedial striatum anterior to the injection site. As nigrostriatal fibers are oriented rostrocaudally within the striatum (Tulloch et al., 1978), and bear varicosities along their length (DiFiglia et al., 1978), boutons of these fibers may have taken up WGA-HRP within the injection site and transported it to more anterior portions of the striatum.

Caudal to the striatal injection site, labelled fibers were seen to gather into the fiber bundles coursing toward the globus pallidus. Experience gathered from a large number of WGA-HRP transport experiments in various systems leads to the conclusion

that labelled projection fibers are prominent when labelled by anterograde transport but are very seldom seen when labelled retrogradely, and even then only when quite near the cell body. Direct evidence that the labelling seen within these fiber bundles in the striatum was striatofugal is provided in Experiment 4.

#### CORTEX

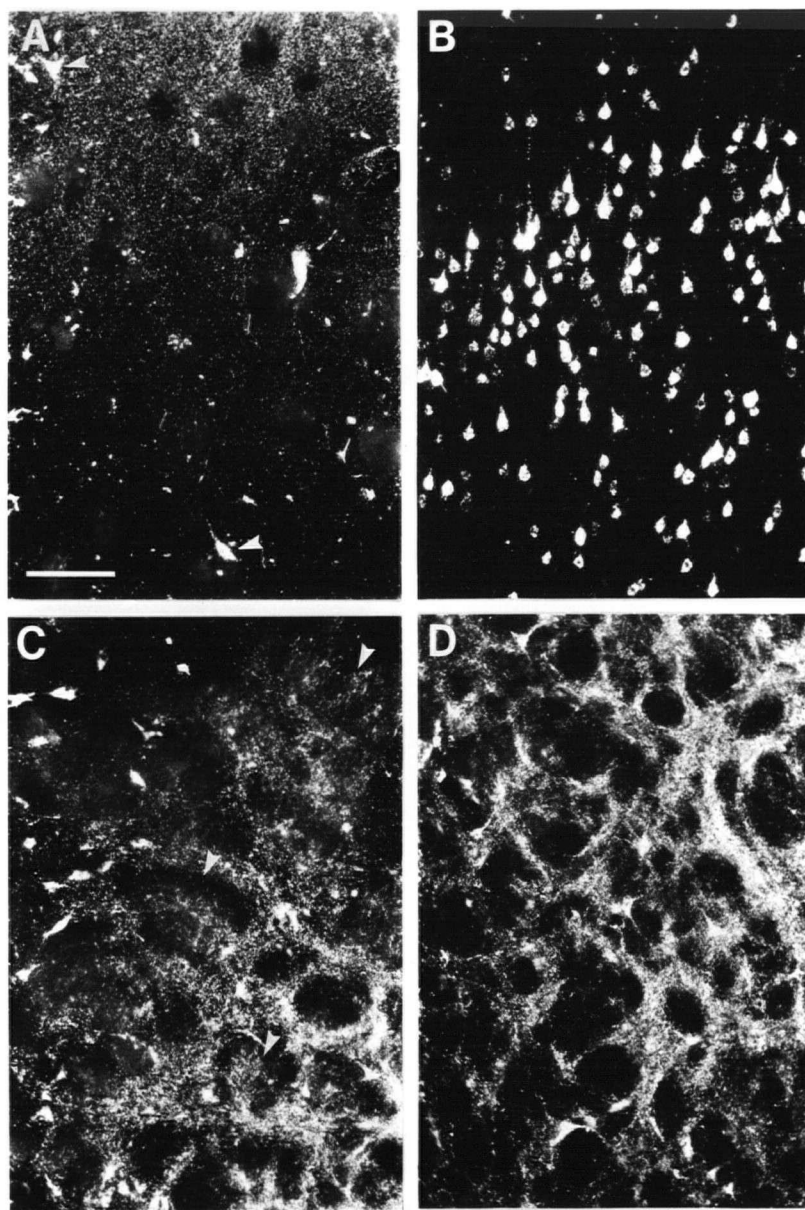
The distribution of cortical cells resulting from the large striatal injection is depicted in Figure 1. Labelled cells were clearly restricted to layer V in areas which contained a granular layer (layer IV). Laterally located neocortical cells formed a continuum with cells in layer II of the archicortex. A few labelled cells were seen in layer VI near the injection tract. The prefrontal cortex, around and anterior to the forceps minor, was not examined in case CP-1, but examination of material from other cases indicated that this area gives rise to the heaviest cortical innervation of the head of the striatum.

Fewer and less densely labelled cells were found in the cortex contralateral to the injection and their distribution mirrored that in the ipsilateral cortex. All labelled cells appeared to be typical medium sized pyramidal cells, with cell bodies roughly 14 by 20  $\mu$ m. No large pyramidal cells were seen labelled. Thick, apical dendrites can be seen in most of the cells in Figure 3B.

#### GLOBUS PALLIDUS

At the anterior tip of the globus pallidus the labelled striatal fiber bundles merged into the much larger fiber bundles

Figure 3. (A) Dark field photomicrograph of the ventral edge of the striatal injection site. Note the labelled striatal neurons (arrows). Other large bright features are peroxidase labelled vascular pericytes or the expression of the endogenous peroxidase activity of red blood cells. (B) Peroxidase-labelled cells in layer V of the cortex ipsilateral to the striatal injection site. (C) Anterograde labelling of the anterior globus pallidus. Note the labelling of fiber bundles (arrows) at this anterior level, although the labelling is predominantly within the neuropil. (D) The distribution of anterograde labelling at a more caudal level of the globus pallidus than that shown in C. Note the labelling of the neuropil and the absence of labelling of the fiber bundles. The whole of the field of this photograph is within the globus pallidus. Bar = 100 um.

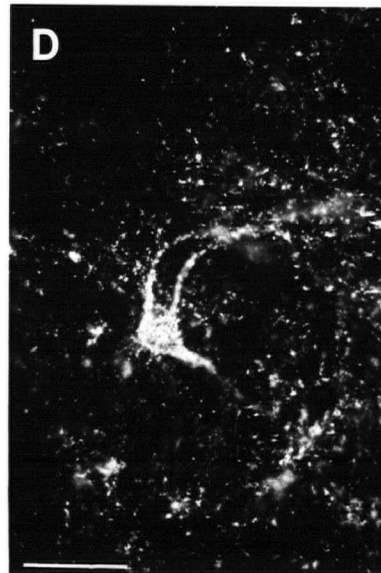
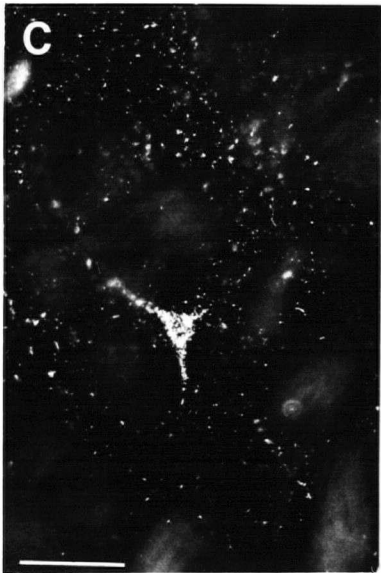
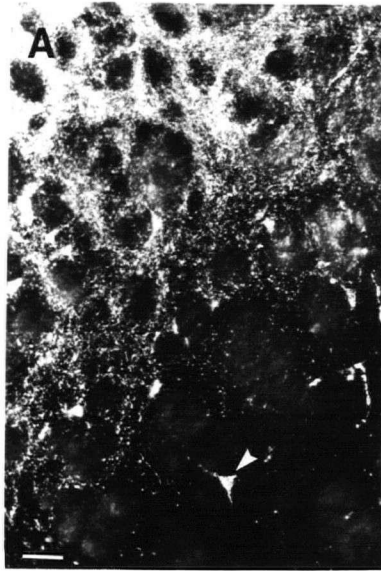


that traverse the globus pallidus, but a heavy, punctate field of reaction product was seen within the pallidal neuropil as well (Fig. 1, Fig. 3C), indicating termination of the striatopallidal projection. At more caudal levels of the globus pallidus (Fig. 3D), the majority of the peroxidase reaction product within the globus pallidus was found within the neuropil and the labelling within pallidal fiber bundles was scant. This observation leads to the conclusion that the labelled striatofugal fibers, including those which continue on to more caudal structures, innervate the globus pallidus rather than just passing through it.

The globus pallidus also contained labelled cell bodies. A few of these, outside the zones of striatopallidal termination, were clearly visible under dark field conditions but the majority could be seen only as small dark areas within the labelled terminal field (Fig. 4A and 4B). Those cells that were clearly visible were triangular or spindle shaped and measured approximately 25  $\mu$ m along their long axes (Fig. 4C). In one section through the globus pallidus in case CP-1, 58 neurons were counted, and this figure is probably an underestimate due to the difficulty in identifying neurons within the stained neuropil. Neurons were very occasionally found embedded in the fibers of the internal capsule near the caudal medial pallidum. These appeared to be somewhat larger and to stain more intensely than the cells within the globus pallidus proper (Fig. 4D).

Findings similar to those described above were seen in every case of WGA-HRP injection into the striatum. Both the area of

Figure 4. Dark field (A) and light field (B) photomicrographs of the labelled terminal field and cell bodies (arrows) within the globus pallidus. (C) Higher magnification of the cell in the terminal free area of (A) and (B). The cell shown in (D) was located medial to the globus pallidus, within the fibers of the internal capsule. Bar = 50  $\mu$ m.



the globus pallidus displaying terminal labelling and the number of retrogradely labelled pallidal neurons was found to be proportional to the size of the striatal injection. While smaller striatal injections led to the labelling of fewer pallidal neurons (eg. a maximum of 35 cells per section in the GP of CP-4), the intensity with which individual cells were labelled did not appear to be affected, arguing against a diffuse projection of individual pallidal cells over wide areas of the striatum. In the pallidum of CP-2 both the anterograde and retrograde labelling took up a much more medial distribution. In contrast, in CP-3 the globus pallidus was predominantly labelled in its lateral extreme, indicating an ordered, mediolateral topography. Neither CP-2 or CP-4 showed labelling of cells within the internal capsule.

#### AMYGDALA

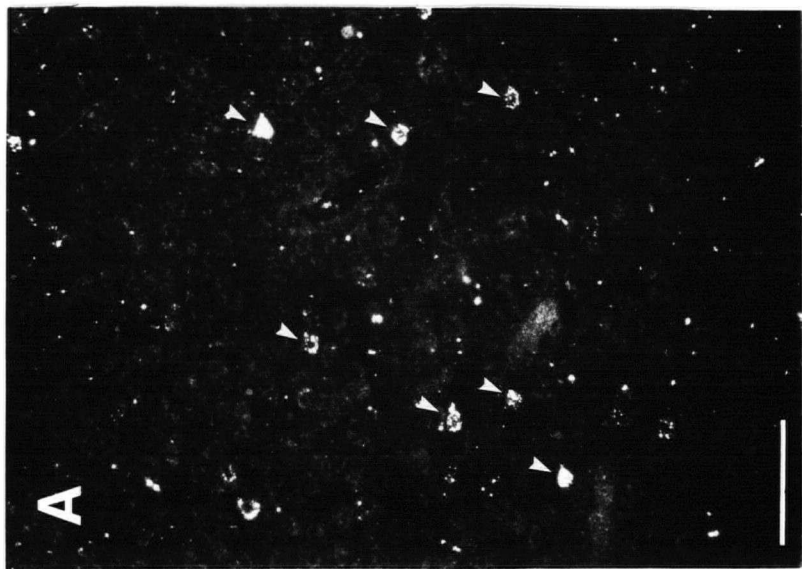
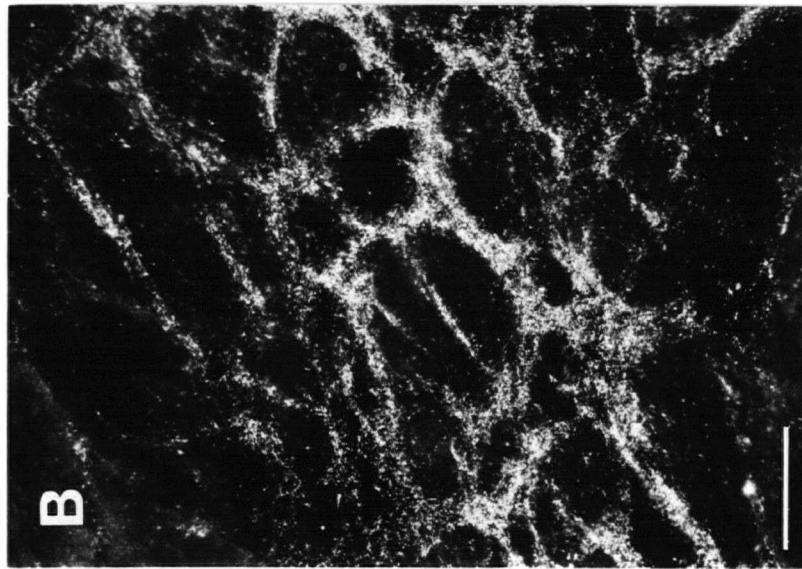
Each case examined revealed weakly staining cell bodies in the basolateral nucleus of the amygdala (Fig. 1, Fig. 5A). The intensity of labelling did not decrease in the smaller injections but labelled cells were far fewer in number. No anterograde labelling was seen within the amygdala.

#### ENTOPEDUNCULAR NUCLEUS

Caudal to the globus pallidus, labelled fibers were apparent within the internal capsule. These were far more numerous than those observed in fiber bundles within the globus pallidus. As depicted in Figures 1 and 5B, the entopeduncular nucleus was heavily labelled with peroxidase stained terminals, but retrogradely labelled cell bodies were not seen. This was a



Figure 5. The arrows in (A) point out the weakly-labelled cell bodies found within the basolateral nucleus of the amygdala. (B) Darkfield photomicrograph of the anterograde labelling of the neuropil of the entopeduncular nucleus. Note the virtual absence of labelling within the fiber bundles at this level. Bar = 100 um.



consistent feature of all cases. The smaller injections led to a more diffuse staining of the entopeduncular nucleus. As with the globus pallidus, labelling within fiber bundles was far less prominent at the level of the entopeduncular nucleus than rostral or caudal to it.

#### SUBTHALAMIC NUCLEUS

Caudal to the entopeduncular nucleus, labelled fibers could clearly be seen in the medial tip of the cerebral peduncle. In the subthalamic nucleus, which lies directly dorsal to this fiber bundle, a small number of very faintly labelled cell bodies were seen in case CP-1. These numbered only 4-6 per section and were restricted to the main body of this nucleus (Fig. 6A). Of the other cases, only CP-2 had labelled cells in the subthalamic nucleus and these were similarly very faintly labelled and few in number. There was no evidence of anterograde transport to this nucleus from the striatum.

Two sets of labelled axons were seen at caudal levels of the subthalamic nucleus (Figure 1). One of these, known from other work to be the striatal efferents (see Experiment 4), remained within the medial tip of the cerebral peduncle to the level of the substantia nigra. The other proceeded dorsomedially into the lateral hypothalamus (Fig. 6A) where they ran until the level of the substantia nigra. At this level they curved ventrolaterally into the substantia nigra pars compacta. These latter fibers were likely the proximal axons of the retrogradely labelled nigrostriatal neurons which run for a short distance in the medial forebrain bundle, and are close enough to the cell body to

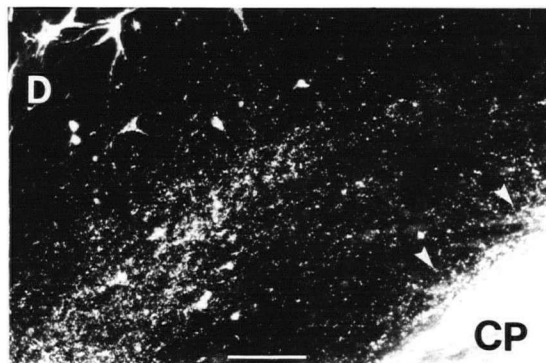
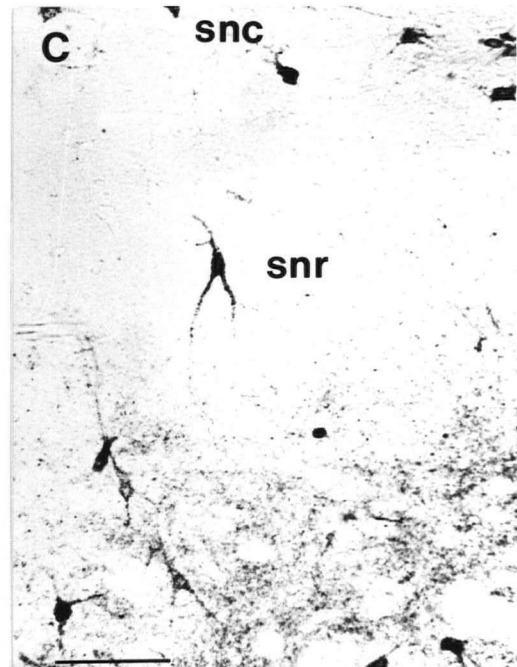
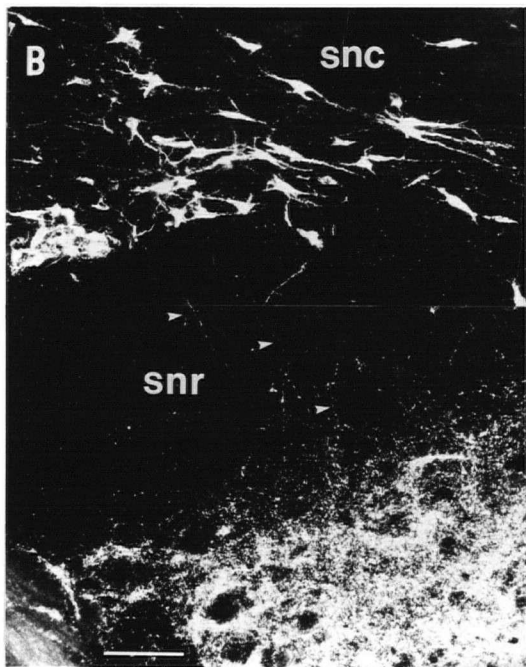
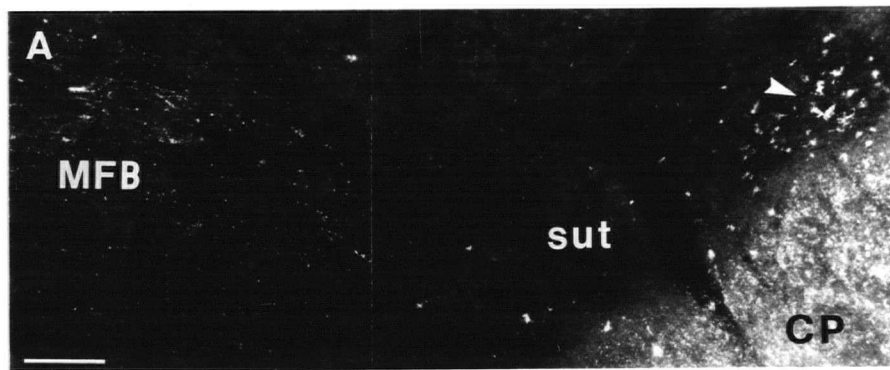
contain detectable levels of peroxidase.

#### SUBSTANTIA NIGRA

Cell bodies and proximal dendrites of neurons in the pars compacta and ventral tegmental area were heavily labelled with peroxidase, as were terminals in the ventral half of the pars reticulata. This terminal field was distributed evenly and was without apparent feature. However, with smaller injections (CP-4) the terminal field in the caudal pars reticulata split into two tiers (Fig. 6D). These two distinct fields of terminal labelling were separated by a band of unreactive neuropil. Both of the labelled fields were confined to the pars reticulata. It was of note that no anterograde labelling was seen in the pars compacta and that at most levels there was a very clear separation between the retrogradely labelled pars compacta cells and the terminal field in the pars reticulata (Fig. 6B). At more caudal levels, a few retrogradely labelled cells were found within the terminal field in the pars reticulata (Fig. 6C). At the caudal end of the substantia nigra there was no evidence of labelled projection fibers, but a cell group continuous with caudal pars compacta was seen to curve dorsolaterally into the retrorubral pons.

In case CP-3, labelled pars compacta cells were found in the extreme rostral part of the substantia nigra. The labelled terminal field in the pars reticulata, however, started at a much more caudal level. In sections in which the striatonigral terminal field was most prominent, only a few very faintly-labelled cells were found in the pars compacta, although

Figure 6. (A) Retrogradely labelled cell bodies within the subthalamic nucleus (arrow). Labelled fibers can be seen within the medial forebrain bundle (upper left) and the bright granular appearance of the cerebral peduncle is due to labelled fibers within this bundle. (B) Photomontage of retrogradely labelled cell bodies within the pars compacta and the terminal field within the pars reticulata of the substantia nigra. A few labelled dendrites of pars compacta cells can be seen descending into the pars reticulata (arrows). (C) Lightfield photomicrograph of labelled cell bodies in the pars compacta (upper fifth) and pars reticulata of the substantia nigra. The striatonigral terminal field can be seen in the lower third of the photograph. Note the presence of retrogradely labelled cell bodies within the pars reticulata. (D) Micrograph from the caudal part of the substantia nigra showing that at this level the labelled terminals form two separate fields within the pars reticulata. The arrows point out a narrow band of densely-labelled terminals lying adjacent to the cerebral peduncle. Bar = 100  $\mu$ m.



the occasional cell in the pars reticulata was heavily labelled. Thus, this case exhibited a negative correlation between the distribution of labelled striatonigral terminals and labelled pars compacta cells. In all cases examined an occasional (one or two per animal) labelled cell was seen in the contralateral pars compacta of the substantia nigra.

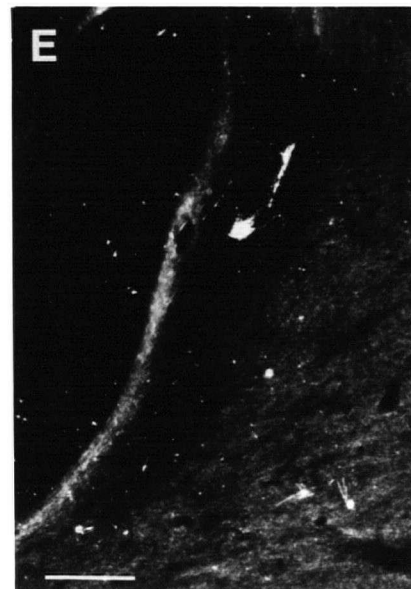
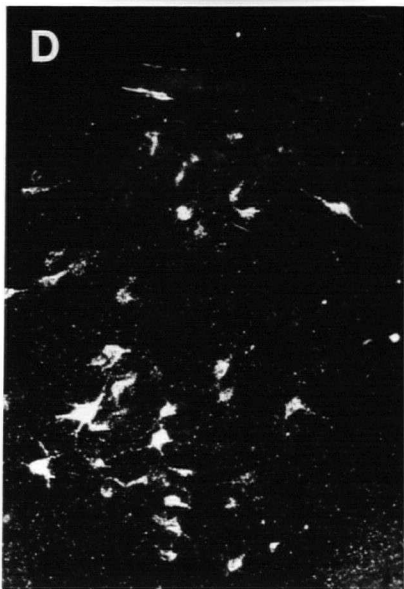
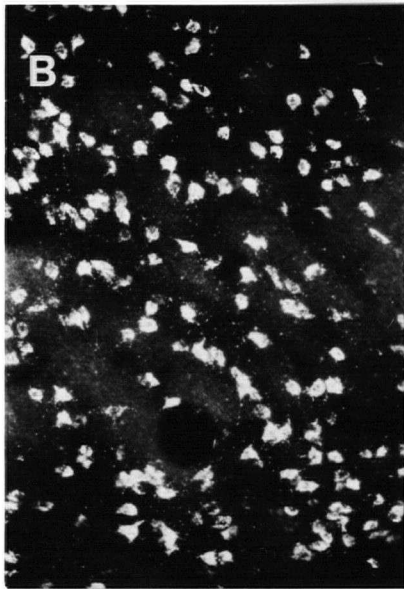
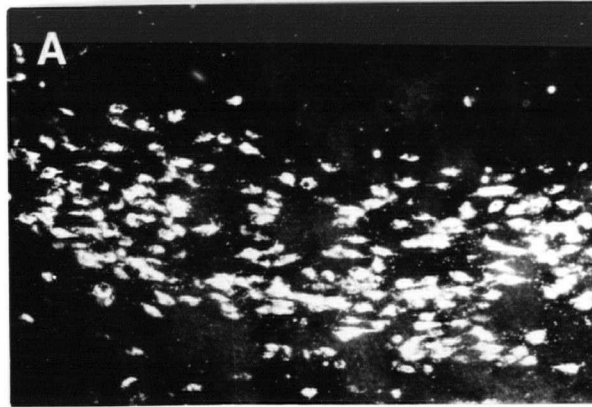
#### THALAMUS

As depicted in Figure 1, a number of thalamic nuclei contained retrogradely labelled cell bodies. These included all of the intralaminar cell groups (central medial, central lateral, and paracentral; after Jones and Leavitt, 1975) and the parafascicular nucleus. A few cells were observed in the anterior and medial parts of the ventrolateral nucleus and the ventromedial nucleus contained a large number of labelled neurons. Cells in the ventral thalamus were ovoid (20  $\mu$ m in diameter), those in the intralaminar regions were distinctly spindle-shaped (10  $\mu$ m by 25  $\mu$ m) and labelled parafascicular neurons were polymorphous (20  $\mu$ m maximum diameter) with two to four thick primary dendrites (Fig. 7A, 7B and 7C).

Labelling of neurons in the parafascicular and intralaminar nuclei was seen in all of the cases examined and smaller injection sites resulted in a less intense labelling of neurons rather than markedly decreasing the number of cells labelled. The reverse was true of labelling of cells in the ventral thalamus, where far fewer cells were seen in cases with small striatal injection sites. These data suggest that intralaminar/parafascicular neurons provide a rather diffuse

Figure 7. Retrogradely labelled cells within the (A) intralaminar nucleus of the thalamus, (B) ventromedial nucleus of the thalamus, (C) parafascicular nucleus, (D) dorsal raphe and (E) locus coeruleus. Bar = 100  $\mu$ m.





innervation of the striatum but that the projections from the ventromedial thalamus is more discrete. Some failures to label ventromedial neurons were encountered in cases where labelling of other cell groups was weak and survival times were shorter. This likely reflects a relatively small number of terminals per fiber on the axons of this projection. Diffusion of the injection site into the cortex was not a prerequisite for the labelling of cells in the ventromedial nucleus and it is doubtful that these cells were labelled by uptake into fibers of passage (see Discussion).

The only marked topographical contrast noted was within the parafascicular nucleus. An abundance of cells medial to the fasciculus retroflexus was noted in the parafascicular nucleus of CP-2 in contrast to the virtual absence of labelled cells in this region in case CP-3, in which labelled cells were lateral to the fasciculus retroflexus. A mediolateral topography of the projection of this nucleus to the striatum is therefore indicated.

#### OTHER AREAS

A small number of neurons was seen in the dorsal raphe nucleus in each case examined (Fig. 1, Fig. 7D), and in cases where the locus coeruleus had been sectioned three or four labelled neurons were found in total (Fig. 7E).

#### DISCUSSION

Careful consideration must be given to potential procedural or technical pitfalls that could influence the interpretation of these results. This is particularly true as the use of WGA-HRP for anterograde and retrograde tracing studies is a relatively

new procedure. Although only a modification of the HRP technique which has been in use for some time, there are theoretical reasons for expecting some of the problems that are associated with the use of HRP to be less in the present procedure and others to be worse.

Although HRP (Nagy et al., 1978; Herkenham and Nauta, 1977) and WGA-HRP itself (Gerfen et al., 1982) have demonstrated a capacity for retrograde labelling of cells or anterograde labelling of terminal fields (Walberg et al., 1980) whose axons merely project through an injection site, this phenomenon does not appear to have occurred with the pressure injections employed in this study. Evidence of this is provided by the lack of generalized labelling expected if afferents to or efferents from the cortex which pass through the head of the striatum had taken up peroxidase. If WGA-HRP had been taken up by projection fibers passing through the striatum, retrogradely labelled neurons would have appeared in the mediodorsal nucleus of the thalamus (Jones and Leavitt, 1975; Keefer et al., 1980), extensive retrograde labelling in the ventral anterior and ventrolateral thalamic nuclei would have occurred (Jones and Leavitt, 1975) and neurons in layer VI of widespread areas of the cortex would have been labelled. Widespread anterograde labelling of the thalamus and cortex would have been expected as well, but none of these were observed in the present experiment. Furthermore, striatal cells anterior to injections into the striatum were not labelled.

In some systems, transport markers have been shown to be transported transneuronally, resulting in the possibility of

apparent retrograde labelling of neurons which are innervated by labelled terminals (Triller and Korn, 1981; Itayama and van Hoesen, 1982). Anterogradely transported marker may also pass from labelled terminals into unlabelled terminals, in which case a heavily labelled terminal field may act like a second injection site (C.R. Gerfen, personal communication). Transneuronal transport, however, has only been reported to occur after injection of very large amounts of material and after survival times in excess of four or five days, much longer than the maximum two day survival time used in this study. Although it is conceivable that pallidal and subthalamic cells may have been labelled by some transneuronal process, the absence of any anterograde labelling in the subthalamus, which receives a dense innervation from the globus pallidus, argues that this phenomenon did not occur to any appreciable extent.

#### STRIATUM

A striking feature of the labelling within the striatum itself is the marked paucity of cell labelling outside the injection site. Coordination of activity in widespread areas of the striatum must, therefore, either be unnecessary to the physiological function of this nucleus or occur through polysynaptic mechanisms. The few large cells seen outside the bounds of the injections site fit the description of the cholinergic striatal interneuron (Fibiger, 1982). Local stimulation of striatal tissue produces excitatory potentials up to a distance of 0.5 to 1.0 um which have been characterized as cholinergic (Misgeld et al., 1982). Other local effects are very

rarely seen, indicating that other striatal cell types have much shorter intrastriatal connections.

#### STRIATAL EFFERENTS

The observation of anterograde peroxidase labelling in the present work is in complete accord with a number of previous studies (Kemp, 1970; Tulloch et al., 1978; Nagy et al., 1978a). The globus pallidus, entopeduncular nucleus and substantia nigra are still recognized as the only areas in receipt of the output of the striatum. It is of interest to review the anatomical picture presented here in terms of neurochemical findings after lesions of the corresponding striatal region.

Large lesions of the head of the striatum reduce substance P, enkephalin (to a small extent) and the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), in the globus pallidus but reduce only substance P in the entopeduncular nucleus and substantia nigra (Brownstein et al., 1977; Jessel et al., 1978; Staines et al., 1980a). Although lesions of the head of the striatum do not decrease GAD levels in the entopeduncular nucleus and substantia nigra, lesions of the main body and tail of the striatum are effective. This biochemical evidence suggests that substance P could be contained in collaterals innervating all three structures but that GABA-containing collaterals would be limited to the innervations of the substantia nigra and entopeduncular nucleus. This is consistent with the anatomical observations presented here. Comparisons with the available biochemical literature indicate that the labelled terminals seen in case CP-1 represent GABA, substance P and

enkephalin-containing terminals in the globus pallidus, but represent only substance P-containing terminals in the entopeduncular nucleus and substantia nigra. For the most part, the GABAergic innervation of the latter two structures arises from areas of the striatum caudal to the injection site in case CP-1.

Firm evidence for collateralization requires electrophysiological or alternate anatomical techniques, but the present data, specifically the observation that striatal efferents left the fiber bundles to ramify within the neuropil of the globus pallidus and entopeduncular nucleus, suggest that there is a cascade of efferents from the head of the CP such that the majority of innervation of each successively caudal structure is via a collateral. Fox and Raffols (1975) made a similar proposal on the basis of their observations of Golgi material. Some electrophysiological data have been presented upholding the idea of collateralization but disagree with the biochemical data in asserting that the transmitter involved is GABA (Yoshida et al., 1972). As nearly 70% of striatal neurons can be shown to project to the substantia nigra (Bolam et al., 1981a), the likelihood of collateralization of these fibers to the globus pallidus and entopeduncular nucleus is high. Recently a dynorphin-containing projection from the striatum to the substantia nigra has been demonstrated (Vincent et al., 1982c). Indications are that this opioid peptide has a similar distribution within the basal ganglia to that of substance P, and may occur within the same fibers (Vincent et al., 1982b).

Striatal cell bodies, demonstrated by immunohistochemical means to contain these substances or, in the case of GABA, the synthetic enzyme, are all of the medium spiny class (Ribak et al., 1979; Pickel et al., 1980; Ljungdahl et al., 1978; Somogyi et al., 1982). Indeed, only medium spiny neurons appear to innervate the globus pallidus (Woolf and Butcher, 1981) and very few cells not of this class project to the entopeduncular nucleus or substantia nigra (Parent et al., 1980; Bolam et al., 1981b). Direct demonstration of the innervation of the globus pallidus by chemically characterized neurons has been obtained in the case of enkephalin (Brann and Emson, 1980; Del Fiaccio, 1982).

#### STRIATAL AFFERENTS

Extensive treatment has been given previously to the observation of striatal afferents from the cortex (Hedreen, 1977; Yeterian and van Hoesen, 1978; Fallon and Ziegler, 1979; Oka, 1980; Donoghue and Kitai, 1981; Royce, 1982) amygdala (Royce, 1978; Kelly et al., 1982), intralaminar and parafascicular nuclei (Jones and Leavitt, 1975; van der Kooy, 1979; Veening et al., 1980), dorsal raphe (van der Kooy, 1979; Loughlin and Fallon, 1982) and locus coeruleus (Mason and Fibiger, 1979).

The evidence obtained for a very sparse projection of the subthalamic nucleus to the striatum agrees with the results of an autoradiographic study in the monkey (Nauta and Cole, 1978) in which a sparse innervation of the putamen was observed. Alternatively, peroxidase activity may have been taken up from a marginal intrusion of the injected WGA-HRP into the globus pallidus, although this was not apparent. Neurons of the

subthalamic nucleus recently have been shown to innervate the globus pallidus in addition to having collateral projections to the entopeduncular nucleus and substantia nigra (Denaiu et al., 1978a; van der Kooy and Hattori, 1980a). From the results of the present study, and in agreement with others (van der Kooy and Hattori, 1980a; Ricardo, 1980) it must be concluded that the subthalamic nucleus probably has, at most, a minor influence on the activity of cells in the head of the striatum, although this projection was at least as significant as that seen from the amygdala. It remains to be determined if the subthalamic innervation of the tail of the striatum, which corresponds more closely to the putamen, is more significant. Alternatively, the apparent sparse innervation of the striatum by the subthalamic nucleus may reflect the presence of the occasional neuron of the globus pallidus within the anatomical boundaries of the striatum. A few examples of a second type of striatonigral neuron have been found which are morphologically very similar to the pallidonigral neuron (Bolam et al., 1981b). Similarly, a small number of striatal neurons of similar size show very dense innervation by enkephalin-containing terminals, a feature not general to the striatum but commonly seen in the globus pallidus (Somogyi et al., 1982).

There is little evidence in the literature supporting a projection of the globus pallidus to the head of the striatum. In fact, many studies on striatal afferents or pallidal efferents have been carried out which might have been expected to have made this observation previously. Among factors which may have



contributed to this failure are lack of sensitivity of previous methods, diffusion of the injection site to the pallidum and the presence of terminal labelling within this structure. Golgi and autoradiographic studies of the globus pallidus have shown axons projecting to the striatum (Nauta, 1979a; Iwahori and Mizuno, 1981), but it was not clear if these observations merely reflected the projection of fibers from peripallidal cells bound for the cortex (Divac, 1975; Lehmann et al., 1980; Parent et al., 1981c). The interstitial cells labelled medial to the globus pallidus may have belonged to this population. The projection of the globus pallidus to the striatum is examined in detail in a number of subsequent experiments (Expts. 4-8).

The observation of labelled cells in the ventromedial nucleus of the thalamus confirms the autoradiographic observations of Herkenham (1979) who found a sparse distribution of silver grains in striatal neuropil after injections of tritiated amino acids into the ventromedial nucleus. Additional evidence has come from a recent retrograde transport study (Veening et al., 1980), but significant negative data exist as well (van der Kooy, 1979). For reasons presented above it is not thought that this represents labelling by uptake into fibers of passage. The ventromedial nucleus receives innervation from the substantia nigra (Clavier et al., 1976; Experiment 2), the deep cerebellar nuclei (Herkenham, 1979; Sugimoto et al., 1981) and possibly the entopeduncular nucleus (Carter and Fibiger, 1978). It projects heavily to layer I of most of the neocortex and to layers III and V of the motor and premotor cortices (Herkenham,

1979). Terminal fields in the deeper layers correspond remarkably to the distribution of cortical cells found to project to the striatum (Fig. 1).

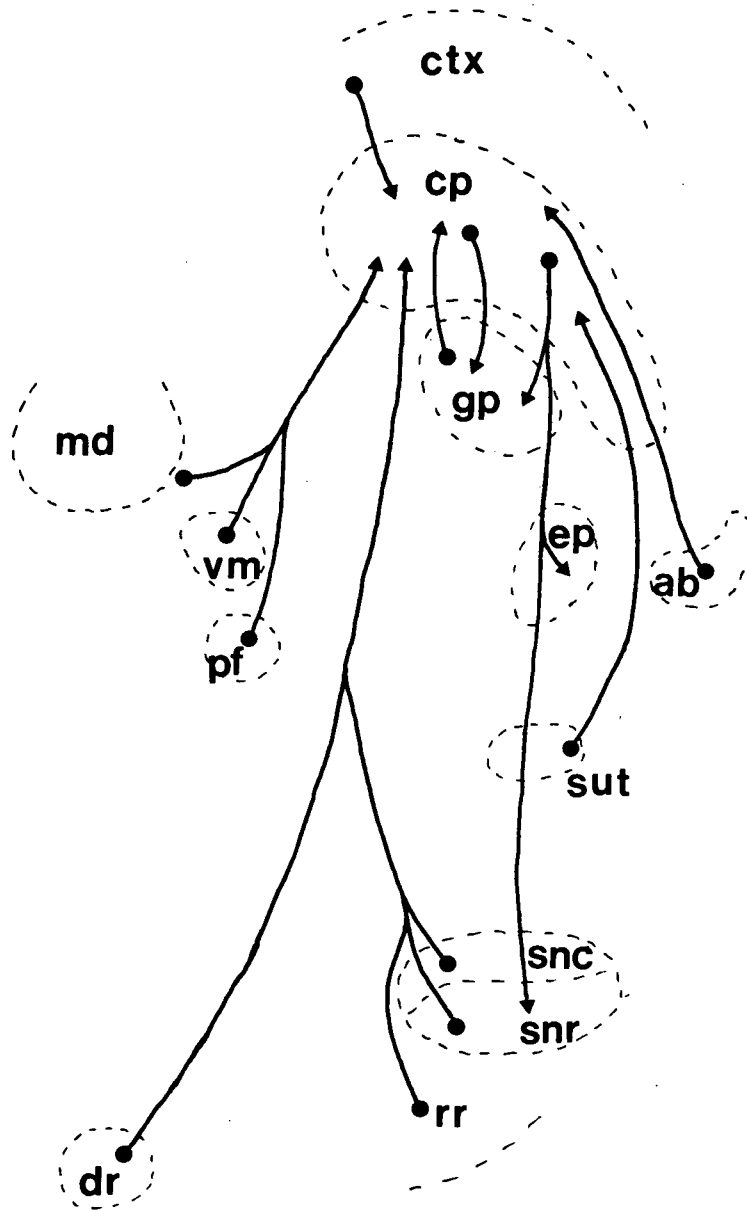
A number of considerations indicate that the ventromedial thalamic nucleus in the rat is a heterogenous nucleus composed of cells with specific and nonspecific cortical innervations (see Carter and Fibiger, 1978; Herkenham, 1979). Corroboration of this is supplied by the observation that the ventromedial nucleus in the cat projects solely to layer I (Glenn et al., 1982). It is not yet apparent which of these two cell types were labelled after striatal injections in the present material. If it were the nonspecific cell type, the sparse innervation of the striatum inferred here would stand in contrast to the reportedly dense innervation of the cortex. The reverse is true of the projection pattern of the intralaminar-parafascicular complex which has a dense innervation of the striatum but a more sparse cortical projection (Jones and Leavitt, 1975).

A few cells were also seen in the ventrolateral thalamus. It seems likely that these neurons were outlying members of the ventromedial population, but others have found large numbers of cells in this area after striatal HRP injections which produced much less labelling in the ventromedial nucleus (Veening et al., 1980). As both of these thalamic regions act as relays for the output of information from the basal ganglia, the possibility that one or both of them project to the striatum deserves more detailed consideration.

The present data fit in well with the impressive literature

on the striatal projections of the dopaminergic cell groups in and around the substantia nigra (Lindvall and Bjorklund, 1974; Carter and Fibiger, 1977; Faull and Mehler, 1978; van der Maelin et al., 1978; Beckstead et al., 1979; Veening et al., 1980). It has been shown that virtually all cells projecting to the striatum from this region, including those found within the pars reticulata, are dopaminergic (van der Kooy et al., 1981b). There are, however, a few nondopaminergic pars reticulata neurons which project to the striatum and have collaterals to other regions in receipt of nondopaminergic nigral innervation (Deniau et al., 1978b; Steindler and Deniau, 1980). The present investigation indicates that there are problems associated with the concept of a direct striatonigral-nigrostriatal feedback loop (see Dray, 1979). Neurons within the pars compacta send descending dendrites into the pars reticulata, largely by running along vascular elements (Schiebel and Tomiyasu, 1980). In fact, Golgi studies in the monkey indicate that most of the dendrites of these cells distribute within the pars reticulata, as far ventrally as the cerebral peduncle (Schwyn and Fox, 1974). It is obvious from Figures 6B and 6C that, although the labelled compacta neurons could receive striatal afferents, most would do so only on the distal parts of their dendrites. A more proximal input would be expected to arise from more ventrally located neurons than were labelled in case CP-1 (Tulloch et al., 1978; Nauta et al., 1978). This suggests at best a rather vague reciprocity of connections between the substantia nigra and striatum. Even more striking is the observation that in some

Figure 8. Schematic diagram summarizing the findings of this study and others mentioned in the discussion. The arrows are schematic and not meant to represent collaterals or monosynaptic connections.



animals the labelling of neurons in the pars compacta and terminals within the pars reticulata occurred at very different rostrocaudal planes. The labelled cells found within the pars reticulata may represent a unique population of nigrostriatal neurons. Their codistribution with the labelled terminal field, even after very small injections, was significant. Hattori et al. (1975) estimated that only 3.5 % of striatonigral terminals end on dopaminergic neurons. A more recent report confirms an innervation but does not supply quantitative data and is clouded by an almost certain labelling of the nigral afferents descending from the globus pallidus as well. (Wassef et al., 1981).

An exceedingly sparse crossed nigrostriatal projection has been mentioned previously (Loughlin and Fallon., 1982). The importance of this pathway appears to arise from its instructive value to neuroanatomists on the question of significance.

#### SUMMARY

The demonstration of a pallidostriatal pathway suggests that the striatum may be reciprocally connected with the globus pallidus but topographical considerations cast doubt on the concept that the striatonigral and nigrostriatal projections constitute a similar reciprocal system. The only other nucleus receiving striatal outputs, the entopeduncular nucleus, does not project back to the head of the striatum.

The striatum receives a massive cortical input from the frontal cortex and a less massive input from other cortical areas including the archicortical entorhinal cortex and the paleocortical amygdala. The other major inputs to the striatum

arise from the dopaminergic neurons of the substantia nigra and the intralaminar/parafascicular neurons of the thalamus. Comparatively minor inputs to the striatum arise from the locus coeruleus, dorsal raphe, subthalamic nucleus, ventrolateral thalamus and ventromedial thalamus. These latter three regions each receive innervation from nuclei to which the striatum projects. These findings are represented diagrammatically in Figure 8.

## EXPERIMENT 2: EXAMINATION OF THE EFFERENT AND AFFERENT CONNECTIONS OF THE SUBSTANTIA NIGRA

### INTRODUCTION

The substantia nigra is a major recipient of efferents of the striatum (Grofova and Rinvik, 1970; Hattori et al., 1975; Tulloch et al., 1978; Experiment 1). It also receives input from other basal ganglia nuclei such as the globus pallidus and subthalamic nucleus and afferents from outside this system including those from the peribrachial area, dorsal raphe, parafasicular nucleus, hypothalamus and prefrontal cortex (Kanazawa et al., 1976, Bunney and Aghajanian, 1976; Nauta and Domesick, 1978). It in turn projects to the medial prefrontal cortex, striatum, ventromedial, mediodorsal and parafascicular thalamic nuclei, superior colliculus and peribrachial region (Beckstead et al., 1979).

In the present study, the connections of the substantia nigra were reexamined using anterograde and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) (Staines et al., 1980b). A marked topographical relationship was observed for both striatal and pallidal connections with the substantia nigra. Bilateral innervation of thalamic nuclei and the superior colliculus is demonstrated and a totally crossed input from the posterior lateral hypothalamus is described for the first time. In addition, a marked difference was seen in both retrograde and anterograde labelling from dopaminergic and nondopaminergic regions of the substantia nigra. Portions of the results in this report have been published



(Gerfen et al., 1982).

#### METHODS

Male albino rats, anesthetised with pentobarbital, received 50-100 nl pressure injections of a 1 to 2.5% WGA-HRP solution into the substantia nigra. Injections were made using a 1 ul Hamilton syringe with a 31 gauge cannula. In a few of the nigral injections the solution also contained kainic acid at a concentration of 10 mM. After a 24 hr survival period the animals were reanesthetised and fixed by transcardial perfusion with 1.0% formaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature, followed by a transcardial rinse with buffered ice cold sucrose. The brains were removed and stored in this latter solution until sectioned. Slide-mounted 50 um sections were reacted for peroxidase activity using tetramethylbenzidine as substrate as described in Experiment 1. In some cases, free-floating sections were reacted for peroxidase using diaminobenzidine as substrate (Graham and Karnovsky, 1966) and then counterstained for cresyl violet to allow morphometric analyses.. In these instances sections were incubated at room temperature in 50 mM Tris-HCl buffer (pH 7.4) containing 0.025% diaminobenzidine (DAB) for 10 minutes. The reaction was started by the addition of 3% hydrogen peroxide to a final concentration of 0.0075% and allowed to proceed for 20 to 30 minutes. Sections were rinsed in Tris buffer and mounted onto subbed slides. After air drying, sections were counterstained with cresyl violet, dehydrated through a graded series of alcohol solutions, cleared in xylene and coverslipped with Permount.

Control injections of WGA-HRP were made along the angled approach to the substantia nigra and into the regions rostral, caudal and dorsal to it. Additional WGA-HRP injections were made into the parafascicular thalamic nucleus, the lateral dorsal thalamic nucleus, and the peribrachial area. Iontophoretic WGA-HRP injections into the posterior lateral hypothalamus were made through a micropipette (20  $\mu$ m tip diameter) filled with a 1% WGA-HRP solution in 0.9% saline using a 2  $\mu$ A positive current for 5 minutes.

## RESULTS

Most of the results of nigral injections presented below were obtained from the case illustrated in Figure 9 (SN-1), in which the injection site was predominantly localized to the pars reticulata. Seven other cases with injections into various regions and subdivisions of the substantia nigra were examined in detail and the important differences are noted in the appropriate sections. Some of these other injection sites are depicted in Figure 10.

### NIGRAL INJECTIONS

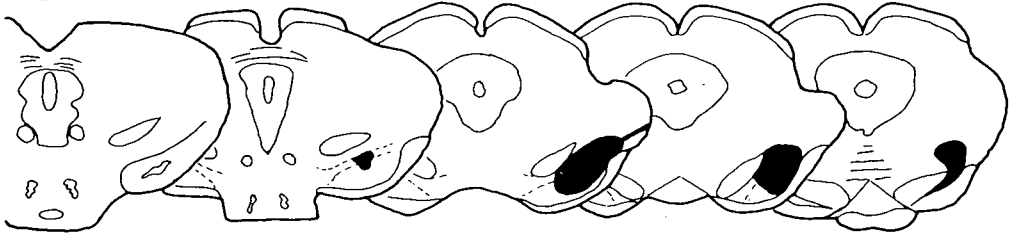
Anterograde and retrograde labelling of neuronal elements resulting from an injection of WGA-HRP confined to the substantia nigra are shown in Figure 9. The injection was centered in the pars reticulata of the substantia nigra and led to dense labelling of neurons, presumably by direct somal or dendritic uptake, around the injection center in both the pars reticulata and pars compacta (Fig. 11A and 11B). These neurons are marked with triangles in Figure 9. That the injection did not spread

Figure 9. Line drawings depicting the WGA-HRP pressure injection into the substantia nigra and the resultant anterograde and retrograde transport of peroxidase in case SN-1. Labelled perikarya are represented by filled circles, except those in the immediate vicinity of the injection site which are represented by filled triangles. Labelled projection fibers and terminal fields are drawn in. Abbreviations in this and subsequent figures: AC, anterior commissure; BC, brachium conjunctivum; ce, central nucleus of the amygdala; cem, centromedial nucleus of the thalamus; cg, central grey area; cl, centrolateral nucleus of the thalamus; cp, striatum; CP, cerebral peduncle; dr, dorsal raphe nucleus; F, fornix; FM, forceps minor; FR, fasciculus retroflexus; g, nucleus gelatinosus; gp, globus pallidus; ic, inferior colliculus; IC, internal capsule; ip, interpeduncular nucleus; ld, laterodorsal nucleus of the thalamus; lh, lateral habenula; lhp, posterior lateral hypothalamic area; md, mediodorsal nucleus of the thalamus; MFB, medial forebrain bundle; ML, medial lemniscus; MT, mammillothalamic tract; pc, paracentral nucleus of the thalamus; ppn, nucleus tegmenti pedunculopontis; r, red nucleus; sc, superior colliculus; snc, substantia nigra pars compacta; snr, substantia nigra pars reticulata; sum, supramammillary nucleus; sut, subthalamic nucleus; vm, ventromedial nucleus of the thalamus; vta, ventral tegmental area; zi, zona incerta.

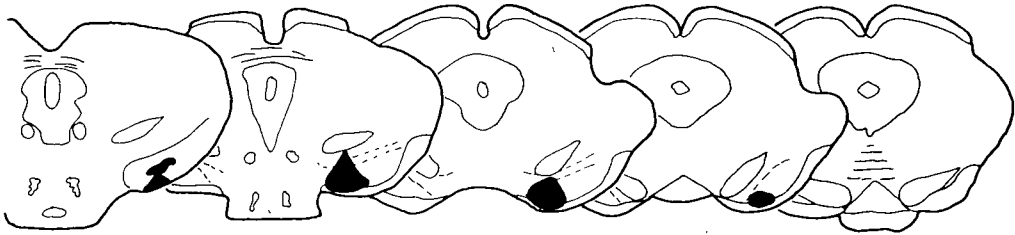


Figure 10. Line drawings depicting the maximum extent of the injection sites (as visualized with tetramethylbenzidine) in cases SN-2 to SN-6. Results of these injections are referred to in the text.

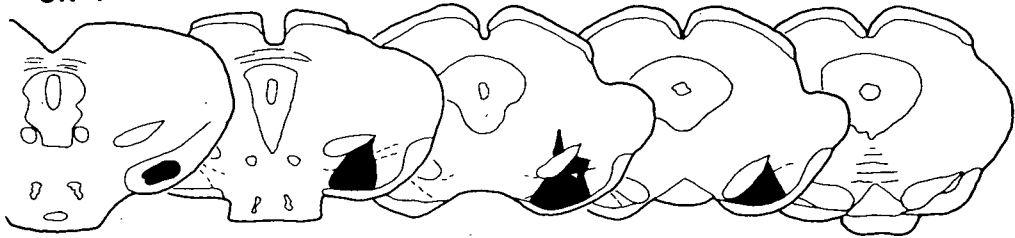
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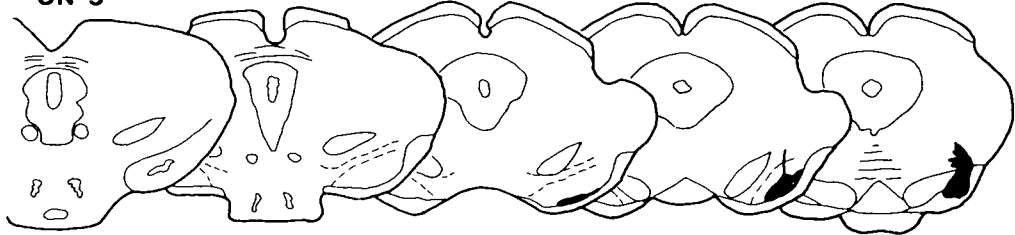
SN 3



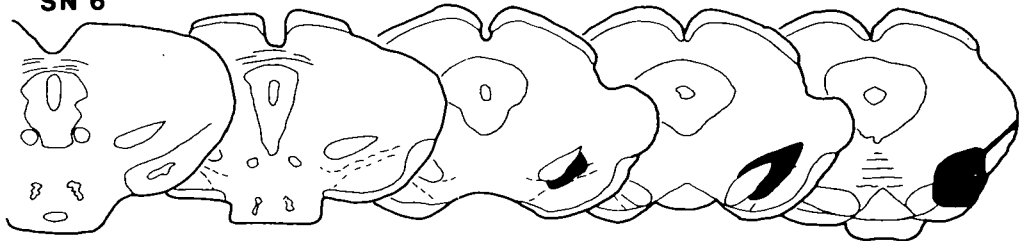
SN 4



SN 5



SN 6



into the medially adjacent ventral tegmental area can be inferred by the absence of direct labelling of these cells and by the absence of anterograde transport to the nucleus accumbens known to receive a massive input from that area (Beckstead et al., 1979). In the case depicted there was negligible diffusion of WGA-HRP along the injection tract. There was no evidence that the tracer had spread into the cerebral peduncle ventral to the pars reticulata.

#### RETROGRADE LABELLING

The distribution of retrogradely labelled neurons, mapped in Figure 9 as large dots, confirms previous descriptions of the sources of nigral afferents in the rat (Hattori et al., 1975; Bunney and Aghajanian, 1976; Kanazawa et al., 1976; van der Kooy and Hattori, 1980a). These include ipsilateral inputs from the prefrontal cortex, striatum, globus pallidus, subthalamic nucleus, dorsal raphe and minor ipsilateral inputs from the hypothalamus, central nucleus of the amygdala, and lateral habenula. In addition, three other regions contain retrogradely labelled neurons. These were the contralateral posterior lateral hypothalamic region, the ipsilateral parafascicular nucleus of the thalamus and the ipsilateral and contralateral peribrachial region, including the pedunculo pontine nucleus (Saper and Loewy, 1982). In contrast with an earlier report (Grofova, 1975) no retrograde labelling of the entopeduncular nucleus was observed.

#### ANTEROGRADE LABELLING

Terminal labelling was identified in the following brain regions: 1) the ipsilateral striatum; 2) the ipsilateral globus

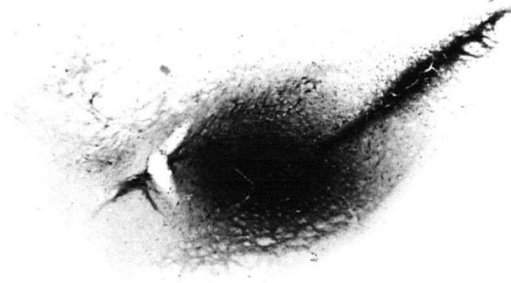
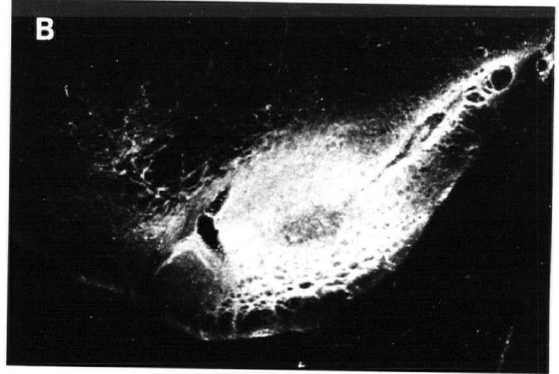
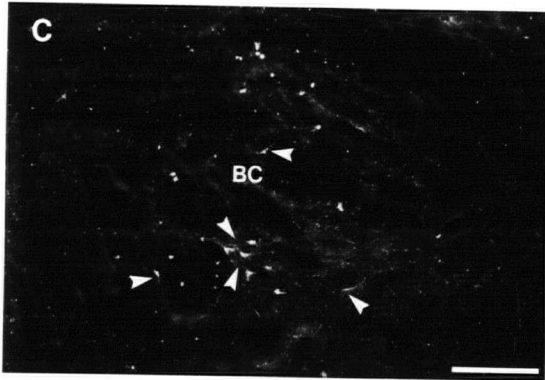
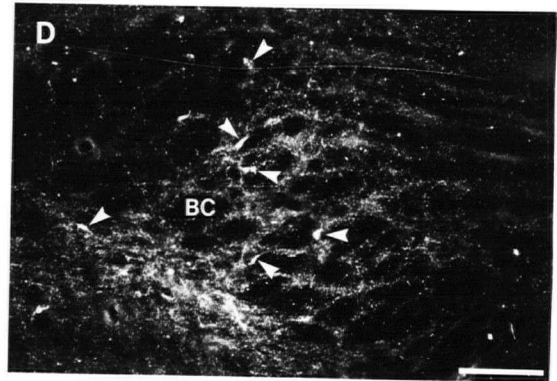
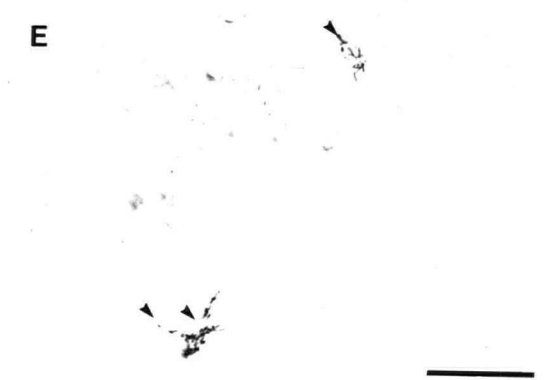
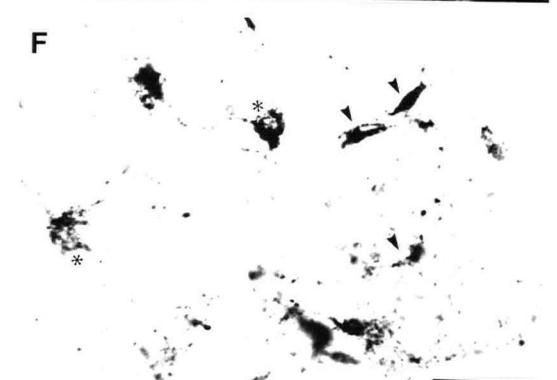
pallidus; 3) thalamic nuclei including the lateral dorsal , paralamellar mediodorsal , and ventromedial nuclei, bilaterally and the ipsilateral parafascicular nucleus; 4) the subthalamic nucleus ; 5) a discrete area of the most ventral and rostral portion of the midbrain central grey; 6) the ipsilateral dorsal midbrain tegmentum; 7) the ipsilateral and contralateral superior colliculus; 8) the central grey region through the midbrain and pons, bilaterally; and 9) the ipsilateral peribrachial region. These results are inclusive of previous reports on nigral projections (Beckstead et al., 1979).

#### NIGRAL CONNECTIONS WITH THE PERIBRACHIAL REGION

WGA-HRP injections into the substantia nigra labelled neurons in the peribrachial region, bilaterally. While it is not possible to identify anterograde labelling with certainty when retrograde labelling is present in the same area, there appeared to be terminal labelling of the ipsilateral but not contralateral peribrachial region (Fig. 11C and 11D). It was noted that in injections with more extensive involvement of the pars compacta, pars lateralis and retrorubral area (SN-2 and SN-6) there was far more retrograde labelling of neurons in the peribrachial region. In cases SN-3 and SN-5 very few cells were labelled. Furthermore, while cells labelled in case SN-1 were found in the region near the decussation of the brachium conjunctivum, those in SN-2 and SN-6 were seen dorsal and ventral to this fiber tract at planes as caudal as the motor nucleus of the trigeminal. As depicted in figure 9, anterograde labelling of the whole of this region was apparent in those cases with involvement of pars



Figure 11. Lightfield (A) and darkfield (B) photomicrographs of the injection site in the substantia nigra in case SN-1. (C) Peroxidase labelled perikarya (arrows) in the contralateral and (D) labelled cell bodies and terminals in the ipsilateral pedunculopontine nucleus in case SN-1. (E) Peroxidase labelling of neurons in peribrachial region of case SN-2 contralateral and (F) ipsilateral to the injection. Note that there appears to be two types of labelled cells on the ipsilateral side, a small cell type (arrows) and a larger cell type (asterisk), but only the smaller cell type on the contralateral side. Bar = 200  $\mu$ m in (B) and (C), bar = 50  $\mu$ m in (E) and (F).

**A****B****C****D****E****F**

reticulata in the injection site. In case SN-6, in which the injection was restricted to the pars compacta, only labelled cell bodies were seen.

Evidence was obtained that two cell types in the peribrachial region project to the substantia nigra. The majority of labelled cells were medium sized, spindle-shaped neurons (10 by 18  $\mu$ m) but larger, multipolar cells (13 by 25  $\mu$ m) were seen as well (Fig. 11F). Although not examined in great detail, it seemed that only the smaller type of cell was labelled in the contralateral nucleus (Fig. 11E).

Injections of WGA-HRP into the peribrachial region, resulted in the labelling of neurons in the ipsilateral but not contralateral pars reticulata. Additionally, these injections resulted in an apparent bilateral labelling of terminals in the substantia nigra and subthalamic nucleus, as well as retrograde labelling of neurons in the entopeduncular nucleus and hypothalamus.

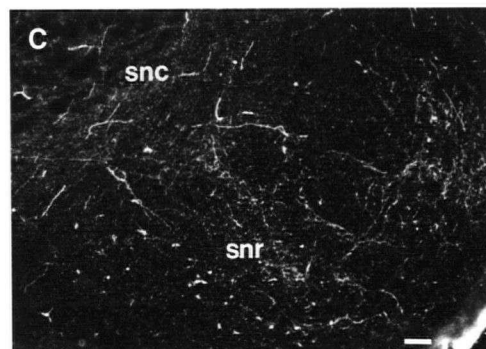
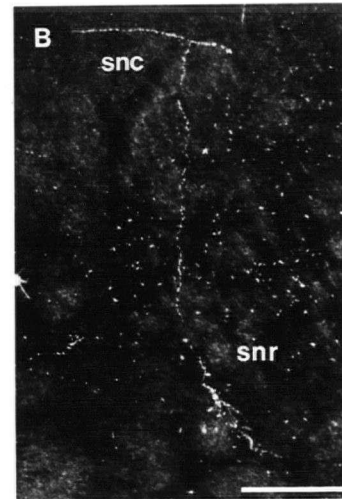
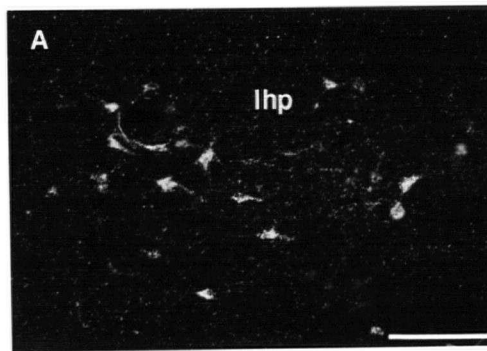
#### NIGRAL AFFERENTS FROM THE CONTRALATERAL HYPOTHALAMUS

Peroxidase labelled neurons located in the contralateral posterior lateral hypothalamus were confined to a discrete area just lateral to the mammillothalamic tract as it descends into the mammillary nuclei (Fig. 9F, G; Fig. 12A). As many as 14 labelled neurons were seen per 50  $\mu$ m section. The largest nigral WGA-HRP injections labelled a total of 120 of these neurons. Only WGA-HRP injections into the rostral half of the substantia nigra labelled these cells. To investigate the possibility that contralateral posterior hypothalamic neurons were labelled by

uptake into fibres of passage running through the substantia nigra or by uptake of tracer that spread beyond the substantia nigra (although this was not apparent), control injections were made into a number of extranigral midbrain areas. A comparison of the distribution of labelled neurons in the hypothalamus after control injections with that seen after nigral injections showed (1) nigral injections labelled cells occupying a region containing very few (3 or 4) neurons labelled by extranigral injections, and (2) while most of the hypothalamic neurons labelled after control injections were ipsilateral to the injection, nigral injections labelled only contralateral neurons in the posterior hypothalamus (although ipsilateral hypothalamic neurons were labelled in a much more rostral region; see Discussion).

An injection of WGA-HRP into the region of the posterior lateral hypothalamus in which labelled cells were found gave rise to anterograde labelling within the contralateral substantia nigra (Fig. 12B and 12C). The fibers crossed in the supramammillary decussation, progressed laterally and caudally into the substantia nigra pars compacta, and then coursed ventrolaterally from the pars compacta into the pars reticulata. Occasionally a labelled fiber in the pars compacta was observed with a collateral that branched into the pars reticulata, where it appeared to terminate. This pattern of transport was most evident in the rostral parts of the substantia nigra and became progressively less apparent in more caudal sections. Other labelling contralateral to the injection appeared in the form of

Figure 12. (A) Peroxidase labelled perikarya in the posterior lateral hypothalamus after a WGA-HRP injection into the contralateral substantia nigra (see Fig. 9G). (B) A single peroxidase labelled fiber in the substantia nigra pars compacta with a collateral descending into the substantia nigra pars reticulata after an iontophoretic WGA-HRP injection site into the contralateral posterior lateral hypothalamus. (C) A larger field showing labelled fibers in the anterior substantia nigra pars reticulata. No labelled perikarya were found in the substantia nigra pars reticulata. Calibration bar =200um.



labelled fibers, terminals, and cell bodies in the dorsal midbrain tegmentum, and labelled terminals and cell bodies in both the peribrachial area and in the laterodorsal tegmental nucleus.

Ipsilateral to the hypothalamic injection, labelled neurons were observed in the ventral tegmental area and the pars compacta. In addition, the pars reticulata contained a moderately dense distribution of punctate reaction product throughout its rostrocaudal extent. These results may indicate connections between these areas and the lateral hypothalamic injection area. It is noted that the neurons located in this region lay directly in the path of the dopaminergic projection fibers in the medial forebrain bundle (Fig. 13E) and may receive innervation by boutons en passant. The labelling in the ipsilateral pars reticulata is not thought to have arisen from the posterior lateral hypothalamic neurons and may have been due to labelling of subthalamonigral fibers.

#### NIGROTHALAMIC CONNECTIONS

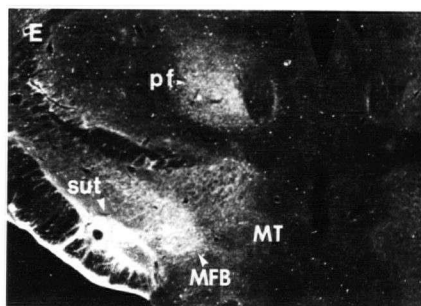
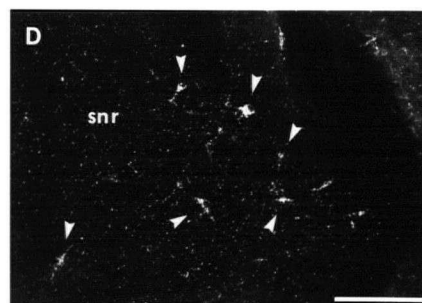
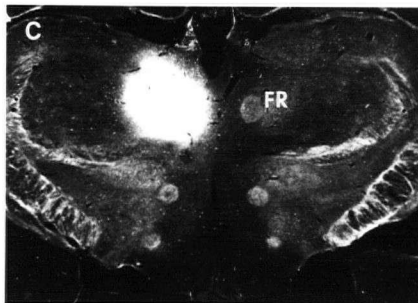
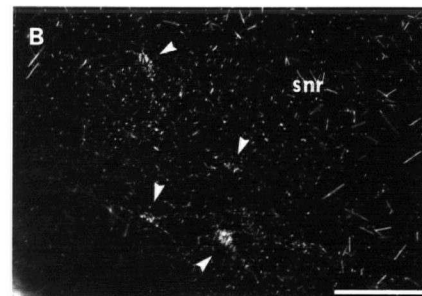
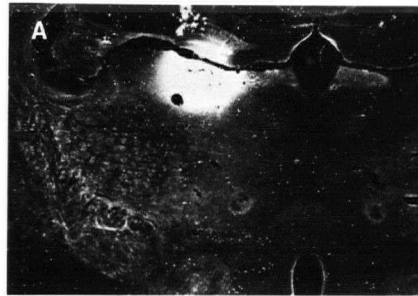
##### Parafascicular nucleus

Neurons in the ipsilateral parafascicular thalamic nucleus were retrogradely labelled after nigral WGA-HRP injections. These injections also resulted in an apparent terminal labelling within the same region.

Injections into the parafascicular nucleus itself resulted in retrograde labelling of over 100 neurons in the ipsilateral pars reticulata of the substantia nigra (Fig. 13C and 13D). Only rarely were neurons labelled in the contralateral pars reticulata

Figure 13. (A) An iontophoretic WGA-HRP injection into the laterodorsal nucleus of the thalamus. Some labelling of the fimbria can be seen as well. (B) Peroxidase labelling of perikarya (arrows) in the substantia nigra pars reticulata ipsilateral to the injection shown in (A). (C) An iontophoretic WGA-HRP injection into the parafascicular nucleus and (D) the resultant labelling of perikarya in the ipsilateral substantia nigra pars reticulata. (E) Peroxidase labelling in the caudal diencephalon after a WGA-HRP injection into the substantia nigra. The anterograde transport of peroxidase to the parafascicular nucleus is apparent in this section. Note that the posterior lateral hypothalamus is directly in the path of ascending medial forebrain bundle, which can be seen due to the presence of peroxidase transported in the anterograde direction.



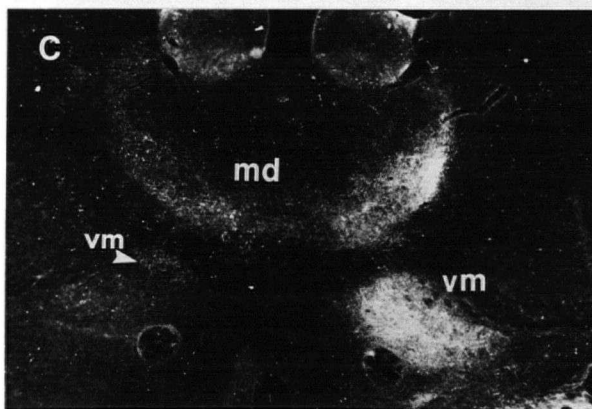
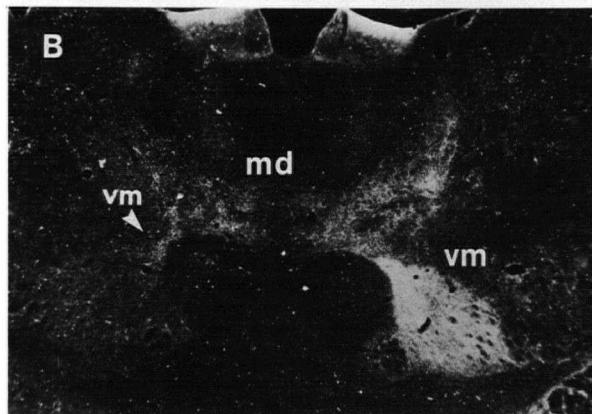
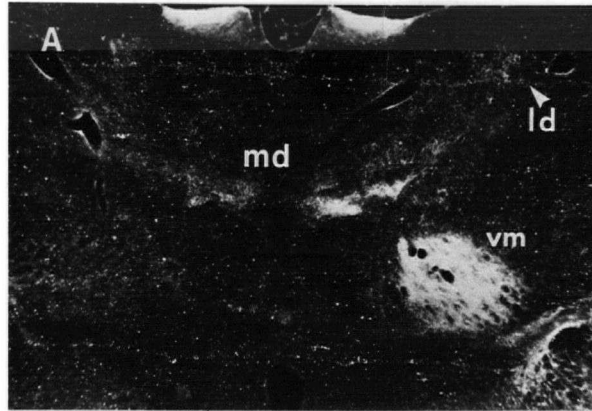


after parafasicular injections. Additionally, there was evidence of terminal labelling in the ipsilateral pars reticulata, striatum, subthalamus and interpeduncular nucleus. Labelled cell bodies were seen bilaterally in the entopeduncular nucleus, hypothalamus, superior colliculus and peribrachial area and unilaterally in the lateral habenula and laterodorsal tegmental nucleus. Previous studies have identified all of these connections except for those implied by the labelling seen in the lateral habenula and interpeduncular nucleus (Ahlenius, 1978). As the tip of the injection cannula was located in the core of the fasciculus retroflexus, peroxidase labelling of these latter two areas probably represents transport of tracer by damaged fibers of passage.

#### Ventromedial thalamic nucleus

Substantia nigra WGA-HRP injections resulted in dense terminal and fiber labelling in the ipsilateral ventromedial thalamic nucleus (Fig. 14). Additionally, there was evidence of a more sparse distribution of terminal labelling in the contralateral ventromedial nucleus, particularly in its most dorsal aspect. Evidence was obtained for a topographical distribution of terminals to the ventromedial nucleus. In very ventrally placed nigral injections the anterograde labelling of this nucleus appeared confined to rostral regions and in the lateral nigral injection (SN-2) terminal labelling was restricted to the dorsolateral half of the the ventromedial nucleus, leaving that portion nearest the mammillothalamic tract free of reaction product. After an injection confined to the pars compacta and

Figure 14. Anterograde peroxidase labelling in the thalamus at three coronal planes after a WGA-HRP injection into the substantia nigra in case SN-1. Labelling of the laterodorsal and ventromedial nuclei and bilateral labelling of the paralamellar portion of the mediodorsal nucleus is apparent in (A). The white appearance of the stria medularis is an artifact of dark field microscopy. Note the bilateral component to the terminal field in the ventromedial thalamus in (B) and (C).



retrotrubral areas of the substantia nigra, the ventromedial nucleus was virtually unlabelled.

#### Mediodorsal thalamic nucleus

There was distinctive labelling of terminals in the paralamellar region of the mediodorsal thalamic nucleus, bilaterally, after nigral WGA-HRP injections (Fig. 14). Fibers appeared to enter the mediodorsal nucleus through the intralaminar nuclei, however, it was not possible to determine whether labelling within the intralaminar region arose from labelled projection fibers alone or whether terminals were also labelled. Injections into the mediodorsal nucleus led to labelling of over 400 cell bodies in the pars reticulata of the substantia nigra. Some of the other areas found to project to this nucleus were the contralateral pars reticulata of the substantia nigra, superior colliculus, peribrachial area, magnocellular nuclei of the basal forebrain and the contralateral posterior lateral hypothalamus. Neurons in this latter region had an identical distribution to those labelled contralateral to injections into the substantia nigra.

#### Lateral dorsal thalamic nucleus

After nigral WGA-HRP injections, labelled fibers were observed exiting in a laterodorsal direction from the intralaminar nuclei. These fibers continued into the lateral dorsal thalamic nucleus in which a sparse terminal-like distribution of label was apparent. (Fig. 14). A similar pattern, although less distinct, was seen contralateral to the injection. Injections of WGA-HRP centered in the laterodorsal

nucleus spread into most of the lateral tier of thalamic nuclei but did not appear to spread more than a minimal amount into the mediodorsal or ventromedial nuclei. Such injections resulted in retrograde labelling of cells in the most caudal medial aspect of the pars reticulata of the substantia nigra (Fig. 13A and 13B).

#### NIGROTECTAL CONNECTIONS

A distinctive puff-like distribution of labelled terminals along the entire mediolateral extent of the stratum album intermediale of the superior colliculus was observed ipsilateral to nigral WGA-HRP injections (Fig. 15). Additionally, numerous labelled fibers were observed in the superior collicular decussation and there was marked terminal labelling in the contralateral superior colliculus. In contrast to the distribution of terminals on the ipsilateral side, that in the contralateral superior colliculus was restricted primarily to the most ventrolateral aspect of the stratum griseum.

Iontophoretic applications of WGA-HRP were made into either the rostral or caudal half of the superior colliculus. Both injections deposited tracer into the intermediate and deep layers of the lateral half of the tectum and into the most dorsal aspect of the midbrain reticular formation. Each injection resulted in distinctly different patterns of retrograde labelling in the substantia nigra (Fig. 16). The caudal injection labelled a large number of ipsilateral pars reticulata neurons located in the rostral half of that nucleus and confined primarily to a ventral area just above the cerebral peduncle. Only two neurons were labelled in the contralateral nucleus. The rostral

Figure 15. (A) Anterograde transport to the anterior superior colliculus and dorsal midbrain tegmentum in case SN-1 shown in relation to the injection site into the substantia nigra. (B) A higher magnification of the anterograde labelling within the ipsilateral and contralateral superior colliculus. Essentially the same pattern of labelling is seen at more caudal levels of the superior colliculus as well (C).

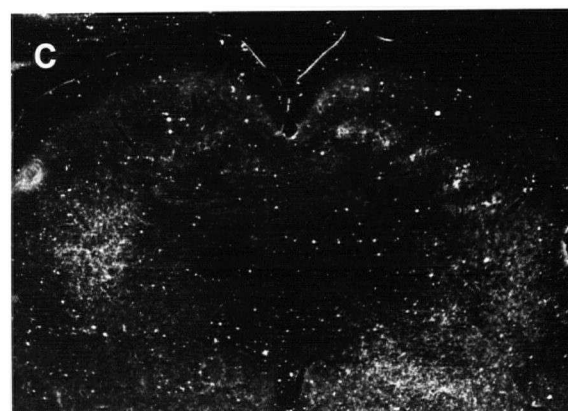
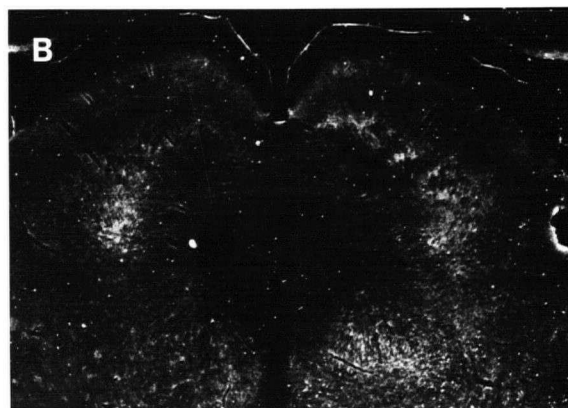
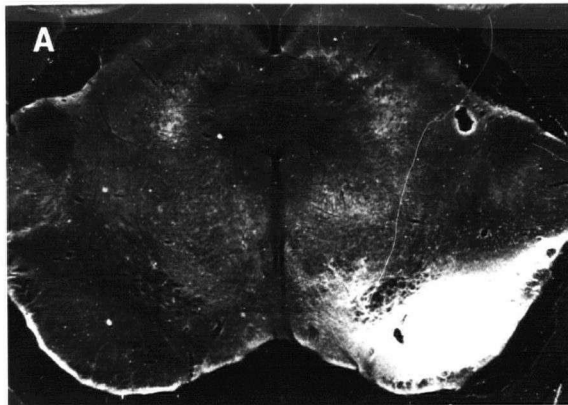
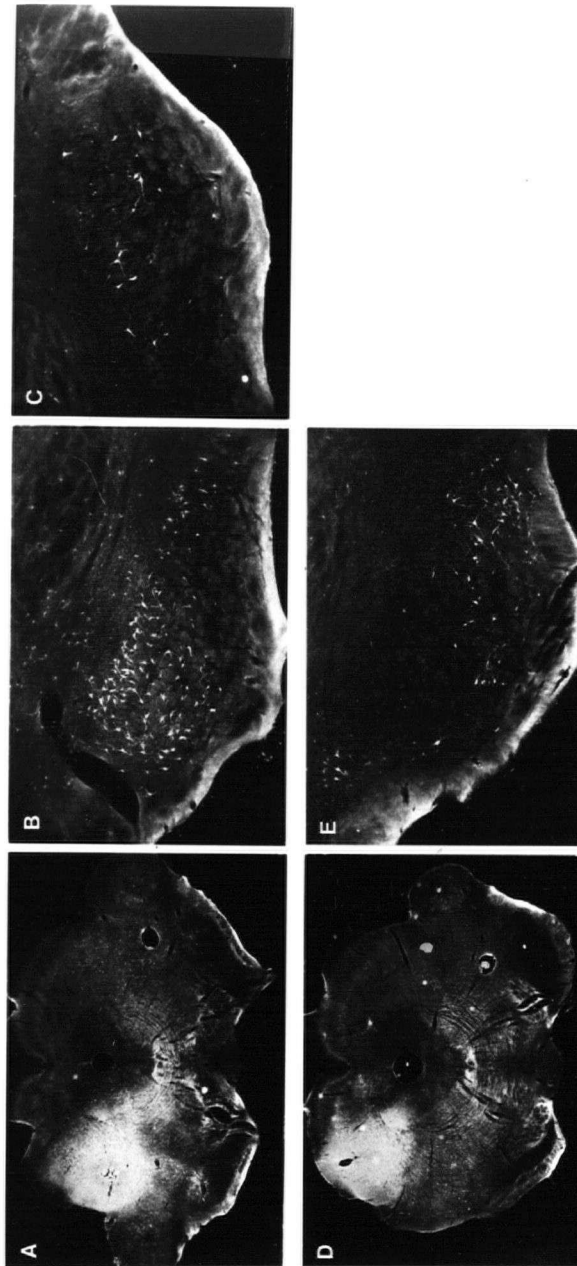




Figure 16. A WGA-HRP injection site (A) into the ventrolateral aspect of the anterior superior colliculus and the resultant labelling of perikarya in the substantia nigra pars reticulata ipsilateral (B) and contralateral (C) to the injection. (D) A WGA-HRP injection site into the ventrolateral aspect of the caudal superior colliculus and (E) the perikaryal labelling it produced within the ipsilateral substantia nigra. There was no significant labelling of cells contralateral to the injection shown in (D).



injection labelled an even greater number of ipsilateral nigral neurons than did the caudal injection. Again these neurons were located in the rostral half of the nucleus; however, in this case, labelled perikarya were dispersed in the dorsal three fourths of the pars reticulata. The contralateral pars reticulata contained in excess of 200 labelled perikarya, fewer than on the ipsilateral side, primarily confined to rostral and dorsal regions. The possible inclusion of the midbrain reticular formation in the injection area precludes a strict identification of all neurons labelled after these injections as exclusively part of the nigrotectal pathway. However, since there was no evidence of crossed fibers terminating in the midbrain reticular formation after nigral WGA-HRP injections, the labelling of contralateral neurons in the pars reticulata after the injections into the superior colliculus is presumed to reflect a crossed nigrotectal connection.

#### SUBTHALAMIC NUCLEUS

Retrograde labelling of neurons in the subthalamic nucleus was a prominent feature of all nigral injections with involvement of the pars reticulata (Fig. 17). In case SN-6, where the injection was confined to the pars compacta, no labelled cell bodies were found within the subthalamic nucleus. Although heavily labelled fibers were observed coursing dorsally and medially to the subthalamic nucleus, no apparent innervation of this structure could be discerned. Dissimilarities were seen between the distributions of labelled neurons in the subthalamic nuclei after the virtually complementary nigral injections, SN-2

and SN-4. The caudolateral injection labelled mainly rostrally located neurons whereas, after the rostromedial injection, labelled cells were confined predominantly to caudal regions. At the level of greatest cell labelling in each case, positive neurons were seen throughout the whole mediolateral extent.

#### STRIATUM AND GLOBUS PALLIDUS

As depicted in Figure 9, nigral injections gave rise to retrograde labelling of neurons within both the striatum and globus pallidus. A marked topographical relationship with the nigra was noted for both. In case SN-1 most labelled striatal and pallidal neurons were found in the core region of these structures. Caudolateral nigral injections (SN-2) gave rise to labelled cells along the lateral borders of both nuclei, more prominently at caudal levels, and, with rostromedial placements, the reverse was true (SN-3; Figs. 18A and 18B). An injection into the ventral extreme of the pars reticulata labelled neurons in the most dorsal parts of the striatum and globus pallidus (SN-4). An injection into the pars compacta, apparently devoid of significant involvement of the pars reticulata (SN-6), virtually failed to label neurons in the striatum (only 10 or 20 were seen in total; Fig. 19). Far fewer cells were seen in the globus pallidus of SN-6 than in other case, and these were distributed widely throughout the nucleus. Except for this last case, the density of labelled pallidal neurons was very high in those regions containing them, leading to the impression that most pallidal neurons project to the substantia nigra. The pallidal cells were generally triangular or spindle shaped and ranged from

Figure 17. Labelled cell bodies in the subthalamic nucleus in case SN-2. Note the absence of anterograde labelling of this nucleus. Note also that the medial aspect of the nucleus (to the left of the field) is well labelled despite the fact that the injection site placement in this case was very lateral.

Figure 18. Labelled neurons in the medial striatum (A) and globus pallidus (B) after the nigral injection in case SN-3. This animal was coinjected with kainic acid which has abolished the anterograde labelling in contrast to that seen in the dorsal globus pallidus of case SN-5 in which a light anterograde labelling of globus pallidus was seen as well as the retrograde labelling of cell bodies (C). Bar = 100 um.

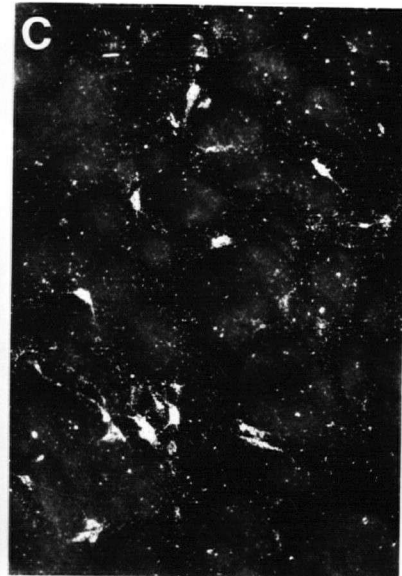
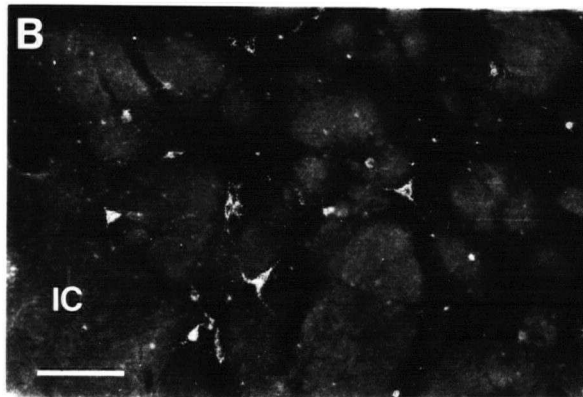
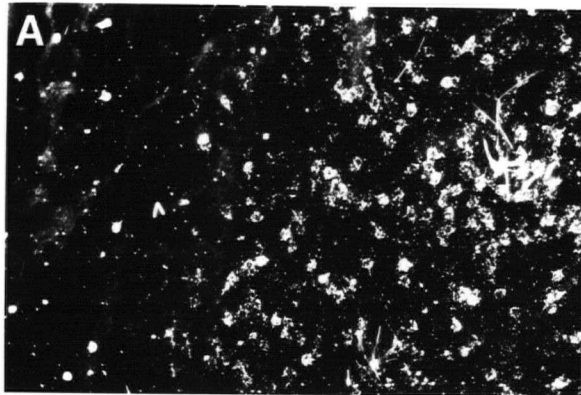
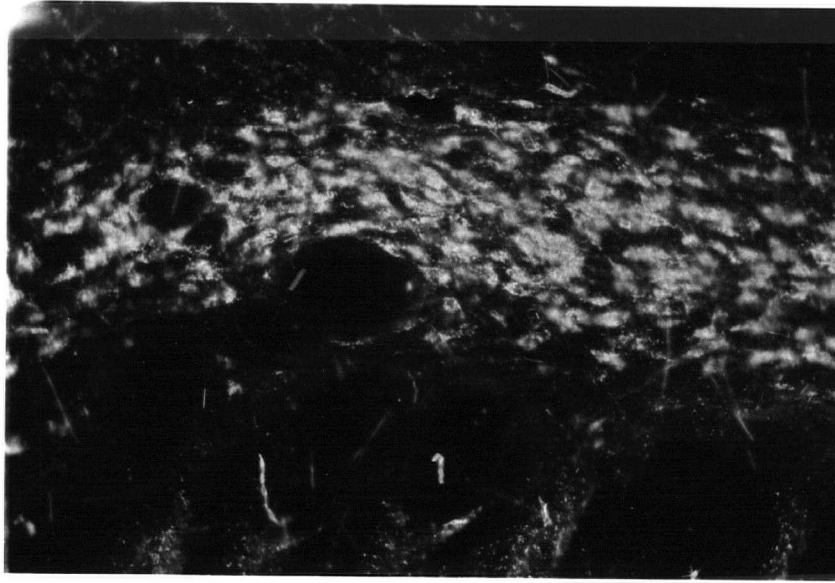
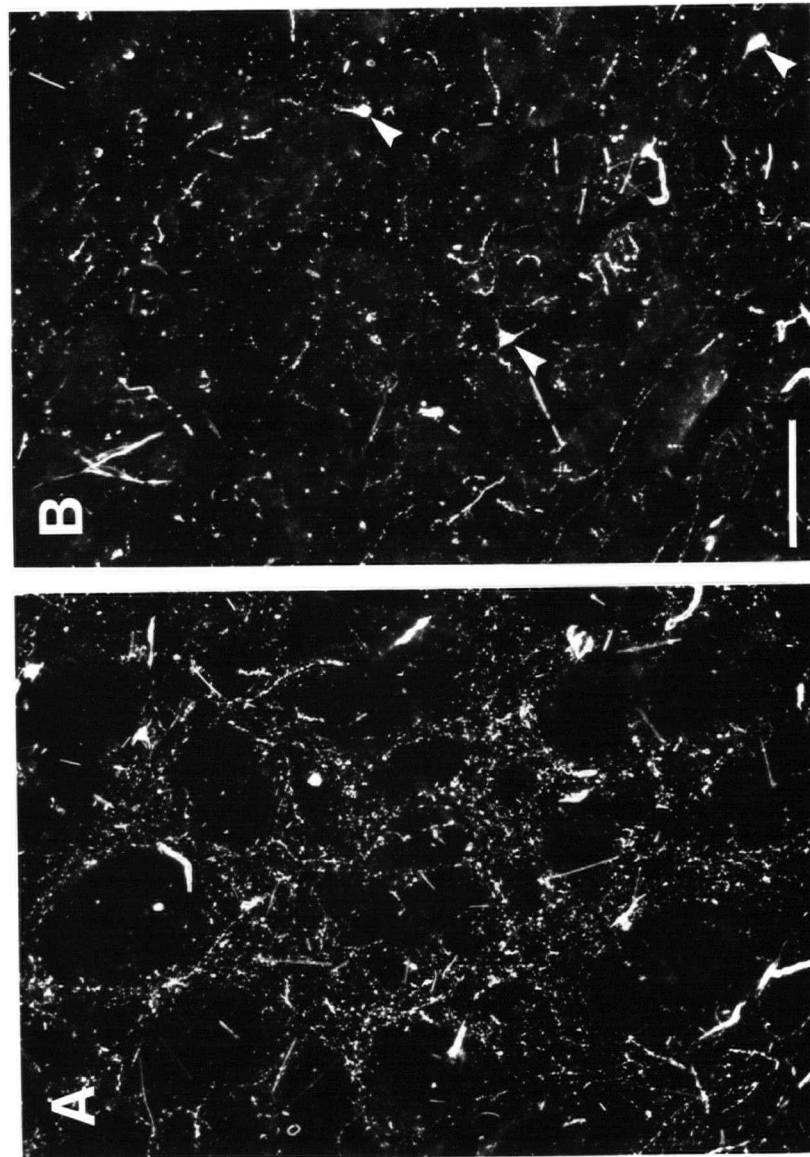


Figure 19. Labelling in the striatum (A) and globus pallidus (B) after a nigral injection restricted to the pars compacta (case SN-6). The large bright features seen within the striatum are artefactual, no labelled cells were seen in this field, although many labelled fibers can be seen within the striatal neuropil. Three labelled cells are shown by arrowheads within the globus pallidus. Labelled fibers can be seen within this structure as well. Bar = 100 um.





19  $\mu\text{m}$  to 28  $\mu\text{m}$  in diameter (mean = 23.7  $\mu\text{m}$ , sem = 1.0  $\mu\text{m}$ , n = 20). This was significantly greater than that of labelled striatal cells which ranged from 12  $\mu\text{m}$  to 21  $\mu\text{m}$  (mean = 15.6  $\mu\text{m}$ , sem = 0.6  $\mu\text{m}$ , n = 20). In addition, pallidal cells were more intensely labelled with reaction product (Fig. 18).

Anterograde labelling of both the striatum and globus pallidus was noted in all cases except those in which kainic acid had been coinjected with the WGA-HRP (Figs. 18A and 18B). The heavy field of punctate reaction product, characteristic of labelled terminal fields, was less apparent in the globus pallidus. Rather, the anterograde labelling took the form of heavily labelled single fibers. It was noted that a pars compacta injection (SN-5) produced prominent anterograde labelling throughout the striatum and globus pallidus (Fig. 19).

#### DISCUSSION

The present data demonstrate that the substantia nigra in the rat receives afferents from the peribrachial area bilaterally and projects efferents to the ipsilateral peribrachial region. Complementary anterograde and retrograde data were obtained after both nigral and peribrachial injections. This semireciprocal system has been reported previously in the cat (Moon-Edley and Graybiel, 1980; Nomura et al., 1980) and this report confirms the previous demonstration of an efferent projection to the ipsilateral peribrachial region in the rat (Beckstead et al., 1979). The correspondence with observations in the cat suggest that the distribution of labelling seen within the peribrachial region represents labelling of the nucleus tegmenti

pedunculopontis pars compacta described in other species (Kim et al., 1976; Larsen and McBride, 1979; Nauta, 1979a). Results from the present study indicating that the projections of this nucleus end predominantly in the substantia nigra pars compacta are in agreement with a study of the projections of the pedunculopontine tegmental nucleus in the rat (Saper and Loewy, 1982). The distribution of cells giving rise to this projection is similar to that of a diffuse group of neurons which stain intensely for acetylcholinesterase in the rat (Fibiger, 1982) and for choline acetyltransferase in the cat (Kimura et al., 1981). A cholinergic input to the dopaminergic cells in the substantia nigra is suggested from biochemical and neuropharmacological studies (Lichtensteigler et al., 1982; James and Massey, 1978) and lesion studies have previously ruled out a forebrain source for such an innervation (McGeer et al., 1971). However, some electrophysiological studies have found neurons of the pars compacta to be insensitive to iontophoretically applied acetylcholine, and instead, point to the marked excitatory effect on pars reticulata neurons as indicative of the relevant post-synaptic elements (Collingridge and Davies, 1981; Pinnock and Dray, 1982). It has been suggested that central cholinergic neurons are generally large cells (Fibiger, 1982) and therefore would more likely represent the less numerous cell type found in the pedunculopontine nucleus which appear to have a unilateral input to the substantia nigra.

It is interesting to note that, although the pedunculopontine nucleus projects predominantly to the pars

compacta, it receives afferents from the pars reticulata of the substantia nigra. A few cells in the pars compacta have been noted to project to this area by other authors (Jackson and Crossman, 1981b) but this may be attributed to the presence of some nondopaminergic cells within the pars compacta (see Beckstead et al., 1979).

The peribrachial region is particularly well connected with other components of the extrapyramidal system. It receives afferents from the ipsilateral and possibly contralateral entopeduncular nucleus (Nauta, 1979a; Moon-Edley and Graybiel, 1980; Larsen and McBride, 1979) via collaterals of the entopedunculothalamic pathway which arises from the "motor" portion of this nucleus (van der Kooy and Carter, 1981). A reciprocal relationship with the subthalamic nucleus has been suggested (Jackson and Crossman, 1981a). Additional inputs include those from the amygdala, hypothalamus and ventrocaudal regions of the striatum and globus pallidus (Jackson and Crossman, 1981b). As will be discussed in Experiment 3 this latter projection probably reflects afferents from nucleus basalis magnocellularis. Efferent projections from the pedunculo pontine nucleus have been described to all of the areas from which it receives afferents (Tohyama et al., 1978; Moon-Edley and Graybiel, 1980; Saper and Loewy, 1982). Some of the widespread connections presently attributed to this nucleus may, in fact, arise from the nucleus parabrachialis, the cell bodies of which surround the brachium conjunctivum and correspond almost exactly to the distribution of terminal labelling shown in

Figure 9G. This nucleus is a relay for second order gustatory and visceral afferents and has been shown to have afferents to the insular cortex, which others have attributed to the pedunculo pontine nucleus (Shiply and Sanders, 1982), and projections to the amygdala and ventromedial nucleus of the thalamus (Voshart and van der Kooy, 1981).

The present report indicates for the first time a totally crossed input to the nigra from a discrete region of the posterior lateral hypothalamus. Additional significance is attached to this finding in light of the observation that these same hypothalamic neurons appear to project to areas of the contralateral mediodorsal thalamus which are in receipt of nigral terminals. Previous studies have described nigral afferents from the ipsilateral lateral hypothalamus (Bunney and Aghajanian, 1976; Nauta and Domesick, 1978). In our material, when WGA-HRP injections were centered in the pars reticulata with spread to but not dorsal to the pars compacta, only two or three neurons were retrogradely labelled in the ipsilateral lateral hypothalamus, at levels considerably more rostral than the contralateral hypothalamic neurons labelled after pars reticulata injections. More dorsally placed injections, including the area just dorsal to the pars compacta labelled a considerably larger number of these ipsilateral anterior hypothalamic neurons.

Previous studies have demonstrated the existence of nigral input to the ipsilateral parafascicular and ventromedial thalamic nuclei, and to the ipsilateral and contralateral paralamellar portion of the mediodorsal thalamic nucleus (Faull and Carmen,

1968; Carpenter and Peter, 1972; Carpenter et al., 1976; Clavier et al., 1976; Faull and Mehler, 1978; Herkenham, 1979; Beckstead et al., 1979; Bentivoglio et al., 1979). The present data confirm and extend these reports with the demonstration of a minor nigral afferent connection from the ipsilateral parafasicular nucleus and a crossed nigral input to the ventromedial thalamic nucleus. Pritzel and Huston (1980) have reported that unilateral nigral lesions induce the contralateral substantia nigra to send fibers to the ventromedial nucleus ipsilateral to the lesion. However, the present findings and those of Herkenham (1979), who reported an occasional labelled neuron in the contralateral pars reticulata after HRP injections into the ventromedial nucleus, point to an existing crossed input to the ventromedial thalamus from the nigra in the unlesioned animal. Thus the report of an induction of this crossed system after unilateral nigral lesions may in fact have been a demonstration of the expansion of an already existing projection.

Particularly problematical among the present findings was the observation of terminal labelling in the laterodorsal thalamic nucleus after substantia nigra WGA-HRP injections. This nigral connection has not been described previously. The laterodorsal thalamic nucleus has been reported to receive afferents from the pretectal nuclei, subicular and presubicular cortex, dorsal mesencephalic nucleus, and subcuneiform nucleus (Ryska and Heger, 1979; Robertson et al., 1980). The latter three areas are in close proximity to the nigral WGA-HRP injection site. In the present study, neurons in each of these

areas were labelled after laterodorsal nucleus WGA-HRP injections. Although such injections also labelled cells in the pars reticulata, the possibility of minor diffusion of the thalamic injection site into the adjacent mediodorsal nucleus precludes a definitive identification of a nigral efferent system to the laterodorsal nucleus.

The connections of the thalamic nuclei that are in receipt of nigral input include ascending projections to the striatal and cortical regions that in turn provide descending input to the substantia nigra (Akert et al., 1979; Gerfen and Clavier, 1979; Herkenham, 1979; Krettek and Price, 1979; Beckstead, 1979a; Bentivoglio et al., 1981; Experiment 1), the superior colliculus and peribrachial areas that receive nigral input. While striatal afferents originating in the mediodorsal and ventromedial thalamic nuclei may be considered light, the parafascicular nucleus provides a massive input to the striatum. This thalamic nucleus is particularly well connected with regions involved in motor function and, as well as projecting to the cortex and striatum, the parafascicular nucleus projects to the inferior olive (Walberg, 1981), thereby allowing it to influence both the basal ganglia and cerebellar motor systems.

Using electrophysiological techniques, Deniau et al. (1977) demonstrated the existence of a crossed nigrotectal projection in the rat. Anatomical techniques have been used to demonstrate bilateral innervation of the superior colliculus by efferents from the substantia nigra in the cat and monkey (Hopkins and Niessen, 1976; Rinvik et al., 1976; Beckstead et al., 1981).

However, previous HRP injections into the rat superior colliculus have been reported to result in the labelling of only a few (1 or 2) neurons in the contralateral substantia nigra. These differences in distribution have prompted some authors to suggest species differences in the basic function of the basal ganglia (Beckstead et al., 1981). The present data, using complementary WGA-HRP injections into the superior colliculus and substantia nigra indicate that the nigrotectal projection is, in fact, bilateral in the rat. The contralateral nigral projection to the superior colliculus appears to be less extensive, in terms of both its distribution and the number of neurons contributing to the pathway, than its ipsilateral counterpart. However, the present labelling of over 200 neurons in the contralateral pars reticulata after collicular WGA-HRP injections suggest that this crossed pathway is much more extensive than previously suspected. The distribution of the crossed system, primarily into the lateral and ventral aspect of the rostral tectum, probably accounts for the failure of previous investigations, using more superficial and caudal injections, to label this pathway as extensively as was the case in the present study. Of further note is the suggestion of a topographic organization to the nigrotectal pathway; i.e. more dorsally located pars reticulata neurons projecting to the rostral superior colliculus and more ventrally located nigral neurons projecting to the caudal superior colliculus. For the most part previous studies have identified only ventral nigral neurons as contributing to the nigrotectal pathway (Faull and Mehler, 1978; Beckstead et al.,

1981), consistent with the fact that these studies employed injections of retrograde tracer into the caudal superior colliculus.

Some reports have parcelled the pars reticulata into separate regions which project to the superior colliculus or thalamus (Faull and Mehler, 1978), but recent double retrograde tracing and electrophysiological studies have shown that a large number of nigral neurons project to both areas (Bentivoglio et al., 1979; Anderson and Yoshida, 1980). In light of the present data it is likely that most, if not all nigral neurons which innervate the thalamus send a collateral to the tectum as well. A similar collateralization has recently been proposed between the nigrocollicular and nigropedunculopontine pathways in the monkey (Beckstead and Frankfurter, 1982). This conclusion was arrived at by indirect means however, and following the same line of reasoning would force the authors to conclude from their data that in the monkey there is no collateralization to the tectum and thalamus. Rather than point to species difference, this is likely a commentary on the limitations of the protocol.

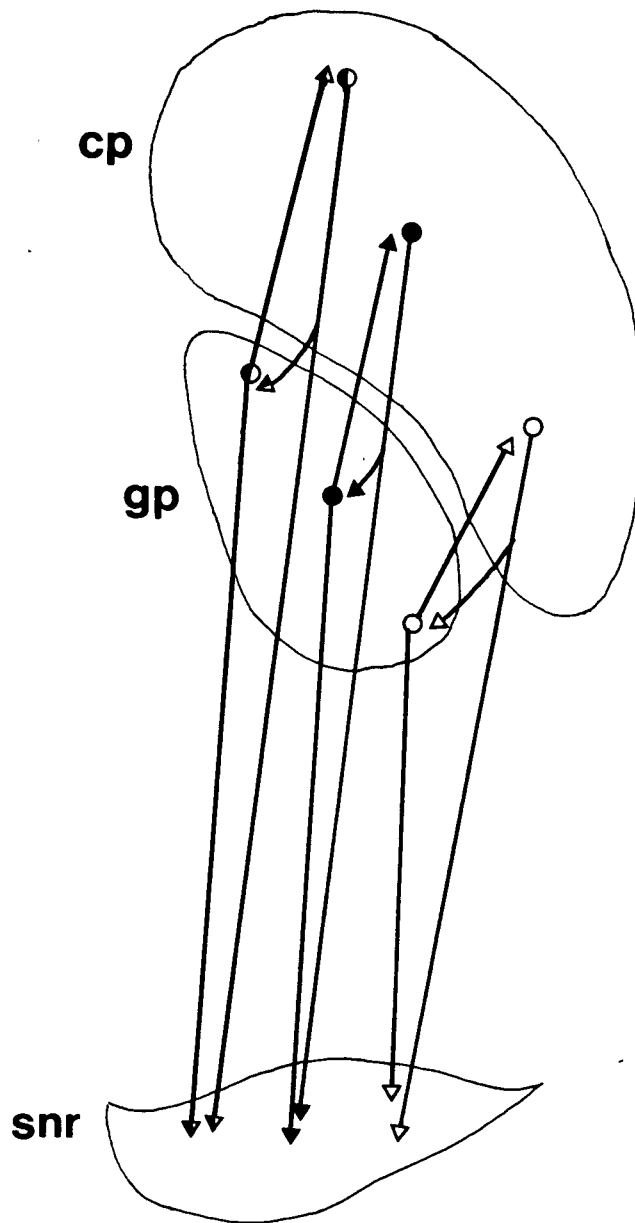
A point that has received little attention in the past is the remarkable similarity between the projections of the pars reticulata and those of cerebellar efferents ascending in the brachium conjunctivum (said to exclude those arising from the fastigial nucleus; Faull and Carman, 1978). These include the superior colliculus, dorsal midbrain tegmentum, parafascicular nucleus and ventromedial thalamus. The thalamic field innervated by the fastigial nucleus itself is virtually confined to the



ventromedial nucleus (Haroian et al., 1981), and, therefore, completely overlaps with that portion of the nigrothalamic projection. These overlapping inputs, together with the divergent inputs from the parafascicular nucleus, may allow for the coordination of basal ganglia and cerebellar influences on motor control.

The recognition that the subthalamic nucleus projects to the substantia nigra has been relatively recent (Kanazawa et al., 1976). Although it might be inferred from these authors' work that the subthalamic nucleus projects primarily to the pars compacta, in the present study the projection was determined to be predominantly to the pars reticulata. This partitioning agrees with a number of autoradiographic studies (Nauta and Cole, 1978; Carpenter et al., 1981). The subthalamic nucleus also projects to the nucleus pedunculopontinus, the globus pallidus and the entopeduncular nucleus (Nauta and Cole, 1978; Larsen and Suttin, 1978; Perkins and Stone, 1980; Jackson and Crossman, 1981). All of its connections to other basal ganglia nuclei are via collaterals from the same neurons (van der Kooy and Hattori, 1980a; Deniau, 1978a). Although not apparent from the present material there is convincing histochemical and biochemical evidence for a dopaminergic innervation of the subthalamic nucleus (Brown et al., 1979; Meibach and Katzman, 1979). Its major input arises from the globus pallidus (see Experiment 3) and it also receives afferents from the nucleus tegmenti pedunculopontis, a somatotopically organized input from the motor cortex and separate input from the prefrontal cortex

Figure 20. Diagrammatic representation of the topographical relationship between striatonigral pallidonigral and pallidostriatal projections. Collateralization is not implied.



(Hartmann-von Monakow et al., 1978).

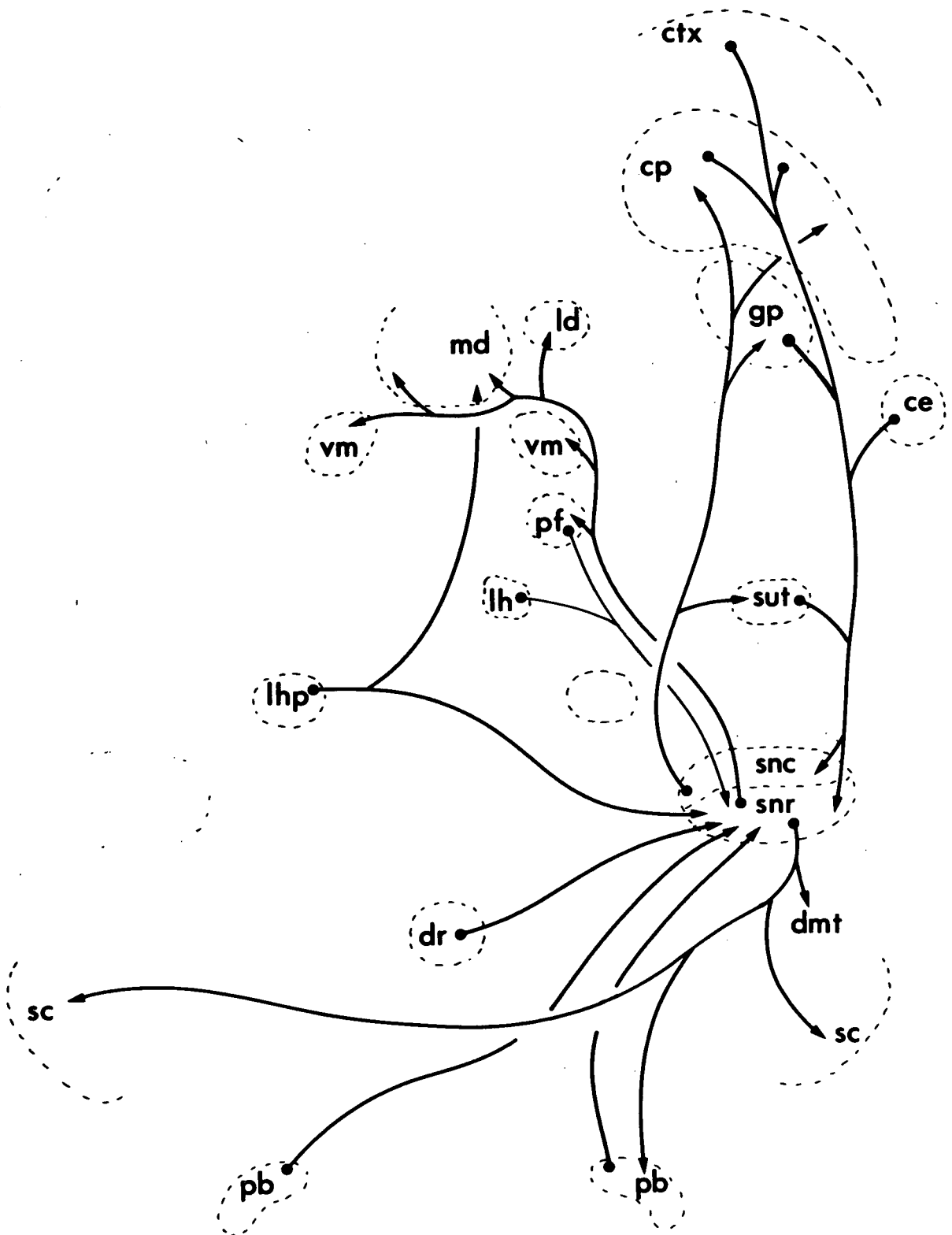
A topographical projection of the striatum to the substantia nigra has been demonstrated previously by both autoradiographic and HRP techniques (Tulloch et al., 1978). The present data confirm this observation and expand it to include a similar topography for the pallidonigral projection. In fact, the two distributions seem to be related to the pallidostriatal topography presented in Experiment 1. Pallidal regions projecting to a restricted region of the striatum also appear to project to the area of the substantia nigra to which that region of the striatum projects (see Figure 20).

#### SUMMARY

The substantia nigra pars reticulata receives a major topographically organized projection from the striatum and a topographically related projection from the globus pallidus. The pars compacta of the substantia nigra projects back to both of these structures, but anatomical evidence for any but a very low resolution feedback loop is lacking. Other major inputs to the substantia nigra include those from the subthalamic nucleus, prefrontal cortex and dorsal raphe. Moderate to light nigral innervation arises from the central nucleus of the amygdala, peribrachial region, lateral habenula, parafascicular nucleus, lateral hypothalamus and an anatomically distinct region of the contralateral hypothalamus.

The efferent connections of the substantia nigra are similarly quite divergent, with major innervation of the ventromedial and mediodorsal nuclei of the thalamus, the dorsal

Figure 21. Diagrammatic summary of the findings.



midbrain tegmentum, peribrachial region and superior colliculus arising from nondopaminergic pars reticulata neurons and a major projection to the striatum arising from the dopaminergic pars compacta neurons. Other nondopaminergic nigral projections include those to the parafascicular and laterodorsal nuclei of the thalamus. Minor dopaminergic innervations of the globus pallidus and subthalamic nuclei have been described.

These observations are summarized diagrammatically in Figure 21.

### EXPERIMENT 3: EXAMINATION OF THE EFFERENT AND AFFERENT CONNECTIONS OF THE GLOBUS PALLIDUS

#### INTRODUCTION

Although the connections of the internal segment of the globus pallidus (entopeduncular nucleus in the rat) have been the subject of a great deal of anatomical research (see van der Kooy and Carter, 1981) there have been relatively few studies conducted to examine the efferent and afferent connections of the external segment (globus pallidus in the rat). Some authors have concluded that this nucleus projects fibers solely to the subthalamic nucleus (Kim et al., 1976); others state that it innervates both the subthalamus and substantia nigra (Hattori et al., 1975; Carter and Fibiger, 1978) and still others describe possible additional connections to the pons, the neocortex and the reticular nucleus of the thalamus (Nauta, 1979a; McBride and Larsen, 1980). The difficulties represented by this lack of consensus lie largely in the lack of sensitivity of past techniques in revealing diffuse projections and in the inability to restrict the injection site to the nucleus of interest (McBride and Larsen, 1980; DeVito et al., 1980). Furthermore, to date there has been no systematic study of the afferents to the globus pallidus in the rat, although it is known from anterograde studies to receive fibers from the subthalamic nucleus, substantia nigra, dorsal raphe and a massive innervation from the striatum.

The present study reports the distribution of the efferents and afferents of the globus pallidus revealed using injections of



WGA-HRP into this nucleus. Particular attention was paid to an examination of ascending efferents to the striatum predicted from the results of Experiment 1.

#### METHODS

Rats were anesthetized with pentobarbital and the skull trephaned for either a vertical or angled approach to the globus pallidus. WGA-HRP was injected stereotaxically through a 5 ul syringe. Injections of 0.1 ul of 2% WGA-HRP were made over a 5 minute period and the cannula left in place for a further 5 minutes. After a survival time of from 24 to 48 hrs, animals were reanesthetized and fixed by transcardial perfusion as described in Experiment 1. Tetramethylbenzidine was used as a peroxidase substrate for the reaction of slide mounted or free-floating sections as described in Experiment 1. In some cases, free-floating sections were reacted for peroxidase with diaminobenzidine as substrate (Graham and Karnovsky, 1966), as detailed in Experiment 2.

#### RESULTS

Four cases involving different pallidal injections were examined and related to the injection locus. The majority of the results presented pertain to the large pallidal injection (Case GP-1) depicted in Figure 22. The WGA-HRP deposition in the cortex in this animal was negligible and the majority of the labelling along the cannula tract was due to an accumulation of erythrocytes. The distribution of labelling seen in this case is depicted in Figure 23. A second case (GP-2) occupied the same coordinates but was much smaller. In case GP-3 a large injection

Figure 22. Photomicrograph of a counterstained section showing WGA-HRP injection into the globus pallidus in case GP-1. Peroxidase reaction using diaminobenzidine substrate.

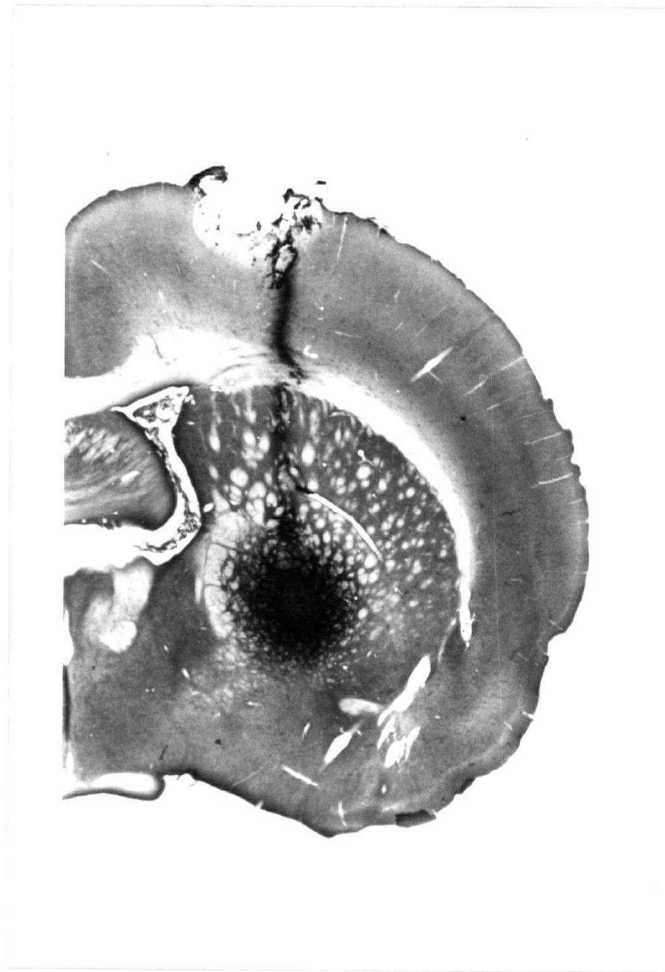


Figure 23. Line drawings depicting the anterograde and retrograde labelling in case GP-1. Retrogradely labelled cells are denoted by filled circles. Anterogradely labelled fibers and terminals are drawn in. Abbreviations used in this and subsequent figures; AC, anterior commissure; BC, brachium conjunctivum; cp, striatum; CP, cerebral peduncle; dr, dorsal raphe nucleus; ep, entopeduncular nucleus; gp, globus pallidus; F, fornix; FM, forceps minor; FR, fasciculus retroflexus; IC, internal capsule; h, habenular complex; md, mediodorsal nucleus of the thalamus; ML, medial lemniscus; MT, mammillothalamic tract; ntp, nucleus tegmenti pedunculopontis; pf, parafascicular nucleus; rr, retrorubral area; rt, reticular nucleus of the thalamus; snc, substantia nigra pars compacta; snr, substantia nigra pars reticulata; sut, subthalamic nucleus; vl, ventrolateral nucleus of the thalamus; vm, ventromedial nucleus of the thalamus.



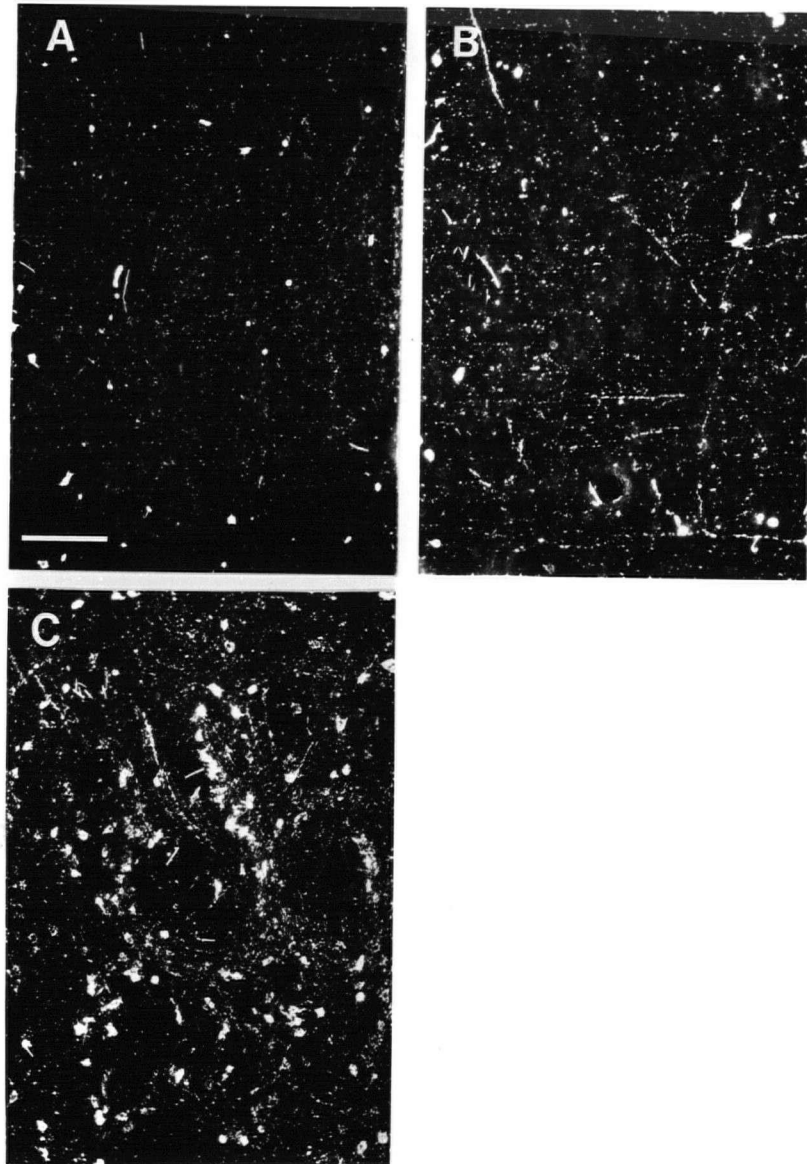
site spread throughout the caudal globus pallidus, internal capsule and anterior extreme of the entopeduncular nucleus and the injection site of GP-4 involved the caudal globus pallidus, internal capsule, entopeduncular nucleus and part of the reticular nucleus of the thalamus.

#### EFFERENT PROJECTIONS

The results of the anterograde transport are largely in agreement with previous work (Carter and Fibiger, 1978). By far the heaviest labelling was seen in the subthalamic nucleus. The substantia nigra and entopeduncular nucleus showed moderate anterograde labelling, and light labelling of the reticular and mediodorsal nuclei of the thalamus were seen. All cases gave rise to retrograde labelling of cells in the striatum, within which moderate levels of fine extraperikaryal reaction product were also seen, indicative of a sparse terminal field labelled by anterograde transport (Fig. 23).

In cases GP-1 and GP-2 a field of faint terminal labelling was seen throughout layers III to V of the dorsal cortex anterior to the level of the globus pallidus (Fig. 24A). This feature was far more prominent when the pallidal injection sites were centered caudally and medially in the nucleus (GP-3 and GP-4). In these cases, fibers could be seen very clearly throughout large areas of the neocortex. Cortical fibers were observed running along the dorsal surface of the corpus callosum in layer VI, radiating out through the cortical layers at right angles to the cortical surface and branching out parallel to the cortical surface in all cortical layers (Fig. 24B). In animals showing

Figure 24. (A) Sparse anterograde labelling in the cortex of GP-1. All of the larger bright features are red blood cells. (B) Anterograde labelling in the cortex in case GP-3. Note that in this case heavily labelled fibers can be seen. The field depicted shows layers VI (at bottom) through III. (C) Heavily labelled fibers can be seen running through the striatum in case CP-3. Some anterograde and retrograde labelling of striatum can be seen as well, but this field does not portray the main site of striatopallidal interconnections. Bar = 100 um.





heavy and distinct cortical labelling, heavily labelled fibers were seen projecting through the striatum (Fig. 24C) and traversing the corpus callosum to branch within the cortex. The characteristics of labelled fibers found in the cortex in these rats corresponds closely to a recent description given to the cortical projection of the nucleus basalis magnocellularis (Fibiger, 1982).

In case GP-1 the head of the striatum contained a field of terminal labelling in and around the area that contained retrogradely labelled cell bodies (Fig. 25). No heavily labelled fibers were seen traversing the striatum in this case; such features appeared only in animals with dense labelling of the afferents to the cortex. The reaction product within the striatal neuropil in case GP-1 was much more dense than that found in experiments in which anterograde transport to the CP had been blocked by the coinjection of kainic acid with the WGA-HRP (Fig. 25). In case GP-1, neither retrograde nor anterograde labelling was seen in the tail of the striatum, but both were seen in this area in cases GP-3 and GP-4.

Figure 26A depicts the labelling seen in the reticular nucleus in case GP-1. This was a consistent feature of all cases examined. The light labelling followed the contours of this nucleus throughout its rostrocaudal extent. Wide areas of the ventrolateral thalamus also contained a punctate reaction product somewhat above background levels, possibly indicating a very sparse innervation of this region (Fig. 23 and 26A). A very restricted field of moderately intense terminal labelling was

Figure 25. (A) Light field photomicrograph of the striatum in case GP-1. Retrogradely labelled neurons were found within fields of diffuse neuropil staining thought to represent peroxidase activity transported into pallidostriatal fibers and terminals. A similar distribution of labelling within the striatum is found after injections into the substantia nigra (B) but note that after blocking anterograde transport by coinjection of kainic acid into the substantia nigra the diffuse labelling of the neuropil is greatly reduced (C). Bar = 20  $\mu$ m.

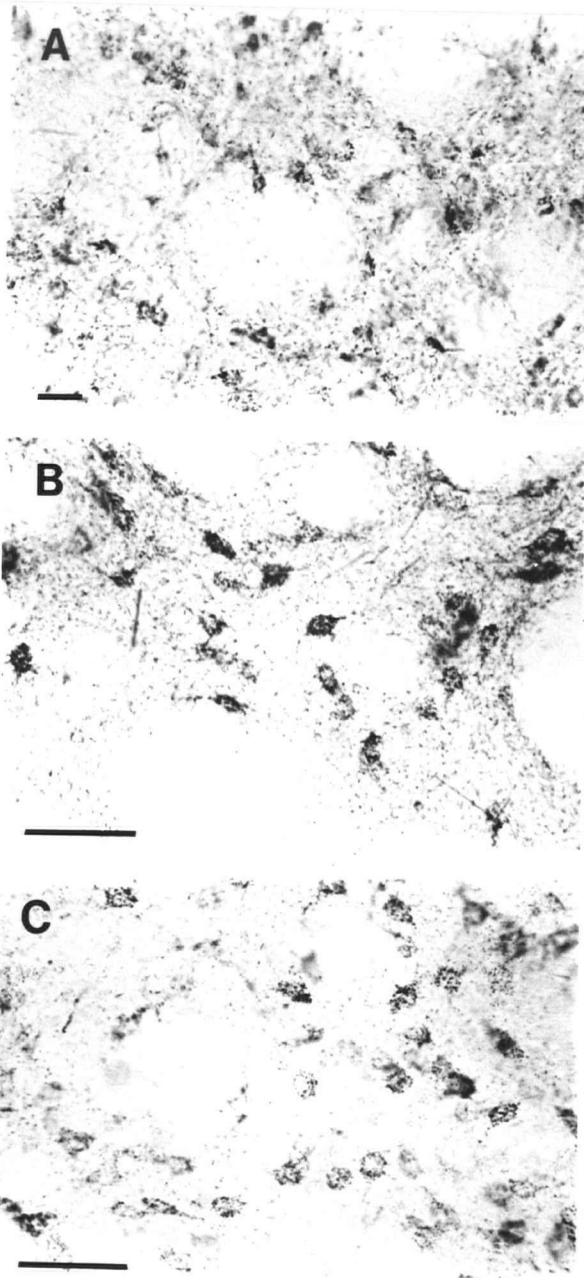
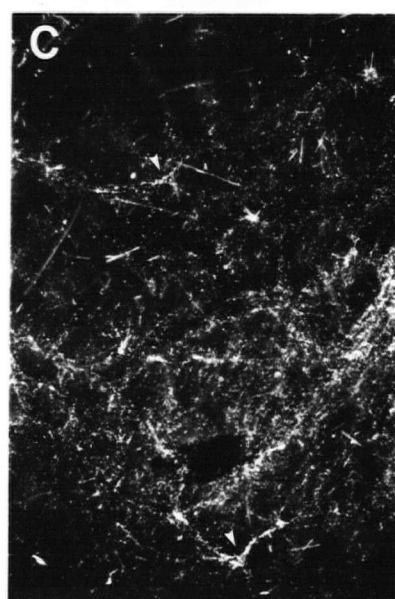
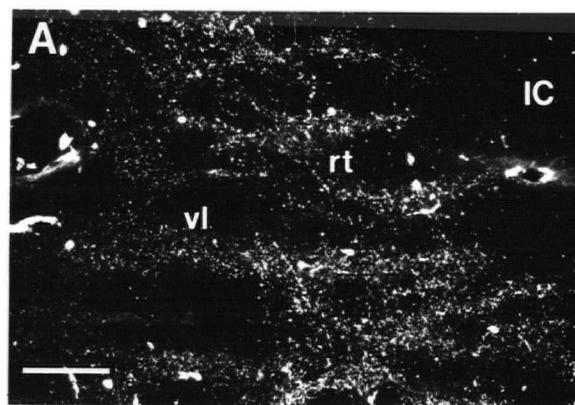


Figure 26. (A) Anterograde labelling of the reticular nucleus of the thalamus in case GP-1. The dark region at the far right is the internal capsule, and the ventrolateral thalamus occupies the left third of the field. (B) Anterograde labelling in the mediodorsal nucleus of the thalamus in case GP-1. Note the restricted terminal field and its reticular appearance. The lateral habenula can be seen at the top of the field. (C) Anterograde labelling in the entopeduncular nucleus in case GP-1. Some of the positive fibers in this field are within internal capsule fiber bundles but many are within the neuropil. The reticular appearance of the labelling within this nucleus contrasts markedly to that seen after striatal injections. The arrows denote heavy labelling outlining neurons in the entopeduncular nucleus; these cells are not retrogradely labelled but rather appear to receive a dense innervation around the region of their cell body. Bar = 100  $\mu$ m.



seen within the mediodorsal nucleus of the thalamus (Fig. 26B). A remarkable feature of the labelling in this region was its irregular or reticular appearance rather than the diffuse field seen after the labelling of striatal efferents (see Experiment 1). A similar pattern of terminal labelling was seen in the other cases except that GP-4 showed dense termination in the medial part of the ventrolateral nucleus and in the lateral habenular nucleus and the zona incerta, undoubtedly reflecting the involvement of the entopeduncular nucleus in the injection site (Carter and Fibiger, 1978).

In two cases (GP-1 and GP-2), the WGA-HRP injection site was confined to the globus pallidus with no spread to the entopeduncular nucleus. In both of these cases, anterograde labelling was apparent within the neuropil of the entopeduncular nucleus which did not appear to derive merely from labelled projection fibers passing through this nucleus. This putative terminal field had a very irregular appearance, and in some instances, seemed to outline the cell bodies of neurons in the entopeduncular nucleus (Fig. 26B).

By far the heaviest terminal field in case GP-1 was seen in the subthalamic nucleus (Fig. 27). A few retrogradely labelled cell bodies could be seen within this nucleus as well (Fig. 27B). In case GP-4 terminal labelling was far less dense while the number of cell bodies labelled was much greater (Fig. 27C).

A faint, diffuse terminal field was seen in the parafascicular nucleus in all cases (Fig. 29A). This field corresponded with the distribution of labelled cell bodies that

Figure 27. (A) Anterograde labelling of the subthalamic nucleus and its intrusion into the cerebral peduncle. (B) Higher power bright field photomicrograph of the subthalamic nucleus in case GP-1 showing the presence of retrogradely labelled cell bodies (arrows). (C) Bright field photomicrograph of the subthalamic nucleus in case GP-4. Note that in this case the anterograde labelling of the nucleus is much more sparse. Bar = 50  $\mu$ m.

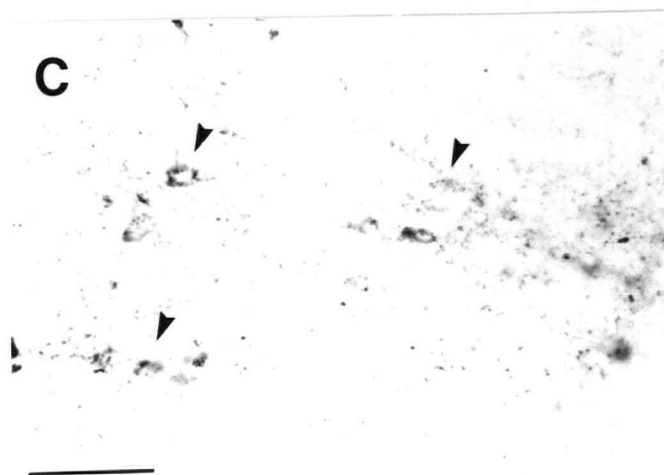
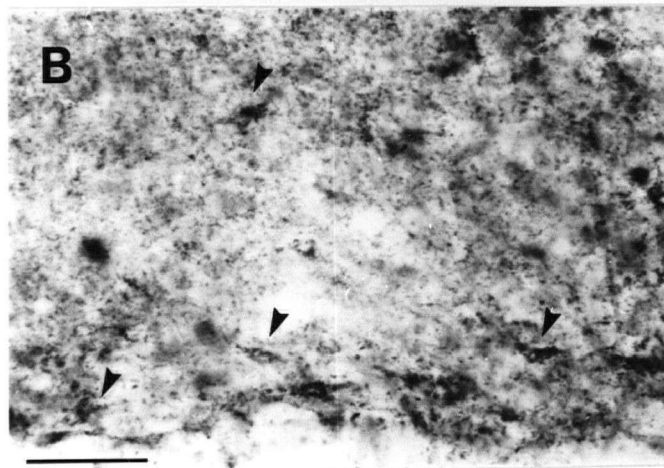
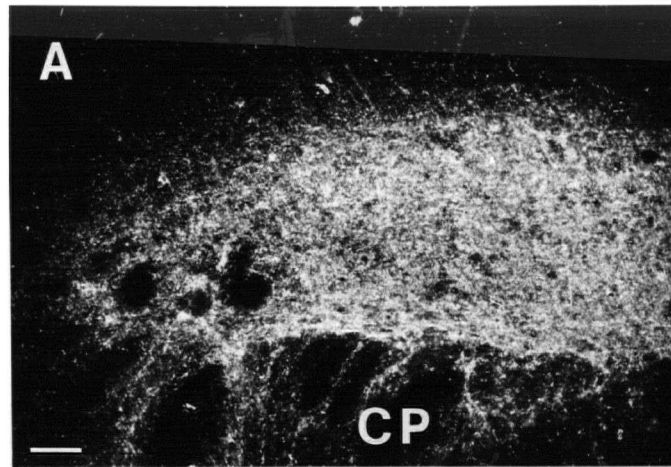
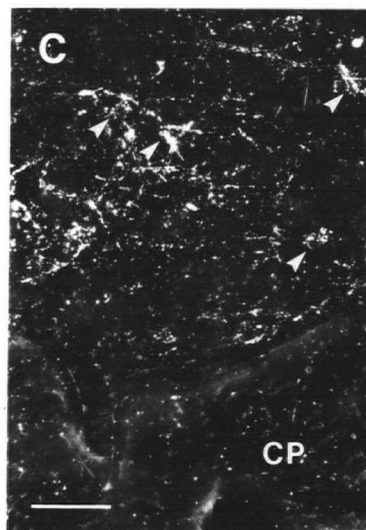
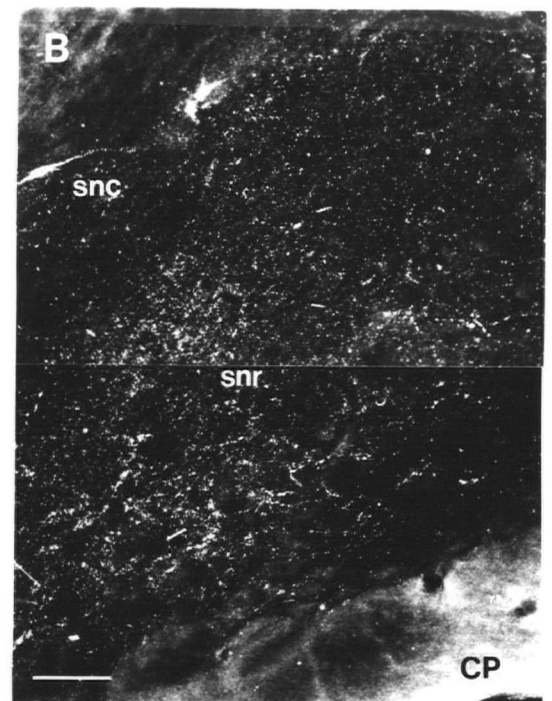
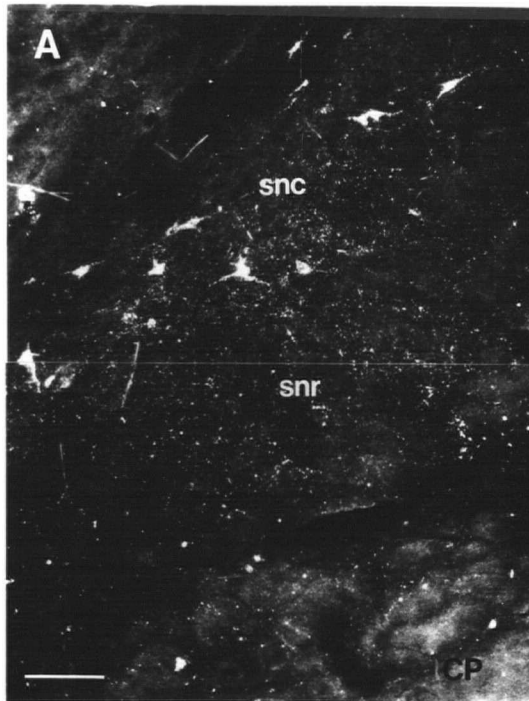




Figure 28. (A) Substantia nigra of GP-1. Note the presence of labelled cell bodies in pars compacta and anterograde labelling in pars compacta and pars reticulata. (B) At a more caudal level in the same case, all of the anterograde labelling is seen in pars reticulata. (C) The appearance of the anterograde labelling in the pars reticulata in case GP-3 in darkfield and (D) lightfield after counterstaining with cresyl violet. The features suggestive of dense termination around cell bodies are denoted by arrows in (C) and by asterisks in the corresponding field in (D). Bar = 100  $\mu$ m.



were sometimes found in this region (see below). The terminal field in this region appeared to be a caudal continuum of the labelling seen in the mediodorsal nucleus of the thalamus but it did not share the same irregular appearance.

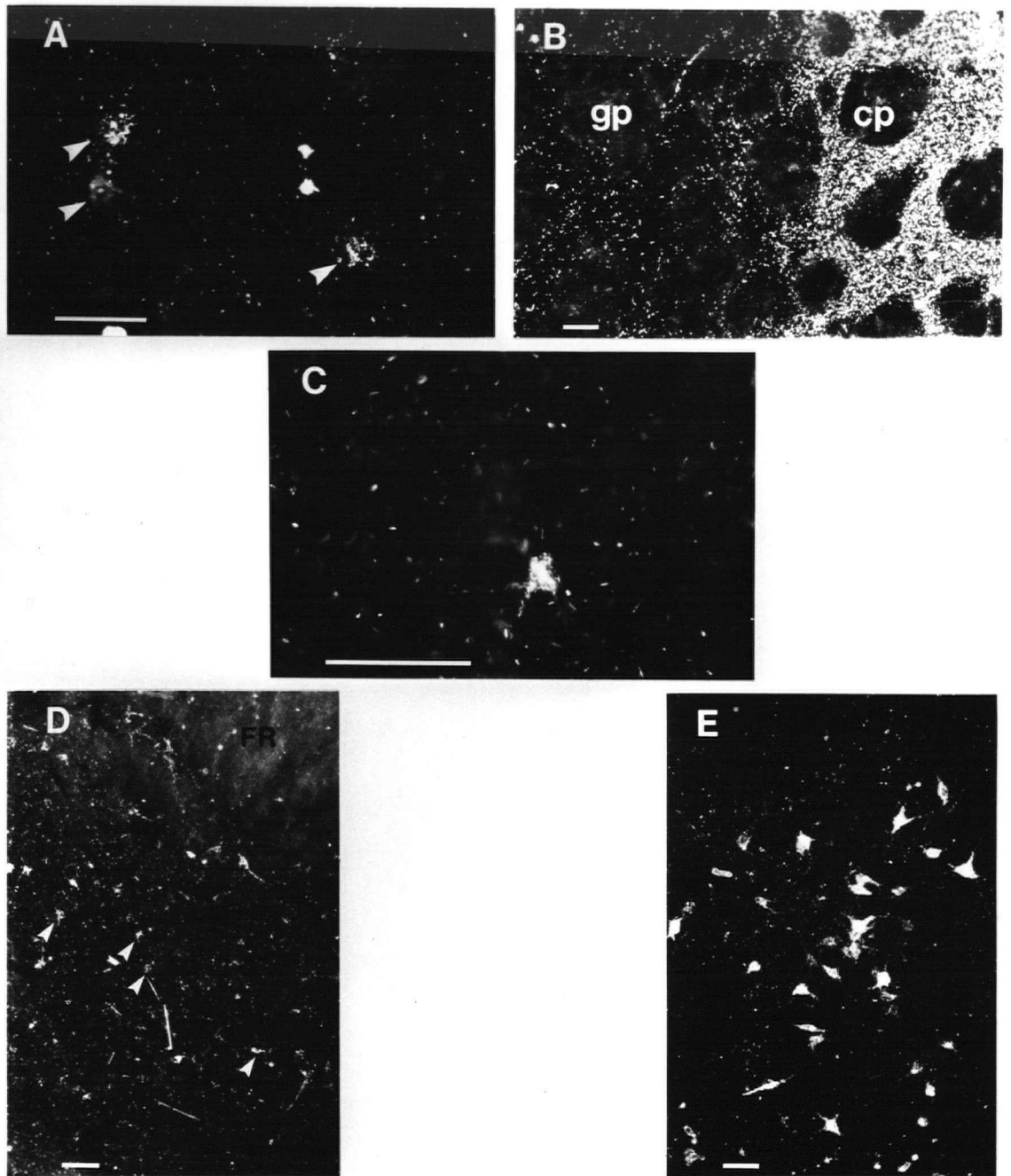
Within the substantia nigra the predominant terminal labelling was found in the pars reticulata. Some anterograde labelling of the pars compacta was seen at rostral levels (Fig. 28A) but more caudally, the labelling within this division was almost exclusively within cell bodies (Fig. 28B). The terminal labelling in the substantia nigra was very different from that seen after striatal injections (see Expt. 1), but similar to the labelling of the entopeduncular nucleus. It seemed that labelled elements had distinct targets within the neuropil of the pars reticulata. The labelling was very reticular and uneven, some labelled elements seemed to form bundles and others were concentrated around perikarya and proximal dendrites of unlabelled neurons within the pars reticulata (Fig. 28C and 28D) and pars lateralis. Although difficult to ascertain, there did not appear to be a similar concentration of labelled fibers around the retrogradely labelled neurons.

Anterograde labelling caudal to the substantia nigra was found only in case GP-4 in which a moderately dense terminal field was seen in the region of the brachium conjunctivum on the side ipsilateral to the injection.

#### AFFERENT PROJECTIONS

A few faintly staining neurons were found in the prefrontal cortex after all pallidal injections (Fig. 29B). No cortical

Figure 29. (A) Very faintly labelled cortical cells (arrows) in case GP-2. (B) Anterograde labelling of the striatum (right) and globus pallidus (left) after a WGA-HRP injection into the frontal cortex. Heavily labelled fiber bundles (presumably corticofugal) were seen within the globus pallidus but are outside the field of this photomicrograph. (C) Retrogradely labelled cell in the ventromedial nucleus of the thalamus in case GP-1. (D) Weakly labelled cells (arrows) and fibers (asterisk) in the parafascicular nucleus in case GP-1. (E) Retrograde labelling of cell bodies in the dorsal raphe nucleus of case GP-1. Bar = 50 um.



cells were found caudal to the rostral tip of the striatum. WGA-HRP injections into the prefrontal cortex led to a dense and diffuse labelling of the striatum but also revealed a light labelling of the globus pallidus (Fig. 29C).

Many neurons in the striatum were labelled. A few lightly stained cell bodies were seen in the ventrolateral, ventromedial, parafascicular and intralaminar nuclei of the thalamus (Fig. 23D to 23F and Fig. 29) of case GP-1. Comparison with other cases confirmed the observations in the ventral thalamus but not those found in the intralaminar group. A few labelled neurons were found in the caudal extreme of the entopeduncular nucleus but it was felt that these cells may have been part of the rostral subthalamic nucleus in which labelled neurons were numerous. Within the substantia nigra labelled cell bodies were found mainly in the pars compacta. At more caudal levels cells were seen within the pars reticulata and pars lateralis as well. In the caudal extreme of this nucleus, cells formed a continuum with labelled cells in the retrorubral area (Fig. 23H). This distribution was similar in all cases. The dorsal raphe nucleus contained a large number of labelled cell bodies (Fig. 23H, Fig. 29D).

The region around the brachium conjunctivum was examined carefully in all cases but no labelled cells could be found in cases GP-1 or GP-2. One neuron was located in this region in case GP-3 and in GP-4 distinct groups of labelled cells were found both ipsilateral and contralateral to the injection corresponding to the nucleus tegmenti pedunculoopontis.

## DISCUSSION

The greater morphological detail provided by the use of anterograde peroxidase compared with the autoradiographic technique (due to the localization of precipitate within the tissue rather than in an overlying emulsion) proved to be of great value in the present study. The distribution of pallidal efferents depicted in Figure 23 is in good agreement with the results obtained by Carter and Fibiger (1978). However, whereas they were unable to conclude from their data that the globus pallidus innervates the reticular nucleus of the thalamus or the entopeduncular nucleus, the present data indicate both connections. The labelling of these structures was beyond the diffusion boundaries of the injection site and differed markedly in appearance from that which would be attributed to diffusion of tracer. Previous autoradiographic (Hattori et al., 1975) and retrograde HRP studies (DeVito et al., 1980) have given indications of a pallidal projection to the entopeduncular nucleus (or its counterpart in the primate). The previous literature on a projection to the reticular nucleus of the thalamus is controversial (Hattori et al., 1975, McBride and Larsen, 1980). The reticular nucleus of the thalamus has not been previously thought to be related to the basal ganglia, but it does receive cortical input from the same region which projects to the substantia nigra (Gerfen and Clavier, 1979; Reep and Winans, 1982) and receives collaterals of the thalamostriatal projection originating from the intralaminar cell groups (Nguyen-Legros et al., 1982).

A very sparse anterograde labelling of parts of the ventral thalamus and mediodorsal thalamus has been detected previously (Carter and Fibiger, 1978) but was not regarded as pallidal in origin. In agreement with these studies, injections of retrograde fluorescent tracers into the thalamus failed to label neurons in the GP of the cat (Parent and de Bellefeuille, 1982) or the rat (van der Kooy and Carter, 1981). On the other hand, Herkenham (1979) did observe neurons in the ventral globus pallidus after HRP injections into the ventromedial nucleus of the thalamus and raised the possibility that neurons of the nucleus basalis magnocellularis (see Fibiger, 1982) rather than pallidal neurons may project to the thalamus. Similar conclusions have been reached regarding the innervation of the mediodorsal nucleus of the thalamus (Sapawi and Divak, 1978; Gerfen et al., 1982). However, it has been suggested that the term nucleus basalis magnocellularis be reserved for neurons staining intensely for acetylcholinesterase (Lehmann et al., 1980), and those neurons within the globus pallidus that project to the mediodorsal nucleus of the thalamus do not appear to contain high levels of this enzyme (Hardy et al., 1976). As most of the cells in this region which project to the thalamus are localized ventral to the globus pallidus (Carter and Fibiger, 1978, Gerfen et al., 1982), it seems likely that the neurons found within the boundaries of the globus pallidus represent outlying members of a second (noncholinergic) population of basal forebrain neurons.

Controversy also surrounds the innervation of the substantia



nigra by the globus pallidus. Early reports of a projection to the substantia nigra indicated that the majority of the innervation was confined to the pars compacta (Hattori et al., 1975). More recent work has suggested that the globus pallidus innervates the pars reticulata as well (Carter and Fibiger, 1978) or exclusively (Grofova, 1975; McBride and Larsen 1980). The present data are consistent with a major innervation of the pars reticulata and, furthermore, indicate an innervation of the soma and proximal dendrites of neurons within this area. This is in agreement with morphological studies showing a large number of axosomatic terminals of pallidal origin in the substantia nigra (Hattori et al., 1975).

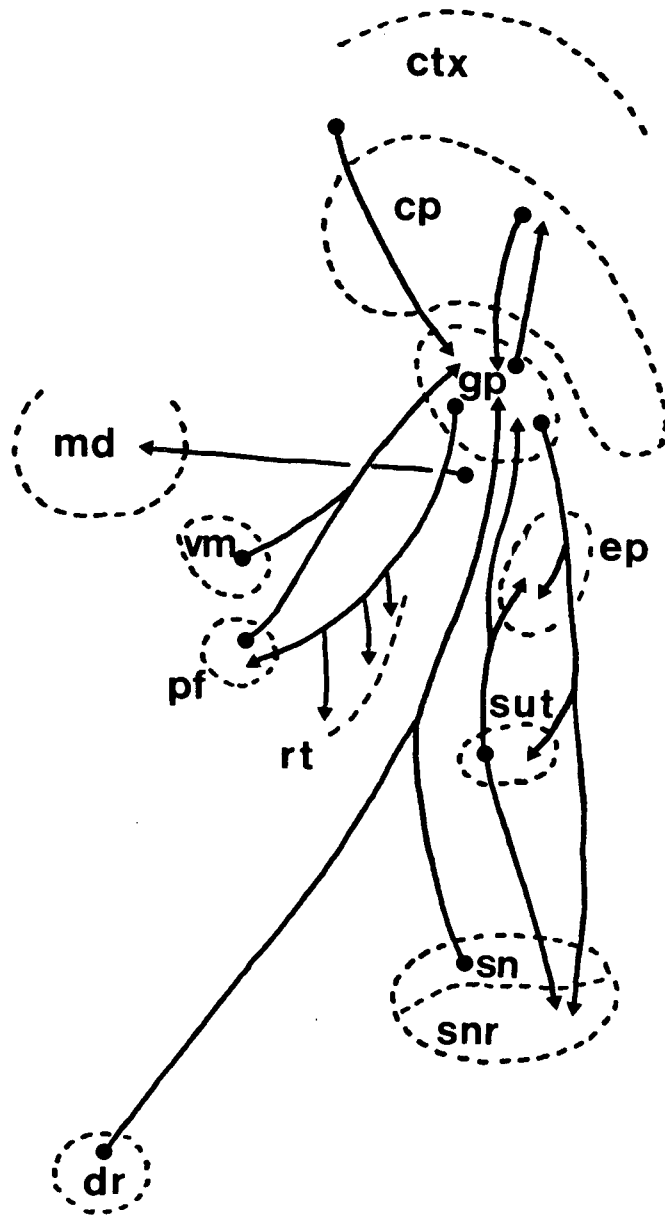
The observation of presumed anterograde labelling in the striatum in and around the regions containing retrogradely labelled neurons lends support to evidence for a pallidal projection to the caudate-putamen in the rat (see Experiment 1). Previously, similar observations were attributed to diffusion of tracer or the labelling of the cortical projection arising from neurons of the nucleus basalis magnocellularis, some of which occupy pallidal or peripallidal regions (Carter and Fibiger, 1978; Nauta, 1979a; McBride and Larsen, 1980). This phenomenon was shown to occur here with caudal pallidal injections of tracer. However, the anterograde labelling of the cortex in case GP-1 was negligible compared to that seen within the striatum. The present data therefore add support to the proposal that a pallidostriatal projection exists and indicates that it may have a reciprocal topographical relationship to the striatopallidal

projection. Conclusive evidence for a pallidostriatal projection awaits the demonstration of labelled terminals on pallidostriatal fibers (see below).

It has been claimed on the basis of retrograde transport studies that the globus pallidus, like the entopeduncular nucleus, projects to the nucleus tegmenti pedunculopontis (Jackson and Crossman, 1981b). Like others (Carter and Fibiger, 1978; Nauta, 1979a; McBride and Larsen, 1980), the present experiments did not provide any indication of this. The distribution of neurons portrayed by Jackson and Crossman (1981b) is very similar to that of the histochemical designation of the nucleus basalis magnocellularis (Fibiger, 1982). A caudally directed projection from this cell group to the hindbrain has been indicated (Divak, 1975), and it may be these neurons, rather than those of the globus pallidus, that project to the nucleus tegmenti pedunculopontis or adjacent regions.

There is now little doubt of a dopaminergic input, albeit light, to the globus pallidus from the substantia nigra (Lindvall and Bjorklund, 1979; DeVito et al., 1980). Pallidal inputs from the subthalamic nucleus, dorsal raphe, and striatum also have a large literature (Nauta and Cole, 1978; van der Kooy and Hattori, 1980a; Ricardo, 1980; Carpenter et al., 1981; Nagy et al., 1978a; DeVito et al., 1980; Brann and Emson, 1980; Parent et al., 1981b; Pasik et al., 1981). However, no previous report of a cortical projection to the globus pallidus has appeared. Diagrammatic representations of the data in a recent paper (Reep and Winans, 1982) appear to provide support for a minor input to the globus

Figure 30. Schematic diagram summarizing the efferent and afferent connections of the globus pallidus.



pallidus. This cortical region also projects to the substantia nigra and the reticular nucleus of the thalamus (Gerfen and Clavier, 1979; Reep and Winans, 1982). Mutually confirmatory anterograde and retrograde demonstrations of this projection were obtained in the present study but both indicated that it is minor.

Some previous indications of a thalamic projection to the globus pallidus can be cited in support of the observations presented here (Herkenham, 1979; DeVito et al., 1980), but for the present these data should be taken only as encouragement for further study.

#### SUMMARY

In summary, the present results confirm a massive projection from the globus pallidus to the subthalamic nucleus and a more modest innervation of the pars reticulata of the substantia nigra. Additional efferents of the globus pallidus include those to the striatum, entopeduncular nucleus and the reticular and parafascicular nuclei of the thalamus. Major inputs to the globus pallidus arise from the striatum, subthalamic nucleus, dorsal raphe nucleus and substantia nigra. Evidence was obtained for minor inputs to the globus pallidus from the prefrontal cortex, the parafascicular nucleus and the ventrolateral and ventromedial nuclei of the thalamus. These observations are summarized schematically in Figure 30.

EXPERIMENT 4: LIGHT AND ELECTRON MICROSCOPIC DEMONSTRATION OF A  
PROJECTION FROM THE GLOBUS PALLIDUS TO THE STRIATUM  
IN THE RAT

INTRODUCTION

Evidence obtained from retrograde transport studies on the connections of the striatum, described in Experiment 1, indicated that this nucleus receives a projection from the globus pallidus (GP). This finding is quite startling both in terms of the implications it has to present concepts of basal ganglia function and the fact that such a connection has gone previously undetected in a system that has been subjected to such intense neuroanatomical research. Previous studies examining the efferents of the globus pallidus (McBride and Larsen, 1980; Kim et al., 1976; Carter and Fibiger, 1978; Carpenter et al., 1981) or the afferents of the striatum (Royce, 1978; Oka, 1980; Veening et al., 1980; Royce, 1982) have made no mention of this projection. The following experiments were designed to investigate further the possibility that the globus pallidus does indeed project to the striatum, and to gather anatomical data about this projection which might serve as a basis for speculation concerning the role of this pathway in the functional organization of the basal ganglia.

In the present study, the phenomenon of retrograde labelling of pallidal neurons after injection of WGA-HRP into the striatum was examined in detail to (1) confirm that the apparent labelling of these neurons was due to intracellular accumulation of peroxidase, (2) rule out the possibility that neurons were

labelled trans-synaptically, and (3) investigate the relationship between the pallidostriatal and striatopallidal pathways. Some of the observations reported in this experiment have been previously published (Staines et al., 1981).

#### METHODS

Male Wistar rats (Woodlyn Farms, Guelph, Ontario) were anesthetised with pentobarbital and injected stereotaxically with 0.05 to 0.10  $\mu$ l of 0.25 to 1.0% solutions of WGA-HRP in saline or sterile saline containing 10 mM kainic acid (KA, Sigma). WGA-HRP was synthesized as described previously (Staines et al., 1980b). Four to forty-eight hours after the injection the animals were anesthetized with pentobarbital and perfused transcardially with 100 mM sodium phosphate buffer (PB) containing 0.9% NaCl, followed by a 1/2 hr perfusion with glutaraldehyde and formaldehyde (1%:1%) in PB at room temperature. The aldehydes were washed out by perfusion with ice cold 10% sucrose in PB and brains were stored 3 hrs in this solution before cutting. Sections 50  $\mu$ m thick were cut on a freezing microtome and processed for HRP by the tetramethylbenzidine (TMB) method (Mesulam 1978; Experiment 1). Alternate sections were counterstained with 1% neutral red (NR). Some unreacted sections at the level of the injection site were stained with cresyl violet for histological examination. Measurements of the dimensions of labelled neurons were made using a grid eyepiece to observe sections which had been reacted for peroxidase activity using diaminobenzidine (DAB) as substrate (see Experiment 2) and counterstained with cresyl violet.

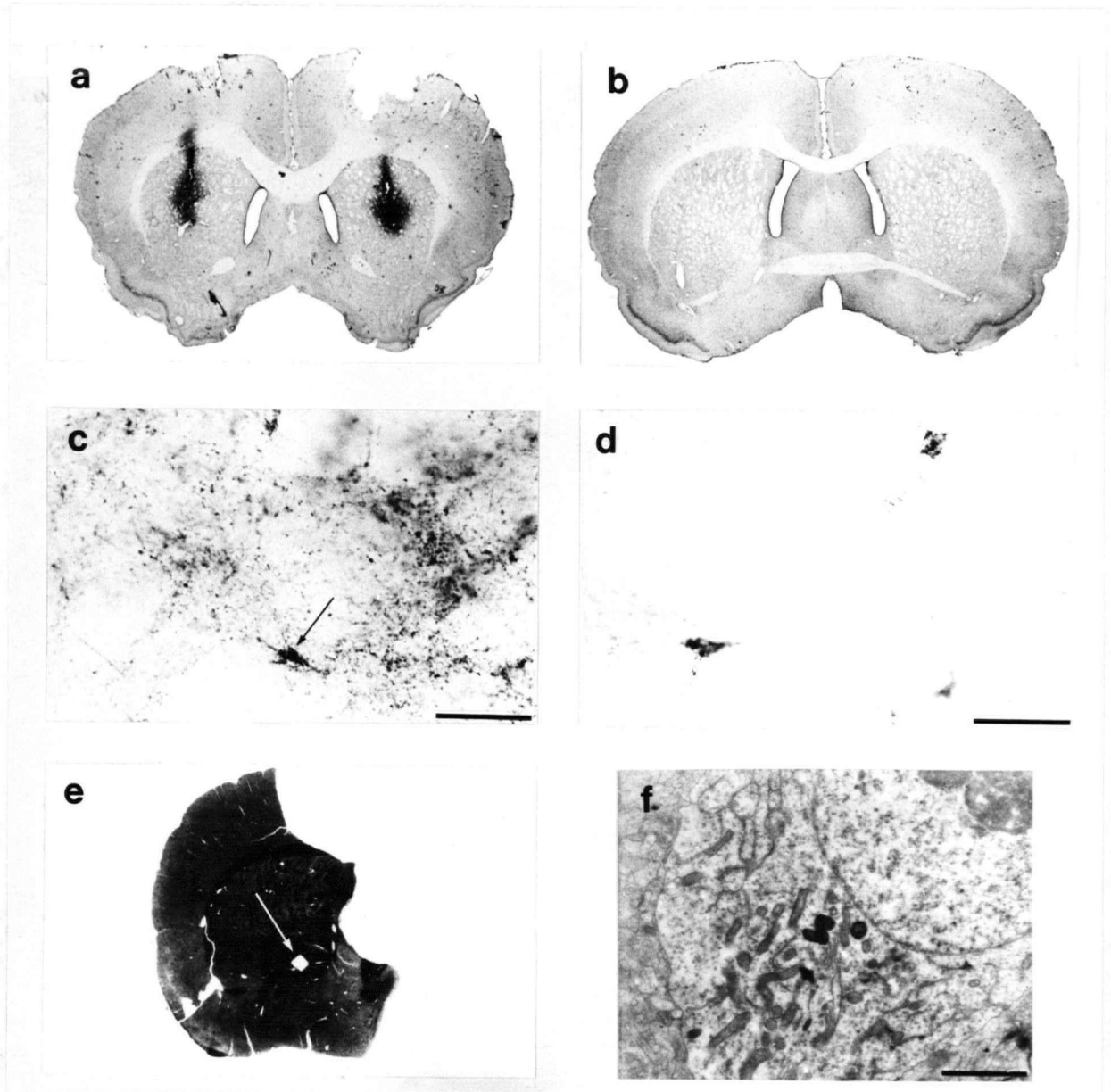
For electron microscopy, animals were perfused as above except that the aldehyde mixture was 1% formaldehyde and 2% glutaraldehyde. Sections were cut at 50  $\mu$ m on an Oxford vibratome and alternate sections were reacted for HRP using TMB or DAB. TMB reacted sections were examined to insure that the injection sites were confined to the head of the striatum. DAB reacted sections were treated with 1% osmium tetroxide, dehydrated, and flat embedded in Epon as described by Wilson and Groves (1979). Labelled pallidal neurons were located under the light microscope and dissected out of the Epon-embedded material. The resultant block was mounted, and pale gold sections cut for examination on a Philips model 201 electron microscope without prior heavy metal counterstaining.

#### RESULTS

In the case illustrated in Figure 31a, both the WGA-HRP and WGA-HRP-KA injections were confined to the head of the striatum. As shown in Figure 31b, in neither injection did the WGA-HRP diffuse as far caudally as the GP. At the caudal pole of the WGA-HRP injection site, reaction product could be seen in fibre bundles in the ventromedial aspect of the striatum. The appearance of this reaction product suggested that it was localized to fibers but these fibers differed markedly in appearance from those labelled by uptake into damaged axons. Axons labelled through damage most often appear to be stained heavily in a Golgi-like manner. The fiber bundles in this instance however showed a faint, discontinuous reaction deposit arranged in linear array. Within the GP, reaction product was



Figure 31. (a) Bilateral injection of WGA-HRP (50 nl, 0.25%) into the striatum. The solution injected on the left also contained 10 mM KA (WGA-HRP-KA). Survival time 24 hrs. Reacted with tetramethylbenzidine (TMB), counterstained with neutral red (NR). (b) Section caudal to (a) showing that the injection sites have not spread to the level of the anterior commissure. Reacted with TMB, counterstained with NR. (c) Section from anterior GP ipsilateral to the WGA-HRP injection ((a) right) showing retrogradely labelled cell body (arrow) surrounded by anterograde reaction product. Reacted with TMB, uncounterstained. Bar = 50  $\mu$ m. (d) Same section as (c) but showing cells in the GP ipsilateral to the WGA-HRP-KA injection ((a) left). Note that the anterograde reaction product is absent. Reacted with TMB, uncounterstained. Bar = 50  $\mu$ m. (e) Epon-embedded section through the GP of a rat receiving an injection of WGA-HRP (100 nl, 1.0%) into the ipsilateral striatum. Reacted with diaminobenzidine (DAB). The arrow denotes the area of GP taken for electron microscopy. (f) Electron micrograph of a pallidal neuron showing multivescicular bodies stained with DAB. Serial sections revealed the nucleus of this neuron to be deeply indented. Uncounterstained. Bar = 2  $\mu$ m.

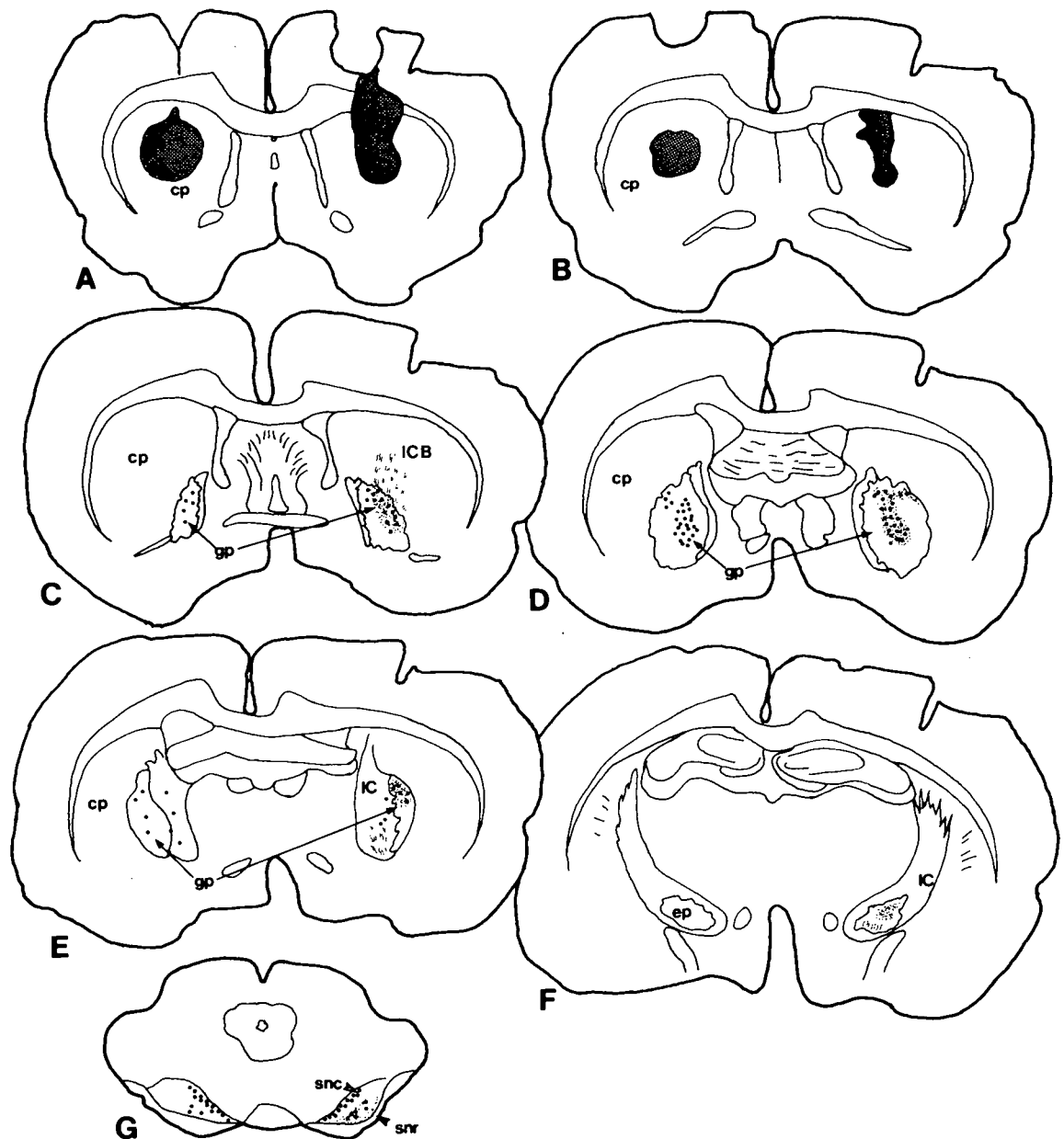


seen in the pallidal neuropil between the capsular bundles. As shown in Figure 31c, in addition to this anterograde label, the anterior GP contained what appeared to be large retrogradely labelled neurons (mean maximum diameter = 22.1 $\mu$ m, sem = 1.1 $\mu$ m, n = 10). The electron micrograph (Fig. 31f) taken from the pallidal section seen in Figure 31e clearly shows that GP perikarya contain WGA-HRP reaction product within multivesicular bodies, as is characteristic for neurons retrogradely labelled in conventional HRP experiments (Somogyi et al., 1979). Results from a limited number of electron microscopic profiles that were obtained from serial sections revealed that all labelled neurons have deeply indented nuclei, sparse axosomatic connections, and are 10  $\mu$ m by 20 to 30  $\mu$ m in size.

Coinjection of WGA-HRP or HRP with KA has already been shown to abolish anterograde labelling of the striatonigral pathway while sparing the retrograde labelling of nigrostriatal neurons (Staines et al., 1980b). Results consistent with this were obtained in the present study. After striatal injections of WGA-HRP together with KA, no reaction product was seen in fibre bundles caudal to the injection site or in the neuropil of the GP. However, labelled pallidal cell bodies could still be clearly seen (Fig. 31d). This feature was utilized to map the distribution of pallidal neurons projecting to the head of the striatum (Fig. 32).

The presence of anterograde label often makes it difficult to visualize retrogradely labelled cells. In the case depicted in Figure 32, the coinjection of KA with WGA-HRP abolished

Figure 32. Line drawings depicting the distribution of labelling after striatal injections of 100 nl of 2% WGA-HRP with (left) and without (right) 10 nM kainic acid in the injection buffer. Labelled neurons are represented by filled circles. Areas of terminal labelling are shown with a fine stippling and projection fibers are drawn in. Abbreviations in this and subsequent figures: cp, striatum; ep, entopeduncular nucleus; gp, globus pallidus; IC, internal capsule ICB, bundles of internal capsule fibers; snc, substantia nigra pars compacta; snr, substantia nigra pars reticulata.



anterograde transport of tracer from the injection site, as seen by the absence of labelled fibers emanating in a caudal direction from the injection site and the absence of terminal labelling in the GP, EP or SN (Fig. 33). Sections from the same animal stained for cresyl violet revealed early degenerative changes on the KA injected side that involved an area of striatum only slightly larger than the extent of the peroxidase injection site. Illustrated in Figure 32 is the distribution of labelled neurons in the presence and absence of labelled striatopallidal terminals. The number and distribution of these cells on either side of the brain is very similar and the slight differences easily attributable to differences in the two injection sites. Neurons within the whole rostrocaudal extent of the GP project to the head of the striatum but as shown in Figure 34, there is an obvious restriction to their mediolateral distribution. At rostral levels both labelled pallidal neurons and labelled afferent terminals occupy an extreme lateral position within the GP. At more caudal levels both positive soma and terminals are seen in a much more medial position. Rather than a progression in the medial direction there appears to be discontinuity in the distribution of labelled cells and terminals. From the examination of a large number of cases in both coronal and horizontal section it seems that two cell groups are labelled within the GP after injections into the head of the striatum. One tier of cells extends from the rostral pole of the GP, through the lateral GP in rostral sections and ends in a slightly medial position at midpallidal levels. Another begins at

Figure 33. Darkfield photomicrographs of the labelling seen in the GP (A and B), EP (C and D) and SN (E and F) after the coinjection of KA and WGA-HRP (A, C and E) or WGA-HRP by itself (B, D and F) into the head of the striatum. Note that there is no terminal labelling in the GP, EP or SN on the kainic acid injected side (A, C and E) although retrogradely labelled cells are seen in the GP and SN (A and E). Bar = 100 um.

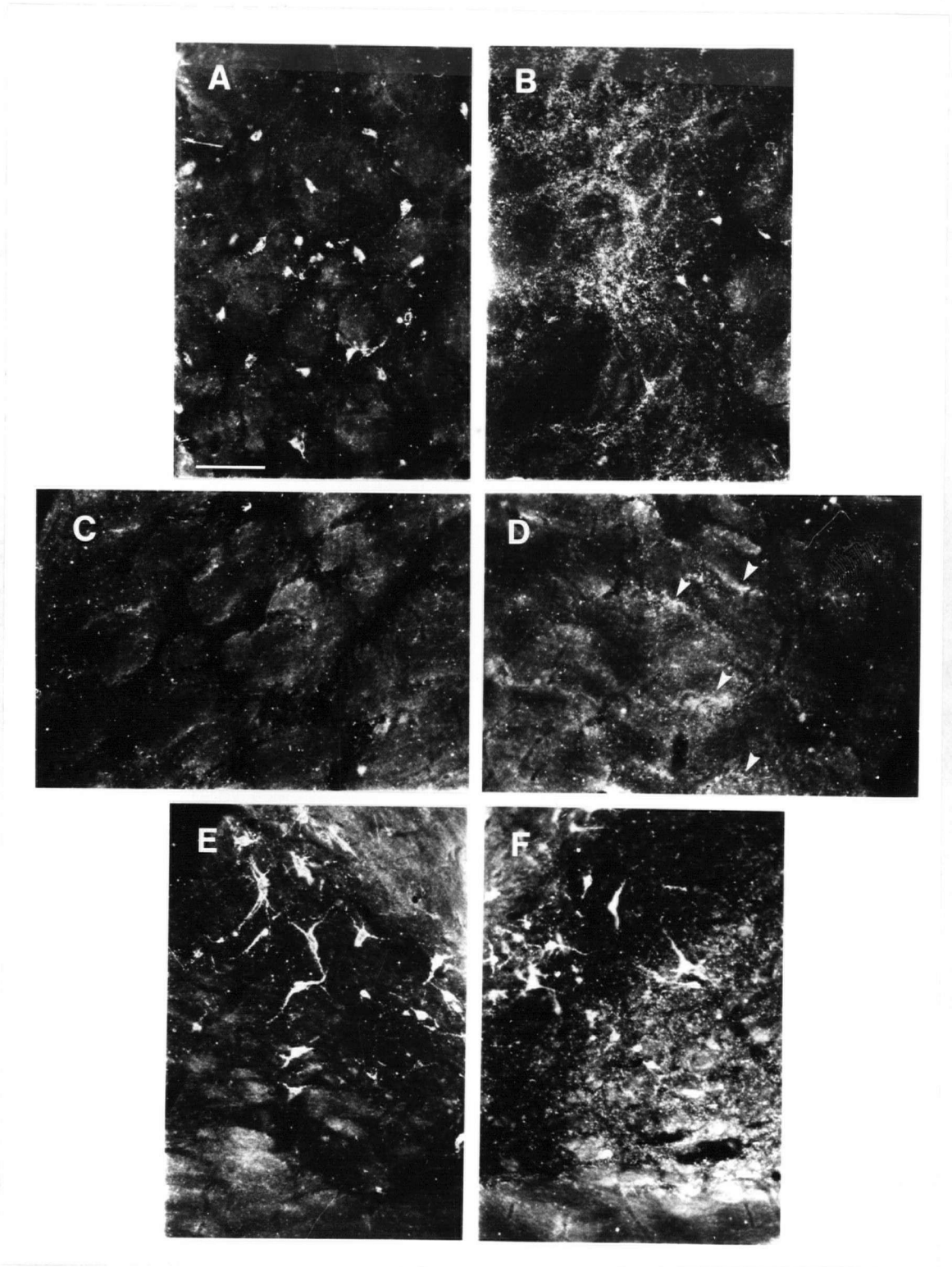
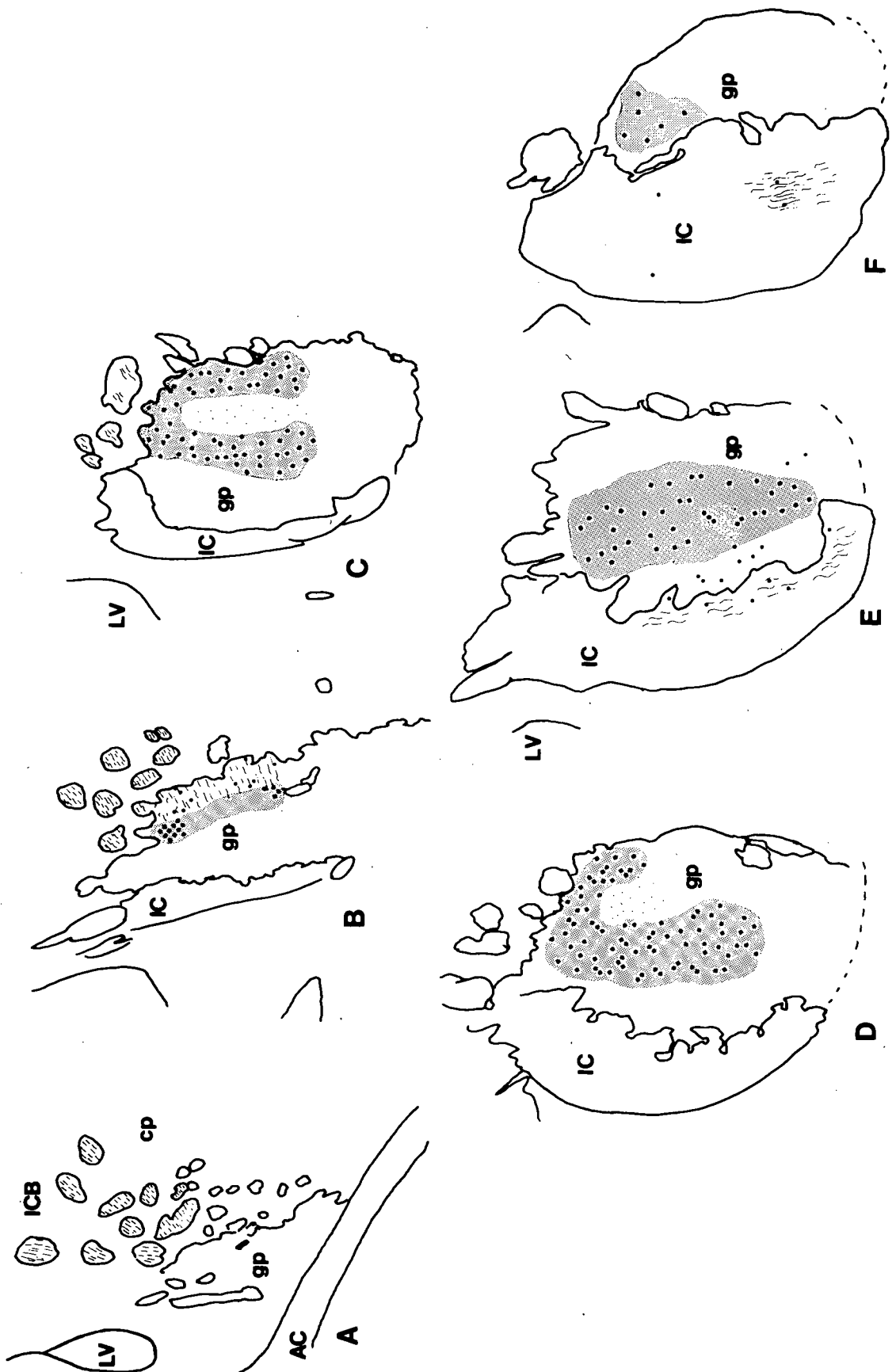




Figure 34. Detailed topography of anterograde (stippling) and retrograde labelling (filled circles) of the globus pallidus after a striatal injection of 100 nl of 2% WGA-HRP into the head of the striatum. Each cell recognized is plotted out in projection drawings of the section. Abbreviations: AC, anterior commissure; LV, lateral ventricle.



midpallidal levels, in a position more medial than the first cell group, and extends as a continuous band of cells into the caudal extreme of the GP. Thus, pallidostriatal neurons can be seen to be distributed into two tiers. The terminal labelling of the striatopallidal pathway shows an identical distribution.

As indicated in Figure 32 and from observations of a large number of other cases, injections into the head of the striatum do not lead to labelling of neurons within the entopeduncular nucleus, although anterograde labelling of the striatoentopeduncular projection pathway and terminal field are clearly visible. Near the caudal pole of the GP, however, a few neurons are occasionally seen embedded within the internal capsule. These cells, although generally of a similar morphology, differed from neurons found within the confines of the pallidum in that they were larger and often were more heavily labelled for peroxidase. It was noted that their presence was correlated to diffusion of the injection site into the cortex overlying the striatum. No neurons were found within this region in five cases where the injection site did not spread into the cortex.

#### DISCUSSION

The results reported here represent a consistent finding so far observed in over twenty animals. Anterograde and retrograde reaction product in the GP can be seen as early as four hours after WGA-HRP injections wholly confined to the striatum. The injections depicted in Figure 31a led to the labelling of roughly 17 cells/50  $\mu$ m section, indicating that this is not a minor

projection. As is the case for the nigrostriatal pathway, the number of retrogradely labelled neurons is not affected by the coinjection of KA. The injection coordinates used here consistently led to labelling of neurons in the lateral GP at rostral levels of this nucleus. At more caudal levels the labelled neurons took up a position in the medial GP. Anterograde label followed a similar distribution. In fact, labelled cells were seldom seen outside areas labelled by anterograde transport as well. The congruence shown by the two forms of label is suggestive of a reciprocity between striatopallidal and pallidostriatal connections.

The present results confirm and expand on the demonstration of a projection from the globus pallidus to the striatum presented in Experiment 1. An important observation added in the present work is that the labelling of pallidal cell bodies is independent of the anterograde transport of WGA-HRP to the GP. As is the case with other tracers, WGA-HRP can cross the synaptic cleft and be taken up by cell bodies and dendrites postsynaptic to heavily labelled terminals (Itaya and van Hoesen, 1982). As discussed in Experiment 1 it is unlikely that this process occurs to any appreciable extent under the conditions employed here. However, the dendrites of pallidal neurons are often described as being covered with a layer of synaptic endings (Fox et al., 1974; DiFiglia et al., 1982a) and as most of the terminals of this immense input would be expected to contain WGA-HRP it was felt necessary to repeat the observation in the absence of anterograde transport.

Used by themselves, each of the approaches employed here to demonstrate the pallidostriatal projection could lead to erroneous conclusions. However, the combination of light and electron microscopic techniques as well as the use of coinjections of kainic acid with WGA-HRP rule out the possibilities that perikarya in the GP appeared to be labelled due to a dense input of anterogradely labelled pallidostriatal fibers surrounding unlabelled pallidal neurons or that labelling of pallidal perikarya in the GP was due to trans-synaptic transport of WGA-HRP.

Although previous investigations of the projections of the GP have failed to identify this pathway, some support for the present finding has recently been provided by Nauta (1979). He reported that injections of tritiated amino acids into the GP of the cat resulted in sparse and widespread appearance of autoradiographic grains in the caudate nucleus and putamen. However, on the basis of his results, Nauta concluded that it was not possible to determine whether these grains reflected pathways which originated in the GP or whether they represented transport from regions adjacent to the GP. The present results provide clearcut evidence for a pallidal origin.

Since the original report of this projection (Staines et al., 1981) it has been confirmed by retrograde fluorescent marker transport in the rat (Arbuthnott et al., 1982a) and retrograde WGA-HRP transport in the cat (Buchwald et al., 1981). Furthermore, Arbuthnott and coworkers (1982b) have reported electrophysiological evidence consistent with a pallidal

projection to the striatum. In a recent Golgi study of the mouse GP, axons of laterally located pallidal cells were traced into the striatum although their termination within this structure could not be ascertained (Iwahori and Mizuno, 1981). It has also been reported that pallidal lesions lead to the degeneration of striatal terminals recognized at the ultrastructural level (Chung and Hassler, 1982).

At present, the precise population of pallidal neurons that project to the striatum is not known. One possibility is that this population represents the cholinergic neurons of the nucleus basalis magnocellularis (nBM). These neurons, many of which are found in the ventromedial aspect of the GP (Lehmann et al., 1980), may project through the striatum to the cortex (Nauta, 1979a; Staines et al., 1980a). This hypothesis is dealt with (and rejected) in Experiment 5, although it is likely that some of these were seen in the present study and represent those neurons found embedded within the internal capsule fibers (Fig. 32).

The nature of the pallidal neurons that give rise to the pallidostriatal projections, therefore, requires further investigation. For example, it would be of considerable interest to determine if the same or different populations of pallidal neurons give rise to the pallidostriatal, pallidosubthalamic, and pallidonigral projections. The general morphology and cell size of the pallidal neurons labelled after striatal injections are very similar to those labelled after nigral injections (see Experiment 2). Two types of cells are seen within the GP of the

monkey in Golgi preparations. A large cell type (20-50  $\mu\text{m}$ ), with a deeply indented nucleus, predominates and is thought to be the projection neuron. A more scarce, medium-sized cell (12  $\mu\text{m}$ ) has been suggested to be a pallidal interneuron (Fox et al., 1974; DiFiglia et al., 1982). The projection neuron in the mouse is much smaller (25  $\mu\text{m}$ ) (Iwahori and Mizuno, 1980) and corresponds closely to those retrogradely labelled from the striatum.

The detailed description of pallidostriatal connections given above indicates a discontinuity in both the anterograde and retrograde labelling of the GP. This is more apparent when striatal injection sites have a limited mediolateral extent. One explanation of this feature could be that a toxic reaction developed to the WGA-HRP at the core of the injection site (along the cannula tract) which inhibited transport from this region. However, a dual topographical representation of striatal efferents within the GP has previously been indicated in autoradiographic studies (Wilson and Phelan, 1982) and after intracellular injection of striatal neurons with horseradish peroxidase (Chang et al., 1981). It would appear that there may also be a dual topographical representation within the pallidostriatal projection as well. Additional significance is attached to this finding in light of the observation of morphological differences found between laterally located pallidal cells and those located within the core of the GP (Iwahori and Mizuno, 1980; Park et al., 1982) and the fact that the lateral zone and core of the GP in the monkey show differential immunoreactivity for substance P and enkephalin

(Haber and Elde, 1981). As mentioned in Experiment 1, two distinct fields of labelled striatonigral terminals can often be seen in caudal regions of the substantia nigra as well, suggesting that there may be some degree of duality to the topography of other striatal efferents.



## EXPERIMENT 5: COMPARISON OF CORTICAL AND STRIATAL PROJECTIONS OF THE GLOBUS PALLIDUS AND PERIPALLIDAL AREAS

### INTRODUCTION

It has been demonstrated by retrograde transport studies in a number of species that a rather disperse group of basal forebrain neurons has a direct projection to the cortex (Kievit and Kuypers, 1975; Parent et al., 1981c; Reinoso-Suarez et al., 1982). In most species, including the rat, there is an infiltration of some of these neurons into the anatomical boundaries of the globus pallidus. Although most authors agree that these cells are restricted to the medial and caudal portions of the globus pallidus (Divak, 1975; Lehmann et al., 1980; Wenk et al., 1980), in contrast to the distribution of pallidostriatal neurons, it was necessary to clearly distinguish the pallidostriatal projections from those of these basal forebrain cells. This distinction would address the objection that retrograde labelling of pallidal neurons after striatal injections results from uptake of tracer by fibers of passage or striatal collaterals of the projection of basal forebrain neurons to the cortex. Indirect evidence from biochemical studies exists which can be interpreted as indicating that these neurons project through the striatum on their way to the cortex (Staines et al., 1980a) and a similar conclusion was arrived at from data presented in Experiment 3.

Four approaches were taken to achieve this differentiation. The first made use of the observation that the majority of the basal forebrain neurons projecting to the cortex stain intensely for acetylcholinesterase (AChE) (Mesulam and van Hoesen, 1976;

Lehmann et al., 1980; Parent et al., 1981c; Ribak and Kramer, 1982). The histochemical visualization of acetylcholinesterase activity may be used as a reliable and reproducible marker for forebrain cholinergic neurons in animals which have been pretreated with the irreversible acetylcholinesterase inhibitor diisopropylfluorophosphate (DFP). At short survival times, intense acetylcholinesterase activity is seen in the cell bodies of neurons which have a high rate of de novo synthesis of this enzyme (Lehmann et al., 1980, Fibiger, 1982). The distribution of pallidal neurons labelled after striatal injections of the fluorescent tracer true blue (Tb) (Kuypers et al., 1980) was compared to the distribution of AChE-positive cells in the same sections and the colocalization of these two markers was evaluated. In the second approach, retrograde transport of fluorescent tracer from the striatum to the globus pallidus was examined in rats which had been previously subjected to cortical ablation. Under these conditions projection axons passing through the striatum to the cortex would be expected to degenerate and the striatum would become the most distal structure, obviating the problem of uptake into fibers of passage. In the third approach, a direct comparison was made of the distribution and morphology of neurons retrogradely labelled by injections of tracer into the striatum with those seen following cortical injections. Finally, the acetylcholinesterase staining of neurons retrogradely labelled by cortical injection of the fluorescent tracer nuclear yellow were examined.

Taken together, the results of these studies support the proposition that neurons in the globus pallidus proper do in fact project to the striatum in the rat.

#### METHODS

Male Wistar rats, anesthetized with pentobarbital, received stereotaxic injections of True Blue (Tb). Pressure injections of 100 to 300 nl of Tb as a 5% suspension in distilled water were made through a 5 ul Hamilton syringe into the striatum. Some rats receiving striatal injections had been subjected to cortical ablation by suction one week or three months previous to the administration of tracer. Three days after Tb injection, animals were injected intramuscularly with diisopropylfluorophosphate (DFP; 1.5 mg/kg in peanut oil) and intraperitoneally with atropine (1.0 mg/kg). After a survival time of four to six hours rats were anesthetized with pentobarbital and perfused transcardially with normal saline, followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB; pH 7.4). Brains were removed and postfixed in the same fixative containing 10% sucrose overnight at 4 C.

Sections were cut on a freezing microtome at a thickness of 15 and 30 um. The latter were mounted immediately onto glass slides from 50 mM PB, air dried and coverslipped with paraffin oil. Sections to be reacted for AChE (the 15 um section) were collected into ice cold PB containing 10% sucrose, rinsed in this solution for 1 hr, and mounted onto subbed slides from 50 mM PB. They were then rinsed in ice cold acetate buffer for 15 min and incubated at room temperature in Karnovsky's reaction medium for

the visualization of AChE. The progress of the reaction was monitored closely by microscopic examination of the sections and terminated when cells became clearly visible in the striatum and nucleus basalis magnocellularis (Lehmann et al., 1980) (generally 20-40 min).

Nuclear Yellow (NY: a gift from Prof. O Dann) was injected into the cortex as a 1% solution and after a survival time of 24 hrs the rats were treated as for Tb.

Injections of WGA-HRP into striatum (single injection) or frontal cortex (double injection) were also made and the animals processed for tetramethylbenzidine visualization of peroxidase activity as described in preceding sections.

#### RESULTS

Scrutiny of sections throughout the brains of animals receiving striatal injections of Tb confirmed the observations of striatal afferents presented in Experiment 1. Experience with both tracers led to the conclusion that in terms of retrograde transport, Tb is superior to HRP and WGA-HRP both in sensitivity and ease of application. Cell groups clearly labelled by WGA-HRP were also well labelled by Tb, but those neurons weakly labelled by small injections of WGA-HRP (such as those in the cortex and amygdala) were strongly labelled by Tb. The injection site obtained with small injections of Tb was easily controlled and rivalled in size those obtained with WGA-HRP. NY, on the other hand, produced a larger, more diffuse injection site which made it the tracer of choice for the cortical injections. Another advantage of Tb is its diffuse cytoplasmic localization in

labelled neurons, allowing description of the cell body and proximal dendrites. Evidence was obtained that Tb undergoes anterograde transport but this merely had the effect of increasing the background in the GP and never obscured the retrogradely labelled cell bodies. Fluorescent labelling with both Tb and NY was found to be compatible with AChE staining if the latter was controlled and not allowed to progress farther than necessary. The high fluorescent background reported by others (Nagai et al., 1982a) was not found to be a problem if sections were examined soon after they were reacted.

The distributions of retrogradely labelled pallidostriatal neurons seen after a Tb injection into the CP and intensely stained AChE-positive cells in the same animal are presented diagrammatically in Figure 35. Each neuron is characterized as AChE-positive (triangle), Tb-positive (circle) both (asterisk) and mapped onto a projection drawing of the section. At rostral pallidal levels AChE-positive neurons were seen in the striatum and subcommissural regions (labelled ventral pallidum by Lehmann et al., 1980) but not within the GP itself. The reverse was true of the Tb fluorescent neurons which are confined to the GP proper. At midpallidal levels (Fig. 35D) a few AChE-positive cells could be seen within the extreme ventromedial GP, a region where the retrogradely labelled GP neurons were very scarce. A few AChE-positive neurons were found in the lateral GP, which contained a large number of fluorescent cells, but no colocalization of the two markers was detected. At caudal levels of the GP, the number of AChE-positive neurons increased markedly

Figure 35. Distribution of fluorescent neurons labelled from a striatal Tb injection (open circles) and neurons showing intense AChE activity 4 hrs. after a DFP injection (triangles) at various levels through the globus pallidus. Each positive cell has been plotted on projection drawings of the section. Neurons showing both fluorescent labelling and AChE activity are indicated (asterisk). Abbreviations in this and following figures: AC, anterior commissure; cp, striatum; gp, globus pallidus; IC, internal capsule.

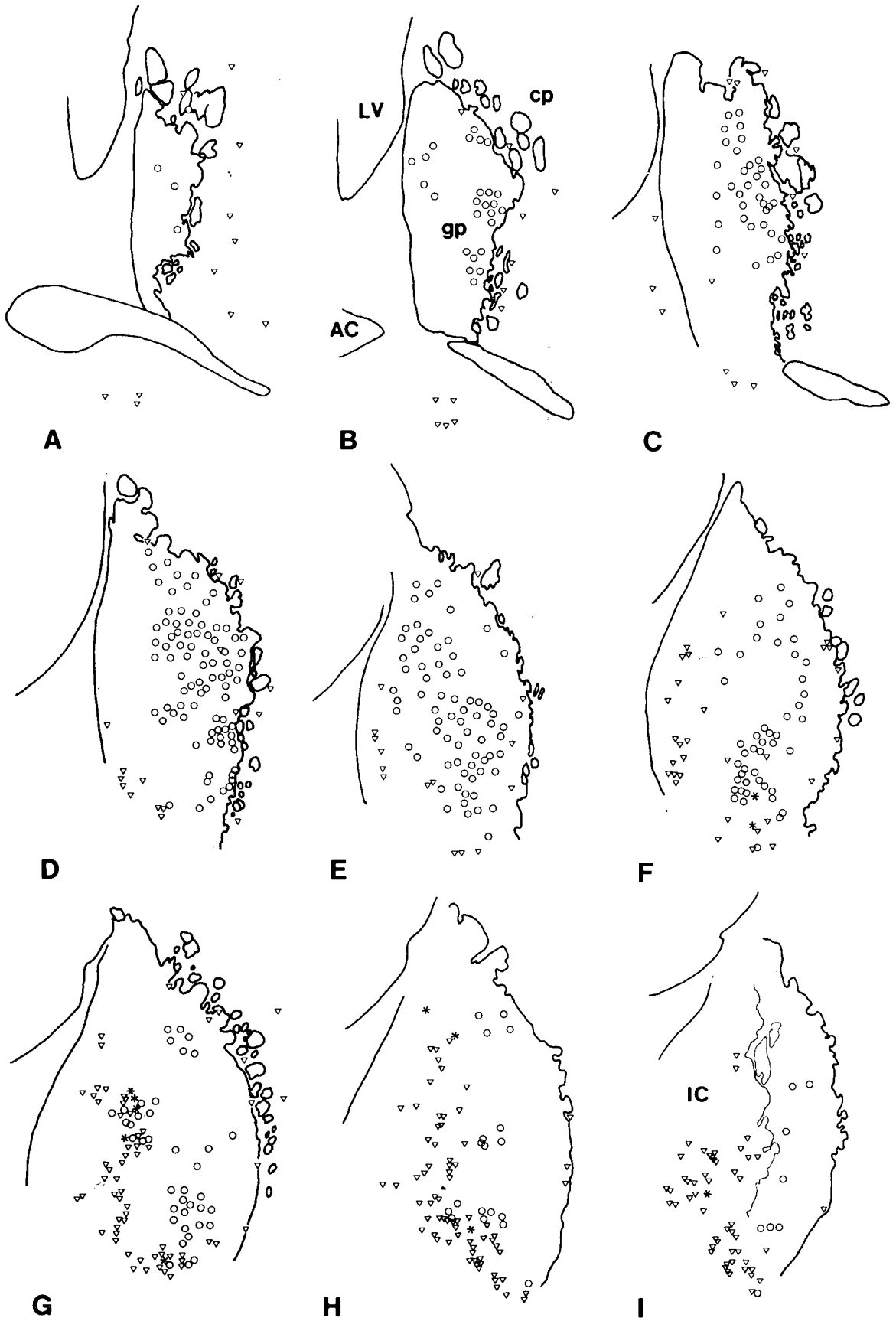
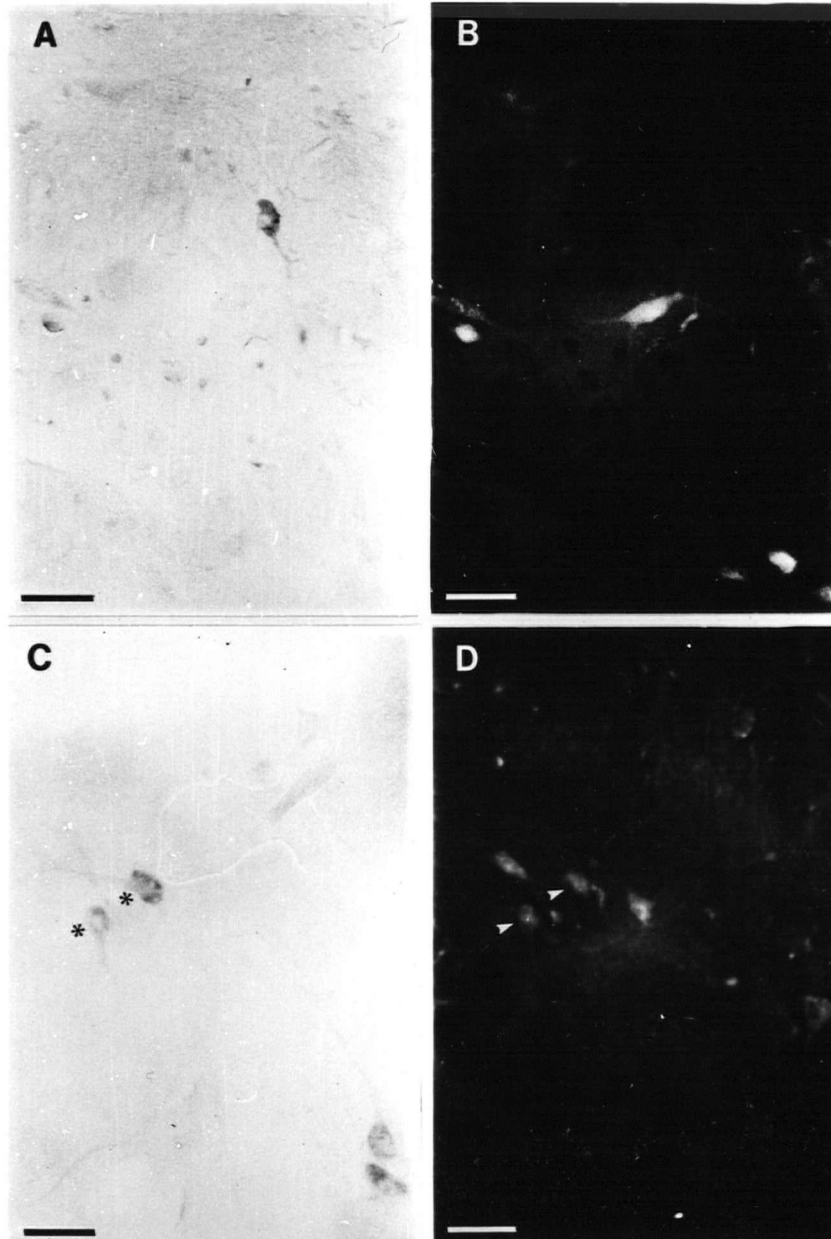


Figure 36. (A) Lightfield photomicrograph of an AChE stained neuron in the globus pallidus. (B) The same field showing Tb labelled neurons. Note that the Tb labelled neuron at the center of the field has no visible AChE reactivity. (C) Lightfield photomicrograph of AChE stained neurons at a more caudal level of the GP. The two AChE positive cells denoted by asterisks are labelled retrogradely labelled with Tb as indicated by the arrows in the fluorescence micrograph (D). Bar = 50 um.



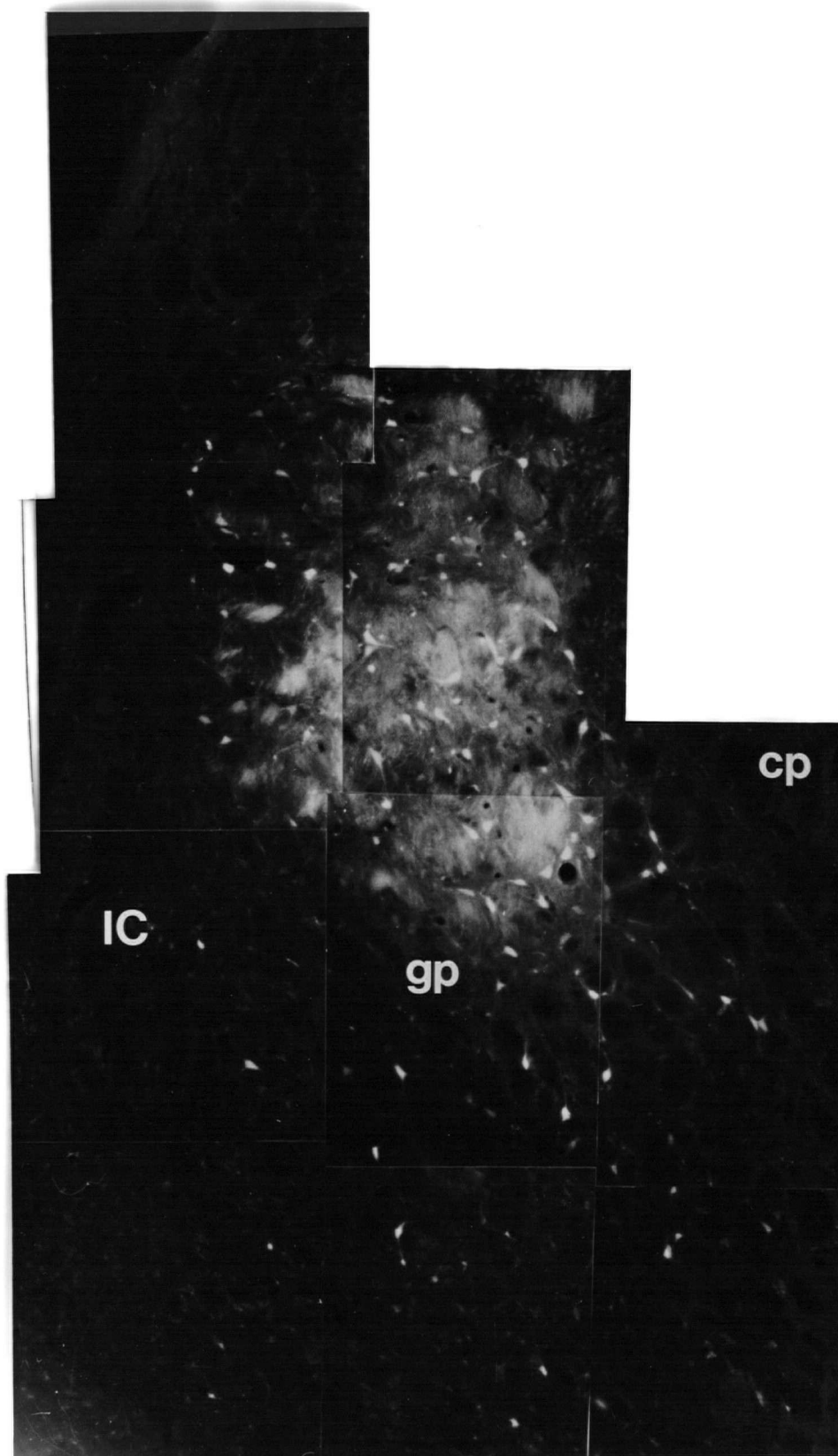


in the medial and ventral portions of the GP and the population of Tb neurons took up a more medial position in the GP, such that these two populations of neurons partially overlapped in their distributions. Within overlapping regions a small number of neurons showed both intense acetylcholinesterase activity and Tb fluorescence (Fig. 35H-K, Fig. 36). Quantitative analysis (cell counts) in unreacted sections indicated that there were more fluorescent cells in these regions than were seen in AChE-reacted material (even after correcting for differences in section thickness). This was no doubt due to the screening of fluorescence in some AChE stained neurons, and was found to result in a 50% underestimate of the number of double labelled cells. This discrepancy was confined to regions containing double labelled cells, in all other regions comparisons of the two sets of sections were quantitatively similar.

A virtually identical distribution of fluorescent labelled GP neurons was obtained in an animal which had undergone an extensive ablation of the cortex dorsal and rostral to the striatum three months prior to the injection of Tb (Fig. 37). Acetylcholinesterase reaction of sections from this brain indicated that a large number of AChE-positive cells survived in and around the GP (distributed as Figure 35), but in this material only three double labelled neurons could be found.

The general distribution of cell bodies labelled in the pallidal and peripallidal regions after injections of WGA-HRP injections into the frontal cortex was similar to that seen with NY injections into the same area (refer Fig. 38). A larger

Figure 37. Photomontage of the anterior globus pallidus in an animal injected with Tb into the striatum 3 months after extensive cortical ablation. The diffuse pale field represents a minor anterograde transport of Tb.



number of cells were seen within the boundaries of the globus pallidus than reported in previous studies in the rat (Divac, 1975). Labelled cell bodies were found mainly within the internal capsule and caudomedial globus pallidus but some were found along the lateral and medial borders as well (Fig. 38A and 38B). In addition, the main body of the globus pallidus contained a few very lightly labelled neurons which appeared to belong to a population of a smaller sized cell (Fig. 38C and 38D). Morphometric analysis was not attempted as labelled cells were polymorphous and highly isodendritic, but the fact that the qualitative difference apparent in Figure 38 was correlated with a distributional difference was felt significant. The smaller, weakly-stained cells were restricted to the GP and, although few in number, were as common in this region as the larger cells. The size, appearance and distribution of heavily labelled cells contrasts dramatically to that already shown for pallidal neurons retrogradely labelled from the tracer injection into the striatum.

In rats receiving injection of NY into the cortex, fluorescent neurons were found in the diagonal band, ventral pallidum, ventrocaudal globus pallidus, and within and ventral to the internal capsule (Fig. 39). The number found in the GP was much less than in the other areas and much fewer than typically seen in this nucleus after a striatal injection (ie. 6 cells per section as apposed to 60 cells per section with striatal tracer injections). In all areas the majority of cells were also found to be AChE reactive, but a few fluorescent neurons in both

Figure 38. Labelled neurons in globus pallidus after a frontal cortical injection of WGA-HRP. Cells shown in (A) and (B) were on the pallidostriatal border and adjacent to the internal capsule respectively. A few smaller, less intensely labelled cells were seen in the body of the globus pallidus as well (C and D). Bar = 50 um.

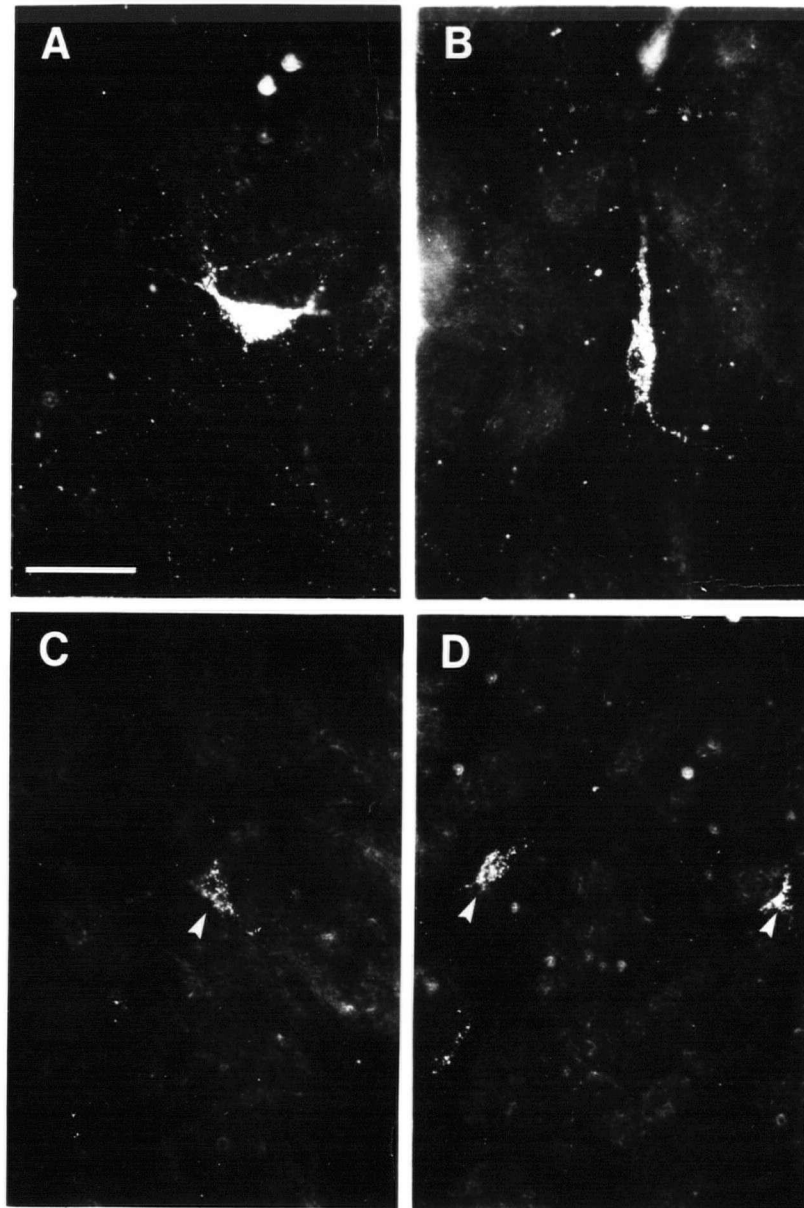


Figure 39. Line drawings depicting the NY injection sites (fields of open circles in A and B) and the resultant retrograde fluorescent labelling of neurons in and around the globus pallidus. Sections were reacted for AChE activity and NY labelled cells showing intense AChE activity are represented by triangles. NY labelled cells not showing AChE activity are represented by open circles. Abbreviations: FM, forceps minor.



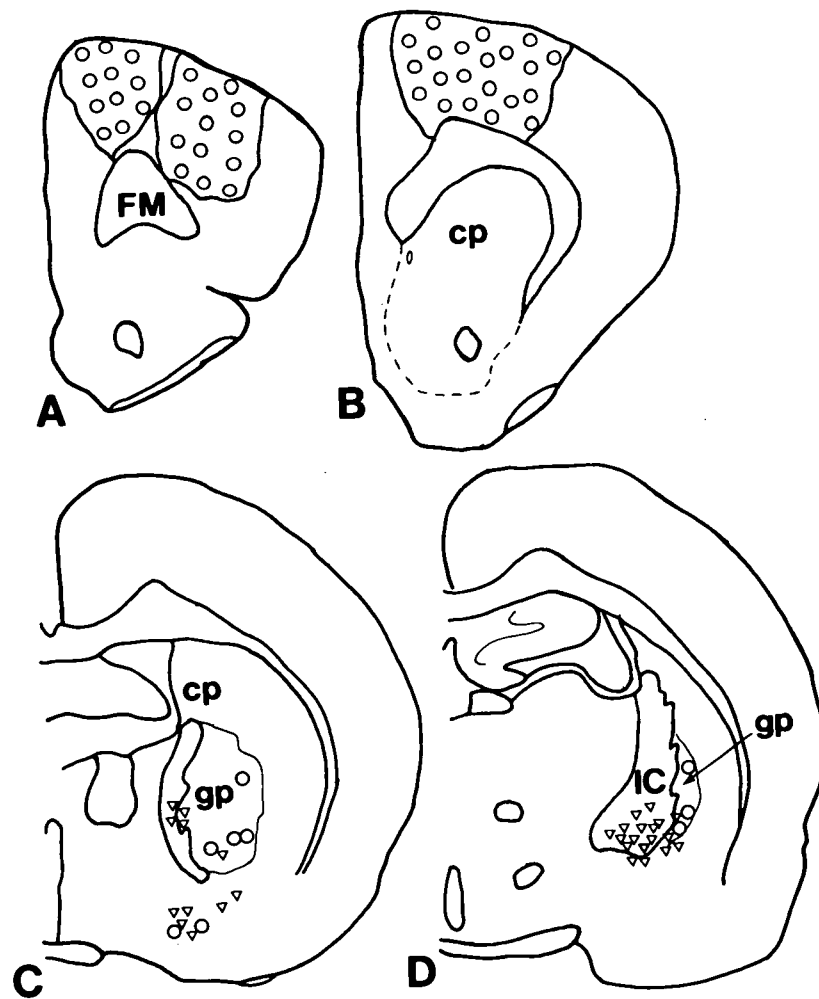
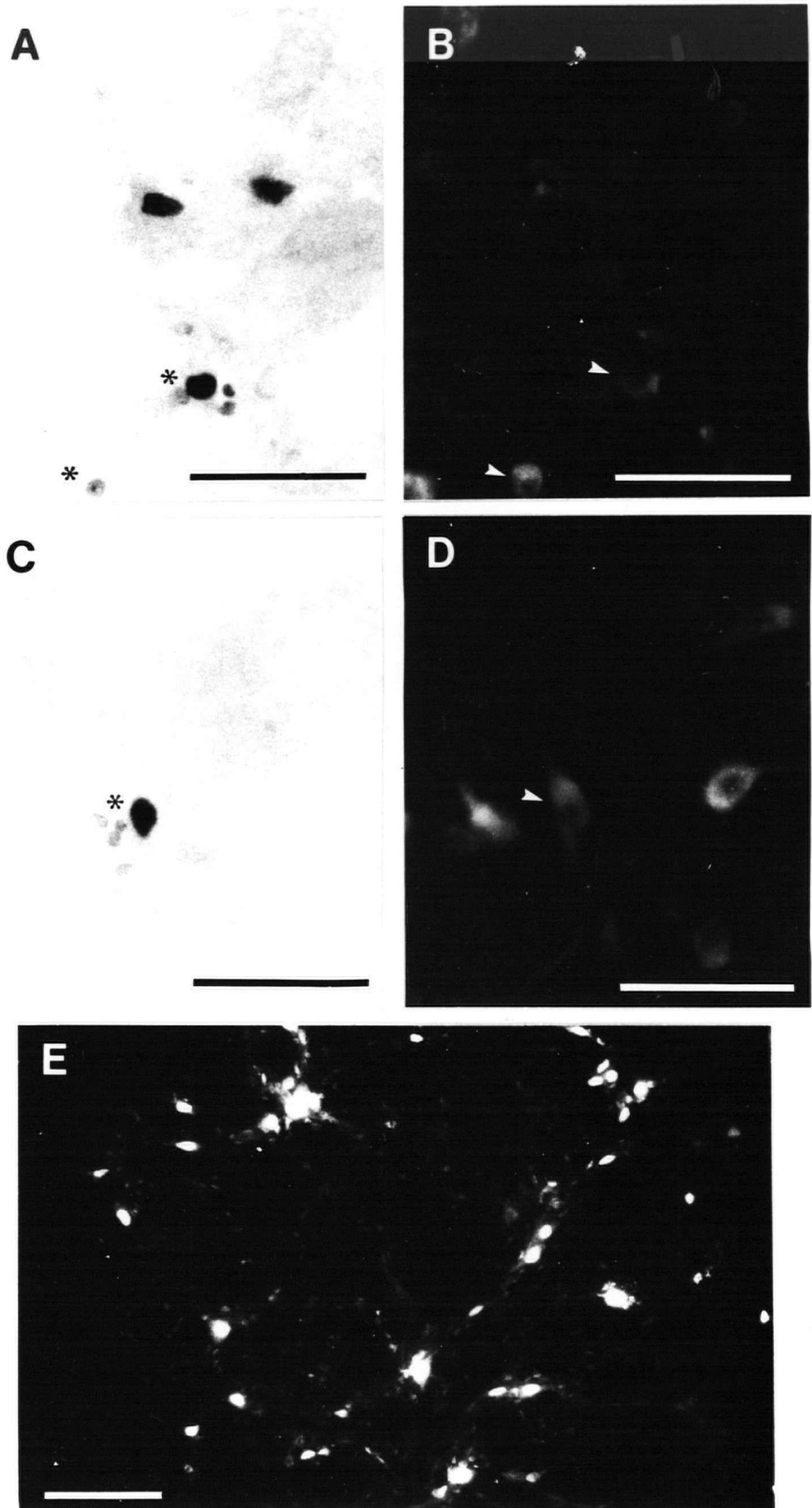


Figure 40. (A) Fluorescent NY labelled neurons in the globus pallidus after a NY injection into the frontal cortex. Fluorescent nuclei appear in negative image (dark on a pale background). Only the two neurons denoted by asterisks were AChE-reactive and are shown by arrows in the negative image lightfield photomicrograph (B). In the caudomedial globus pallidus AChE positive neurons were far more abundant but not all were retrogradely labelled (C and D). (E) A positive image of the NY labelled cells within the internal capsule. Bar = 100 um.



pallidal and peripallidal regions did not show significant AChE activity (Fig. 39 and 40).

#### DISCUSSION

The neuropathology of Alzheimer's disease is now seen to include a loss of basal forebrain cholinergic neurons which have projections to the neocortex (Nagai et al., 1982b; Whitehouse et al., 1982; Rosser, 1982; Pearson et al., 1983). The distribution of these cells overlaps the boundaries of the globus pallidus (and putamen) and has led to claims of a pallidocortical cholinergic projection (Edstrom and Phillis, 1980; Kelley and Moore, 1980). These (magnocellular) cells can, however, be differentiated from typical parvocellular (to use the terminology of Parent et al., 1981c) pallidal neurons by their larger size, intense acetylcholinesterase activity, as revealed by the pharmacohistochemical procedure, and by their projections (Lehmann et al., 1979; Parent et al., 1981c). The major pallidal cell type stains neither for AChE (Lehmann et al., 1980) nor choline acetyltransferase (Kimura et al., 1981). The present data clearly indicate that the majority of the pallidal neurons found to project to the striatum are parvocellular. There is insufficient evidence in this or previous experiments to indicate any significant collateralization of the magnocellular projection within the striatum on its way to the cortex.

The third and fourth sections of the present work confirm observations in the cat that the frontal cortex also receives a non-magnocellular projection from the globus pallidus and subpallidal regions (Ribak and Kramer, 1982). Whether or not

those seen within the GP are parvocellular neurons (as defined by projections to other basal ganglia nuclei) has yet to be established. It is noted with interest that this second type of neuron is not seen after injections into other cortical areas (Parent et al., 1981c), but has so far only been observed after injections into the frontal cortex.

EXPERIMENT 6: COLLATERAL PROJECTIONS OF THE NEURONS OF THE GLOBUS  
PALLIDUS TO THE STRIATUM AND THE SUBSTANTIA NIGRA

INTRODUCTION

With the completion of Experiment 5, the retrograde demonstration of a pallidostriatal pathway is considered confirmed. The globus pallidus (GP) also has a massive projection to the subthalamic nucleus (SUT) (Carter and Fibiger, 1978; van der Kooy et al., 1981a; Nauta, 1979; McBride and Larsen, 1980) and a lighter projection to the substantia nigra (SN) (Hattori et al., 1975; Kanazawa et al., 1976; Bunney and Aghajanian, 1976, Experiments 2 and 3). The density of neuronal cell bodies within the GP is not great and the observations from Experiment 1 and 2 indicate that in some regions at least a majority of neurons within the GP project to either SN or CP. Topographical considerations discussed in Experiment 2 suggest that pallidal cells projecting to an area of striatum are in close proximity to those projecting to the portion of the substantia nigra which is innervated by that area of striatum. Taken together, therefore, previous experiments have provided data suggesting that at least some pallidal neurons innervating SN have collaterals to the CP. In this experiment, the proposal was tested directly using double retrograde transport of fluorescent tracers (van der Kooy, 1979; Bentivoglio et al., 1979; Kuypers et al., 1980). This technique was also used to confirm topographical claims made in Experiment 1 concerning the pallidostriatal projection.

## METHODS

Male Wistar rats (150 gm) were anaesthetized with pentobarbital and injected stereotaxically with 100 to 200 nl of True Blue (Tb; yields blue fluorescent cytoplasm in retrogradely labelled neurons) into the CP as described in Experiment 5. One to two days later, an injection of 50-100 nl Nuclear Yellow (NY; yields yellow fluorescent nuclei in retrogradely labelled neurons) was made into the substantia nigra, SUT or a separate region of the CP. In cases of striatal NY injection, the injection buffer contained 10 mM kainic acid. Twelve to twenty-four hours after the second injection, animals were fixed with paraformaldehyde and sections cut and visualized for fluorescence as described in Experiment 4. In some cases nigral injections of another tracer, primuline (Pri; yields golden granules in cytoplasm of retrogradely labelled neurons) were made in animals also receiving a striatal injection of Tb. Survival time in these cases were three days for both tracers.

## RESULTS

Short survival times are required with the use of NY as at larger intervals it may diffuse out of retrogradely or anterogradely labelled neuronal elements and into neighbouring neurons not projecting to the site of injection. The occurrence of this is readily apparent by the labelling of glial nuclei in areas containing retrogradely labelled neurons (Bentivoglio et al., 1980a; Bentivoglio et al., 1980b). Results obtained with the use of these tracers provided support for all of the retrograde observations contained in Experiments 1 and 2,

including striatal inputs from ventral thalamus, SUT, and amygdala, although the invariable labelling of the cortex along the cannula tract made it impossible to determine with assurance that the labelling reflected striatal connections. Coinjection with KA was found to be a necessary adjunct to the use of short survival times to prevent labelling of glial nuclei in the globus pallidus.

#### PALLIDAL COLLATERALS TO THE CP AND SN

Confirmation was obtained of the topography of pallidal projections to CP and SN presented in Experiments 1 and 2. When the striatal injection sites were located outside of those areas showing retrograde labelling with the nigral tracer, there was little overlap in the distribution of the differentially labelled pallidal neurons. However, when striatal injections of Tb fell within areas containing NY labelled cell bodies, there was coincidence of Tb and NY labelled pallidal cells and, moreover, a large number of cells were double labelled (Fig. 41 and 42). The incidence of double labelled neurons varied with the locations of the injection sites and their size and ranged from 20% to 50% of the smaller population. Although not studied in detail, the occasional double labelled cell was noted in the frontal cortex but not in the parafascicular or intralaminar nuclei of the thalamus.

#### PALLIDAL COLLATERALS TO CP AND SUT

Injection of Tb into the CP and NY into the SUT gave rise to double labelled neurons in the GP, but invariably produced retrograde labelling of some striatal neurons as well. This



Figure 41. Line drawings (after Konig and Klippel, 1968) depicting a striatal Tb injection site and resultant neuronal labelling (open circles) and a nigral NY injection site and its resultant retrograde neuronal labelling (open triangle). Neurons showing both Tb and NY fluorescence are denoted by filled circles. Abbreviation in this and subsequent figures: ac, nucleus accumbens; cp, striatum; gp, globus pallidus; snc, substantia nigra pars compacta; snr, substantia nigra pars reticulata.

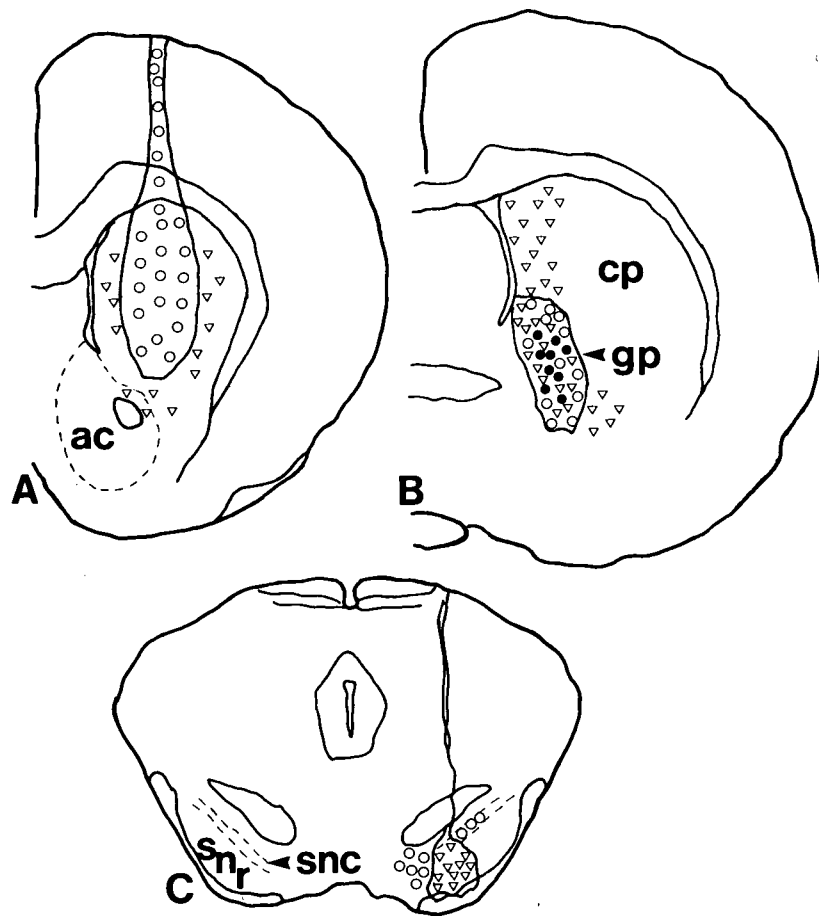


Figure 42. (A and B) Photomicrographs of the fluorescent labelling of pallidal neurons after NY injection into the substantia nigra and Tb injection into the striatum. (A) A pallidal neuron near the pallidostriatal border is labelled for both NY and Tb (arrow). Note the NY labelled striatal neurons at the top of the field. In (B) a double labelled neuron (arrow) is shown along with a single labelled pallidonigral cell demonstrating the differential intracellular labelling of the two tracers. Note the absence of glial labelling. (C) Pallidal neurons labelled by a nigra primuline injection and a striatal True Blue injection. Some primuline-containing neurons are singly labelled but the majority of True Blue neurons are double labelled (arrows).

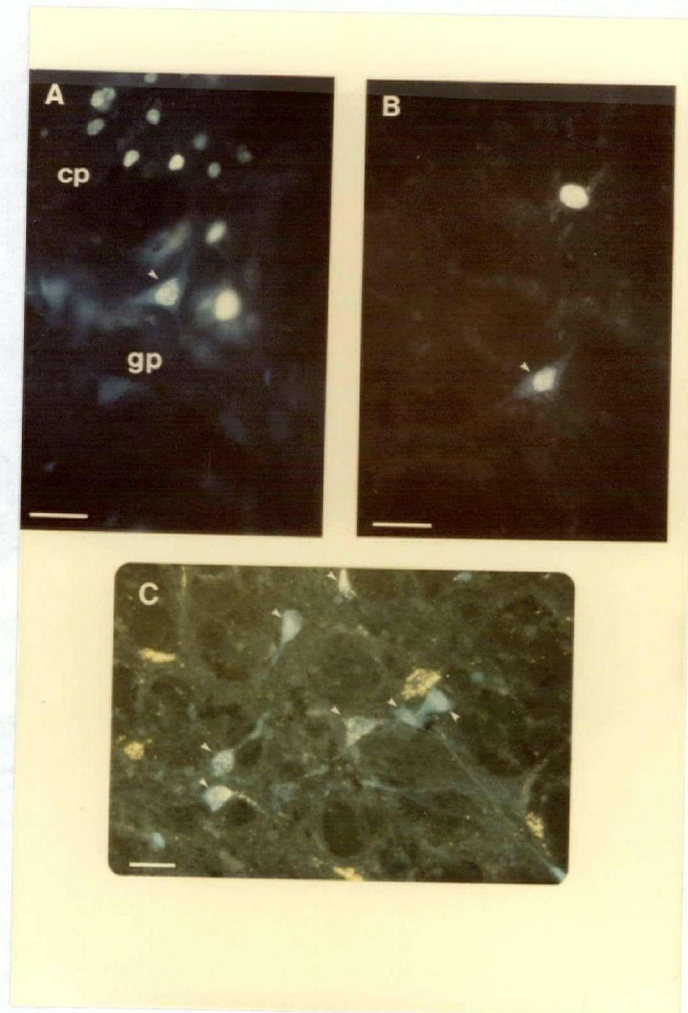
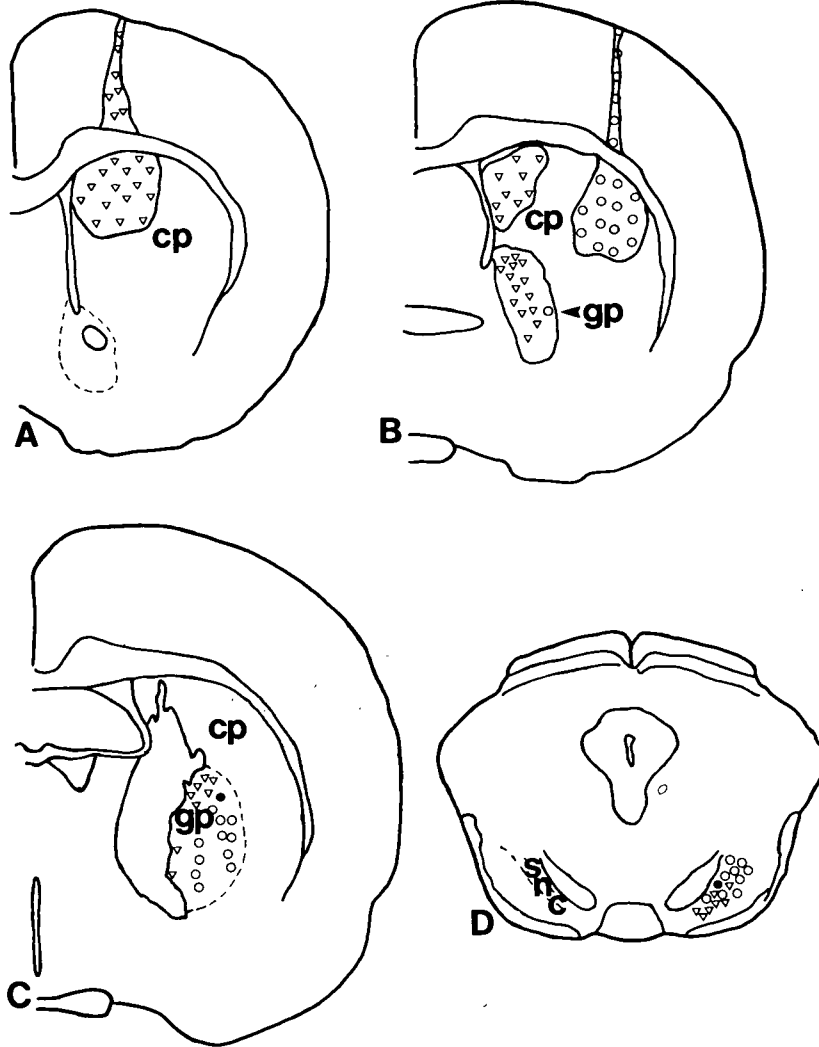


Figure 43. Line drawings of the striatal injection sites and resultant retrograde labelling of NY (triangles) and Tb (open circles) in the globus pallidus and substantia nigra. One double labelled cell was identified in each of the globus pallidus and substantia nigra and these are indicated by filled circles.



indicates that NY was taken up into fibers of passage and therefore double labelled neurons could have arisen through labelling of pallidonigral collaterals.

#### PALLIDAL COLLATERALS TO DIFFERENT AREAS OF STRIATUM

As seen in Figure 43, non-contiguous striatal injections gave rise to the labelling of separate populations of pallidal neurons. A mediolateral topography is maintained and only a few (1 or 2) double labelled cells were seen. In agreement with van der Kooy (1979), no double labelled cells were seen in the thalamus and very few (only 3) in the SN.

#### DISCUSSION

The present report demonstrates that some pallidal neurons which innervate the striatum also project collaterals to the substantia nigra. It was not possible to determine with assurance whether neurons of the GP innervate the striatum and subthalamus via collaterals as well. Furthermore, individual neurons do not appear to have widespread connections within the striatum.

These data suggest that the GP may play a more direct role in the overall regulation of activity in basal ganglia than previously thought. It is known to have a dense innervation of the SUT, a nucleus with widespread collateral innervation of many of the component nuclei of the basal ganglia (Deniau et al., 1978a; van der Kooy and Hattori, 1980a; Jackson and Crossman, 1981). The present results indicate that at least some of the projections of the GP are similarly collateralized. Activity in the pallidal neurons may modify the firing rate of SN neurons

both directly, via the pallidonigral pathway, and indirectly, through the topographically related striatonigral projection (see Fig. 20).



## EXPERIMENT 7: MORPHOLOGICAL CHARACTERIZATION OF THE EFFERENTS OF THE GLOBUS PALLIDUS

### INTRODUCTION

Evidence has been presented in the preceding experiments for a projection from the globus pallidus to the striatum. Implicit in these demonstrations is termination within the striatum. In the past, conclusive demonstration of termination has usually entailed ultrastructural observation of a pathway marked by means of a lesion (eg. Kemp, 1970). A new technique developed by Gerfen and Sawchenko (1983), which utilizes a unique lectin as a neuroanatomical tracer, allows the visualization of projection fibers and their associated terminal elements in their entirety and with morphological detail rivalling the Golgi technique. This procedure was applied in the present study to provide a morphological description of the projections of the globus pallidus to the striatum, thalamus, subthalamic nucleus and substantia nigra.

### METHODS

Male Wistar rats were anesthetized with pentobarbital and secured in a stereotaxic instrument. Glass microcapillaries, broken back to a tip diameter of 10 to 20  $\mu$ m, were filled with a 2.5% solution of Phaseolus Vulgaris - Leucoagglutinin (PhA-L, Vector Labs) in 100 mM sodium phosphate buffered saline, 0.9%/pH 7.4 (PBS), and centered within the globus pallidus using empirically determined coordinates. In one animal an injection was made into the striatum to serve as a control. Lectin was injected by iontophoresis into the striatum or globus pallidus

using a current of 5 uAmp pulsed (7 sec. on/7 sec. off) for from 5 to 15 min. After a 5 to 8 day survival time animals were reanesthetized and perfused sequentially with ice cold normal saline, 4% paraformaldehyde in ice cold sodium acetate buffer (100 mM/pH 6.5), and finally, 4% paraformaldehyde and 0.05% glutaraldehyde in sodium tetraborate buffer (100 mM/pH 9.5). Brains were postfixed overnight in a cold 10% sucrose solution made up in the basic fixative. Sections 30 um in thickness were cut on a freezing microtome and collected serially in ice cold potassium phosphate buffer, pH 7.6 (KPBS). A series consisted of one section out of every six.

Sections for immunohistochemistry were transferred through the following : (1) Incubation in rabbit anti-PhA-L antibodies (Vector Labs), at a 1:2000 dilution in KPBS containing 1% Triton-X 100 and 2% normal goat serum, for 24 to 48 hrs. at 4 C. (2) Rinse for 15 min in a solution of normal saline buffered with Tris-HCl (50 mM/pH 7.4; TBS) containing 1% Triton X 100 and 2% normal goat serum. (3) Incubation for 45 minutes in biotinylated goat antirabbit antibodies (Vector Labs) at an 1:250 dilution with TBS containing 1% Triton-X 100 and 2% normal goat serum. (4) Rinse for 15 min. in TBS. (5) Incubation for 60 min. in avidin-dh/HRP complex (Vector Labs) 1:250 dilution (6) Rinse in TBS for 10 min. (7) Incubation for 10 min in 0.5% cobalt chloride in TBS. (8) Rinse in TB for 5 min. (9) Rinse in 100 mM sodium cacodylate buffer (CB), pH 5.1, for 3 min. (10) Incubation in CB containing 0.05% diaminobenzidine (DAB) and 0.04% hydrogen peroxide for 10 min. (11) Rinse in TBS for 10 min. (12) Mount

sections from 10 mM PB, dehydrate through graded series of alcohols and coverslip.

Some series were processed from step 7 for the HRP reaction protocol using DAB as substrate outlined in Experiment 2. Both procedures yield comparable results in terms of enzyme visualization but differ in the background staining of the tissue. With the procedure outlined above, nonreactive tissue stains lightly in the manner of cresyl violet. To avoid confusion, this reaction will be referred to as CoDAB in figure captions to differentiate it from the noncounterstaining reaction (DAB).

## RESULTS

The PhA-L injection site for case Ph-1 (from which most of the following observations were made) is depicted in Figure 44A. Unless otherwise stated it will be understood that all data presented derive from this case. A second pallidal injection (Ph-2) was much larger and involved part of the striatum as well, and a third pallidal injection (Ph-3) was virtually identical to that in Ph-1. The area of involvement of the injection site in case Ph-1 is obviously well confined to the globus pallidus (GP). Under higher magnification it took the appearance of a diffuse peroxidase reaction in the pallidal neuropil and a much more intense peroxidase reaction within the cell bodies and dendrites of a limited number of pallidal neurons which fell within the bounds of stained neuropil. Axons could be seen to ramify within the GP at short distances from the injection site, and axons leaving the GP could be followed. In addition, fibers and

terminals were found within other brain nuclei including the striatum, entopeduncular nucleus, subthalamic nucleus, substantia nigra and thalamus. Labelled fibers appeared to be totally filled with reaction product and to be visible throughout their extent, including varicosities and terminals. Their appearance was virtually identical to the staining of axons and terminals seen in Golgi material, or after intracellular HRP injections (Chang et al., 1981). The only similarities with the anterograde labelling produced by WGA-HRP injections were in terms of distribution and, as the distribution of the efferent projections was virtually identical to that reported in Experiment 3, the mapping of pallidal efferents will not be repeated in this experiment.

In case Ph-1, only two retrogradely labelled neurons were observed in the striatum even though virtually every striatal section obtained was reacted for the presence of PhA-L. These two were very weakly reactive and immunoreactivity appeared limited to the cell body. No retrogradely labelled neurons were seen in any of the other brain sections. These observations are in keeping with previous reports that PhA-L, when used under the present conditions, is almost exclusively an anterograde transport marker (Gerfen and Sawchenko, 1983). The naming of structures as axons, axon varicosities and terminals follows the precedent set in the Golgi literature. In some cases, light microscopic observations suggestive of synaptic contact with an identified postsynaptic element were made. Rather than repeat modifiers such as "terminal-like" or "suggestive of" it will be

acknowledged at this point that they are not proven explicitly as such. With this proviso in mind, the detailed observations made on the efferents of the GP revealed using this technique are reported below.

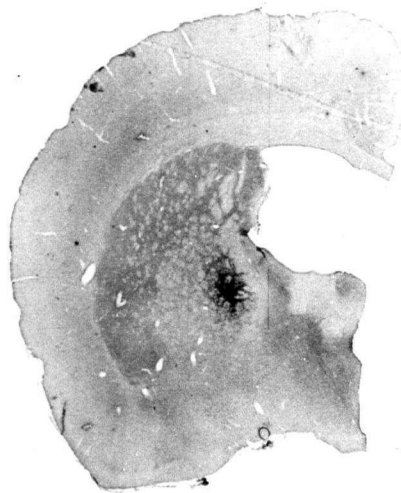
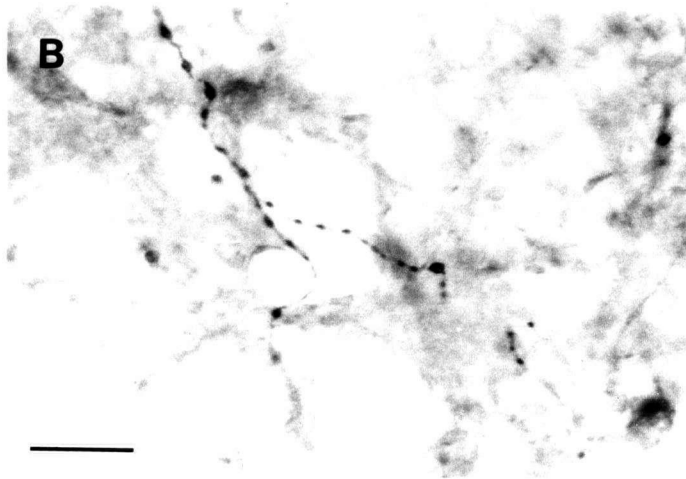
#### GLOBUS PALLIDUS

The PhA-L injection site occupied approximately 10% of the volume of the GP and was situated medially at midlevels of this nucleus. The main features seen emanating from the injection site at this level were varicose fibers which ramified quite locally within the the GP itself (Fig. 44B). The majority of the GP lateral to the injection site remained free of labelled structures. In the GP rostral to the injection site, single, labelled projection fibers were commonly observed. These smooth, straight fibers had a predominantly rostrocaudal orientation and were seen both within fiber bundles and running freely within the pallidal neuropil between the fiber bundles.

#### STRIATUM

At the rostral pole of the GP, fibers were seen to radiate rostrally and laterally within striatal fiber bundles and occasionally in the striatal neuropil. More commonly observed within the striatal neuropil, however, were randomly oriented, twisting, varicose axons. A fairly dense plexus of the varicose fibers was seen in the medial 1/4 of the striatum rostral to the GP but similar features could be observed throughout the medial 1/2 of the caudal head of the striatum (Fig. 45A and 45B). They were also apparent throughout the whole of the rostral half of the head of the striatum (Fig. 45C). Varicosities were sometimes

Figure 44. (A) Low power photomicrograph showing the PhA-L injection site in the GP of case Ph-1. Reacted for CoDAB. (B) Higher magnification showing an immunoreactive varicose axon or dendrite within the GP ventral to the injection site. Bar = 25 um.

**A****B**

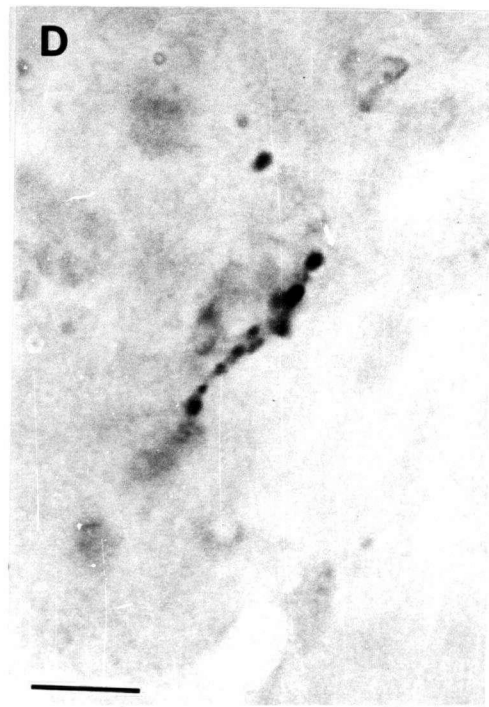
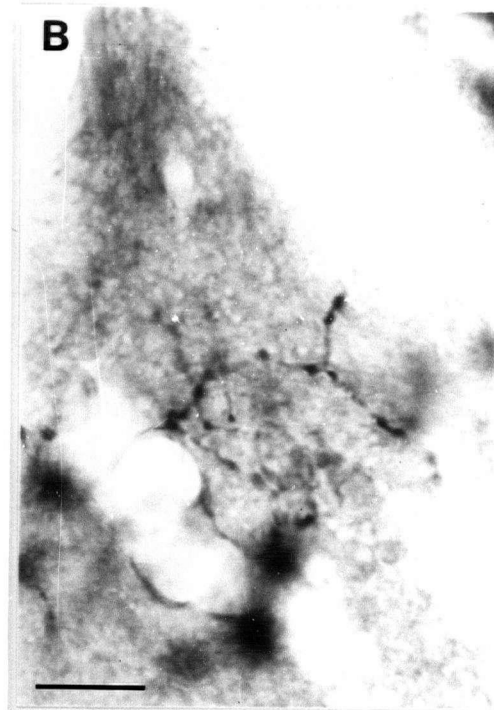
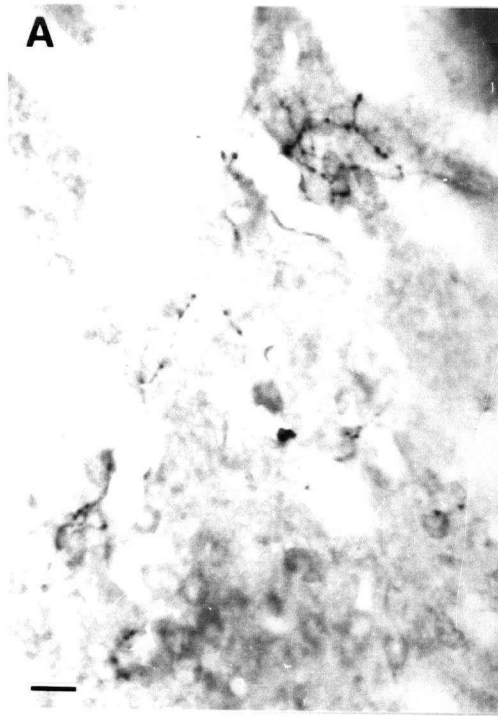
very large (2 or 3  $\mu\text{m}$  by 1  $\mu\text{m}$ ) and elliptical in shape, but were more commonly only slightly elliptical and somewhat smaller (1.5-2.0  $\mu\text{m}$  x 1.5  $\mu\text{m}$ ). Within varicose segments of an axon, the varicosities appeared at roughly regular, 4-5  $\mu\text{m}$  intervals. Some individual fibers could be followed in 1 section for several hundred  $\mu\text{m}$ , through which distance they were seen to give off four to six beaded collaterals. However, with the section thickness employed, the most commonly observed elements were short segments of beaded fibers. It was not uncommon to observe these in close apposition to striatal cell bodies (Fig. 45D), but little significance should be attached to this finding alone.

#### CORTEX

Several smooth PhA-L labelled projection fibers were found within the fiber bundles in the rostral pole of the striatum and were seen within the fibers of the forceps minor at still more anterior levels. Very rarely, labelled fibers could be seen ascending through the corpus callosum overlying the head of the striatum. Examination of the cortex surrounding and anterior to the head of the striatum revealed 8 - 10 segments of stained fibers bearing terminal like varicosities. These appeared to be of two types. The first consisted of fine, straight fibers which radiated perpendicular to the cortical laminae (Fig. 46A). They were very sparsely varicose during their passage through cortical layers V and III and bore very short collateral processes ending in terminal end bulbs of 0.5  $\mu\text{m}$  in diameter which were identical to the axonal varicosities. One of these fine caliber fibers was traced as far as layer II. In another part to the cortex of the



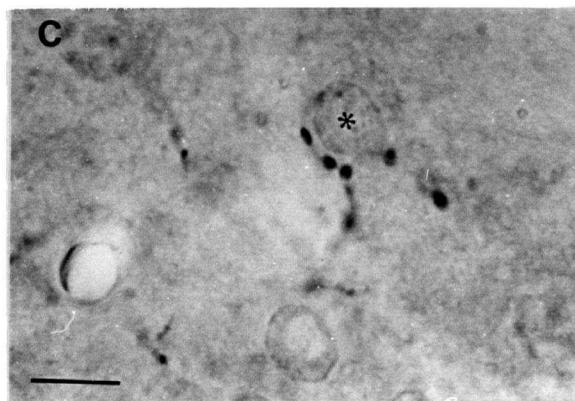
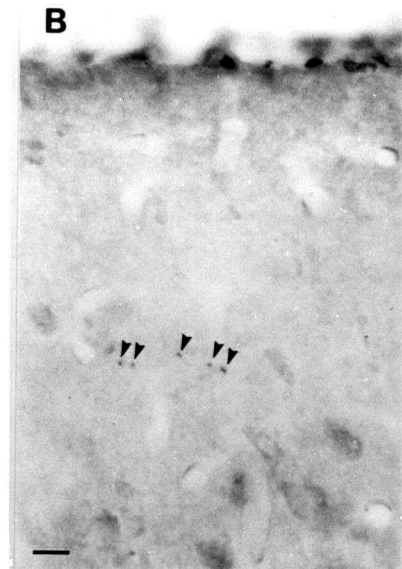
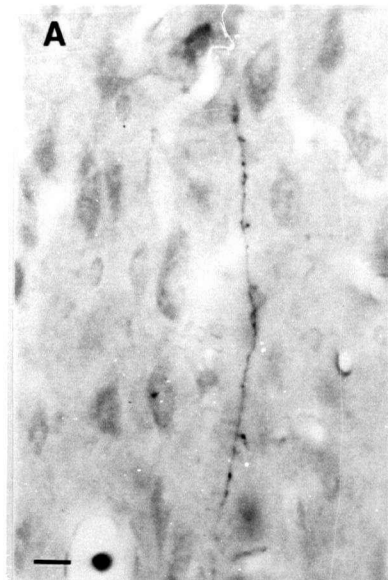
Figure 45. (A) PhA-L positive fibers in the striatum. Note the varicosities distributed along the fibers and the extensive branching of that portion in the upper right of the field. Reacted for CoDAB. (B) Higher magnification of a portion of the field in (A). (C) PhA-L positive fiber in the rostral extreme of the striatum in case Ph-2. Reacted for DAB. (D) Segment of labelled varicose axon lying just ventrolateral to a medium-sized striatal neuron in case Ph-1. The cell body is slightly darker than the surrounding neuropil and the nucleus is pale. Reacted for CoDAB. Bar = 10  $\mu$ m.



same section, a fiber of similar caliber was seen to bifurcate and end in small boutons within layer I (Fig. 46B).

The second, more commonly observed immunoreactive elements were short, randomly oriented, segments of beaded fibers as depicted in Figure 46C. These were judged to be morphologically similar to those described in the striatum but differed from the fine caliber fibers seen in the cortex in that the varicosities were distributed along the axon rather than on short branches off the main axon and the varicosities were much larger. Segments of beaded fibers were sometimes found within a few hundred microns of the fine caliber radiating fibers but insufficient material was collected to determine if they originated from the radiating fibers. Fragments of beaded fibers were most commonly observed in the agranular cortex, dorsomedial and ventrolateral to the forceps minor. Their distribution was much broader than that of the fine caliber fibers. In sections anterior to the striatum, the major orientation of these fibers was within the coronal plane of the section, but in more caudal sections (at the level of the rostral pole of the CP) their orientation was predominantly rostrocaudal. Terminals of this fiber type were found predominantly within layer V in the cortex anterior to level 9650 of Konig and Klippel (1968). Labelled fibers of either type were not detected caudal to this plane and were therefore restricted to the agranular frontal cortex. It should be emphasized that these fibers were not a predominant feature of the labelling seen in any of the cases studied. The total labelling of cortex appeared to arise from only six or eight

Figure 46. Labelling in the cortex in case Ph-1. (A) Projection fiber within layer III of the cortex oriented perpendicular to the cortical surface. This fiber was traced through layers V to II. (B) Fine caliber fiber ending in small boutons (arrows) within layer I of the cortex. (C) A segment of beaded axon bearing large varicosities found in layer V in apparent axosomatic contact with the cell whose nucleus is denoted by an asterisk. Reacted for CoDAB. Bar = 10 um.



projection fibers.

#### THALAMUS

Caudal to the PhA-L injection site in the GP, fibers were seen to traverse the internal capsule (IC) in a dorsomedial direction. No fibers passed laterally into the tail of the striatum. Once the fibers had crossed into the reticular nucleus of the thalamus, they branched quite extensively and displayed spheroid varicosities at regular intervals along the axonal branches (Fig. 47C). These fibers were most commonly observed in the anterior regions of this nucleus. Identical immunoreactive fibers were seen in more caudal portions of the nucleus as well, but at these levels occupied its dorsal extreme. The fibers and their varicosities could not be distinguished from those seen in the striatum. Medial to this nucleus very rare (three in total) projection fibers were seen within the ventral thalamic tier. Each was quite long and straight and they all projected in the direction of the stria medullaris. Sections from case Ph-1 were examined in detail, but neither continuation nor termination could be found. In case Ph-2, however, a few labelled varicose fibers were found in a restricted region within the mediodorsal nucleus of the thalamus. No labelling was seen within this region in case Ph-3, but a few labelled varicose fibers were seen within the parafascicular nucleus.

#### ENTOPEDUNCULAR NUCLEUS

Immunoreactive projection fibers with a predominantly rostrocaudal orientation were followed within the internal capsule to the anterior portion of the entopeduncular nucleus.

At this level, beaded axons appeared within the neuropil inserted within the capsular fiber matrix (Fig. 48). Most often these beaded fibers had a featureless, random orientation, but at times appeared to outline cellular profiles. Beaded axons were found more frequently within anterior portions of this nucleus. At the level where the inclusions of entopeduncular neuropil into the IC become large, the fibers were once again only visible as projection fibers with a mainly rostrocaudal orientation.

#### SUBTHALAMIC NUCLEUS

The subthalamic nucleus received a much more dense innervation than any of the other nuclei examined. Varicose axons were evident throughout the medial part of the nucleus and along the dorsal border of the lateral area (Fig. 47A). They could also be seen within those segments of the nucleus which protruded into the internal capsule. The morphological description of the beaded axons is as that given for those found in the striatum (Fig. 47B). It appeared that a mediolateral orientation of the beaded segments of fibers predominated within the body of the subthalamic nucleus. It was noted that very few projection fibers were seen within the internal capsule at the level at which innervation of the SUT was most dense, far fewer than observed within this fiber bundle either rostral or caudal to this nucleus. In the caudal SUT, the network of labelled varicose fibers was much less dense. Long, single beaded axons were seen running parallel to the cerebral peduncle and within the plane of the section while the majority of fibers were found projecting caudally within the peduncle. This distribution was

Figure 47. (A) PhA-L labelling in the subthalamic nucleus. The cerebral peduncle is in the lower right of the field. (B) Higher magnification of the labelling in the subthalamic nucleus. Reacted for CoDAB. (C) Photomontage of a labelled varicose fiber in the reticular nucleus of the thalamus. Reacted for CoDAB. Bar = 100 um.



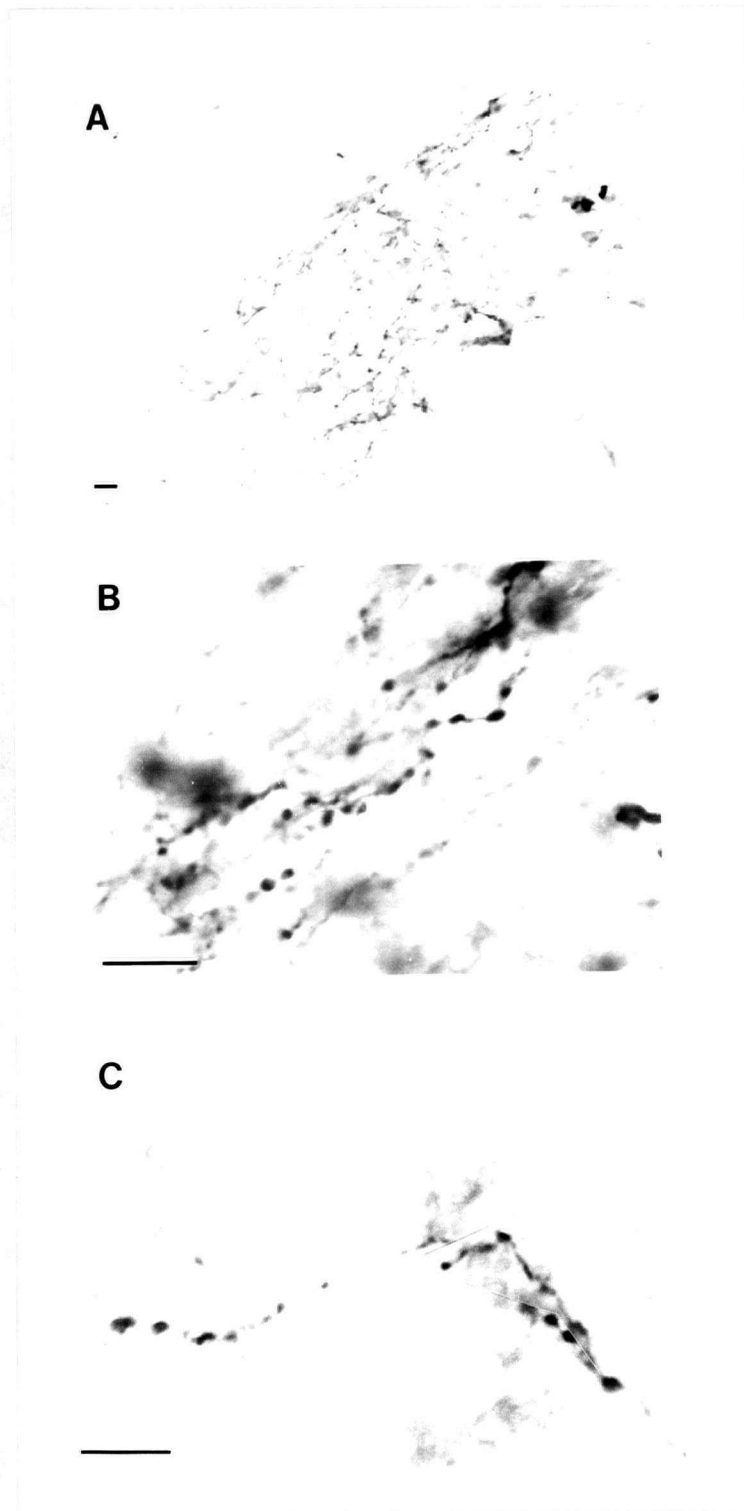
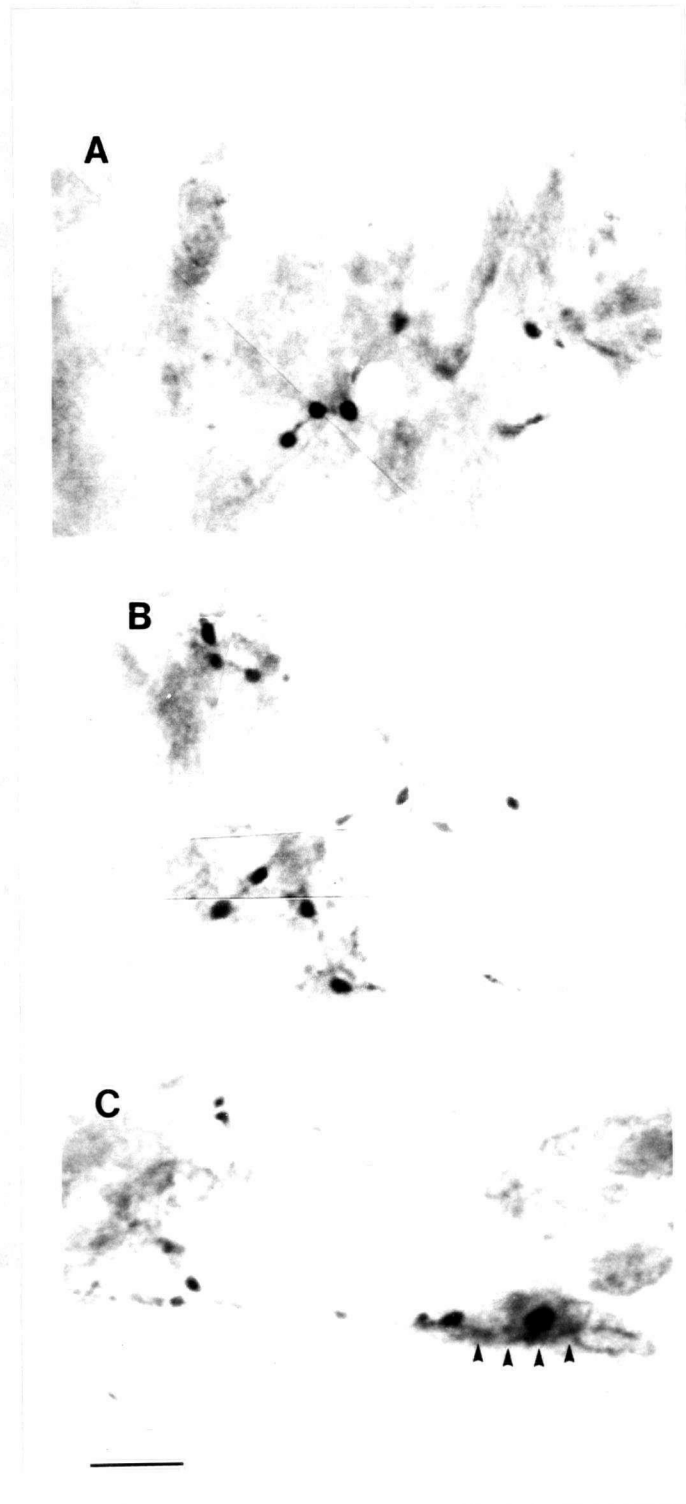


Figure 48. Photomontages of PhA-L immunoreactive varicose fibers in the entopeduncular nucleus. Note that some varicosities are in close apposition to cell bodies (arrows in C). Reacted for CoDAB. Bar = 10  $\mu$ m.



seen to continue back to the rostral tip of the substantia nigra, in agreement with the cytological delineation of the SUT provided by van der Kooy and Hattori (1980).

#### SUBSTANTIA NIGRA

Projection fibers ran within the medial portion of the internal capsule from the subthalamic nucleus to the substantia nigra. From this point to the caudal reaches of the SN, projection fibers followed a path through the pars compacta. Varicose axons as described in the striatum were seen within the pars compacta but were far more common in the pars reticulata (Fig. 49A). This was true throughout the whole rostrocaudal extent of this nucleus. Although the morphology of labelled axons and varicosities was as described in all previous regions except for the cortex, their distribution in the substantia nigra was remarkable. Single varicose axons were relatively rare and instead several segments of beaded axons would associate in tight plexes. This occurred in its simplest form as two varicose segments wound around one another. However, many instances were noted in which four to six fibers would form elaborate arrays, apparently ensheathing neuronal cell bodies within the pars reticulata (Figs. 49B, 50A, 51 and 52). Similar, although infrequent and not so marked, examples of this were also found within the pars compacta (Fig. 50B). It appeared, from the light counterstaining afforded by the CoDAB reaction that fully 50% of the varicose fibers in the pars reticulata were in intimate contact with cell bodies and the proximal portions of their dendrites. In each instance, cresyl violet counterstaining

Figure 49. (A) PhA-L immunoreactivity in the substantia nigra. Labelled varicose fibers can be seen throughout much of the pars reticulata (snr) but only one or two fibers with varicosities on them can be seen in the pars compacta (snc). The region denoted by the arrow is shown in higher magnification in (B) and in the drawing in Figure 50A. The area denoted by the asterisk is shown on Figure 50B. Reacted for DAB. Bar = 10 um.

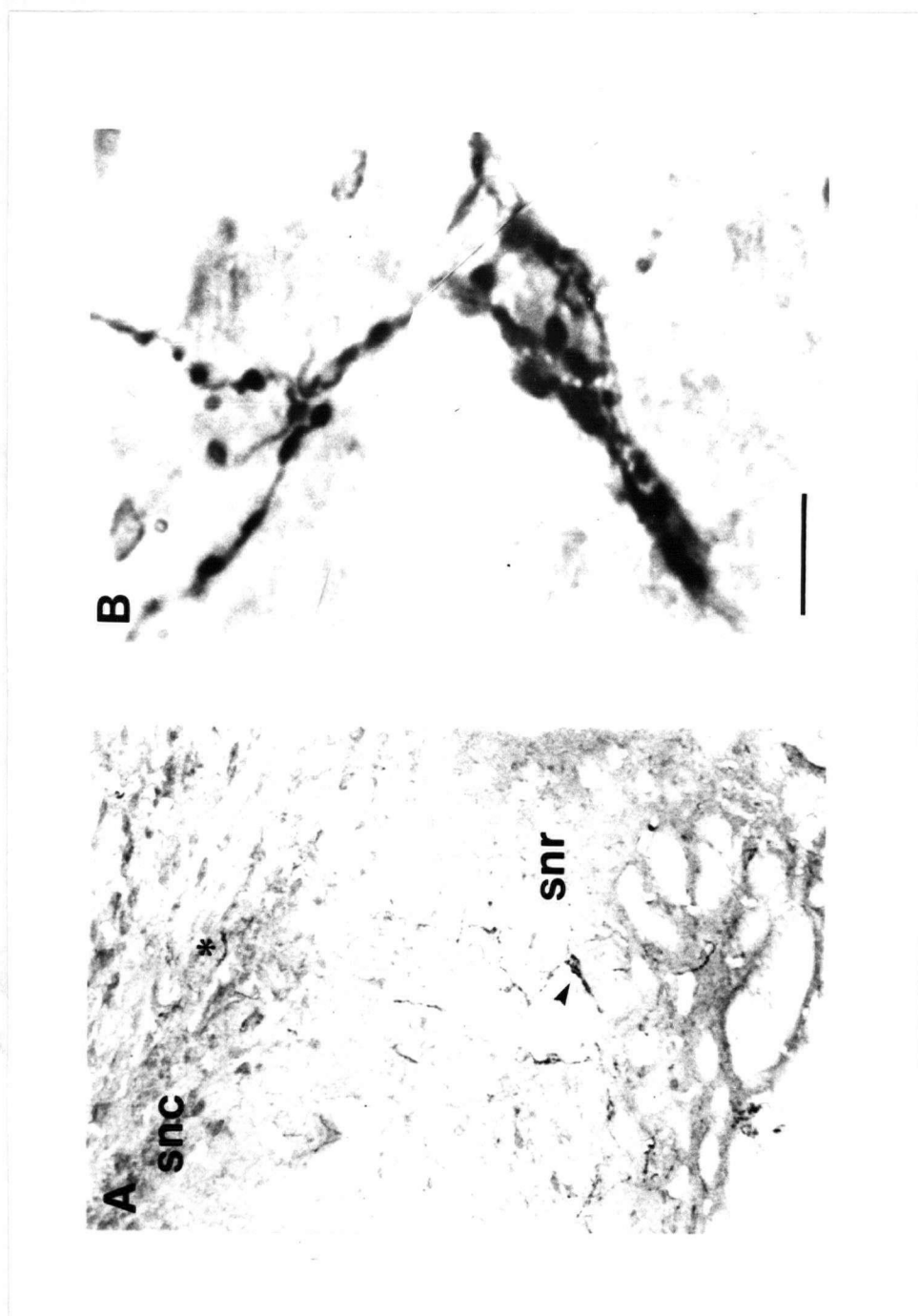


Figure 50. Drawings of labelled varicose fibers in the pars reticulata (A) and pars compacta (B) of the substantia nigra (refer to Figure 49A). Bar = 10  $\mu$ m.

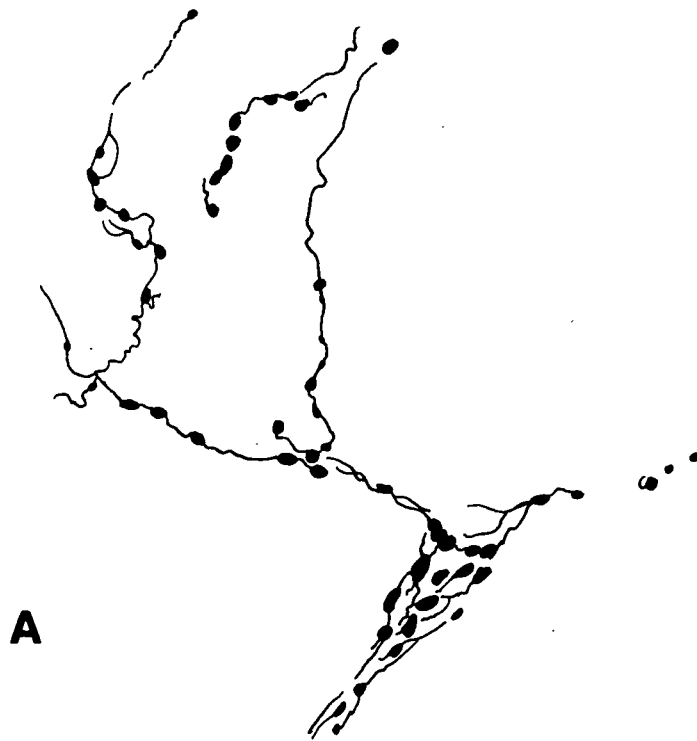




Figure 51. Photomontages of selected features of the labelling in the pars reticulata of the substantia nigra. Note that there appears to be a neuronal cell body at the center of the fiber plexus in (A). This was confirmed by counterstaining with cresyl violet. The same was true of the plexus shown in (B) (refer to Fig.53). (A) reacted for CoDAB, (B) reacted for DAB. Bar = 10  $\mu$ m.

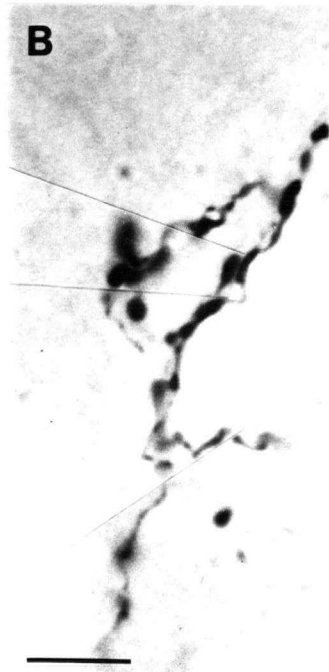
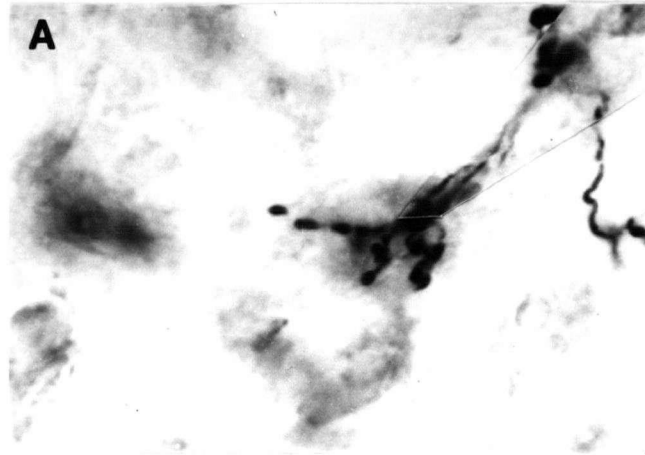
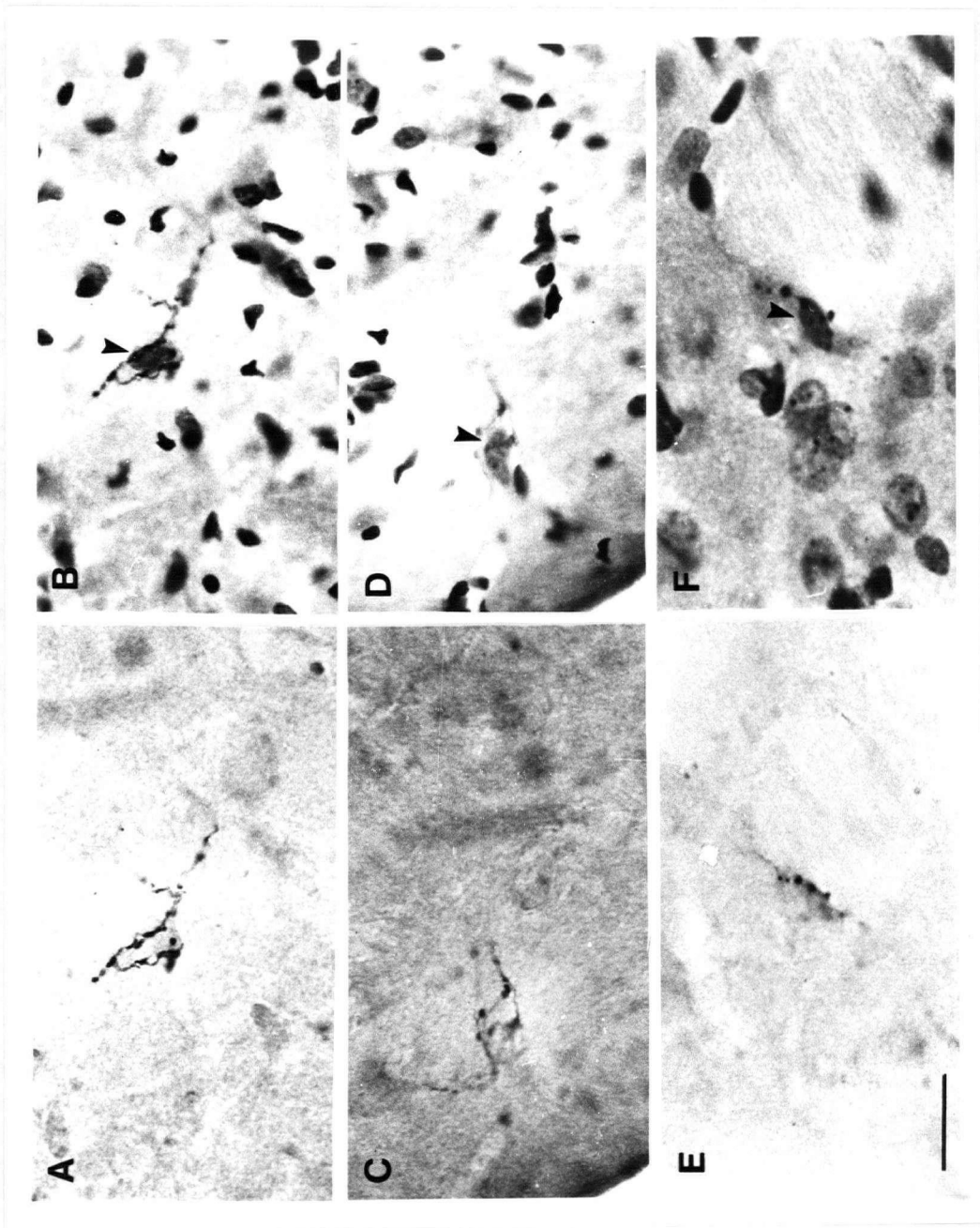


Figure 52. Apparent axosomatic connections in the (A and C) substantia nigra and (E) striatum. The corresponding field after cresyl violet counterstaining is shown at the right. Bar = 25  $\mu$ m.



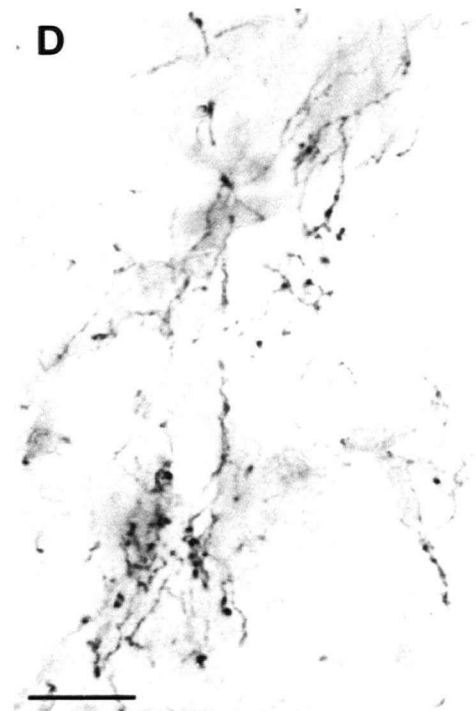
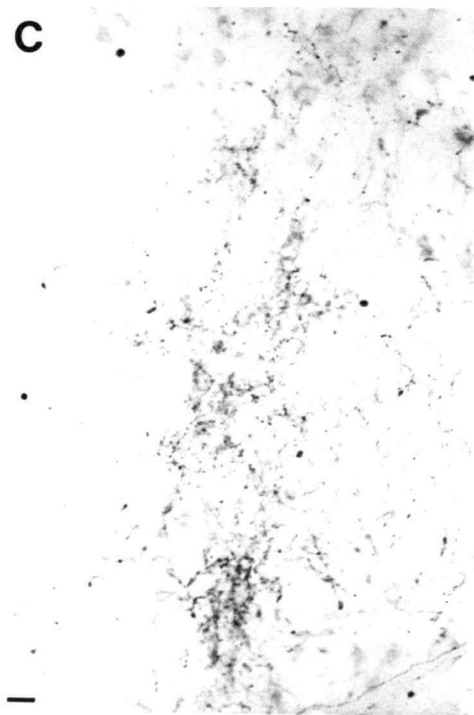
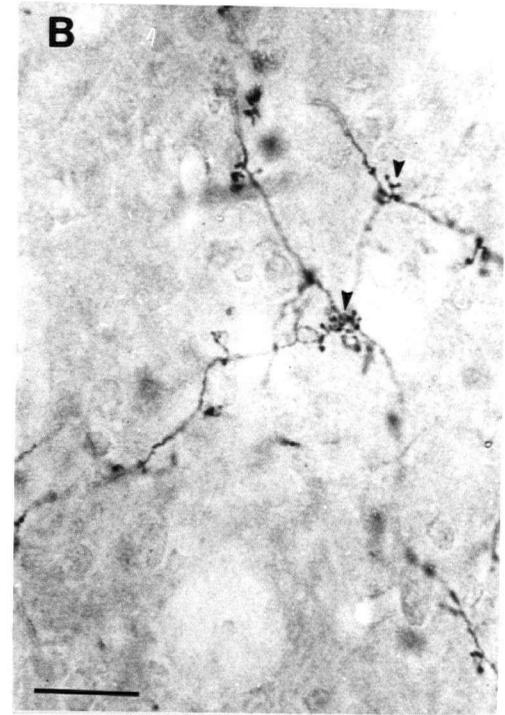
revealed the presence of a neuronal nucleus within these arrays of labelled varicose fibers (Fig.52). In light of the prevalence of this type of contact within the SN, sections from the striatum were examined after cresyl violet counterstaining and, although individual examples were not as dramatic, apparent axosomatic contacts were common in this nucleus as well (Fig. 52F).

Projection fibers, virtually perpendicular to the plane of sectioning, were observed in the caudal extreme of the SN and could be traced through a few sections into the pons, at which point they were lost. Available sections back to the level of the locus coeruleus were scrutinized, but neither fibers nor varicosities were observed.

#### STRIATAL INJECTION

The iontophoretic injection of PhA-L into the head of the striatum (Ph-4) gave rise to a minor collateral labelling within the striatum, and labelled striatal projections to the GP, EP and SN (Fig. 53). Both the distribution and morphology of striatal projections contrasted dramatically with that seen after injections into the GP. Far fewer labelled fibers were seen within the striatum in case Ph-4 than in any of the cases involving injection of the GP and the boutons associated with these fibers appeared as terminal end-bulbs rather than varicosities along the length of the fiber (compare Figs. 45 and 53). These characteristics also applied to the labelling seen in the GP, EP and SN in case Ph-4. Furthermore, the labelled fibers seen in the SN after the striatal injection of PhA-L appeared to be evenly and randomly distributed within the pars reticulata.

Figure 53. Anti-PhA-L immunoreactivity after a PhA-L injection into the striatum (case Ph-4). (A) Labelling around the injection site in the striatum. Note the relative scarcity of labelled fibers around the injection site (arrows). (B) Higher magnification of one of the labelled fibers around the injection site. Note the marked difference in axonal and terminal (arrows) morphology compared to that seen in the striatum after a pallidal injection. (C) Low power photomicrograph of the labelling in the globus pallidus after a striatal injection. (D) Higher magnification of the labelled fibers and terminals in the globus pallidus after a striatal injection of PhA-L. Bar = 25 um.



There was no evidence of the obvious convergence of terminals around nigral cell somata as was seen after injections into the GP.

#### LARGE PALLIDAL INJECTION

Observations of material resulting from a large pallidal injection which also involved part of the striatum (Ph-2) are of some additional interest. The terminal labelling in the substantia nigra reflected the fact that two morphologically distinct inputs had been labelled (morphological heterogeneity was not an obvious feature after injections of the striatum alone). It was noted that all areas receiving pallidal projections showed a much more profuse labelling, due no doubt to the larger injection site, but the shorter survival time in this case - five days rather than eight - seemed to have led to a less complete labelling of individual elements).

#### DISCUSSION

Before discussing the present observations in terms of the projections of the globus pallidus a number of possible sources of misinterpretation must be examined. Perhaps the most serious source of error would be that arising from the uptake and transport of lectin by fibers from other sources passing through the pallidal injection site. This would force the admission that terminals in the substantia nigra could in fact have arisen from labelling of the striatonigral pathway, and that the features in the striatum could be explained in terms of any number of ascending inputs.

The characteristics of PhA-L transport have been rigourously



investigated in the hippocampus and it was concluded that this lectin, under the conditions used here, does not give rise to significant uptake by fibers of passage (Gerfen and Sawchenko, 1983). Furthermore, the morphological detail afforded by this technique allows one to assess this possibility through a comparison of axonal and terminal morphologies. Both the distribution pattern and morphology of labelling in the substantia nigra after pallidal injections differed markedly from those seen after striatal injections. This shows that, although striatonigral fibers pass through the globus pallidus, they do not take up appreciable amounts of tracer and, furthermore, indicates that it is unlikely that any of the labelling attributed to projections of the GP arose instead from labelling of fibers that originated outside the injection site.

The morphologies of a number of striatal afferents have been described in the monkey using the Golgi technique (DiFiglia et al., 1978). Examination of the photographs and drawings of striatal afferents thought to arise from the cortex, substantia nigra and dorsal raphe nucleus reveals striking differences in morphology with the striatal fibers labelled after pallidal injections of PhA-L. Although similar in some respects, the morphology of immunoreactive elements in the striatum also differs significantly from the Golgi characterization of afferents suggested to arise from the thalamus (DiFiglia et al., 1978). The pallidal afferents differ from the thalamic afferents in having a much more consistent axonal diameter throughout the length of the fiber, a far greater prevalence of long varicose

segments as opposed to short varicose or end-bulb collaterals and a much more regular shape of varicosities. Thus, not only is it very unlikely that fibers passing through the GP were labelled, but the morphology of the labelled axons within the CP differed from that of any of its other known afferents.

Another phenomenon with the potential to confound the interpretation of the present observations would be the retrograde labelling of local collaterals of neurons projecting to the injection site. In agreement with previous work (Gerfen and Sawchenko, 1983), the retrograde labelling of cell bodies after the injection in case Ph-1 and Ph-3 was virtually nonexistent. Direct comparison of the morphology of pallidostriatal fibers (Fig. 45) with that of local collaterals of striatal neurons seen after PhA-L injections into the striatum (Fig. 53) or by intracellular HRP injections into striatal efferent neurons (Wilson and Groves, 1980; Preston et al., 1980) also serves to indicate that the terminal labelling studied did not arise from retrograde labelling of local collaterals of striatal efferent neurons. It appears, therefore, that this novel tracing technique can provide reliable data on the efferent projections of the injected nucleus. Some examples can be found of local collaterals of striatal neurons which do resemble the pallidostriatal fibers (Somogyi et al., 1981), but it would not be anticipated that the morphology of the pallidostriatal fibers be totally unique.

The distribution of terminal labelling found in this experiment is in total accord with that implied by the

anterograde labelling of pallidal efferents by WGA-HRP presented in Experiment 3, including a minor efferent projection to the mediodorsal and parafascicular nuclei. The fact that labelling of these latter two nuclei was not a consistent feature after pallidal injections may have been due to the small number of efferents labelled in each case, such that minor connections might be missed in a single animal. In addition, many of the other observations presented in previous sections seem to be borne out by observations made in the present study. As the literature in support of the existence of these projections has been cited previously, the present findings will be discussed in terms of the conclusions of the previous experiments.

It is of special note that the examination of cortical material provoked a heterogeneous description of afferent morphology. All of the other nuclei receiving afferents from the globus pallidus showed the same beaded, axon en passant type of synaptic formation (Figs. 54 and 55). The labelling within the cortex can in part be attributed to the fact the injection site in case Ph-1 undoubtedly involved some neurons of the nucleus basalis magnocellularis. As the fine-caliber axon with small varicosities and end bulbs were unique to the cortex, it is likely that the observation of a small number of these fibers in case Ph-1 represented cortical afferents arising from the magnocellular neurons of the nucleus basalis. The fragments of larger, beaded fibers seen within the cortex were indistinguishable from the pallidal efferents to other nuclei and could represent the cortical terminals of the small number of

parvocellular pallidal neurons which were demonstrated in Experiment 5 to project to the frontal cortex.

It is noted with interest that the morphology of the local collaterals of the lateral pallidal neuron described by Park et al. (1982) is identical to that which has been seen in the CP, EP, SUT and SN in the present study after pallidal PhA-L injections. The same is true of the local collaterals illustrated by DiFiglia et al. (1982a) for pallidal neurons in the monkey studied by the Golgi technique. In fact, in all areas except for the cortex, the labelled pallidal efferents shared the same rather unique axonal morphology (Fig. 54). The remarkable maintenance of morphological homogeneity seen within all areas receiving input from the GP supports the conclusion drawn in Experiment 6 on the basis of double retrograde labelling that the pallidostriatal and pallidonigral pathways arise at least partly as collateral outputs. The present results suggest that this concept might be expanded to include the projections to the subthalamic nucleus and thalamus as well.

As described in Experiment 5 there is evidence that the GP is not a homogeneous nucleus. The injection site, depicted in Figure 44, in the more well studied case in this report was situated in the medial part of the GP. This region is distinguished from the lateral GP in that (1) its neurons do not collaterize within the GP, (2) its neurons have a different dendritic morphology and orientation and (3) in the monkey it demonstrates a much heavier enkephalin immunoreactivity and a much lighter substance P immunoreactivity (Park et al., 1982;

Figure 54. Drawings of typical labelled fibers and varicosities in the striatum (A), reticular nucleus of the thalamus (B), entopeduncular nucleus (C), subthalamic nucleus (D) and substantia nigra pars reticulata (E). Note the marked similarities in the appearance of labelled features in these five nuclei. Bar = 10  $\mu$ m.

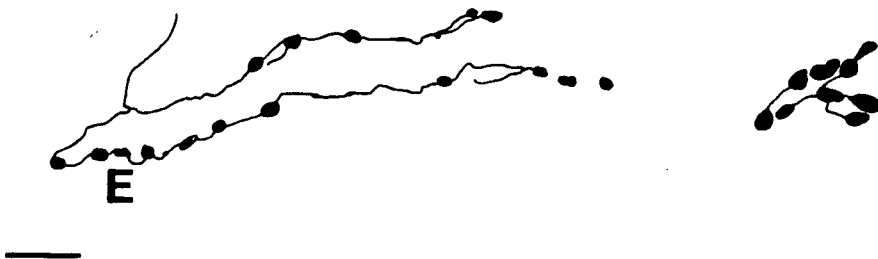
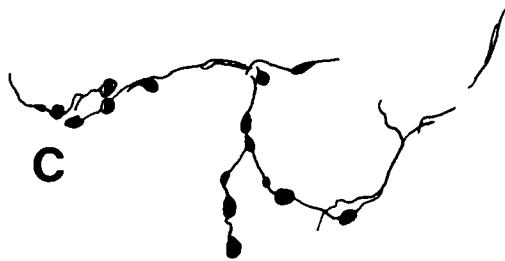
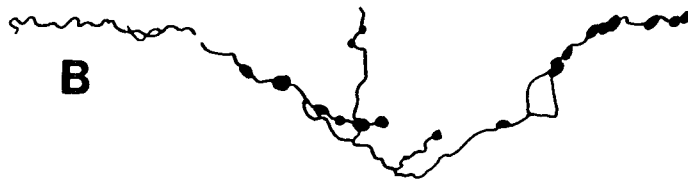
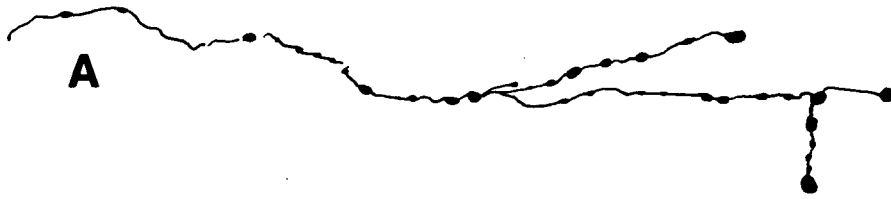
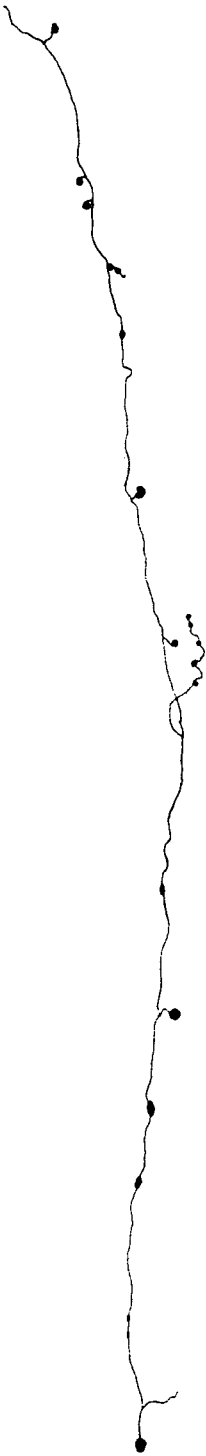
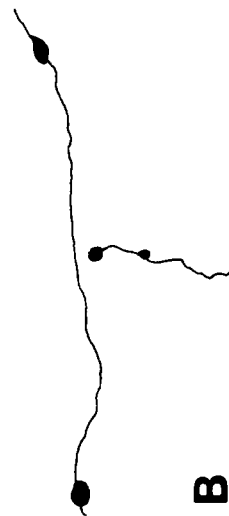
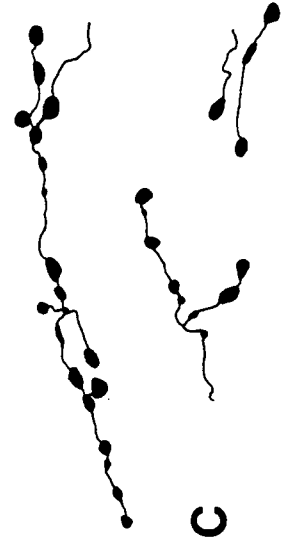


Figure 55. Camera lucida drawings of some of the PhA-L labelled fibers in the cortex of case PhA-1. (A) An example of the fine-caliber, radiating fiber. (B) Example of the short segments of beaded fiber found within the prefrontal cortex. Compare this latter type to the beaded fibers in the subthalamic nucleus (C). Bar = 10  $\mu$ m.

**A****B****C**



Haber and Elde, 1981). However, as the distribution of efferents of the GP did not differ in any significant way between the small pallidal injection (which labelled only the medial GP) and the large pallidal injection (which labelled the medial GP, lateral GP and the bordering striatal tissue) it would appear that the basis for nonhomogeneity within the GP does not include its efferent projections.

As shown in Experiments 2 and 3, the major pallidal projection to the SN ends within the pars reticulata rather than compacta and differs markedly from the striatal input to the pars reticulata in its concentration around cell bodies and primary dendrites, and in its morphology. Previous demonstrations of the projection of the globus pallidus to the substantia nigra have described it as light. While this may be true in some contexts, it is obvious from Figure 49 that the pallidal innervation of some pars reticulata neurons is heavy indeed. The large varicosities studding the cell soma and primary dendrites are likely to have a significant functional advantage over more distally located afferent elements. Axosomatic contacts of pallidal terminals were also suggested by observations made in the CP and EP, but were not as numerous or obvious as those seen in the SN. Neither, however, were those seen in the SUT but recent ultrastructural observations of the pallidal efferents to this nucleus show that in fact, pallidal terminals do end predominantly on the cell bodies and proximal dendrites of neurons in the SUT (Romansky et al., 1980). It may be that techniques better able to answer this question will show that

proximal innervation of target cells (termination predominantly on the cell soma and proximal dendrites) by pallidal terminals is generalizable to nuclei other than the SN and SUT.

The major finding of significance in the present report is the observation of varicose fibers projecting from the globus pallidus to the striatum. This represents the first clear demonstration of anterograde transport from the GP to the CP and serves as final confirmation of the existence of a pallidostriatal projection in the rat. A recent electron microscopic study reports the morphology of a reputed pallidostriatal projection. After electrocoagulation of the external globus pallidus in the monkey, degenerative changes were observed in a myelinated afferent which made broad, asymmetric, axospinous, synaptic contacts in the CP (Chung and Hassler, 1982), characterised as plump axospinous type III. Degenerating terminals ranged from 0.5 to 2.0  $\mu\text{m}$  in diameter and contained large round vesicles. The large varicosities seen on labelled pallidostriatal fibers in the present study are certainly within this size range but it is probable that Chung and Hassler were examining the degenerating processes in a whole range of striatal afferent populations due to their choice of lesioning technique. Anterograde labelling of this pathway with a tracer or with a fiber sparing lesion of the GP is required before reliable ultrastructural conclusions can be reached.

## EXPERIMENT 8: DEMONSTRATION OF A PALLIDAL INNERVATION OF STRIATAL SOMATOSTATIN CONTAINING NEURONS

### INTRODUCTION

Recent immunohistochemical studies have demonstrated that the neuropeptide somatostatin is contained within a population of striatal neurons (DiFiglia and Aronin, 1982; Vincent et al., 1982d). Somatostatin (SST)-containing neurons found within the striatum can also be reliably and selectively stained by using a histochemical technique to visualize nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) activity (Vincent et al., 1982e). Thus, within the striatum there is a perfect correspondence between perikaryal SST immunoreactivity and NADPH-d activity, indicating that this enzyme can be used to identify striatal SST-containing cells. The histochemical technique is, in fact, superior to the immunohistochemical method in that it provides much greater morphological detail and is more readily amenable to combinations with other histochemical procedures (Vincent et al., 1983). In the present report, NADPH-d activity was combined with anterograde labelling of the pallidostriatal projection to demonstrate that pallidal terminals innervate the proximal dendrites of SST-containing neurons within the CP.

### METHODS

Sections through the striata of the cases discussed in Experiment 7 (the small pallidal injections, PhA-1 and PhA-3, and the large pallidal injection, PhA-2) were used in the present study. Therefore, the procedure for injection of PhA-L, fixation

and sectioning are as described in Experiment 7. Sections which had been stored for six days, in ice cold potassium phosphate buffer (100 mM, pH 7.6) containing 0.9% NaCl, after cutting were reacted for NADPH-d activity using a modification of the method of Scherer-Singler et al. (see Vincent et al., 1982e). Sections were incubated at 37 C for 30 min. in Tris-HCl buffer (100 mM, pH 8.0) containing 1.0 mM NADP, 0.2 mM nitro blue tetrazolium and 15 mM monosodium malate. After a 10 min. rinse in phosphate buffered saline, the sections were reacted for the immunohistochemical demonstration of PhA-L. Dehydration through a graded series of alcohols was found to produce crystalline artifact within the sections so some sections were simply air dried and coverslipped with paraffin oil. Sections were observed and photographed using a Zeiss universal microscope fitted with a 100x oil immersion objective and drawings were made with a Wild microscope equipped with a drawing tube.

## RESULTS

The appearance of NADPH-d stained neurons within the striatum was as described previously (Vincent et al., 1982e; Vincent et al., 1983). Dark blue formazan precipitate filled the perikarya and dendrites of medium sized polymorphic neurons distributed throughout the striatum (Fig. 56). The striatal neuropil also contained numerous beaded fibers stained dark brown for the immunoperoxidase reaction revealing anterogradely transported PhA-L as described in Experiment 7 (Fig. 56). However, the clarity of the staining was somewhat diminished compared to sections reacted for PhA-L alone. In all three

Figure 56. Photomicrographs of NADPH-d-stained neurons (blue) in the striatum showing apparent innervation by terminal varicosities of pallidostriatal fibers labelled by the immunohistochemical procedure for anterogradely transported PhA-L (brown). All examples are from case PhA-2. The neuron in (A) is shown in higher magnification in (B). Bar = 10  $\mu$ m.

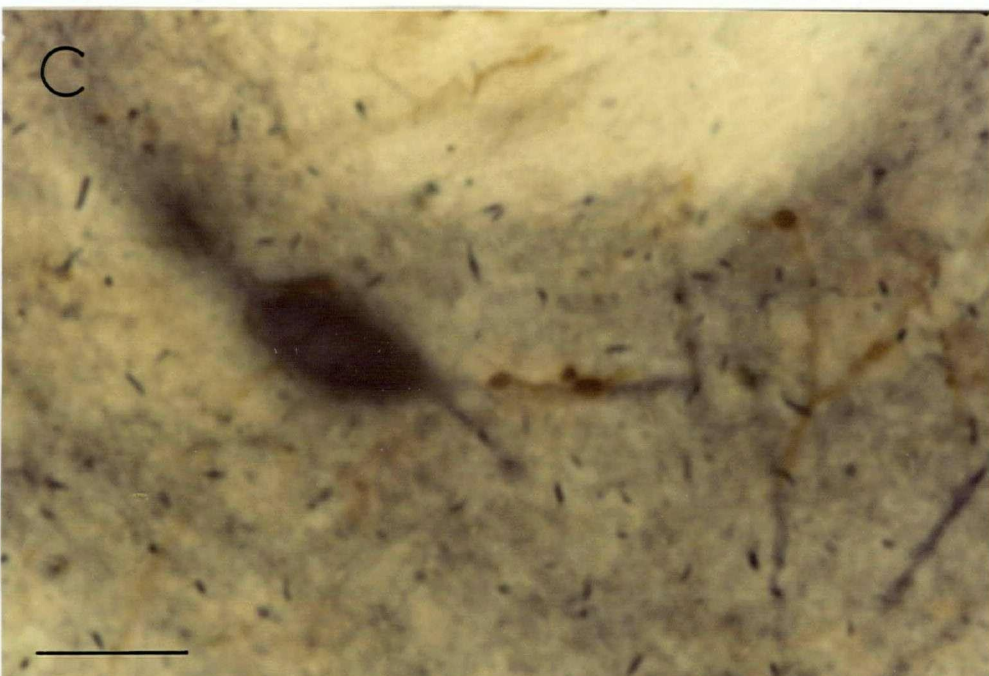
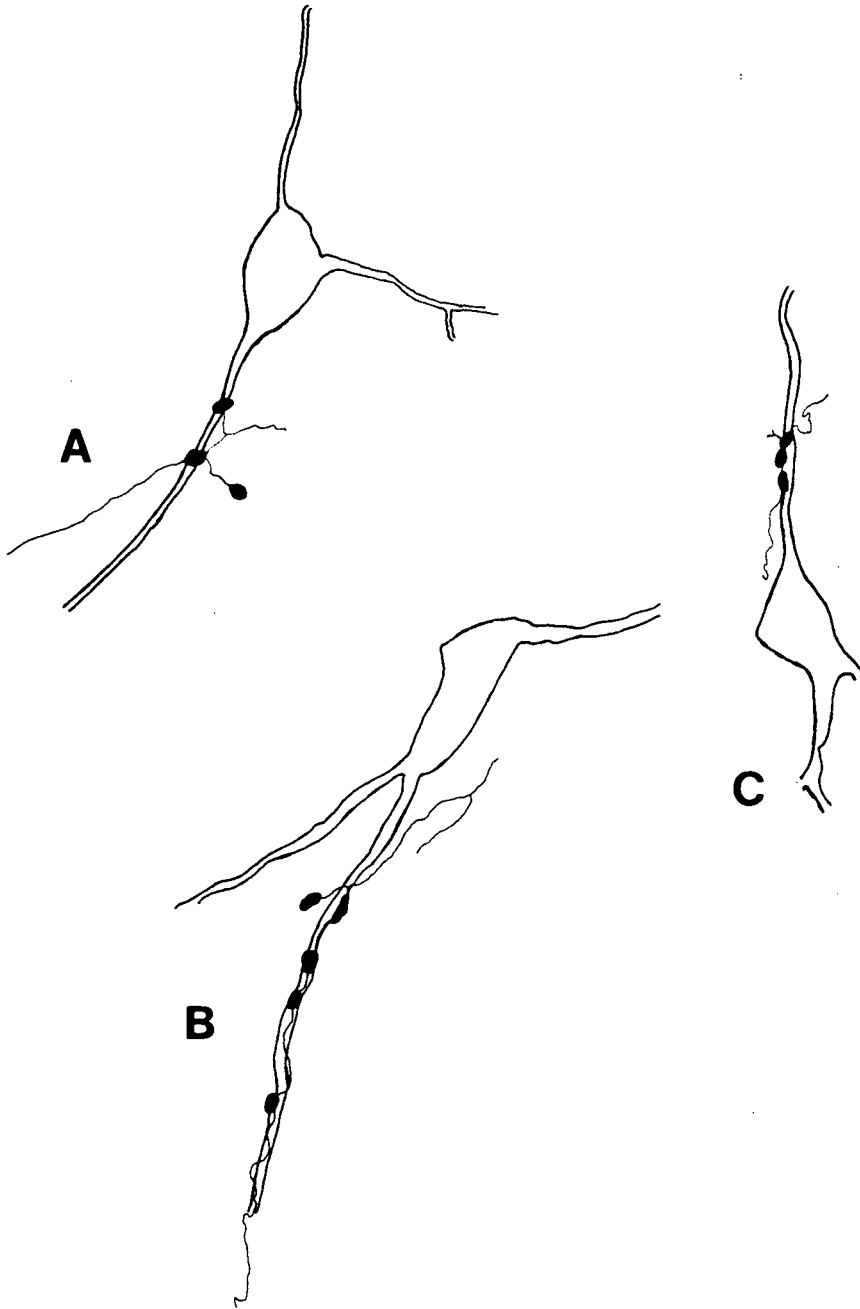


Figure 57. Camera lucida drawings of NADPH-d-stained neurons and labelled pallidostriatal fibers. The neuron shown in (A) is from case PhA-1, the other two are from PhA-2. Note in particular the correspondence between the orientations of the afferent fiber and one of the dendrites of the neuron in (B). Bar = 10  $\mu$ m.





cases, instances were found in which NADPH-d reactive neurons had beaded fibers stained for PhA-L running along their dendrites (Fig. 56 and 57). In some of these cases, the fibers and the varicosities they bore followed the dendrites for some distance and matched it in focal plane (Fig. 57B), suggestive of a longitudinal axodendritic innervation. In areas containing labelled pallidostriatal fibers, an apparent apposition to NADPH-d-labelled dendrites was noted for approximately 5% of the terminal varicosities.

#### DISCUSSION

Although the association of a few varicosities with an identifiable post-synaptic element is of questionable significance, the results of this experiment showed a congruence between labelled axon and primary dendrite which could hardly be attributed to a random process. Labelled terminal varicosities were rare within the fields from which the examples presented above were taken, and thus, the probability of coincidental observation of apposition was small. As many other immunoreactive fibers and varicosities were found which were devoid of identifiable association with NADPH-d reactive perikarya or dendrites, it would seem that only one of the targets of the pallidal efferents within the striatum is the primary dendrite of the striatal somatostatin containing neuron. This neuron is thought to correspond to the aspiny medium sized type III neuron described as an interneuron in the Golgi studies of Dimova et al., (1980).

In recent ultrastructural studies, the SST-containing cell

of the striatum has been described as receiving only two or three types of synaptic endings (DiFiglia and Aronin, 1982; Takagi et al., 1983). Large synaptic boutons were frequently seen on proximal dendrites and represent an excellent candidate for the pallidostriatal terminals. These boutons were seen to form symmetrical synapses and to contain pleomorphic vesicles, features often associated with GABAergic terminals (Ohara et al., 1983). Another, smaller type of bouton was seen to form asymmetrical synaptic contacts with more distal portions of the dendrites of SST-containing CP neurons and contained small round vesicles such as those found within terminals originating in the cortex or thalamus (Kemp and Powell, 1971b).

Although the observation of innervation of SST-containing striatal neurons by pallidostriatal terminals bears confirmation by electron microscopic techniques, the present data provide strong evidence for such an association. Similar combinations of the PhA-L tracing technique with retrograde labelling and histochemistry for acetylcholinesterase have already proved successful (Staines and Gerfen, unpublished observations) and these strategies will undoubtedly enhance our understanding of the circuitry underlying brain function.

## GENERAL DISCUSSION

As the major thrust of the research presented above has been an investigation of the connections of the globus pallidus within the context of the circuitry of the basal ganglia, this discussion will concentrate mainly on the role of the GP in the function of this system. In speculating about this function, a number of primary and secondary assumptions have been made.

(i) Neurons of the GP have axon collaterals which innervate the CP, SUT and SN.

In the double retrograde fluorescent tracing experiment, it was shown that at least a portion of pallidal neurons innervate the CP and SN via collaterals (Expt. 6). The extension to include collateralization to the SUT as well is based on observations made using PHA-L to trace the efferent projections of the GP. With this method, similar terminal morphology was seen in the CP, SUT, and SN. Furthermore, although beaded axons were seen within the subthalamic nucleus, descending projection fibers could not be found at this level. Projection fibers were clearly visible rostral and caudal to this nucleus. These observations suggest that the innervation of the substantia nigra arose from a caudal extension of fibers giving off terminals in the SUT.

(ii) Pallidal endings within the CP, SUT and SN are inhibitory.

This assertion is based on the assumption of collateralization and the observations from electrophysiological studies on the pallidosubthalamic projection. The effects of

pallidal stimulation on the firing of rate of neurons in the SUT were reported in earlier work as a mixed inhibitory-excitatory response but more recent studies have shown that this input is purely inhibitory (Tsubokawa and Sutin, 1972; Ohye et al., 1976; Rouzaire-Dubois et al., 1980; Kita et al., 1983).

(iii) GABA is (one of) the neurotransmitter(s) used by the efferents of the GP.

At present, although there is no consensus as to the transmitter(s) used by neurons of the GP in any of their connections, the majority of available evidence points to GABA as the major pallidal neurotransmitter. The data bearing on this point are reviewed briefly below.

In early immunohistochemical work, substance-P positive cell bodies were found in the GP (Kanazawa et al., 1977), but other studies, employing colchicine to inhibit axonal transport and increase the concentration of the peptide in the perikarya, failed to confirm this observation (Ljungdahl et al., 1978). Other data indicate that the majority of the substance-P immunoreactivity in the GP is contained within afferents from the CP (Staines et al., 1980a; but see Hong et al., 1977).

Projections from the SNr to the superior colliculus, ventromedial thalamus, dorsal midbrain tegmentum and peribrachial region all appear to contain GABA (Vincent et al., 1978a; DiChiara et al., 1979; Childs and Gale, 1983). Similarly, the projection of the EP to the lateral habenula is also GABAergic (Gottesfeld et al., 1977; Nagy et al., 1978b; Starr and Kilpatrick, 1981; Vincent et al., 1982a) and that to the ventral

tier of the thalamus may be as well (Uno et al., 1978). Frequent references to the remarkable similarities between the GP, EP and SNr (see Nauta, 1979a) suggest that the possibility of a GABAergic component to the outputs of the GP deserves consideration.

An immunohistochemical study on the distribution of the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), in the forebrain contrasted the numerous positive cell bodies seen within the CP with the relative rarity of perikaryal staining in the GP (Ribak et al., 1979). The few stained cells located within the GP were medium sized (15-20  $\mu$ m) and the more numerous large cells received a heavy innervation of GAD-positive terminals but were themselves unreactive. On the other hand, recent studies from another laboratory, reported in abstract form, state that nearly all of the neurons in the GP, EP and SNr show perikaryal staining for GAD (Oertel et al., 1982).

There are conflicting biochemical data concerning the possibility of a GABAergic projection from the GP to the SUT. Fonnum and his colleagues (1978) found decreased levels of GAD in the SUT of the cat following electrolytic lesions of the GP. Kainic acid lesions in the rat GP however failed to significantly reduce GAD activity in the SUT although they were effective in blocking the anterograde labelling of the pallidosubthalamic projection (van der Kooy et al., 1981a).

Nagy and Fibiger (1980) reported that there was no significant decrease in the GAD activity in the SN after kainic acid lesions of the GP and a portion of the surrounding CP. This

observation has been confirmed, but with the addendum that at shorter post-lesion survival times a significant decrease in nigral GAD can be seen (van der Kooy et al., 1981a). GAD activities have been shown to increase in some brain areas after lesions of non-GABAergic inputs (Vincent et al., 1978b; Gilad and Reis, 1979), probably as a result of sprouting of GAD-containing fibers within the target locus. It is possible, therefore, that at longer survival times, a similar post-lesion increase in nigral GAD activity due to sprouting may mask a small post-lesion decrease in nigral GAD levels due to a loss of pallidonigral terminals.

It remains to be determined if lesions of the GP cause a decrease in the GAD activity of the CP but this would be difficult to discern, given the high levels of this enzyme contained within intrinsic elements of the striatum (McGeer and McGeer, 1976; Nagy et al., 1978a) and the comparatively few neurons projecting to it from the GP. It has been noted that kainic acid lesions of the GP produce significant decreases in the GAD within this nucleus (Nagy and Fibiger, 1980). An almost identical percentage of the GAD activity in the SN is lost after nigral kainic acid lesions (Nagy et al., 1978c). In the case of the SN, and perhaps the GP as well, these data reflect the fact that, although most of the activity of this enzyme is located within afferent terminals, a portion of it derives from neurons found within that nucleus.

The inhibitory effect of GP stimulation on the firing rate of SUT neurons is mimicked by iontophoretic application of GABA

or muscimol (a GABA agonist) and is antagonized by the GABA antagonists bicuculline and picrotoxin (Rouzaire-Dubois et al., 1980). Furthermore, the inhibitory postsynaptic potentials recorded from neurons in the SUT after pallidal stimulation result from an increase in chloride ion conductance (Kita et al., 1983). The inhibitory action of GABA is well known to act via this ionic mechanism (see McGeer et al., 1978).

In a recent series of papers, the histochemical demonstration of GABA-transaminase (GABA-t) activity has shown potential for the identification of GABAergic projections. Lesions of nuclei containing GABAergic projection neurons lead to decreases in the histochemical reaction for GABA-t within the corresponding terminal regions. Thus, lesions of the CP decrease GABA-t in the GP, EP and SN; lesions of the EP decrease GABA-t in the ventral thalamus and the lateral habenula; and, bearing on the present question, lesions of the GP decrease GABA-t in the SUT (Vincent et al., 1981; Vincent et al., 1982a). These findings suggest that pallidal efferents, like those of the CP and EP, have a GABAergic component. The ultrastructural immunohistochemistry of GABA-t has been examined in the cerebellum and this enzyme has been shown to occur within GABAergic neurons, on the membrane postsynaptic to GABAergic nerve terminals, and within glial elements associated with GABAergic perikarya (Chan-Palay et al., 1979). The histochemical findings cited above suggest that GABA-t is localized within the terminals of GABAergic projection neurons as well. Using combined immunofluorescence and retrograde fluorescent tracing

techniques (see van der Kooy and Sawchenko, 1982) it has been determined that all of the pallidal neurons projecting to the SN and CP display moderate GABA-t immunoreactivity (Staines and Vincent, unpublished observation). In fact, virtually every pallidal neuron, and a significant population of pallidal glial cells stain for GABA-t. Although hardly definitive, this observation further correlates features of the pallidal neurons with those of other cells known to be GABAergic, ie. the SNr neuron (Vincent, personal communication).

There is, therefore, evidence both for and against the proposal that GABA is a neurotransmitter used by the efferents of the GP. Under these circumstances one either draws no conclusions or adopts a tentative working hypothesis. The working hypothesis supported by the majority of the available data and employed in the succeeding discussion is that GABA is a major transmitter of the efferents of the GP.

(iv) Pallidal terminals predominantly innervate the nondopaminergic neurons of the substantia nigra.

Observations of the retrograde and anterograde transport of WGA-HRP (Experiments 2 and 3), studies of pallidal efferents using PHA-L (Experiment 7) and the literature cited within these experiments show that the GP projects predominantly to the pars reticulata of the SN. This does not mean that pallidal terminals do not innervate the ventral dendrites of pars compacta neurons or the dopaminergic neurons located within the pars reticulata. Although it is of importance to distinguish between inputs to dopaminergic and nondopaminergic neurons of the SN this cannot be



accomplished simply by noting differential input to the two zones of the nigra. There is a dopaminergic cell type whose cell body and dendrites are thought to be confined to the pars compacta, but these neurons project to the allocortex (olfactory bulb and amygdala) rather than the striatum (Fallon et al., 1978).

The functional implications of the pallidal input to the substantia nigra differ radically for a major termination on dopaminergic SNc as opposed to nondopaminergic SNr neurons. Not only do their neurotransmitters differ, but these two cell types have markedly different efferent projections. Those of the nondopaminergic neurons constitute outputs from the basal ganglia, while those of the dopaminergic cells, for the most part, remain intrinsic to this system. As described in Experiments 3 and 7, fibers from the GP terminate mainly in the SNr, and appear to contact the cell bodies of neurons within this region. Although a few dopaminergic cells are found within the SNr, these are confined to the caudal half of the nucleus, a more restricted distribution than that observed for the apparent axosomatic features seen in the anterograde tracing experiments. Another commonly observed feature of the pallidal termination in the SNr was that of several fibers running in a bundle. This might be related to the bundling of ventrally directed dopaminergic dendrites described within the SNr. However, whereas these dendritic bundles have a predominantly vertical orientation in coronal sections (Scheibel and Tomiyasu, 1980), the axonal bundles displayed no such preferred orientation. It seems more likely that these fibers were engaged in longitudinal

axodendritic association with the dendrites of pars reticulata neurons. The conclusion drawn from this work (that the GP predominantly innervates the nondopaminergic neurons of the SNr) is in accord with the findings of Hattori et al., (1975) who concluded that although the pallidonigral projection ends in part on dopaminergic nigral neurons, for the most part it innervates nondopaminergic neurons.

#### PALLIDAL INNERVATION OF THE SUBSTANTIA NIGRA

In order to consider the possible function of the pallidal innervation of the substantia nigra it is first necessary to examine the present understanding of the striatonigral projection.

##### (i) Function of the striatonigral projection.

The projection of the CP to the SN is neurochemically heterogeneous, with at least three transmitter candidates being carried in different descending fibers of this system, ie. GABA, substance P and dynorphin. All end predominantly within the pars reticulata region but could presumably innervate the ventral dendrites of the dopaminergic neurons of the pars compacta. Although according to Hattori and coworkers (1975) SNc neurons are not the major nigral targets of the striatonigral terminals, recent studies have provided morphological evidence for some descending striatonigral termination onto dopaminergic neurons within the SN (Wassef et al., 1981).

Neuropharmacological work has indicated that while substance P produces an activation of SNc neurons (Starr, 1978; Cheramy et al., 1977; James and Starr, 1979), the neurons of the SNr are

much more sensitive to the excitatory effects of iontophoretically applied substance P (Pinnock and Dray 1982). Similarly, an inhibitory influence of descending GABA projections onto dopaminergic nigrostriatal neurons has been described (Bunney and Aghajanian, 1978; Guidotti et al., 1978; Kelly and Moore, 1978b; but see Cheramy et al., 1978). However, the behavioral effects of the injection of GABA agonists or antagonists into the SN are opposite to those which would be predicted from a major direct effect on dopaminergic nigrostriatal neurons (Scheel-Kruger et al., 1980) and on this basis the major action of GABA within the SN is believed to be exerted on SNr neurons. In analogy to the iontophoretic studies on the nigral actions of substance P, SNr neurons are twenty times more sensitive to the application of GABA agonists than are SNc neurons (Grace et al., 1982). Thus, although responses to substance P and GABA can be elicited from both SNc and SNr neurons, SNr neurons are more sensitive. Taking the anatomical data into account as well, it appears that the striatonigral pathway exerts its major influence over the nondopaminergic SNr neurons.

A number of observations have led to the recent conclusion that the influence of nigral afferents on the activity of the dopaminergic nigrostriatal neurons may be mediated through a projection of SNr neurons onto SNc cells (Grace et al., 1982). These observations include the finding that these two cell types show reciprocal alterations in firing rates in response to noxious stimuli or drug application within the SNr.

To summarize to this point, the SNr receives dual excitatory and inhibitory inputs from the CP. The striatal influences on the SNc are much weaker and may act in part through the SNr. As it is the neurons of the pars reticulata which constitute the output elements, effects on the ascending dopaminergic system arising from the SNc will be ignored for the present, except to reiterate that dopaminergic activation of the striatum and nucleus accumbens is facilitatory to movement.

A large body of evidence now indicates that inhibition of SNr neurons leads to an increase in motor behavior. Direct electrical stimulation of the CP can elicit some crude movements in experimental animals (Lee and Slater, 1981) and results in a mixed excitation-inhibition response in the nondopaminergic projection neurons of the SNr, with inhibition as the predominant response (Deniau et al 1976; Collingridge and Davies, 1981). Furthermore, intranigral injection of ethanolamine o-sulphate (EOS), which inhibits degradation of GABA, or nigral injections of the GABA agonist muscimol, increase the level of motor behavior in animals and antagonize the cataleptic effects of dopaminergic antagonists such as haloperidol (Dray et al., 1975; Scheel-Kruger, 1977). Bilateral lesions of the substantia nigra produces chronic stereotyped behavior in animals (Olianas et al., 1978), suggestive of a release of these behaviors from an inhibitory nigral control.

Rats subjected to asymmetrical dopaminergic stimulation of the striatum show turning behavior away from the more stimulated side, contralateral turning (Ungerstedt, 1971; Yamamoto et al.,

1982). Circling resultant from an intranigral injection of GABA agonists is also in the contralateral direction (Kilpatrick et al., 1980). Unilateral lesion of the SN induces contralateral turning (DiChiara, 1976) and nigral injections of tetanus toxin, which remove nigral neurons from descending inhibitory and (to a lesser extent) descending excitatory influences, cause animals to turn towards the injected side (ipsilateral turning) (Collingridge and Davies, 1982). That lesions of the SN or the intranigral infusion of inhibitory substances elicit movement and mimic the effects of striatal stimulation indicates that activity within neurons of the SNr is inhibitory to movement and that movement is correlated with an inhibition of these cells by the descending striatonigral projection.

All of the projections of the SNr, excluding the small population of dopaminergic cells found within this region, have been shown to contain GABA. Stimulation of the SNr produces inhibition in the superior colliculus (Chevalier et al., 1981) and the ventromedial nucleus of the thalamus (Yoshida and Omata, 1978). It would be predicted, therefore, that activation of the GABA receptors within these nuclei, simulating stimulation of the neurons of the pars reticulata, should inhibit movement and, indeed, it has been observed that injection of the GABA agonist muscimol into the ventromedial nucleus produces catalepsy (DiChiara et al., 1979), and the circling produced by unilateral injections is opposite to that of asymmetrical striatal activation (Kilpatrick et al., 1980).

Thus, it appears that movement is correlated with an

inhibition of SNr neurons and furthermore, the motor effects of fiber systems descending to the substantia nigra act mainly through the projection neurons of the SNr. The motor effects attributed to activity of these neurons are disrupted by lesions of the regions receiving afferents from the SNr (Mulas et al., 1981; Garcia-Munoz et al., 1982), specifically, the superior colliculus and dorsal midbrain tegmentum.

(ii) Function of the pallidonigral projection.

In contrast to the substantia nigra, the activity of neurons in the globus pallidus seems to be positively correlated with movement. Although the globus pallidus is analogous to the pars reticulata in its receipt of a massive innervation from the striatum, functionally it appears to be analogous to the CP. Like the CP, activity in the efferents of the GP appears to inhibit cells which are themselves inhibitory to movement.

Injectons of neuroleptic drugs, such as haloperidol, block dopamine receptors and produce a catalepsy in experimental animals which is correlated to some of the extrapyramidal symptoms seen in human patients given these drugs. It has been suggested that this catalepsy arises from an increased inhibition of the activity of cells in the GP by their inputs from the CP (Costall and Olley, 1971). Although this proposal was based on the effects of lesions which undoubtedly disrupted far more than the striatopallidal projection, it has gained support from more selective experiments. Thus, muscimol injections into the GP potentiate the catalepsy induced by haloperidol (Matsui and Kamioka, 1978; Scheel-Kruger et al., 1980) and EOS injections

into the GP, acting to inhibit the degradation of GABA within this structure, block the increase in locomotor behavior elicited by the injection of dopamine agonists into the nucleus accumbens (Pycock and Horton, 1976). This is consistent with the observation that apomorphine administration increases the firing rate of GP neurons (Bergstrom et al., 1982). In addition, GABA turnover, an indicator of synthesis and release of this neurotransmitter, is seen to increase within the GP in animals treated with neuroleptic drugs (Marco et al., 1976). With repeated exposure to dopamine antagonists a tolerance develops to the effects on GABA turnover in the GP which shows a time course similar to the development of tolerance to the cataleptogenic effects of the drugs (Marco et al 1976; Ezrin-Waters and Seeman, 1977). A similar increase in GABA turnover is seen within the nucleus accumbens which does not show tolerance and may therefore be related to the antipsychotic effects of neuroleptics (Marco et al., 1976; Costa et al., 1978).

Effects on motor behavior independent of dopaminergic systems can also be elicited by pharmacological manipulations of the GP. Direct injection of EOS into the GP produces an akinesia which is not reversed by the dopamine agonist amphetamine (Pycock et al., 1976).

Opiate drugs can also produce catalepsy and have a concomitant stimulatory effect on the GABA turnover within the GP (Moroni et al., 1979). Local injection of stable analogues of enkephalin into the GP, however, produce an increase in coordinated locomotor behavior (Joyce et al., 1981) which is

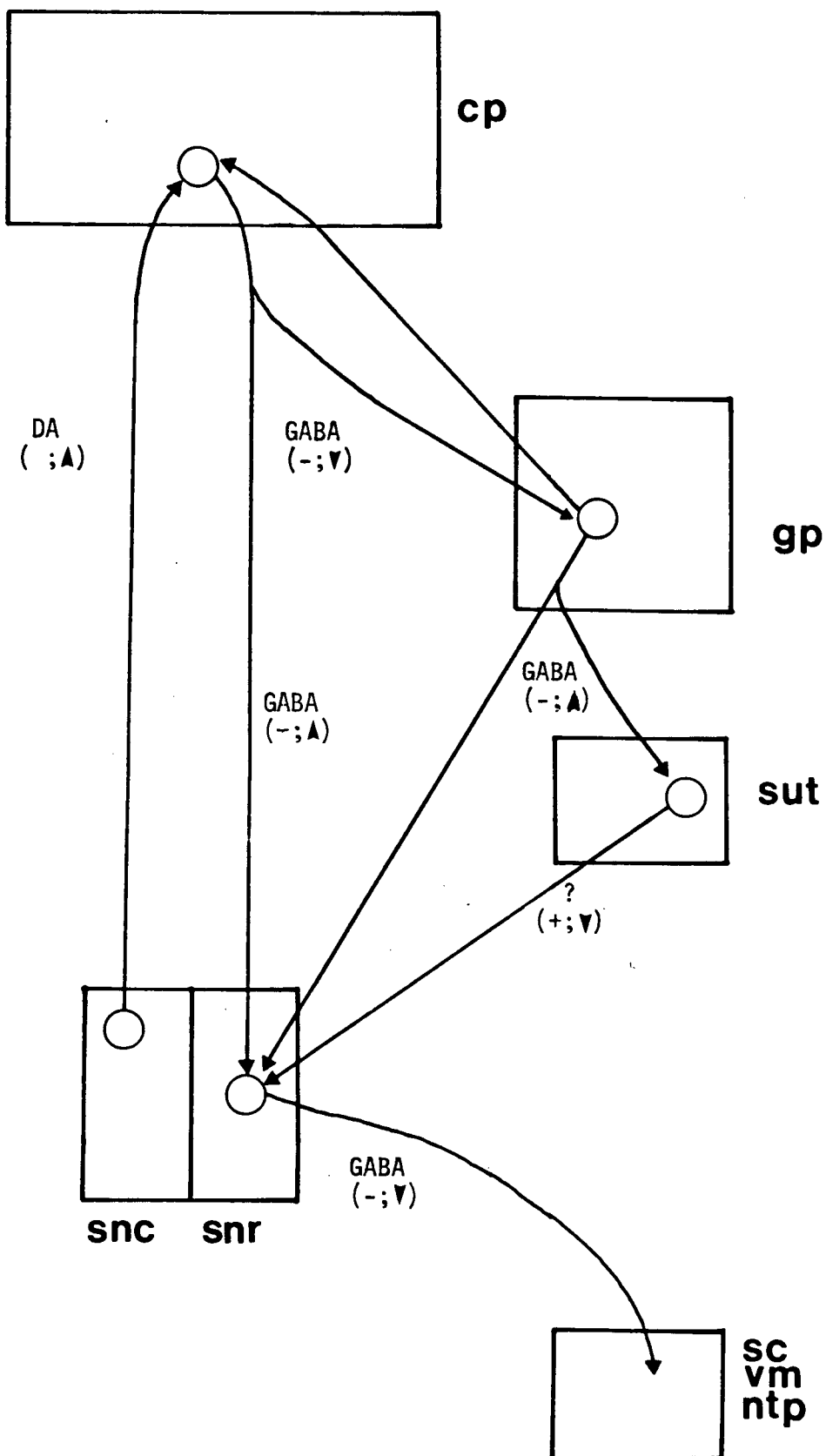
reversed by naloxone but not by dopaminergic antagonists.

It is evident, therefore, that the behavioral consequences of pharmacological manipulation of GABA systems within the GP and SN differ. An increase in GABA receptor stimulation within the SN leads to an increase in motor behaviors and within the GP it leads to a decrease in motor behaviors. It is of interest to note that the administration of apomorphine, at doses having a locomotor stimulant effect, has opposite effects on the metabolic activity in the GP and SN (Kozlowski and Marshall, 1982). Note that a motor stimulant effect rather than motor depression would be expected from pallidal EOS injections if the GP gave rise to a major inhibitory projection to dopaminergic SNc neurons. These findings from pharmacological studies and the pathways which may mediate them are summarized in Figure 58.

Lesion studies indicate that striatal GABAergic projections to the SN and GP are not collaterals (Jessel et al., 1978; Brownstein et al., 1977; Staines et al., 1980a). This independence of the striatal GABA projections to the GP and SN implies that under certain conditions, the striatal and pallidal projections to the SN could act in concert. Experimental evidence of this is provided by neuropharmacological manipulations of the GP in animals subjected to stimulation of the striatum. In rats, CP stimulation causes the animal to turn its head to the side opposite the site of stimulation. These motor effects are not blocked by prior cortical ablation, indicating that they do not arise from activation of corticofugal fibers passing near the stimulating electrode and that this motor



Figure 58. Schematic diagram showing some of the connections of the basal ganglia labelled for the neurotransmitter used, the major postsynaptic response to stimulation (+ or -) and the overall behavioral consequence of stimulation (increase or decrease in motor behaviors; arrows). Collateralization and monosynaptic associations are not implied by the figure.



function is not mediated through a feedback activation of cortical motor centers. It is observed that injections of muscimol into the GP decrease the speed with which animals perform this movement in response to striatal stimulation and that pallidal injections of the indirect GABA antagonist picrotoxin cause the movement to be performed much more quickly (Lee and Slater, 1981). These data indicate that the GP acts in parallel with the striatum in the control of some types of motor behavior. The motor response given in this example is not wholly dependent on the descending projections to the SN as the movement is still performed, albeit slowly, after lesions of the ipsilateral SN. These lesions do, however, abolish the above-mentioned effects that pharmacological manipulation of the GP has on the motor response (Lee and Slater, 1981).

As seen in experiments presented above, the nigral innervation originating in the striatum is quantitatively much greater than that arising from the GP, but the location of pallidal terminals on the cell soma and proximal dendrites of nigral neurons allows for the possibility that the projection from the GP is of an equal or even greater functional significance. As mentioned in Experiment 7, there was no evidence of a perisomal innervation of SNr neurons by striatonigral terminals.

In summary, both the CP and GP have an inhibitory input to the SNr. These two descending projections are topographically, and, therefore, somatotopically linked and activity in both is facilitatory to movement. As activity in the pallidonigral

projection is largely determined by the input that the GP receives from the CP it may be thought of as a more highly integrated form of descending inhibition. It would seem logical in such a system that the more highly integrated input play a relatively greater role in the control of the firing rate of SNr neurons, and the dense concentration of pallidal terminals on the cell soma and proximal dendrites of the neurons in the SNr may in fact reflect just that.

#### PALLIDAL INNERVATION OF THE STRIATUM

As discussed above, a large portion of the GABA within terminals in the CP is assumed to reside within local collaterals of GABA-containing projection neurons (McGeer and McGeer, 1976; Nagy et al., 1978a; Ribak et al., 1979) and local circuit interneurons (Bolam et al., 1983). It must be assumed then that if the pallidostriatal pathway is GABAergic it must have some characteristic not shared by the far more numerous GABAergic local collaterals in order to exert its influence within the striatal neuropil. One way this could come about is suggested by the possibility that pallidal terminals end on the cell soma and proximal dendrites of neurons in the CP, as discussed in Experiment 7. This would make them electrophysiologically more efficacious in controlling the firing of striatal neurons than GABAergic local collaterals, which end predominantly on the dendritic spines of other GABAergic neurons (Ribak et al., 1979).

Alternatively, GABAergic terminals originating in the GP may innervate cell types within the striatum which do not receive input from the local GABAergic collaterals. In Experiment 8

evidence was presented indicating that pallidal endings terminate in part on the proximal dendrites of striatal somatostatin (SST)-containing neurons. SST-containing neurons have extensive local projections within the CP (DiFiglia and Aronin, 1982), and if the GP proved to be the only source of inhibitory input to these cells it could provide the pallidostriatal projection with an effective means of influencing the firing rate of other CP cells.

Evidence for a reciprocal innervation of topographically related portions of the CP and GP was presented in the WGA-HRP tracing studies in Experiment 4. This circuit may function as a point to point reciprocal feedback system regulating striatal efferent activity, the requirements of which do not seem to be met by the circuits connecting the striatum and the substantia nigra.

The collateralization of pallidal projections to the SN and to the regions of the CP which project to that same area of the SN, indicates that the GP may influence the SN not only by direct innervation but also by modulating the activity of the striatonigral projection. Additional access to the SN is provided by the projection of the GP to the SUT which in turn projects to the SN.

#### PALLIDAL INNERVATION OF THE ENTOPEDUNCULAR NUCLEUS

The studies presented above represent the first clear demonstration of an innervation of the EP by the GP. Previous studies have alluded to such a connection but lacked the anatomical resolution to draw definitive conclusions (Hattori et

al., 1975; Carter and Fibiger, 1978). There is very little data on the function of the EP on which to base speculation concerning the involvement of an input from the GP. In many developmental and anatomical ways, the EP is similar to the SNr (Nauta, 1979b). Biochemical data indicates that the EP, like the SN, receives a GABAergic input which is dissociable from the input to the GP, and in fact the GABAergic innervation of the EP and SN may be collaterals of the same neurons (Staines et al., 1980a). Like the nondopaminergic projections of the SNr, the EP sends branched projections to the ventrolateral thalamus, intralaminar thalamus and nucleus tegmenti pedunculopontis which are inhibitory and may use GABA (Uno et al., 1978). Presumably, these connections of the EP function in a manner similar to those of the SNr. Like the SNr, the EP appears to receive dual descending inhibition from the CP and GP.

The majority of the neurons in the EP project to the lateral habenula (van der Kooy and Carter, 1981) and this projection has also been determined to use GABA (see above). The lateral habenula projects to the SNc and the dorsal raphe (Herkenham and Nauta, 1977) and therefore could control both the dopaminergic and serotonergic innervations of the striatum. Although the functional significance of these anatomical findings have yet to be determined, stimulation of the lateral habenula has been shown to decrease serotonin release within the striatum (Reisine et al., 1982).

#### PALLIDAL PROJECTIONS TO THE CORTEX

Findings from both retrograde fluorescent transport studies

(Experiment 5) and anterograde labelling with PHA-L (Experiment 7) point to a small pallidal innervation of the prefrontal cortex. This region of cortex gives rise to the cortical innervation of the SN (Gerfen and Clavier, 1979), and part of the cortical innervation of the CP (Experiment 3). It is tempting to speculate that this pallidal projection contributes to the regulation of these corticofugal pathways.

#### PALLIDAL INNERVATION OF THE THALAMUS

The significance of the findings concerning the pallidal innervation of the reticular and mediodorsal nuclei are difficult to evaluate. The apparent innervation of the mediodorsal nucleus by the GP is likely to have arisen from neurons more properly classified as belonging to the ventral pallidum and will be discussed below. After pallidal injections of WGA-HRP (Experiment 3), an apparent innervation of the whole of the reticular nucleus of the thalamus (RTN) was observed. However, a much more restricted distribution of terminals was found within the RTN when PHA-L was used as an anterograde tracer of pallidal projections (Experiment 7), and this distribution matches very well that seen for cholinergic terminals in the RTN (Kimura et al., 1981). Although the morphology of the anterogradely labelled elements within this nucleus corresponded to that seen in the CP, SUT and SN and did not match that of the putative cholinergic fibers in the cortex, it is possible that neurons of the nucleus basalis magnocellularis rather than those of the GP innervate the RTN. In the absence of retrograde labelling data no definitive claim can be made for an innervation of the RTN by

the parvocellular neurons of the GP.

#### PALLIDAL INNERVATION OF THE SUT

The heaviest output of the GP is its projection to the SUT. Pallidal terminals end on the cell bodies and proximal dendrites of SUT neurons which project back to the GP (Romansky et al., 1980; van der Kooy et al., 1981) and send additional axon collaterals to the EP and SN (Deniau et al., 1978; van der Kooy and Hattori, 1980a; Kita et al., 1983b), and presumably the cortex as well (Jackson and Crossman, 1981). The SUT also receives a very powerful excitatory input from the cortex (Kitai and Deniau, 1981) which is somatotopically organized (Harmann-von Monakow et al., 1978). Although the projection distances of subthalamic neuron axon collaterals to the GP and SN differ considerably, the conduction velocities of descending and ascending fibers are such that impulses arising from the subthalamic nucleus arrive at the SN and GP simultaneously (Kita et al., 1983b).

In a carefully controlled study, stimulation of the SUT was observed to give rise to an excitation of neurons in the substantia nigra (Hammond et al., 1978). Most of the cells activated were located in the SNr but a few cells in the SNc also responded. Other studies (Yoshida et al., 1971; Perkins and Stone, 1980) describe the effects of stimulation of the SUT as producing an inhibitory or a mixed excitation/inhibition response in the GP. These findings appear irreconcilable with the fact that these are collateral projections. Nauta and Cuenod (1982) have suggested that the SUT projections are GABAergic, based on



the retrograde transport of tritiated GABA to the SUT from the GP, but the reliability of this technique has yet to be determined. Use of an inhibitory transmitter by the projections of the SUT would certainly fit the classical impression of the function of the SUT, that of exerting a powerful behaviorally-inhibitory control over several basal ganglia nuclei (see DeLong and Georgopoulos, 1981) but, as discussed below, the observations pointing to an excitatory subthalamic neurotransmitter actually predict the behaviorally-inhibitory role of this nucleus.

Hemiballismus, a violent movement disorder, has long been regarded as due to a loss of the inhibitory influence of the SUT on the output neurons it innervates. The pioneering work of Carpenter and others (1950) on the hyperkinesia resulting from the destruction of the SUT has recently been confirmed using the more selective kainic acid lesioning technique (Hammond et al., 1980). The consequence of this lesion is an episodic, ballistic hyperkinesia of the contralateral extremities. Interepisodic limb function is normal save for a minor bradykinesia. Similar effects have been observed after the infusion of picrotoxin into the internal GP of primates (EP; Crossmann et al., 1980).

Paradoxically, the most reliable electrophysiological data on the projections of the SUT suggests that it has excitatory actions, at least in the SN (Hammond et al., 1978). As represented in Figure 58, the loss of an excitatory input to the SNr would be expected to result in a movement disorder characterized by increased motor activity because decreasing the

firing rate of these nigral cells is associated with increased movement. In this context then, hyperkinesia is the natural consequence of a lesion of excitatory projections arising from the SUT. The types of movements seen with damage of the SUT differ from those associated with SNr efferents, however, and it is likely, considering the lesion placements which counteract the ballistic movements (i.e. the entopeduncular nucleus and the ventrolateral nucleus of the thalamus; see DeLong and Georgopoulos, 1981), that the clinical syndrome is related more to the SUT innervation of EP than of SNr. The hyperkinesia arising from picrotoxin injections into the EP (Crossman et al., 1980) cannot be accounted for in this scheme.

#### GLOBUS PALLIDUS

The major source of afferents to the GP is the CP. The dendrites of pallidal neurons are almost totally covered by GABA or enkephalin-containing terminals derived from this projection (Ribak et al., 1979; Somogyi et al., 1982). Electrophysiological data indicate that the GABAergic input innervation of the GP by the CP is inhibitory (Yoshida and Obata, 1977), as is the enkephalinergic input (Napier et al., 1983). This finding agrees with the observation that inhibition is the major response of GP neurons to CP stimulation. A small (15 %) excitatory component is seen as well (Mogenson et al., 1983), perhaps from the substance P and/or enkephalin-containing projection (Staines et al., 1980a). In some animal preparations, pallidal neurons are noted to have a high firing rate with a periodic pattern (DeLong and Georgopoulos, 1981; Park et al., 1982). This periodic pattern

does not appear to arise from its striatal input and it has not yet been determined if it is due to one of the other afferents to the GP or whether it is an intrinsic property of pallidal neurons (Park et al., 1982).

In the putamen of the monkey, a study of the firing rate of neurons in relation to various movements revealed a somatotopic organization with the hindlimb represented in the dorsal putamen, forelimb representation in the ventrolateral putamen, and representation of the head and neck in the ventromedial putamen (see DeLong and Georgopoulos, 1982). The firing rates of caudate cells were not well correlated to these distal movements. A similar somatotopic distribution was found for the firing of GP neurons associated with these same movements, with the exclusion of the rostromedial GP which is connected to the caudate nucleus. Considerations from other work would suggest that the caudate nucleus, and presumably the rostromedial GP, is associated with activation of axial musculature (see Schneider and Lidsky, 1981). These data, derived from correlations between firing rates and movements, are borne out by the somatotopy suggested by the distribution of the cortical input to the CP (Garcia-Rill et al., 1979; Kunzle et al., 1975; Tanaka et al., 1981). The observation that pallidal firing rate is correlated to movement in the same topographical manner as are striatal neurons points to the active involvement of the GP in movement. Given the largely inhibitory nature of the striatopallidal projection, one might predict that pallidal neurons would be inactive during movement. However, the above data indicate that

this is not the case, and suggest instead that those pallidal neurons relevant to the movement are spared striatal inhibition or actually excited by the CP. It should be mentioned that some of the excitatory influences acting on the GP may derive from the innervation it receives from the SUT, although this is based on inference from the excitatory effects of the SUT on the SN. Direct evaluation of the effects of SUT stimulation on the activity of pallidal neurons has suggested that although excitatory effects occur, the predominant effect is inhibition (Perkins and Stone, 1980); however, a well controlled study may well show the subthalamic projection to the GP to be excitatory.

There is a large but generally inconclusive literature on the effects of pallidal lesions on motor behavior in experimental animals. On the whole, these studies have found that such lesions do not produce obvious alterations in gross motor behaviors (see DeLong and Georgopoulos, 1981). Some workers have attributed this lack of effect to the ability of visual information to allow the animals to compensate for the effects of pallidal lesion. Experimental strategies eliminating this variable reveal greater motor dysfunction subsequent to lesions of the GP (Hore et al., 1977). Another possible reason for these negative findings is that the majority of studies have used electrocoagulation of the GP, which is in fact a multiple lesion, destroying not only the GP but also many of the efferents and afferents of the CP and cortex. It is widely observed that the effects of lesions of basal ganglia nuclei can be reversed by lesions of other brain areas associated with this system. Thus,

the hemiballismus resulting from lesions of the SUT may be partially counteracted by lesions of the EP, ventromedial nucleus of the thalamus, or motor cortex and lesions of the pallidal complex or ventrolateral thalamus can partially reverse some of the symptoms of Parkinson's disease (see DeLong and Georgopoulos, 1981). There is abundant data from experiments using pharmacological manipulation of the GP (discussed above) to suggest that this nucleus is important for normal motor function.

#### GLOBUS PALLIDUS VS. VENTRAL PALLIDUM

As mentioned briefly in the introduction, the nucleus accumbens (NA) is considered one of the components of the basal ganglia. Its similarities to the CP suggest that in some respects it may be viewed as a ventral extension of this nucleus. An impressive array of histochemical and immunohistochemical data indicate that the GP has an analagous rostromedial extension which has been termed the ventral pallidum (VP) (Switzer et al., 1982). This analogy may be applied to many other components of the basal ganglia as well and gives rise to the impression that there are parallel motor and limbic basal ganglia systems.

The CP differs somewhat from the NA in a number of ways, but most notably in that the latter receives telencephalic inputs from the amygdala and hippocampus as opposed to the neocortex (see Nauta et al., 1978), although some input from parts of the frontal cortex and entorhinal cortex are now recognised (Beckstead, 1979b; Krayniak et al., 1981; Reep and Winans, 1982).

The NA receives a dense dopaminergic input from the ventral tegmental area (VTA) and retrorubral nucleus (RR) which lie

adjacent to and partially overlap the dopaminergic SNc neurons innervating the CP (Nauta et al., 1978; Carter and Fibiger, 1977).

Rather than project to the GP, as it is demarcated by most workers, the NA projects to the ventral pallidum, lateral preoptic area and fields within the lateral hypothalamus medial to the entopeduncular nucleus (Nauta et al., 1978). The innervation of the GP and VP by the CP and NA, respectively forms a topographical continuum (Mogenson et al., 1983) and agrees with the proposal that these systems should be considered parallel. Furthermore, in the rat there is some degree of overlap such that pallidal neurons cannot be assumed to belong to the ventral pallidum (defined as in receipt of NA afferents) merely by their location ventral to the decussation of the anterior commissure. Like the response of pallidal neurons to striatal stimulation, the response of VP neurons to NA stimulation is predominantly inhibitory (Mogenson et al., 1983), and there is evidence that GABA is used as a transmitter in both pathways (Nagy et al., 1978a; Fonnum and Walaas, 1979). Injections of retrograde tracers into the ventral parts of the CP (but still not within the NA) results in the labelling of cell bodies in the ventral pallidum (unpublished observation), but it has not as yet been determined if there is a VP projection to NA.

Nauta et al. (1978) commented on the absence of an efferent pathway from the NA analogous to the striatoentopeduncular projection. Considerations of the efferent projections of the EP in the rat, however, suggest that a population of cells medial to

the tip of the internal capsule and a population of cells ventral to the EP, in the nucleus of the ansa lenticularis, might be analogous to the EP (Larsen and McBride, 1979; van der Kooy and Carter, 1981; Parent et al., 1981a). Drawings of the efferent projections of the NA reveal that both of these regions could receive inputs and, therefore, could represent a parallel of the CP innervation of the EP (Nauta et al., 1978; Mogenson et al., 1983). Furthermore, cells in the lateral hypothalamus, medial to the tip of the internal capsule, project to the VTA and RR and this terminal field precisely overlaps the regions giving rise to the dopaminergic innervation of the NA (Nauta and Domesick, 1978). This finding represents a departure from the analogy, as the EP has not been seen to project to the SN.

In keeping with the topography of striatonigral projections, in which successively more ventral striatal areas project to successively more dorsal nigral regions, the NA (as a ventral continuation of the CP) projects heavily to the SNc and upper reaches of the SNr (Nauta et al., 1978). The NA projects to a much greater region of the dopaminergic perikarya than it receives input from. In fact, the efferents of the NA could influence virtually all of the dopaminergic cells innervating the striatum (Nauta et al., 1978). This observation has called into question the concept of the point to point reciprocity in the nigrostriatal feedback loop discussed in Experiment 1.

As mentioned above, the efferent projections of the NA appear to parallel the system of striatal efferents. They deviate however in that the accumbens also projects to the

mediodorsal nucleus of the thalamus (Nauta et al., 1978). It will be recalled that a noncholinergic projection to the mediodorsal nucleus from the ventral GP and VP was discussed in Experiment 2. Thus, it appears that the NA and VP share in a thalamic projection which has no analogy in the connections of the CP and GP, but may share a role similar to the projection from the EP to the ventrolateral nucleus of the thalamus. The mediodorsal nucleus projects to prefrontal cortex which then in turn innervates the NA (Jones and Leavitt, 1975; Beckstead, 1979b) and the ventrolateral thalamus projects to the motor and premotor frontal cortex which form part of the innervation of the CP (Jones and Leavitt, 1975; Kunzle et al., 1975; Jones et al., 1977).

One of the histochemical features characteristic of the GP, EP and VP is their intense reaction for the presence of iron. This feature is also noted in the ventral thalamus and the mediodorsal nucleus. The presence of iron within the ventral thalamus has been suggested to arise from the entopeduncular efferents to it, and it may be that that in the mediodorsal thalamus arises from a projection from VP (Switzer et al., 1982).

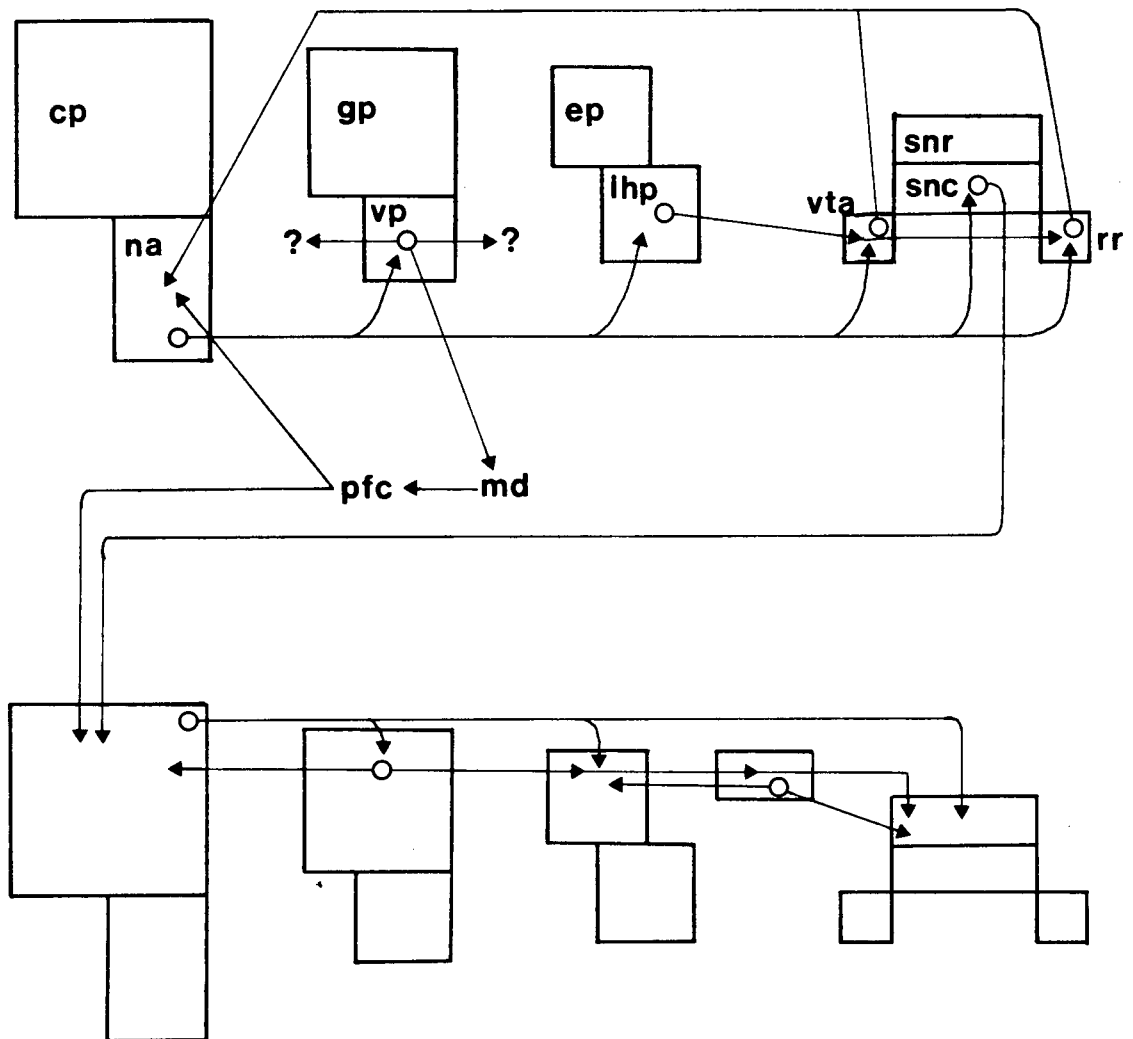
As shown by Lehmann et al., (1980), the VP at rostral levels contains relatively few neurons classified as belonging to the nucleus basalis magnocellularis (nBM), defined by histochemical means. Although both the NA projection to VP and the CP projection to GP could innervate portions of the nBM, neither seem to be specifically targeted on this neuronal population. In primates the nBM forms a more definite nucleus well removed from



the terminal fields of striatal efferents and it is doubtful that these neurons are involved to any great extent with the circuits of the basal ganglia.

There appear, therefore, to be parallel systems within this expanded concept of the basal ganglia related to both motor and limbic areas, (Fig. 59). The anatomical associations of the motor basal ganglion are compatible with modulation of the activity of skeletal musculature but those of the limbic basal ganglia may modulate affect and motivation (see Nauta and Domesick, 1978). In the rat, stimulation of the CP results in orofacial movements and stimulation of the NA gives rise to an increase in locomotor activity (Anden and Johnels, 1977). Somatotopic considerations discussed above however, suggest that representations of the musculature involved in locomotion would reside in the dorsal and lateral CP and that if indeed the NA were simply an extension of the ventral parts of the CP it would be associated with movements of rostral body parts. Furthermore, the NA does not receive sensorimotor cortical input like that projecting to the CP, but instead has inputs arising from limbic structures such as the amygdala, hippocampus and prefrontal cortex. From an anatomical viewpoint the NA appears ill suited to control of the mechanics of locomotion and may instead control the motivational aspects of locomotion. Bearing on this point are experimental data in animals which show that the differential symptoms of Parkinson's disease, akinesia and rigidity, produced in this case by reserpine administration, are obviated by the infusion of dopamine agonists into the NA and CP respectively

Figure 59. Schematic diagram of some the connections of the motor circuit and the limbic circuit of the basal ganglia. Collateralization and monosynaptic connections are not implied.



(Anden and Johnels, 1977). These data fit the corollary that dysfunction of the NA would result in a motivational motor deficit, akinesia, and striatal dysfunction would lead to a mechanical motor deficit, rigidity.

One pathway by which the limbic basal ganglia circuits may influence the motor basal ganglia circuits is, as described above, the possible innervation by the NA of virtually all of the dopaminergic neurons projecting to the CP. The significance of this pathway to the locomotor effects of NA stimulation could be tested by examining the effect that selective lesion of the striatal dopamine input has on locomotion induced by infusion of dopaminergic drugs into the accumbens. Existing psychopharmacological data are consistent with an interaction between these two systems (Kelly, 1975; Kelly and Moore, 1977). Another pathway by which these two circuits could interact is via the projection from the EP and lateral hypothalamus to the lateral habenula which in turn gives rise to the major innervation of the dorsal raphe. The dorsal raphe sends diffuse, collateral, serotonergic projections to the SN and CP, innervates the NA, and is also one of the major sources of input to the GP (van der Kooy and Hattori, 1980b; Parent et al., 1980b; Pasik et al., 1981). Thus, projections of the limbic circuits of the basal ganglia can potentially influence both the serotonergic and dopaminergic inputs to the motor regions of the basal ganglia.

Injection of dopamine into the NA increase locomotor activity in the rat (Anden and Johnels, 1977) as do picrotoxin injections into the ventral tegmental area, which presumably

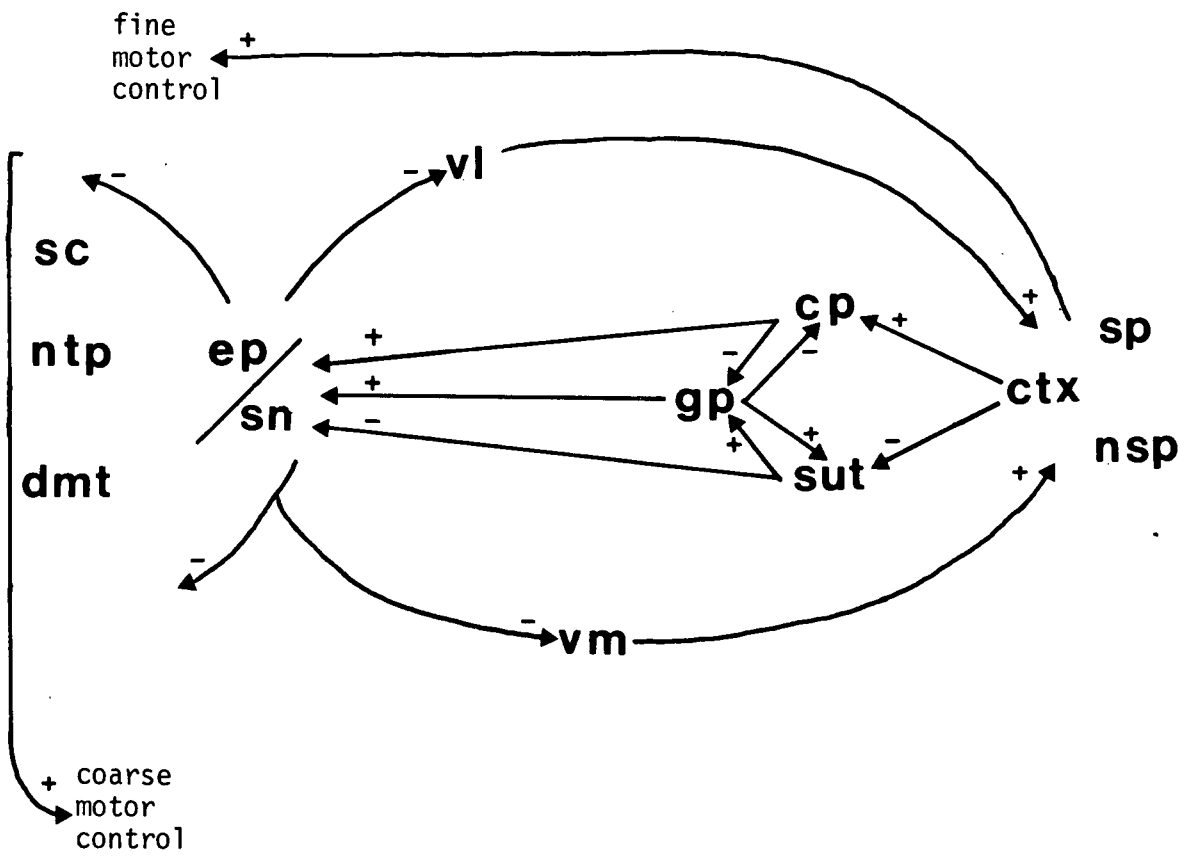
reduce inhibition of these dopaminergic cells projecting to the NA (Mogenson et al., 1980). It has been proposed that this increase in motor activity occurs through a projection from the NA to the GP (Mogenson et al., 1980) as injections of picrotoxin into the GP increase the locomotor activity (an effect most often associated with the NA as opposed to the CP) in otherwise untreated animals, and injections of GABA into the GP antagonize the hyperactivity induced by dopaminergic stimulation of the NA. This proposal however lacks an anatomical basis. The NA does not have a significant projection to the GP (Mogenson et al., 1983; Experiment 3). An alternate hypothesis which could account for these findings and still be compatible with the known anatomical organization would suggest that the reversal of the locomotor activation results not from the GABA induced blockade of the NA effects on the GP but by blocking the CP effects on the GP which arise from a NA stimulation of the CP through the SNc.

#### SUMMARY

The CP has long been considered the only significant fenestra for entry of afferent inputs to the basal ganglia. It has largely inhibitory projections to the output nuclei of the basal ganglia, the EP and SNr. The somatotopically organized cortical input to the SUT would seem to force consideration of this nucleus as an additional channel for input into this system. The SUT appears to have excitatory inputs to the SNr and therefore, because of the collateralization of the outputs from the SUT, probably to the EP as well. The GP has reciprocal connections with both the CP and the SUT and it also innervates

the SN and EP. Therefore, it would appear to be in a position to influence the circuit that facilitates motor function (CP efferents) as well as that which inhibits motor activity (SUT efferents). Specifically, the GP may have the unique property of being excited by the circuit which is ultimately inhibitory and inhibited by the circuit which is ultimately facilitatory to movement. It also has inhibitory terminals advantageously positioned on the output neurons of the SNr and EP which would result in motor facilitation. These concepts are depicted in Figure 60.

Figure 60. Schematic diagram summarizing the major connections of the basal ganglia. Pathways are labelled (+ or -) according to their overall effect on motor behavior (facilitatory or inhibitory). The abbreviations sp and nsp refer to specific and nonspecific thalamocortical projections.





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