ANATOMICAL AND BIOCHEMICAL ORGANIZATION
OF THE BASAL GANGLIA

by

JAMES IMRE NAGY

B.Sc. University of British Columbia, 1973
M.Sc. University of British Columbia, 1976

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in
THE FACULTY OF GRADUATE STUDIES
Interdisciplinary Studies:
(Neurological Sciences)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

July 1979

James Imre Nagy, 1979
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Neurological Sci.

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date August 10/79
ABSTRACT

The biochemistry and anatomy of various nuclei of the basal ganglia of the rat were investigated. The head of the striatum was found to project to the anterior globus pallidus (GP) and to the entopeduncular nucleus (EP). A projection from the anterior striatum to the substantia nigra (SN) was confirmed. The tail of the striatum was found to project to the posterior part of the GP. No anatomical evidence was obtained for a projection from the tail of the striatum to the EP. The posterior striatum was found to project to the lateral SN. Biochemically, the presence of glutamic acid decarboxylase (GAD) in the projection from the head of the striatum to the GP has been confirmed. The head and tail of the striatum were found to project GAD-containing fibers to the EP. While the absence of nigral GAD-containing afferents originating in the anterior striatum has been confirmed, it was found that the SN does receive such afferents from more posterior regions of the striatum. The SN was found to be devoid of a GAD-containing input from the GP. The EP efferents to the habenula were found to be GAD-containing. Preliminary evidence was obtained for the presence of cholinergic fibers in the striatal projections to the GP and EP.

The cellular localization of various enzyme systems in the SN and of the dopamine (DA) receptor in the SN and striatum was investigated using kainic acid and 6-hydroxydopamine lesion techniques. It was concluded that choline acetyltransferase and DA-sensitive adenylate cyclase are contained in nigral afferents, acetylcholinesterase is contained in both nigral afferents and intrinsic neuronal elements and tyrosine hydroxylase is contained in nigral perikarya. The results concerning nigral GAD were inconclusive. Evidence was obtained for the existence of DA receptors on DA-containing neurons in the SN and their terminals in the striatum.
TABLE OF CONTENTS

INTRODUCTION

I General Review of the Basal Ganglia

a) The striatum

1. internal organization
2. striatal afferents
3. striatal efferents

b) The globus pallidus

1. internal organization
2. pallidal afferents
3. efferents of the medial pallidal segment
4. efferents of the lateral pallidal segment
5. acetylcholinesterase staining neurons of the globus pallidus

c) Subthalamic nucleus

d) Substantia nigra

1. internal organization
2. nigral efferents
3. nigral afferents

II Adenylate Cyclase and Dopamine Receptors in the Substantia Nigra and Striatum

III The Present Investigation

a) Methodological considerations
b) Objectives
METHODS AND MATERIALS

I Animal Surgery
   a) 6-Hydroxydopamine lesions of the nigro-striatal pathway 45
   b) Kainic acid lesions 46
   c) Hemitransections 47
   d) Electrolytic lesions 47

II Anatomical Methods 48
   a) Autoradiographic studies 48
   b) Retrograde transport of horseradish peroxidase 48
   c) Dissections 49
   d) Histology 50

III Biochemical Methods 50
   a) Glutamic acid decarboxylase 50
   b) Tyrosine hydroxylase 51
   c) Choline acetyltransferase and acetylcholinesterase 51
   d) Adenylate cyclase 52
   e) Protein assay and scintillation counting 53
   f) Tissue packaging 53

RESULTS 53

I Neuroanatomy 53
   a) Anterior striatal efferents to the globus pallidus, entopeduncular nucleus and substantia nigra 53
   b) Posterior striatal efferents to the globus pallidus, entopeduncular nucleus and substantia nigra 63

II Biochemical Neuroanatomy 68
   a) The contribution of the head of the
striatum to various neurotransmitter enzyme markers in the globus pallidus, entopeduncular nucleus and substantia nigra 68

b) The contribution of the tail of the striatum to various neurotransmitter enzyme markers in the globus pallidus, entopeduncular nucleus and substantia nigra 71

c) The contribution of the globus pallidus to various neurotransmitter enzyme markers in the entopeduncular nucleus and substantia nigra 74

d) Efferents of the entopeduncular nucleus 94

III Biochemical Investigations of Substantia Nigra and Striatum 94

a) Neurotransmitter synthetic enzyme localization in the substantia nigra 94

b) Neurotransmitter receptor localization in the substantia nigra and striatum 104

DISCUSSION 111

I Anatomy of the Striato-Pallidal and Striato-Nigral Projections 111

II Biochemical Neuroanatomy of Striatal and Pallidal Efferent Projections 114

a) Striato-pallidal projections; glutamic acid decarboxylase 114

b) Striato-pallidal projections; choline acetyltransferase 116

c) Striato-nigral projections 121

d) Pallido-nigral projections 123

III Striatal and Pallidal Efferents: Synthesis and Speculation 125

a) Topographic relations 126

b) Striatal projection neurons 129

c) Neurotransmitters in striatal
and pallidal efferents 130

d) Biochemical neuroanatomy of striatal and pallidal efferents 133

IV Preliminary Observations of Nigral and Entopeduncular Efferents 136

V The Localization of Enzymes in the Substantia Nigra 138

VI The Localization of Dopamine Activated Receptors and Adenylate Cyclase 140

a) Dopamine-sensitive adenylate cyclase in the substantia nigra 140

b) Pre- and post-synaptic dopamine receptors in the substantia nigra and striatum 142

c) Dopamine transmission in the substantia nigra and striatum: synthesis and speculation 146

CONCLUSIONS 147

REFERENCES 150
LIST OF TABLES

Table 1  The activity of GAD in various areas after lesions of the head of the striatum.
Table 2  The activity of GAD and CAT in the GP, EP and SN after hemitranssections anterior to the GP.
Table 3  The activity of GAD and CAT in the GP and tail of the striatum after hemitranssections anterior to the GP.
Table 4  The activity of GAD and CAT in various areas after lesions of the tail of the striatum.
Table 5  The activity of GAD and CAT in the EP and SN after electrolytic lesions of the GP.
Table 6  The activity of GAD, CAT and TH in various areas after kainic acid lesions of the GP.
Table 7  The activity of GAD and CAT in the habenula after electrolytic lesions of the EP.
Table 8  The activities of various enzymes after intranigral injections of kainic acid.
Table 9  The effect of 6-OHDA lesions of the NSP and kainic acid lesions of the striatum on various enzymes in the SN and striatum.
Table 10  $^3$H-apomorphine binding in the SN and striatum.
Table 11  $^3$H-neuroleptic binding in the SN and striatum.
LIST OF FIGURES

Figure 1  Injection locus of $^3$H-leucine in the head of the striatum.
Figure 2  Anterograde transport of $^3$H-leucine from the head of the striatum.
Figure 3  Diagram of autoradiographic grain distribution after $^3$H-leucine injections into the head of the striatum.
Figure 4  Retrograde transport of HRP from the GP and EP.
Figure 5  Anterograde transport of $^3$H-leucine from the tail of the striatum.
Figure 6  Diagram of autoradiographic grain distribution after $^3$H-leucine injection into the tail of the striatum.
Figure 7  Diagram of electrolytic lesions of the tail of the striatum.
Figure 8  Diagram of electrolytic lesions of the GP.
Figure 9  Diagram of kainic acid lesions of the GP.
Figure 10 Photomicrographs of electrolytic and kainic acid lesions.
Figure 11 Location of acetylcholinesterase-staining neurons in the GP.
Figure 12 Diagram of the location of labeled neurons in the GP after intranigral injections of HRP.
Figure 13 Photomicrograph of HRP labeled neurons in the GP.
Figure 14 Photomicrograph of electrolytic lesions of the EP.
Figure 15 Histology of normal and kainic acid-injected SN.
Figure 16 Dopamine-sensitive adenylate cyclase in the SN.
Figure 17 Scatchard plot of $^3$H-apomorphine binding in the striatum.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>anterior commissure</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>AChE</td>
<td>acetylcholinesterase</td>
</tr>
<tr>
<td>AMPH</td>
<td>amphetamine</td>
</tr>
<tr>
<td>cAMP</td>
<td>adenosine cyclic 3',5'-monophosphate</td>
</tr>
<tr>
<td>CAT</td>
<td>choline acetyltransferase</td>
</tr>
<tr>
<td>CM</td>
<td>centromedian nucleus</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>DAC</td>
<td>dopamine-sensitive adenylate cyclase</td>
</tr>
<tr>
<td>DBH</td>
<td>dopamine-β-hydroxylase</td>
</tr>
<tr>
<td>DRN</td>
<td>dorsal raphe nucleus</td>
</tr>
<tr>
<td>EM</td>
<td>electron microscope</td>
</tr>
<tr>
<td>EP</td>
<td>entopeduncular nucleus</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory postsynaptic potential</td>
</tr>
<tr>
<td>F</td>
<td>fornix</td>
</tr>
<tr>
<td>FR</td>
<td>fasciculus retroflexus</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>glutamic acid decarboxylase</td>
</tr>
<tr>
<td>GP</td>
<td>globus pallidus</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>IC</td>
<td>internal capsule</td>
</tr>
<tr>
<td>IP</td>
<td>interpeduncular nucleus</td>
</tr>
<tr>
<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>LC</td>
<td>locus coeruleus</td>
</tr>
<tr>
<td>LGP</td>
<td>globus pallidus, lateral segment (primate)</td>
</tr>
<tr>
<td>MGP</td>
<td>globus pallidus, medial segment (primate)</td>
</tr>
<tr>
<td>ML</td>
<td>medial lemniscus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MMT</td>
<td>mammillo-thalamic tract</td>
</tr>
<tr>
<td>MRN</td>
<td>medial raphe nucleus</td>
</tr>
<tr>
<td>NA</td>
<td>noradrenaline</td>
</tr>
<tr>
<td>NSP</td>
<td>DA-containing nigro-striatal pathway</td>
</tr>
<tr>
<td>OT</td>
<td>optic tract</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>6-hydroxydopamine</td>
</tr>
<tr>
<td>PF</td>
<td>parafascicular nucleus</td>
</tr>
<tr>
<td>SC</td>
<td>superior colliculus</td>
</tr>
<tr>
<td>SM</td>
<td>stria medularis</td>
</tr>
<tr>
<td>St</td>
<td>striatum</td>
</tr>
<tr>
<td>SN</td>
<td>substantia nigra</td>
</tr>
<tr>
<td>SNC</td>
<td>substantia nigra, pars compacta</td>
</tr>
<tr>
<td>SNR</td>
<td>substantia nigra, pars reticulata</td>
</tr>
<tr>
<td>SUT</td>
<td>subthalamic nucleus</td>
</tr>
<tr>
<td>TH</td>
<td>tyrosine hydroxylase</td>
</tr>
<tr>
<td>TPP</td>
<td>nucleus tegmenti pedunculopontis</td>
</tr>
<tr>
<td>VAp</td>
<td>ventral anterior thalamic nucleus, principal part</td>
</tr>
<tr>
<td>VM</td>
<td>ventral medial thalamic nucleus (subprimate)</td>
</tr>
<tr>
<td>VLo</td>
<td>ventral lateral thalamic nucleus, oral part</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

Someone once said "I have only myself to blame for my failure but many people to thank for my successes". This is very true in my case. To begin, several years ago, the teaching ability of Dr. Don Clark was second to none. He sparked interest and enthusiasm and brought a scholarly attitude to research which I well remember. Unfortunately, the chemistry department failed to grant Dr. Clark tenure. Even if such is the reward for ability and scholarship, I would be glad to follow in Don Clark's wake.

While I was a Masters' student in biochemistry, Dr. S.C. Sung taught me how to keep a laboratory. This, though, was the least of his contributions to me. His continued, heartfelt concern for my personal well-being and career was always appreciated. His praise, to the point of my embarrassment, was both inspiring and reassuring, especially to the insecure son of an immigrant Hungarian farmer.

I consider myself fortunate that my contemporaries and collaborators, Steven Vincent and William Staines, have the ingredients essential in any working environment. Their interest in their work is authentic. Their keen eye for problem solving is unmistakable. Their dispositions are cheerful. By these qualities they have created an atmosphere in which it is a delight to work. I am glad to have known them. I would also like to express my gratitude to my fellow students for bearing with me on those occasions when I could be a real pain in the ass.

I am grateful to Dr. Stephen Mason for bringing to me from the other side of the Atlantic his measure of encouragement and support. Also for the interesting and endless discussions and liquid consumables; I can't remember which was more interesting and which was more endless.

I freely admit that the technical expertise of Stella Atmadja is far more advanced than mine and because of this her assistance in the present endeavours has proven to be invaluable. Thank you, Stella, for your hard work and
xii

and your patience with me.

I would like to express my thanks to the members of my committee for suggestions that markedly enhanced the literary value of this thesis.

Finally, I owe a great debt to my thesis advisor, Dr. Hans C. Fibiger, without whom the path I might have taken would have been quite different indeed. Dr. Fibiger is exemplary of my conception of an excellent thesis advisor. He has allowed me to make and learn from my own mistakes while at the same time providing guidance when my own resourcefulness was exhausted. He has been receptive and willing to discuss both good and bad ideas, always able, of course, to choose the former. Whether the situation looked bleak or otherwise he has constantly been quick to encourage. The output of personal energy and time, the high quality of work required in research and certain ideals have been taught to Dr. Fibiger's students by example. I have benefited from my association with him enormously and am honored to have been one of his students. I'll never be able to repay Dr. Fibiger for everything he has done for me but hopefully I will be given the opportunity to try. Perhaps the highest compliment a student can give his teacher is to say with certainty that in the future I will be asking myself "Now, how would Chris have handled this?"
Brain research is necessarily an interdisciplinary field. In spite of the tremendous technical advances in each of the disciplines contributing to the growing body of knowledge of the machinery of the brain, there remain precious few hard and fast rules on which to hinge conceptual frameworks and which could provide guidelines for future work of the kind that would provide major insights. For this reason, it is of some advantage that a student of, for example, the basal ganglia be able to draw upon information available in many fields during any consideration of basal ganglia function. While a formulation of a meaningful construct of the function of this part of the brain may, at present, be unsuccessful, an attempt at the condensation and synthesis of available data may at least be worthwhile. Such an attempt has been made in the Introduction of the present thesis.

It has been learned by the present author (reliable sources, 1977) that works such as a doctoral thesis provide a rare opportunity to extend the interpretation of experimental data beyond the constraints of current knowledge. Some speculative licence is therefore taken in the discussion of the present thesis.
I  **General Review of the Basal Ganglia**

a) The striatum

1. internal organization

The apparent homogeneous internal organization of the striatum (caudate-putamen) has made difficult the study of the detailed anatomical relationships between this structure and associated brain areas. Nevertheless, the striatum has yielded to some investigations probing its structural framework. In general, the neurons in the striatum have been classified into three types on the basis of size (Kemp and Powell, 1971a; Hassler, 1978). These are: 1) giant aspiny neurons representing less than 1% of the total neuronal population, 2) small neurons with few spines, also present in numbers less than 1% of the total, and 3) medium sized spiny neurons which constitute greater than 95% of the total neuronal pool of the striatum.

The medium spiny cells can be classified further on the basis of spine density and dendrite and axon morphology (Kemp and Powell, 1971a). Greater than 96% of these neurons have dendrites heavily laden with spines and numerous axon collaterals (Kemp and Powell, 1971a). The aspiny neurons have also been further classified into three types (Difiglia et al, 1976); two of these are of the small variety and the third is the large aspiny neuron described by Kemp and Powell (1971a). With regard to investigations of the arrangement of striatal neurons, although some cell clustering has been observed (Kemp and Powell, 1971a; Mensah, 1977; Chronister et al, 1976), such studies have not revealed any striking features of neuronal organization of the striatum; nevertheless, they do suggest a degree of morphological diversity within this structure. The degree of morphological heterogeneity of the striatum and of any brain region becomes an important consideration not only in the interpretation of the organization and topography of afferents to the striatum but also in neurochemical analysis. For example, after production of any manner of lesion,
particularly an incomplete lesion, the neurochemical consequences of the lesion may be extrapolated to the whole in the case of a homogeneous structure. This clearly is not the case for a heterogeneous structure where each component must be treated separately. This point has some bearing on the present results and will be dealt with further in subsequent sections.

Analogous with the search for some regularity in the arrangement of neurons in the striatum, very little is known of the organization and extent of the intrinsic long- or short-axon connections in this structure. Degeneration of symmetrical and asymmetrical synaptic contacts have been observed only as far as 0.5 and 1.0 mm, respectively, from the site of a small lesion in the striatum (Kemp and Powell, 1971a). Virtual complete surgical isolation of the striatum results in the persistence of symmetrical and asymmetrical synaptic contacts in this structure and it has been concluded that all of the former synaptic type is of intrinsic origin while the latter type is of both intrinsic and extrinsic origin (Kemp and Powell, 1971a; Tennyson and Marco, 1973). Interestingly, rather than being scattered homogeneously throughout the striatal neuropil, intrinsic synapses have been observed to be arranged in groups (Tennyson and Marco, 1973).

It should be mentioned that currently the view is held by some that the nucleus accumbens is a medial extension of the striatum (Nauta et al, 1978; Swanson and Cowan, 1975). This is based on the cytoarchitectonic appearance, connections and development of the striatum and nucleus accumbens. Although these two areas have similar anatomical connections, some differences do exist (Nauta et al, 1978). The apparent grouping of these areas may simply depend on the criteria of classification chosen. Although the nucleus accumbens and striatum may have similar functional and processing capabilities, the information acted on by the two structures may differ. Since evidence bearing on this point is presently not available, the nucleus accumbens and striatum, for the sake of convenience if not accuracy, will be treated as
distinct entities.

The striatum contains numerous putative neurotransmitters and their metabolizing enzymes. These biochemical systems have been attributed to the various afferent or intrinsic neuronal elements of the striatum. Although interneurons containing other transmitters probably exist in the striatum, the least equivocal evidence for an interneuronal localization of specific transmitter systems is that for the synthetic enzymes of acetylcholine (ACh) - choline acetyltransferase (CAT) - and gamma-aminobutyric acid (GABA) - glutamic acid decarboxylase (GAD) (McGeer et al, 1971a; Hattori et al, 1976; McGeer and McGeer, 1975). Correlations of the cell types containing CAT in the striatum have been attempted. Thus, it appears that medium sized neurons stain immunohistochemically (Hattori et al, 1976) and cytohistochemically for CAT (Kaiya et al, 1979).

2. striatal afferents

The striatum receives afferents from a number of diverse areas of the brain. The largest of these is the afferent system from all parts of the cerebral cortex (Webster, 1961, 1965; Heimer and Wilson, 1975; Goldman and Nauta, 1977; Carmen et al, 1963; Kemp and Powell, 1970; Jones et al, 1977). According to Kemp and Powell (1971b) the cortex supplies 30 to 40% of the terminals of extrinsic origin to the striatum. These terminals form synaptic contacts that have membrane thickenings exclusively on the postsynaptic structure. This type of synaptic contact is referred to as asymmetrical. Degeneration studies suggested (Kemp and Powell, 1971b) and autoradiographic transport studies dramatically demonstrated (Goldman and Nauta, 1977; Jones et al, 1977; Yeterian and Hoesen, 1978) a discontinuous nature of the termination of the cortico-striatal fibers. Thus, in the striatum the cortical afferents distribute themselves in clusters, strips and bands. The transmitter of the cortico-striatal system is thought to be glutamate. This has been suggested on the basis of the findings that cortical lesions reduce the high affinity uptake of
glutamate (McGeer et al, 1977; Fonnum and Storm-Mathisen, 1977) and glutamate levels (Kim et al, 1977) in the striatum. Electrophysiological investigations have shown the cortico-striatal pathway to be excitatory; cortical stimulation inducing monosynaptic excitatory postsynaptic potentials (EPSP's) in striatal neurons (Kitai et al, 1976a, 1976b; Kocsis et al, 1977). This is in accord with the excitatory nature of glutamate on striatal cells (Spencer, 1976) and with the hypothesis that glutamate may be the transmitter of this system.

There is also electrophysiological evidence that the cortico-striatal fibers are independent of the cortico-spinal or cortico-bulbar systems (Kitai et al, 1976b). This is in agreement with the different location within the cortical layers of striatal afferents (layer III, Kitai et al, 1976b; layer V, Jones et al, 1977) and spinal and bulbar afferents (layer IV).

The thalamus is second only to the cortex with regard to the density of asymmetrical synaptic terminals it supplies to the striatum (20 to 25%, Kemp and Powell, 1971b). The neurons of origin of the thalamo-striatal system are located primarily in the parafascicular and centromedian intralaminar thalamic nuclei (Jones et al, 1977; Powell and Cowan, 1967; Johnson, 1961; Kuroda et al, 1975) although all of the intralaminar nuclei appear to project to the striatum (Jones and Leavitt, 1974). The termination within the striatum of these thalamic nuclei (Kemp and Powell, 1971b), particularly the centromedian, (Kalil, 1978; Royce, 1978) not to be outdone by the cortical afferents, also exhibit a striking array of configurations from patches and bands to semicircles. There are no candidates for the transmitter of the thalamo-striatal system. A decrease in CAT activity and ACh levels has been observed in the head of the striatum after parafascicular nucleus lesions suggesting a cholinergic component in this projection (Simke and Saelens, 1977; Saelens et al, 1979). This, however, is suspect since the anterior one-third of the striatum receives few, if any, afferents from the parafascicular nucleus (Jones and Leavitt, 1974). Thalamic stimulation evokes depolarization responses in the striatum (Kocsis
et al, 1977; Purpura and Malliani, 1967; Malliani and Purpura, 1967). In addition, evidence has been presented (Kocsis et al, 1977) that there is considerable convergence of cortical and thalamic efferent fibers on medium spiny neurons in the striatum. The implications, if not the means of proof, of the patchy distribution of both cortical and thalamic striatal afferents and the convergence of these systems on the same neurons are obvious. The most intriguing of these is the possibility of a variety of closed loop circuits within and/or between the basal ganglia, thalamus and cortex.

Three monoamine-containing systems have been shown to innervate the striatum. The richest of these is that originating from the substantia nigra (SN), pars compacta (SNC) and will be discussed together with SN efferents.

A second monoamine system innervating the striatum originates from the mesencephalic raphe nucleus (Pasquier et al, 1977; Moore et al, 1978) and, specifically, the dorsal raphe nucleus (DRN) (Miller et al, 1975; Azmitia and Segal, 1978; Jacobs et al, 1978). Consistent with the findings that the raphe nuclei are rich in serotonin-containing neurons, lesions of raphe nuclei (Kuhar et al, 1972) and, more precisely, the DRN reduce serotonin levels and tryptophan hydroxylase - the synthesizing enzyme for serotonin - activity in the striatum (Geyer et al, 1976; Dray et al, 1978). Although anatomical studies indicate a uniform innervation of the striatum by the DRN (Moore et al, 1978), it appears from biochemical studies that serotonin terminals are mainly localized in the ventrocaudal area of this structure (Ternaux et al, 1977). Stimulation of the DRN produces inhibition of neurons of the striatum (Miller et al, 1975; Davies and Tongroach, 1978) and this inhibition is presumably mediated by serotonin since it was abolished by the serotonin antagonist methysergide (Davies and Tongroach, 1978).

A minor, but nevertheless authenticated, noradrenaline (NA)-containing projection to the striatum occurs from the locus coeruleus (LC) (Moore, 1978;
Mason and Fibiger, in press). This projection must indeed be sparse as the level of NA in the striatum is low (Farley and Hornykiewicz, 1977) or non-detectable (Versteeg et al, 1976) and lesions of the locus coeruleus failed to reduce dopamine-β-hydroxylase (DBH) - the synthesizing enzyme of NA - in the striatum (Ross and Reis, 1974).

3. striatal efferents

One of the major efferent projections of the striatum is that to the SN. This fiber system was recognized by early workers (Rundles and Papez, 1937; Riese, 1924; Ranson and Ranson, 1941). Subsequent studies using the Nauta method or its Fink-Heimer modification supported those early observations and indicated that the projection was topographically organized (Niimi et al, 1970; Voneida, 1960; Johnson, 1961; Johnson and Rosvold, 1971; Szabo, 1962, 1967, 1970; Mickle, 1976). These studies all stressed that the site of termination of striatal efferents in the substantia nigra was principally the pars reticulata (SNR), although, Nauta and Mehler (1966) did observe some termination in the SNC. Investigators applying electron microscopy and the more recent autoradiographic and horseradish peroxidase (HRP) tracing methods to the analysis of this projection have confirmed and extended these earlier studies (Hajdu et al, 1973; Kemp, 1970; Grofova and Rinvik, 1970; Kanazawa et al, 1976). It has become evident that striatal efferents to the SN arise from all parts of the striatum except a central core region (Richardson et al, 1977; Bunney and Aghajanian, 1976a). In addition, such studies provided clear evidence of a projection to the SNC (Hattori et al, 1975; Bunney and Aghajanian, 1976a; Tulloch et al, 1978) and further indicated some topographical arrangement. The exact topographical arrangement, however, has been difficult to discern. For example, in the strictest sense, it is impossible to correlate topographical relationships from degeneration or autoradiographic profiles in the SN when lesions or injections of label, respectively, are shifted in more than one plane in the striatum. This, notwithstanding, the dorsal-ventral dimension
in the striatum appears to be superimposed on the ventral-dorsal aspect of the SN; the anterior-posterior striatal plane may be represented in the anterior-posterior as well as medial-lateral plane in the SN. Although obviously not altogether definitive, such topographical relations are of considerable interest, particularly in view of recent findings by Faull and Mehler (1978). They suggest that the functional topography of the cortico-striate system is faithfully conserved in the striato-nigral system, such that striatal areas which receive motor and visual input from the cortex project to nigral regions that have efferents to the thalamus and SC, respectively. Of course, topographical overlap does not necessarily indicate synaptic convergence. If, however, these initial findings are born out by demonstrations of direct synaptic relations either by electrophysiological or multilabeling anatomical techniques, then, in the opinion of the present author, these advances would represent a significant breakthrough in understanding the organization of the basal ganglia.

It is not clear whether the striato-nigral pathway is inhibitory or excitatory or whether it contains fibers mediating both types of these actions. Constant latency, inhibitory as well as excitatory, responses in the SN have been reported to occur following striatal stimulation (Ferger and Ohye, 1975; Dray et al, 1976a). Intracellular observations of short latency EPSP's in nigral neurons led Frigyesi and Purpura (1967) to suggest a monosynaptic excitatory component of the striato-nigral pathway. These striatal evoked excitatory events in the SN are in marked contrast in findings of the exclusively inhibitory monosynaptic response observed by Yoshida and Precht (1971) and Yoshida et al (1972).

In the study by Yoshida et al (1972), evidence was presented that the inhibitory striato-nigral fibers send collaterals to the GP. Kitai et al (1975) have reported that striatal neurons projecting to the SN are not directly innervated by the nigro-striatal fibers. In addition, Richardson et al (1977)
have shown that striatal neurons that are directly innervated by SNC neurons also give rise to GP afferents which may be excitatory. These findings together can be taken to suggest, insofar as it is possible to generalize, that at least the striatal excitatory fibers which project to the SN do not give off collaterals to the GP.

The exact neuronal type(s) in the striatum that give rise to the efferent fiber system of this structure has not been firmly established. However, the literature on the subject makes certain conclusions unavoidable. Fox et al (1971-72) suggested, on the basis of Golgi studies, that the large aspiny neurons were solely responsible for conveying striatal output. They found that the only myelinated axons in the striatum were those belonging to aspiny neurons. Spiny neurons have unmyelinated axons. The striatal efferents are myelinated as they enter the GP. Hence, large aspiny striatal neurons form, at least in part, the efferents to the GP. There is evidence, based on the assumption that these neurons transport AChE down their axons, that they do not contribute to the striato-nigral system (Lehmann et al, 1979). The striatal efferents in the "comb system" are unmyelinated. This suggests their origin to be striatal spiny neurons. Such a conclusion is supported and indeed warranted by recent findings that nigral afferents from the striatum originate predominantly, if not exclusively, from medium sized spiny neurons (Grofova, 1975; Bunney and Aghajanian, 1976a). Although the retrograde transport of herpes simplex virus has not yet been proven a reliable anatomical research tool for the tracing of anatomical pathways, it should be mentioned that large aspiny neurons as well as many medium sized neurons in the striatum were labeled after intranigral herpes injections (Bak et al, 1978). Thus, the possibility must be entertained that the failure to label the large aspiny striatal neurons with HRP after intranigral injections may be due to their inability to accumulate this protein.

From electrophysiological work the striatal medium sized spiny neurons
must necessarily be divided into two populations. Kitai et al (1975) have shown that nigro-striatal fibers do not converge on striato-nigral neurons, but this group has also shown that nigral efferents to the striatum synapse on medium spiny striatal neurons (Kocsis et al, 1977). Hence, there are presumably at least two populations of medium spiny neurons, one which projects to the SN and one which does not. Although this is the conclusion the present author has been led to, it should be pointed out that the reports by Kitai and coworkers are sometimes difficult to comprehend, particularly when they tend not only to contradict themselves from paper to paper (Kitai et al, 1975; Kocsis and Kitai, 1977) but from paragraph to paragraph (Kocsis et al, 1977). If the above line of reasoning is correct, this would invalidate the argument of Fox et al (1975) of extensive collateralization of striatal efferents in the globus pallidus (see next section), before continuing to the SN.

b) The globus pallidus

1. internal organization

Ontogenetically, the globus pallidus is derived from the lateral part of the basal wall of the prosencephalon and is therefore related more to the diencephalon (Zeman and Innes, 1963) than the forebrain with which it is usually associated. In fresh tissue it has a pale appearance due to a rich content of myelinated axons. The globus pallidus in primates is divided into medial and lateral (or internal and external) segments by the lamina pallidallis interna. In non-primates (rat, cat, etc.) a distinct lamina dividing the globus pallidus into two divisions cannot be found. In these species the nucleus commonly labeled the globus pallidus does not appear to be homologous to both the medial and lateral segments of the primate globus pallidus. Rather, there is substantial anatomical and ultrastructural evidence that the internal segment in non-primates is represented by the entopeduncular nucleus (see below for references). Henceforth, the internal and external divisions of the globus pallidus in primates will be referred to as the MGP and LGP, respectively,
and in non-primates the EP and GP. For general purposes, these structures, whether in the primate or non-primate, will be referred to collectively as the globus pallidus.

In the human it has been estimated that there are 540,000 neurons in the LGP and 170,000 in the MGP (Fox and Rafols, 1976). If the ratio of the number of Purkinje cells in the human (Armstrong and Schild, 1970) and rat (Braitenberg and Atwood, 1958) cerebellum parallels that of the ratio of neurons in the globus pallidus, then in the rat these are about 13,000 and 4,000 neurons in the GP and EP, respectively. Neurons in the GP are far apart and dispersed throughout the nucleus. The perikarya are of the large fusiform type which range from 30 to 60 μm in length. The dendrites of these cells are smooth, radiating, slightly branched structures and have been seen to be as long as 900 μm (Fox et al, 1974). The dendrites and dendritic branches are occasionally varicose, according to Fox et al (1974), and commonly so according to Kemp (1970). This is also true of neurons in the EP (Adinolfi, 1969a). Spines on dendrites are sparse (Fox et al, 1974; Kemp, 1970). There are a few short-axon local circuit neurons in the GP (Fox et al, 1974). Similarly, in the EP there are islands of cell bodies and neuropil which are isolated by myelinated bundles of fibers traversing the region (Adinolfi, 1969a; 1969b). Surrounding the globus pallidus are what Das (1971) calls interstitial nerve cells, so named for the reason that they are located in the white matter and distinctly separate from the neighbouring globus pallidus. On the basis of morphological, dendroarchitectonic and histochemical criteria, these neurons are different from those of the globus pallidus (Das and Kreutzberg, 1968).

With regard to the morphology of the afferents to the LGP and MGP, some of these form long sleeve-like plexuses of fine, longitudinally running fibers which ensheath the radiating dendrites of pallidal neurons (Fox et al, 1974). At the electron microscope (EM) level these dendrites can be seen completely covered with a continuous sheet of synaptic endings. Immediately peripheral
to these terminals are the closely packed profiles of fine afferent fibers which bear these endings and which run longitudinally in the direction of the dendrites (Fox et al, 1974; Fox and Rafols, 1976). This configuration, which has been termed the longitudinal axo-dendritic synapse, is characteristic of the synapses climbing fibers form with Purkinje cells. Axo-somatic synapses are less common (Kemp, 1970). The majority of the terminals in the globus pallidus have symmetrical membrane thickenings although asymmetrical synapses are also present and both types are found to be in contact with somata and dendrites (Fox et al, 1974; Fox and Rafols, 1976; Adinolfi, 1969b).

2. pallidal afferents

The globus pallidus receives afferents from the striatum, the nucleus accumbens and subthalamic nucleus. Of these the striatum appears to provide the most dense innervation of the globus pallidus. It has been demonstrated by degeneration tracing techniques that the striatum projects to both the LGP and MGP (Cowan and Powell, 1966; Fox and Rafols, 1975; Johnson and Rosvold, 1971; Kemp, 1970; Szabo, 1962, 1967, 1970; Nauta and Mehler, 1966; Mickle, 1976; Voneida, 1960). The projection is topographically organized such that the head of the striatum projects to the dorsal and rostral parts of the pallidum, whereas the putamen projects to the ventral and caudal parts (Cowan and Powell, 1966; Szabo, 1962, 1967, 1970; Voneida, 1960). The efferents of the striatum run in radially arranged fiber bundles which converge on the GP "like spokes in a wheel" (Papez, 1941). Szabo (1962) likened the whole striatum to a dome, superimposed on the pallidum. These radial fibers are thought to give rise to the bouton-en-passage fibers and the longitudinal axo-dendritic synapses in the globus pallidus since both these and the radial fibers degenerate after lesions of the striatum (Fox and Rafols, 1975; Fox et al, 1975). There is good anatomical evidence that the radial fibers, during their transit of the LGP and MGP, give off extensive collaterals (Fox and Rafols, 1975, 1976; Fox et al, 1975). It is uncertain whether the same radial fibers give
rise to collaterals in both pallidal segments before continuing on to the SN. Fox et al (1975) suggest that they do for the following reason: The radial fibers after converging upon the globus pallidus are thought to be continuous with the "comb" system. The "comb" system, so named because it is arranged in the cerebral peduncle like teeth in a comb, is the route of the striatonigral fibers (Szabo, 1962, 1967, 1970; Nauta and Mehler, 1966). Fox et al (1975) found that the caliber of the radial fibers, before entering the LGP is larger than those entering the MGP which in turn is larger than those in the "comb" system. They suggest that the successive decrease in fiber diameter is due to the substantial loss of axoplasm to extensive collateral ramifications in both segments of the globus pallidus.

In the few studies that have been done employing the autoradiographic tracing technique, the striatum has been shown to project to both the MGP and LGP in the monkey (Kim et al, 1976) and to the GP (Tulloch et al, 1978) and EP in the rat (Hattori et al, 1975). With regard to the striatal-EP pathway there remains a paucity of definitive studies. Niimi et al (1970) report only a few degenerating preterminals in the EP after lesions in the dorsolateral head of the striatum in the cat. In the same species, Adinolfi (1969) reports numerous axodendritic and relatively sparse axosomatic terminal degeneration in the EP following undescribed striatal lesions. Mickle (1976) after placing lesions throughout the head of the caudate of the opossum, observed degeneration in the intrapeduncular nucleus, a structure which is located in a position similar to that of the EP in the rat. Tulloch et al (1978), in an autoradiographic study, made no mention of the involvement of the EP in the striatal efferent projections. The question of a striatal-EP pathway in the rat is further complicated by the presence of corticofugal fibers of passage which are damaged by striatal lesions. Knook (1965) reported slightly more dense degeneration in the EP of the rat after striatal lesions than after cortical lesions suggestive of the existence of a striatal-EP pathway.
Biochemically, the GP presents a highly complicated picture as it contains many putative neurotransmitters and their synthesizing enzymes at fairly substantial levels compared with other brain areas. These include GABA (Okatda et al, 1971) and GAD (Tappaz et al, 1976; Lloyd et al, 1975), ACh (Jacobowitz and Goldberg, 1977) and CAT (Lloyd et al, 1975; Brownstein et al, 1975), serotonin (Saavedra et al, 1974), NA (Farley and Hornykiewicz, 1977; Versteeg et al, 1976), dopamine (DA) (Versteeg et al, 1976), substance P (Kanazawa and Jessel, 1976) and methionine-enkephalin (met-enkephalin) (Hong et al, 1977b; Sar et al, 1978). It is not known to what extent these substances are represented in the GP by virtue of their presence in neurons intrinsic to the GP. There is, however, evidence for the localization of some of them in afferents to the GP. That GAD and met-enkephalin in GP are of striatal origin is indicated by the observation of a decrease in GAD activity (McGeer et al, 1974a; Hattori et al, 1973b; Fonnum et al, 1978a; Jessel et al, 1978), met-enkephalin levels (Hong et al, 1977b) and met-enkephalin immunofluorescence (Cuello and Paxinos, 1978) following lesions of the striatum. There is presently no datum bearing on the source of substance P, ACh and CAT in the GP. Similarly, there have been no comparative measurements of these various enzymes and substances in the EP or MGP. However, supportive of anatomical evidence of a striatal projection to MGP and EP, there is biochemical data for GAD (Fonnum et al, 1978) and immunohistochemical data for substance P (Paxinos et al, 1978) that suggest these to be contained in striatal efferents to the EP.

Studies of the electrophysiological influence of striatal efferents on neurons of the GP and EP have indicated primarily inhibitory effects (Malliani and Purpura, 1967; Levine et al, 1974; Yoshida et al, 1972). It has been further suggested that inhibition in the EP is mediated by striato-nigral fibers which send collaterals to the EP (Yoshida et al, 1972). This is supported by the anatomical evidence obtained by Fox et al (1975, see above). These find-
ings are consistent with biochemical observations demonstrating a GABA-containing projection from the striatum to the pallidum. However, excitatory responses in the GP and EP have also been observed after striatal stimulation (Malliani and Purpura, 1967) and it has been suggested by inference that the striato-pallidal system contains an excitatory component (Richardson et al, 1977). The evidence for a substance P-containing pathway from the striatum to at least the EP (Paxinos et al, 1978) together with the excitatory actions of this peptide on neurons in the CNS (Krnjevic and Morris, 1974; Phillis and Limacher, 1974) are consistent with the suggestion of a striatal excitatory input to the EP. There is presently no transmitter candidate for the mediator of excitation in the striato-GP pathway.

The dorsal raphe nucleus has been shown to be the source of the high levels of serotonin in the GP and EP (Moore et al, 1978). Thus, this structure has been shown to project to both EP and GP and serotonin-histofluorescence is decreased following lesions of the dorsal raphe (Kuhar et al, 1972). The presence of DA in the GP (Versteeg et al, 1976) is consistent with the demonstration of a projection to the GP from the DA-rich neurons of the SN (Fallon and Moore, 1978). It has been reported that lesions of the SN with no apparent involvement of the subthalamic nucleus (SUT) results in some degeneration of fibers in both MGP and LGP (Carpenter and Strominger, 1967; Moore et al, 1971). Examination of the EP at the EM level also revealed terminal degeneration after lesions of the SN (Adinolfi, 1968). With regard to NA, the GP contains levels comparable to most areas of the cortex. However, a projection from the NA-containing neurons of the locus coeruleus has not been observed (Jones and Moore, 1977).

It is now well established that the nucleus accumbens innervates the GP (Nauta et al, 1978; Swanson and Cowan, 1975; Williams et al, 1977; Conrad and Pfaff, 1976), although there is one autoradiographic study reporting the ab-
sence of such a projection (Powell and Leman, 1976). These GP afferents are located mainly in the ventromedial portion of the GP. The accumbens appears not to project to the EP, as all the descending fibers from this structure en route to the SN conspicuously avoid the EP (Nauta et al, 1978).

Both segments of the globus pallidus in the primate and non-primate receive afferents from the subthalamic nucleus (SUT). In the primate a topographical innervation of the globus pallidus was observed and the pattern of termination was in discrete bands oriented parallel to the medullary laminae. Stimulation of the SUT produces inhibition of pallidal neurons (Larsen and Sutin, 1978; Yoshida, 1973). Electrophysiological evidence suggests that SUT neurons simultaneously innervate the GP, EP and SN by way of axon collaterals (Deniau et al, 1978a). Although the transmitter of this system is not known, it has been suggested that glycine may be involved (Yoshida, 1973).

3. efferents of the medial pallidal segment

In contrast to the lateral pallidal segment, the EP has a major projection to the habenula. Only with the introduction of autoradiographic tracing methods has firm evidence been provided for the existence of this pathway (Nauta, 1974), and this has recently been confirmed (Kim et al, 1976; Herkenham and Nauta, 1977; Carter and Fibiger, 1978). The medio-lateral dimension of the EP was suggested to be represented topographically in a similar fashion in the lateral habenula (Herkenham and Nauta, 1977). In this same study, after HRP injections into the lateral habenula, it was reported that virtually all neurons of the EP were labeled with HRP. Since it is known that the EP has other projection areas, this raises the possibility that some connections of the EP arise by way of axon collaterals of EP efferents. Although collaterals of EP efferents have been demonstrated electrophysiologically, little evidence was obtained suggesting that EP neurons which project to the habenula also project to other sites (Larsen and Sutin, 1978; Filion and Harnois, 1978). There is evidence that at least a component of the EP-habenula pathway contains GAD
(Gottesfeld et al, 1977) and therefore utilizes GABA as a transmitter. Depending on the extent of collateralization of the EP efferents, this suggests that the other efferent projections of the EP may also be GABA-containing.

Although an extensive characterization of the EP efferents to the thalamus was not undertaken here, this projection of the pallidum represents perhaps one of the more interesting outputs of the basal ganglia. The globus pallidus sits at the apex of the convergence of fibers from the cortex to the striatum to the pallidum. Intuitively, then, the pallidal efferents may transmit highly integrated information, the nature of which may be a valuable key to the understanding of basal ganglia function. The MGP gives rise to two fiber tracts, the ansa lenticularis and the lenticular fascicularis, that merge into the thalamic fasciculus which contains the pallido-thalamic projection (Grofova, 1970; Kuo and Carpenter, 1973). In non-primates these fiber systems, arising from the EP, are less distinct. It seems fairly clear that the pallido-thalamic projection originates exclusively from the MGP (Kuo and Carpenter, 1973; Kim et al, 1976; Carpenter, 1976) and EP (Larsen and McBride, 1979; Carter and Fibiger, 1978; Hattori et al, 1975; Grofova and Rinvik, 1974). Thus, technical differences (or difficulties) may have contributed to the results of the only recent study suggesting widespread projections from the GP to the thalamus (Severin et al, 1976). Degeneration (Carpenter, 1976; Kuo and Carpenter, 1973; Nauta and Mehler, 1966; Harding, 1973) and autoradiographic (Kim et al, 1976) studies in the monkey have shown that the principal termination sites within the thalamus of pallidal fibers include the ventral lateral nucleus oral part (VLo), ventral anterior nucleus principal part (VAp) and centromedian nucleus (CM). In these studies a topographical organization of pallido-thalamic fibers has been observed such that rostral parts of the MGP project predominantly to VAp, whereas caudal parts of MGP project primarily to VLo. The greatest number of pallido-thalamic fibers ending in CM originate in
the rostral and medial portion of the MGP (Carpenter, 1976). In the rat, EP efferents to the thalamus terminate in the lateral ventromedial nucleus (VM) and immediately lateral to VM (Carter and Fibiger, 1978). It is significant here that studies of the nigral (Rinvik, 1975; Carpenter et al, 1976; Clavier et al, 1976; Faull and Carman, 1968) and cerebellar (Faull and Mehler, 1976) efferents to the thalamus in the rat, cat and monkey suggest that VM in subprimates is homologous to VA and VL in primates, and specifically to VL pars medialis and pars magnocellularis of VA. In addition to these pathways, a further EP projection in the rat and cat has been observed to the parafascicular nucleus (PF) (Carter and Fibiger, 1978) and CM (Nauta, 1974) of the thalamus. A projection from the MGP to PF was not observed in the monkey (Kim et al, 1976) whereas a projection to CM was. Therefore, although there may be some correspondence between the PF in the rat and the CM in the monkey, it is noteworthy, insofar as pallido-nigral interactions in the thalamus are concerned, that whereas a nigro-parafascicular projection has been reported in the rat (Clavier et al, 1976), in at least one study no nigral efferent fibers were found to project to any of the intralaminar nuclei in the monkey (Carpenter et al, 1976).

The EP-thalamic projection in the rat appears to originate throughout the nucleus and there seems to be no topographical organization of the projection (Larsen and McBride, 1979). Such observations seem to be consistent with electrophysiological demonstrations of some degree of collateralization of EP efferent fibers (Larsen and Sutin, 1978; Filion and Harnois, 1978).

For the most part, electrophysiological studies of EP-thalamic relationships have yielded little information. Dormont and Ohye (1971) observed only weak excitation of units in the VL thalamus after EP stimulation and were hard pressed to attribute this solely to stimulation of EP neurons. Stimulation of diencephalic regions "which should include efferent fibers from the EP" (Yoshida et al, 1971) produced monosynaptic IPSP's in the EP. This suggests
an inhibitory component of EP efferents and their recurrent collaterals. The origin or destination of these fibers, however, remained unknown. Stimulating in the brachium conjunctivum and ansa lenticularis Desiraju and Purpura (1969) observed EPSP's in the VL thalamus and concluded the existence of a direct monosynaptic excitatory convergence of fibers from the cerebellum and "corpus striatum" onto a common pool of VL neurons. In contrast, Uno and Yoshida (1975) and Uno et al (1978) observed only monosynaptic IPSP responses in VL neurons upon EP stimulation, found these neurons to form anatomically separate populations from those receiving cerebellar input and concluded that very little, if any, direct convergence of cerebellar or EP fibers on single VL neurons occurs. It is apparent that somewhat more attention to detail is required in electrophysiological investigations in order even to begin to make sense of the synaptic relationships of EP-thalamic fibers and the interaction of these with other thalamic afferents. To emphasize further the lack of knowledge in this area, virtually nothing is known of the biochemistry of this major fiber projection of the basal ganglia.

Other than its existence, very little is known about the pathway from the MGP (Kim et al, 1976; Kuo and Carpenter, 1973; Nauta and Mehler, 1966) and EP (Larsen and McBride, 1979; Carter and Fibiger, 1978; Filion and Harnois, 1978) to the nucleus tegmenti pedunculopontis (TPP). In addition, it has been reported that injection of HRP into areas of the brain stem other than SN or TPP results in labeling of EP and GP in the rat and MGP in the monkey (Hopkins, 1977). These pathways appear to be fairly sparse and therefore further investigations are required before the suggestion can be taken seriously that they provide a means whereby the basal ganglia can directly and significantly exert an influence on, for example, descending motor systems (Hopkins, 1977).

4. efferents of the lateral pallidal segment

The LGP projection to the SUT has been known for some time (Whittier and Mettler, 1949; Nauta and Mehler, 1966) and has recently been demonstrated with
autoradiographic transport methods (Kim et al, 1976; Hattori et al, 1975). This projection is topographically organized such that the efferents of the LGP focus onto the SUT in much the same manner as the striatum projects to the LGP (Carpenter et al, 1968). The MGP and EP appear not to project to SUT as no autoradiographic activity was observed in the SUT after injections of radio-tracer into these structures (Kim et al, 1976; Carter and Fibiger, 1978). Lesions of the GP have been shown to decrease GAD activity in the SUT (Fonnum et al, 1978b). Although the topographical changes observed in GAD were reported to reflect the organization of the pallido-subthalamic projection, this conclusion can be disputed since some of the GP lesions involved the internal capsule which contains all of the descending pallido-subthalamic fibers (Nauta and Mehler, 1966). In the absence of electrophysiological data, the presence of GAD in the efferent fibers of the GP to SUT suggests that this pathway is inhibitory. An excitatory component, however, cannot be ruled out in view of immunohistochemical demonstrations of substance P-positive neurons in the GP (Kanazawa et al, 1977), an excitatory action of ACh on SUT neurons (Ferger et al, 1979), and that the majority of neurons in the SUT respond by initial excitation to stimulation of the contralateral area of the skin in the vicinity of the vibrissae (Hammond et al, 1978).

It has been virtually impossible to demonstrate a projection from the GP to the SN using degeneration techniques. Lesions of the GP invariably involve striatal fibers passing through the GP on their way to the SN. Indeed, Nauta and Mehler (1966) in their review article concluded that the pallido-subthalamic projection "possibly represents the only extrinsic efferent connection of the external segment". The first convincing anatomical demonstration of a fiber system between the GP and SN was an autoradiographic study by Hattori et al (1975). They showed that the GP projects primarily to the zona compacta of the SN. Subsequent autoradiographic studies confirmed the existence of nigral afferents of the LGP (Kim et al, 1976). In neither of these studies
was label found in the SN after the injection of tracer into the EP or MGP. Employing the HRP retrograde transport technique, labeled cells in the GP have been observed after injections of HRP into the SN (Grofova, 1975; Tulloch et al, 1978). In addition, HRP positive cells were found in the EP after injections of HRP into the medial caudal parts of the SNC (Grofova, 1975). The differences obtained between the HRP and autoradiographic techniques with regard to an EP projection to the SN have yet to be reconciled.

The preferential projection of the GP to the SNC was also observed by Kanazawa et al (1976) who found that pressure injections of HRP into the SNC resulted in a greater number of labeled cells in the GP than injections into the SNR. On the other hand, Bunney and Aghajanian (1976a) reported a larger number of labeled cells in the GP after HRP injections into the SNR than SNC. The discrepancy between these results may be due to the fact that in the latter study HRP was delivered to the SN by microelectrophoresis and this procedure has been shown to cause damage to and uptake of HRP by fibers of passage (see section IIIa). Physical and anatomical parameters might be such that the fiber of passage problem posed by electrophoretic injections manifests itself to a greater degree after injections into the SNR than SNC.

The coincidental unequivocal demonstration of the existence of a pallido-nigral pathway with the onset of serious efforts to determine the biochemical nature of the striato-nigral system has led to an inseparable evolution of data on the origin of the neurons containing the neurotransmitters involved in these nigral afferents. For this reason the biochemical neuroanatomy of the striato- and pallido-nigral systems are discussed simultaneously in the following section.

The observation that the SN contains relatively high levels of GABA and GAD activity was made shortly after the discovery of GABA in the CNS (Albers and Brady, 1959; Muller and Langemann, 1962). The SNR, in fact, contains the highest GAD activity of the 74 brain areas measured by Tappaz et al (1976).
In their original observation McGeer et al (1971b) showed that lesions of the GPL caused a reduction of nigral GAD activity. Kim et al (1971) showed that in the rat SN GABA content decreases after hemitransections at the level of the SUT or after striatal lesions and suggested that some of the striato-nigral fibers utilize GABA as a neurotransmitter. From the histology of the lesion site shown in their paper the striatal lesion almost certainly involved the GP. In addition, although not detracting from the qualitative validity of their observation, without taking special precautions, the measurement of GABA levels cannot be used to gain quantitative estimates of the total contribution of nigral GABA from afferent sources since brain GABA content has been shown to undergo post mortem changes (Baxter, 1969). Subsequent studies by the same group (Kataoka et al, 1974) showed that nigral GAD activity can be reduced 70% by hemitransections at the subthalamic level. One study has focused on defining the exact source of GABA-containing afferents to the SN. Hattori et al (1973b) (see also McGeer et al, 1974a) demonstrated that hemitransections anterior to the GP, severing descending projections from about 60% of the striatum, did not reduce nigral GAD activity. On the other hand, hemitransections at the level of the hypothalamus reduced GAD activity in the SN by 82%. These workers suggested that nigral GAD-containing afferents originate in the tail of the striatum and/or the GP. In contrast to these results, Fonnum et al (1974) observed GAD reductions (up to 90% in some instances) in the SN after large striatal lesions, including those confined to the anterior striatum, which led these investigators to conclude "that only a small number of GABAergic fibers to the substantia nigra could be derived from other sources". Fonnum et al (1974) attributed the differences between their work and that of Hattori et al (1973b) to be due to their observation that nigral GAD loss is regional and follows the topography of the striato-nigral projections, and such losses may have been overlooked by Hattori et al (1973b) who employed measurements of the whole SN. Such an explanation for the incongruities be-
tween the results of the two groups, although possible, is doubtful. GAD activity is highest in the medial half of the SN (Fonnum et al, 1974). From the large reductions in GAD activity observed by Fonnum et al (1974) only in the medial half of the SN after "rostral" striatal lesions, it is highly unlikely that such losses of GAD would go unobserved in whole SN assays. These discrepancies are more likely to be due to species differences between the cat (Fonnum et al, 1974) and the rat (Hattori et al, 1973b) or to methodological differences. For example, it is presently not apparent what the equivalent of an anterior hemitransection lesion in the rat would be in the cat.

Following these initial observations and probably due, in part, to the controversy surrounding them, there have been numerous reports dealing with the source of nigral GAD-containing afferents. The earlier work of Hattori et al (1973b) was confirmed (Brownstein et al, 1977; Jessel et al, 1978; Spano et al, 1977). Brownstein et al (1977) found that the rostral three-quarters of the striatum does not send GABA-containing fibers to the SN. In addition, they found that lesions involving the lateral half of the tail of the striatum produce only modest decreases of GAD activity in the SNR without altering it in the SNC. Brownstein and coworkers concluded that GAD containing neurons innervating the SN are located in the GP and in the striatum immediately bordering the GP on its anterior and lateral aspect. Similarly, Jessel et al (1978), after producing lesions which separated the entire striatum from the GP, concluded that the GP contains significant numbers of GABA neurons projecting to the SN. As for Fonnum and coworkers (Fonnum et al, 1978), they have refuted some of their earlier claims and have stated that "a significant decrease in nigral GAD occurred only after hemitransections involving the tail of the caudate-putamen". It should be mentioned here that some workers (Kanazawa et al, 1977; Gale et al, 1977b) still maintain that anterior striatal regions contribute to nigral GAD-containing afferents. Although the possibilities for the anatomical location of the elusive GAD-containing striato-
pallido-nigral neurons are narrowing, it is evident from the foregoing discussion that the exact distribution of these neurons within the striato-GP complex remains unresolved.

Paralleling the above studies, the existence of striato- and/or pallido-nigral substance P-containing fibers has also been demonstrated (Jessel et al, 1978; Brownstein et al, 1977; Mroz et al, 1977; Gale et al, 1977b; Palkovits et al, 1978; Kanazawa et al, 1977; Hong et al, 1977c). Indeed, greater than 90% of nigral substance P appears to be in terminals of these descending fibers. For the greater part of the nigral afferents of the striatum, there appears to be a clear separation of those containing GAD from those containing substance P. Thus, a substantial number of substance P-containing striato-nigral neurons are located in the striatum anterior to the GP (Brownstein et al, 1977; Jessel et al, 1977; Gale et al, 1977b) and according to Brownstein et al (1977) none originate in the tail of the striatum or the GP. However, substance P has been demonstrated immunohistochemically in typical neurons of the GP (Kanazawa et al, 1977) and by inference the existence of pallido-nigral substance P-containing fibers is indicated (Jessel et al, 1977).

For the localization of both substance P- and GAD-containing neurons which project to the SN, it appears that mechanical or electrolytic lesions can provide only a limited degree of resolution which is determined by lesion size, dissection accuracy and assay sensitivity; in the case of pallido-nigral projections such studies are impossible due to the fiber-of-passage problem.

Elucidation of the architectural arrangement of the substance P and GAD-containing projection neurons in the striatum and GP will require a combination of histological, possibly immunohistological techniques, with retrograde transport anatomical techniques, or, in the case of the GP lesions, techniques which do not destroy fibers of passage.

There is currently no electrophysiological datum available on the pallido-nigral pathway.
5. acetylcholinesterase staining neurons of the globus pallidus

A source of confusion with regard to the present experiments (see discussion) and in defining the efferent connections of the globus pallidus are the cells, referred to by Das and Kreutzberg (1968) as interstitial neurons, which surround and intersperse both segments of the globus pallidus. Compared to the smaller triangular and fusiform neurons of the globus pallidus proper, these interstitial neurons are much larger, multipolar and stain more intensely for AChE. Whether these cells should be classified as an integral part of the globus pallidus is doubtful. For example, unlike neurons of the GP, they appear to project to cortical areas (Kelly and Moore, 1978, Divac, 1975; Johnston et al, 1979; Das, 1971). Grouping these interstitial cells with the substantia innominata would not solve matters but merely put the onus of classification on a broader scope, vis à vis, the current controversy of whether the substantia innominata is part of the globus pallidus or a separate entity. The organization of these cells in the existing conceptual and anatomical framework of the basal ganglia must await a more detailed account of their afferents and efferents.

c) Subthalamic nucleus

In a Golgi study of the SUT by Rafols and Fox (1976), three principal neuron types were identified on the basis of size and morphology. These are radiating neurons, elongated fusiform neurons and local interneurons. It may be of some significance that the local interneurons of the SUT bear some similarities to neurons in other nuclei that have been shown to be presynaptic to axon terminals (see Rafols and Fox, 1976). The major connections of the SUT, those with the globus pallidus and SN, are discussed in previous and subsequent sections, respectively. Some sparse fiber projections of the SUT to the VA-VL thalamus, putamen and TPP pars compacta have also been observed (Nauta and Cole, 1978). A detailed documentation of SUT afferents awaits examination with the retrograde HRP technique.
d) Substantia nigra

1. Internal organization

A great deal of anatomical, electrophysiological and biochemical investigatory effort has been brought to bear on the SN. In part, this may be due to the possible involvement of this structure, and in particular, of the nigrostriatal DA neurons in a number of phenomena of psychological, pharmacological and clinical importance. What follows is a brief review of the data available on the SN with emphasis on certain features that pertain to the present work.

The SN is divided into two portions: the neuron dense pars compacta and the neuropil rich pars reticulata. Three types of neurons have been identified in the SN by various techniques (Juroska et al, 1977; Rinvik and Grofova, 1970; Grofova and Rinvik, 1970; Gulley and Wood, 1971; Schwyn and Fox, 1974). The largest nigral neurons, those in the SNR, measure 45 to 74 μm. Medium sized (19–45 μm) heterogeneously shaped neurons, similar in morphological characteristics to the large neurons, are present in both SNR and SNC. The smallest neurons, also present in both SN substructures, measure 11 to 26 μm. Neurons of all types have varying numbers of spines and some degree of varicosities or beading of their dendrites. Medium sized neurons of the SNC project long varicose dendrites into the SNR. These neurons also have restricted dendritic fields in the SNC. Dendrites arising from neurons in the SNR tend not to leave this structure. Axons of many large and medium sized neurons in the SNR have collaterals, some of which are recurrent while others follow the orientation of the principal axon. The medium sized neurons of the SNC, which give rise to the ascending DA-containing projection, stain intensely for DA (Dahlstrom and Fuxe, 1964). The dendrites of these neurons, where they invade the SNR, have also been shown to contain DA (Bjorklund and Lindvall, 1975). It is of interest here that ultrastructural studies have disclosed vesicle-containing dendrites in the SN and these appear to form dendro-dendritic synapses (Hajdu et al, 1973). Moreover, Wilson et al (1977a), using the osmophilic
label 5-hydroxydopamine, have recently reported that labeled vesicle-containing dendrites and axons form en passage synapses in the SNC and dendro-dendritic synapses in the SNR. These observations are significant in that they relate to certain electrophysiological and biochemical phenomena concerning DA-containing neurons which will be discussed in subsequent sections.

Electrophysiologically at least two types of neurons have been identified in the SN (Guyenet and Aghajanian, 1978; Wilson et al, 1977b). Type I neurons, located in the SNC and probably giving rise to the dopaminergic nigro-striatal system, have a slow bursting pattern and a slow conduction velocity. Type II neurons have a higher firing rate and conduction velocity and are located in the SNR. Although most of the Type II neurons could be antidromically activated from the thalamus, some located subjacent to the SNC were antidromically activated from the striatum (Guyenet and Aghajanian, 1978). There is only partial agreement between electrophysiological and anatomical studies as to the exact location within the SNR of neurons projecting to the striatum.

Biochemically, the SN presents a profile that in some respects resembles the GP. The substances and enzymes, relevant to neurotransmission, that have been observed in the SN include NA, DA (Farley and Hornykiewicz, 1977; Versteeg et al, 1976), tyrosine hydroxylase (TH) (Reis et al, 1975), DBH (Reis et al, 1978; Ross and Reis, 1974), adrenaline (van der Gugen et al, 1976), serotonin (Palkovits et al, 1974), GABA (Fahn and Côté, 1968; Okatda et al, 1971), GAD (Tappaz et al, 1976; Lloyd et al, 1975) and substance P (Brownstein et al, 1977; Jessel et al, 1978). A major goal of numerous studies has been to establish the efferent, afferent and intrinsic anatomical systems of the SN that contain these materials and some success has been achieved in these attempts.

In the case of TH, it is probable that this enzyme is located within dopamine containing perikarya and dendrites, and perhaps even terminals although at present there is no evidence of this. The DA-containing neurons in
the SNC stain immunohistochemically for TH (Hökfelt et al, 1976) and after hemitransections anterior to the SN or 6-hydroxydopamine (6-OHDA) injections into the nigro-striatal pathway (NSP), the retrograde degeneration of DA-containing neurons coincides with the reduction of TH in the SN (Reis et al, 1978; McGeer et al, 1973). It is interesting to note that the 6-OHDA lesions result in severe depletions of nigral TH. However, after hemitransection about 20% of nigral TH remains. This suggests that these neurons may have some collaterals which prevent their complete degeneration. Alternatively, the limited capacity to survive transection of their principal axons may be related to the extensive ramifications and specialization of the dendrites of these neurons in the SNR (Reis et al, 1978). In addition, a projection to regions posterior to the hemitransections cannot be ruled out.

With regard to GAD, Ribak et al (1976) observed no immunohistochemical staining of neuronal perikarya in the SN although GAD-positive axon terminals were abundant. Apparently, this absence of cell body staining was "anticipated since there is evidence that another neurotransmitter candidate, dopamine, is concentrated within neurons of the substantia nigra" (Ribak et al, 1976). This explanation, however, is not satisfying. Among other explanations is the possibility that GAD levels in neuronal soma are below the threshold of detection by the immunohistochemical method.

A cholinergic involvement in nigral function is suggested by the presence in the SN of ACh (Jacobowitz and Goldberg, 1977), CAT (Brownstein et al, 1975; Cheney et al, 1975; Lloyd et al, 1975) and AChE (Butcher and Hodge, 1976; Butcher et al, 1975; Palkovits and Jacobowitz, 1974). In addition, ACh has been found to influence the electrical activity of nigral neurons (Dray and Straughan, 1976; Aghajanian and Bunney, 1974a). The localization of cholinergic markers in the SN is at present unclear. Although it has been difficult to attribute a function to the disproportionately high levels of nigral AChE relative to CAT, it has been shown by histochemical (Butcher and Hodge, 1976;
Butcher et al, 1975) and biochemical criteria (Lehmann and Fibiger, 1978) that dopaminergic neurons contain some of the AChE of the SN. With regard to CAT, since hemitransections immediately anterior to the SN fail to reduce the activity of this enzyme in the SN (Kataoka et al, 1974; McGeer et al, 1971) a rostral cholinergic projection to the SN is unlikely. Curiously, both the SN and GP possess ACh levels which are disproportionately high compared to areas containing greater amounts of CAT activity (Jacobowitz and Goldberg, 1977). The reason for this remains unknown.

The other substances mentioned above appear, for the most part, to be present in terminals of afferent fibers to the SN and are discussed in that context.

2. nigral efferents

The nigro-striatal system has been much studied and well documented (Andén et al, 1964; Hökfelt and Ungerstedt, 1969; Hattori et al, 1973a; Fallon and Moore, 1978). These striatal afferents and their terminals are probably the source of the bulk of the remainder of terminals of extrinsic origin to the striatum after the cortex and thalamus (Kemp and Powell, 1971b). That these fibers are dopamine-containing has been well documented. In addition, lesion studies followed by DA measurements in the striatum (Moore et al, 1971; Koob et al, 1975) are in good agreement with anatomical studies (Fallon and Moore, 1978) in demonstrating that both the SN and ventral tegmental area (VTA) project DA-containing fibers to the striatum, albeit the VTA contribution is somewhat less. The organization of the SN/VTA-striatal DA system has been shown to be highly topographic (Fallon and Moore, 1978; Moore and Bloom, 1978). This strict topographical arrangement may provide some insight into the function of the reciprocal connections of the SN and striatum and will be discussed later. The SNC also has widespread projections to cortical and other subcortical areas. Thus, following the initial demonstration of the presence of DA in the cortex (Thierry et al, 1973), many papers quickly appeared demonstra-
ting the SN-VTA to be the source of DA in the cortex, accumbens, olfactory
tubercle, septal nucleus, amygdala, habenula, cerebellum, thalamus, hypothala-
mus and median eminence (Fuxe et al, 1974; Fallon et al, 1978a, 1978b; Lindvall
et al, 1974; Lindvall, 1975; Kizer et al, 1976; Berger et al, 1976; Carter and
Evidence has been provided that the population of DA-containing neurons in the
SNC projecting to the striatum are separate from those projecting to other
brain areas (Fallon et al, 1978b; Moore, 1978).
With regard to the electrophysiological nature of the DA input to the
striatum, the general consensus has shifted from the view that DA is primarily
inhibitory on striatal neurons to the currently held one of an excitatory ac-
tion of DA (Richardson et al, 1977; Kitai et al, 1975, 1976c; Davies and
Tongroach, 1978). Excitatory responses in septal neurons have also been ob-
served by stimulation of the VTA and this was suggested to be mediated by DA
(Assaf and Miller, 1977). The discrepancies in the determination of the elec-
trophysiological action of the nigro-striatal system have also been suggested
to be due to a dual innervation of the striatum by the SN; a DA and non-DA
pathway (Hedreen, 1971, Hedreen and Chalmers, 1972; Davies and Tongroach,
1978; Frigyesi and Purpura, 1967; Fibiger et al, 1972). This possibility has
not been fully resolved.
A nigrotectal projection has been demonstrated in several species includ-
ing the rat, cat and monkey (Hopkins and Niessen, 1976; Hopkins, 1977;
This projection arises exclusively in the SNR and terminates mainly in the
caudal two-thirds of the intermediate gray layer of the ipsilateral superior
colliculus (SC). The nigral fibers in the SC array themselves in remarkably
regular bands running the rostral-caudal length of the SC. From electrophys-
iological and biochemical evidence the SNR-SC pathway appears to have both an
excitatory and inhibitory component. Stimulation of the SN has been shown to
produce excitation of SC neurons (York and Farber, 1977). Support for an inhibitory nigro-collicular pathway has been provided by the finding that GAD in the SC decreases following nigral lesions (Vincent et al, 1978).

The SNR has also been shown to project to the VA and VL thalamus in the monkey (Carpenter and Peters, 1972; Carpenter et al, 1976) and the homologue of these nuclei in the rat (Rinvik, 1975; Clavier et al, 1976; Faull and Mehler, 1978). In addition, although in several studies no nigral-intralaminar projections were observed (Rinvik, 1975; Kultas-Ilinsky et al, 1978; Carpenter et al, 1976), there is one report of a projection to the PF from the SN (Clavier et al, 1976). The nigro-thalamic projection appears to be largely inhibitory as stimulation of the SN has been shown to produce monosynaptic IPSP's in thalamic neurons (Ueki et al, 1977; Anderson and Yoshida, 1977; Deniau et al, 1978b). There are no reports of the biochemical nature of the nigro-thalamic pathway.

Several of the above studies and others (Jayarman et al, 1977; Hedreen and Chalmers, 1972) have reported on the regional distribution within the SN of neurons projecting to the striatum, SC and thalamus. However, the relationship to each other of neurons projecting to these various targets has been most clearly demonstrated in a recent study by Faull and Mehler (1978). These investigators showed that nigral neurons comprising the striatal, thalamic and SC efferents fell into distinct size groups and that within the SNR and SNC there was very little overlap of the location of the neurons projecting to these separate nuclei. This suggests that the various SN projections arise from separate populations of neurons and is therefore inconsistent with electrophysiological observations demonstrating that a significant portion of neurons in the SNR send axon branches to both the SC and thalamus (Anderson and Yoshida, 1977). Deniau et al (1978c), refuting their earlier work (Deniau et al, 1977), have also suggested on electrophysiological grounds the
existence of collaterals of SNR efferents to multiple sites. These discrepancies are reminiscent of those already described concerning the degree of collateralization of projections from the EP to the habenula and thalamus. These incongruities between anatomical and electrophysiological investigations have yet to be reconciled.

A further point regarding the study by Faull and Mehler (1978) is their observation of substantial numbers of nigro-striatal neurons located in the SNR. As mentioned earlier a pathway connecting the SN and striatum in addition to the dopaminergic nigro-striatal system has been proposed (Hattori et al, 1973a; Fibiger et al, 1972; Feltz and DeChamplain, 1972). The non-SNC location of these cells together with the observation of the presence in the SNR of non-catecholamine fluorescent HRP-positive neurons after striatal HRP injections (Berger et al, 1978) suggest that these cells may be the origin of the non-dopaminergic nigro-striatal system. In the context of other possible sources (besides the SNR) of striatal afferents originating in the vicinity of the SN, it is perhaps significant that following HRP injections in the striatum of the cat labeled cells have been observed in the retrorubral nucleus and in cell bridges between this nucleus and the SN (Vandermaelen et al, 1978). Fallon and Moore (1978) also found labeled cells in this area in the cat after striatal HRP injections. They, however, simply referred to this zone as caudal SN. For a more detailed discussion of the relationship of these outlying nigral neurons to nigral efferents and afferents and, in particular, hypothalamo-nigral connections the reader is referred to papers by Nauta and Domesick (1978) and Nauta et al (1978). Further studies are required to determine the exact relation of these neurons to the SN proper.

There are some additional descending projections from the SNR to the central gray area, pons and medulla oblongata (Hopkins, 1977; Hopkins and Neissen, 1976). The terminal fields of these fibers and the cells of origin
in the SN relative to the thalamic, striatal and tectal projection neurons are as yet not well defined.

3. nigral afferents

The SN receives numerous afferents from diverse brain areas. In view of implications of the involvement of the SN and its efferents in the mediation of certain behavioral processes and in the site of action of some pharmacological agents, the nigral afferents take on special significance.

The SN receives monoamine inputs from the LC and raphe nucleus. Although not specifically stated the LC appears to project to the SNR as gleaned from the diagram in a paper by Jones and Moore (1977). That this projection is NA-containing is suggested by the presence in this area of NA (Versteeg et al, 1976) and NA-containing fibers (Ungerstedt, 1971a) and a decrease in DBH following lesions of the posterior midbrain (Reis et al, 1978). Swanson and Hartman (1975) did not observe immunohistochemical staining for DBH in the SN. It should be pointed out, however, that these workers consistently observed little or no staining in areas known to receive NA input. In this context it is perplexing that they reported staining in the frontal cortex which contains lower DBH activity than the SN (Reis et al, 1978).

The SN receives a substantial projection from the midbrain raphe nucleus (MRN) (Moore et al, 1978; Fibiger and Miller, 1978). The SNC appears to receive a heavier projection than the SNR and indeed Azmitra and Segal (1978) report the absence of a projection to the SNR. That this projection is serotonergic is indicated by the findings that serotonin levels decrease in the SN following raphe lesions (Dray et al, 1978; Palkovits et al, 1977; Fibiger and Miller, 1977). Electrophysiological studies have shown the raphe-SN projection to be mainly inhibitory (Fibiger and Miller, 1977; Dray et al, 1978), and this inhibition of SN neurons appears to be mediated by serotonin (Fibiger and Miller, 1977). As in the case of the extent of termination of raphe efferents in the SNR, there is disagreement concerning the exact source of these fibers.
from within the raphe nuclei. Some studies suggest that the projection arises exclusively from the DRN (Azmitia and Segal, 1978; Fibiger and Miller, 1977; Pasquier et al, 1977) while others suggest the existence of serotonergic afferents from both DRN and MRN (Dray et al, 1978; Dray et al, 1976b).

The afferents to the SN from the nucleus accumbens have been adequately demonstrated (Nauta et al, 1978; Swanson and Cowan, 1975; Williams et al, 1977; Conrad and Pfaff, 1976). The terminal area within the SN is heaviest in the medial half of the SNC although there is some termination in the VTA and the SNR subjacent to the SNC. In an autoradiographic study Powell and Leman (1976) also purported to demonstrate an accumbo-nigral projection. However, contrary to the above studies, they failed to confirm accumbens connections with the external segment of the globus pallidus and found an accumbens projection to the caudate nucleus. In the report by Powell and Leman (1976), inasmuch as the label in the caudate looks suspiciously like diffusion of radioactivity up a needle tract, this labeling in the caudate confounds their analysis of the efferent projections of the nucleus accumbens.

Electrophysiological experiments suggest that the nigral and VTA afferents of the accumbens are primarily inhibitory although excitation has also been observed (Dray and Oakley, 1977; Wolf et al, 1978). The inhibition was suggested to be mediated by GABA as it was blocked by bicuculline (Wolf et al, 1978). However, no decreases in GABA levels in the SN were observed following electrolytic lesions of the accumbens (Dray and Oakley, 1977) or GAD in the VTA following hemitranssection at the level of the rostral GP (Fonnum et al, 1977). Lesions of the nucleus accumbens with kainic acid decreased GAD activity in the VTA but not in the SN (Waddington and Cross, 1978). In view of the lack of effect of hemitranssection, it seems possible that the results of the study by Waddington and Cross (1978) may be attributed to diffusion of kainic acid to the striatum and/or GP.
From autoradiographic (Nauta and Cole, 1978) and HRP studies (Kanazawa et al, 1976; Tulloch et al, 1975) it is evident that both the SNR and SNC receive a substantial projection from the SUT. No studies have been conducted to characterize the electrophysiological or biochemical nature of the nigral afferents from the SUT.

Other afferents to the SN arise from various parts of the cortex, the habenula, hypothalamus and amygdala (Bunney and Aghajanian, 1976a). In a recent paper by Phillipson (1978) no less than 37 diverse brain areas were observed to contain labeled neurons following HRP injections into the VTA. Further work, however, is required to substantiate some of these afferents and to determine their importance.

II Adenylate Cyclase and Dopamine Receptors in the Substantia Nigra and Striatum

The likelihood that Parkinson's disease and the possibility that schizophrenia and even some of the symptoms of Huntington's disease have their cause in malfunctions of central DA systems has led to the expenditure of enormous efforts to determine the factors that govern the activity of dopaminergic neurons in the SN. One approach along these lines is the definition by anatomical methods of the nigral afferents that might regulate the activity of these neurons. Inputs to the SN from the striatum have received particular attention in this regard. Another approach is directed toward the concept of the self-regulation of dopaminergic nigral neurons: the importance of this process, the extent to which it occurs and the mechanisms by which it is achieved. What follows is a description of some of the key findings which have prompted the current view that there exist both a striato-nigral feedback system and a feedback system intrinsic to dopaminergic neurons. Both of these systems may aid DA-containing nigral neurons to gauge and adjust their output. An introduction to the emergence of these concepts is best done
within a historical perspective. Consequently, both feedback systems are discussed simultaneously.

More than fifteen years ago Carlsson and Lindqvist (1963) administered haloperidol and chlorpromazine (DA receptor blockers; although even that was not certain at the time) to rats and found that these agents augmented the accumulation of DA metabolites in the brain. They suggested that there is "a compensatory activation of monoaminergic neurons after the blockade of monoaminergic receptors". A decade passed before the insight of this conclusion began to be appreciated. What this conclusion did not explain, however, was whether the feedback system was intrinsic to the DA neurons or mediated through a long axon system. The battle waged by the proponents of each of these mechanisms has been raging from the time it became apparent that two such systems may exist.

The first suggestion that a neuronal feedback loop may account for the regulation of dopaminergic transmission and biosynthesis in the nigro-striatal pathway was provided by Corrodí et al (1967). These workers suggested that some of the effects of d-amphetamine (AMPH) on DA metabolism might be secondary to a decrease in the firing rate of dopaminergic neurons. They further suggested that this decrease of DA neuronal activity might be due to the ability of AMPH to cause, indirectly, an increase in the stimulation of postsynaptic DA receptors, leading to a compensatory decrease in the firing rate of DA neurons mediated by an interneuronal feedback pathway. These suggestions found support through the subsequent work of Bunney, Aghajanian and collaborators (Bunney et al, 1973a, 1973b; Aghajanian and Bunney, 1973, 1974a, 1974b; Bunney and Aghajanian, 1975; Bunney and Aghajanian, 1973, 1976b). These workers found 1) that intravenously administered AMPH causes a depression of DA neuronal activity in the SNC, 2) that postsynaptic cells in the striatum are very sensitive to microiontophoretically applied AMPH whereas nigral neurons are not, and 3) that transection of the brain between the
striatum and SN and more specifically, lesions in the crus cerebri or in the vicinity of the tail of the striatum, greatly attenuate the depressant effects of intravenous AMPH on nigral neurons.

At about the same time that the above work was begun, evidence for an intraneuronal dopaminergic feedback mechanism was provided by the spectacular observations of Kehr et al (1972). Initially, Andén et al (1972) found that, within the first day after hemitranssections between the striatum and SN, there occurs a marked increase in DA levels in DA neuron target areas anterior to the lesion. Kehr et al (1972) (see also Carlsson et al, 1972, 1976) demonstrated that this was due to an increase in DA synthesis after axotomy. They further showed that the increased DA synthesis was reversed by the DA agonist, apomorphine, and that this reversal could be blocked by pretreatment with haloperidol. Since these drugs influenced events in DA-containing terminals even after the above hemitranssections, these results led to the suggestion of the presence of DA receptors on dopaminergic terminals and the term "autoreceptors" was coined for these entities by Carlsson (1975). These experiments, however, could not exclude a postsynaptic effect of apomorphine and haloperidol followed by an action on dopaminergic terminals of a transsynaptic messenger.

Support for the existence of autoreceptors on DA-containing neurons in the SN was provided by Groves et al (1975). Contrary to the above cited findings of Bunney and Aghajanian, these workers found that intranigral infusions of AMPH depressed whereas haloperidol augmented neuronal activity in the SNC. Moreover, intrastriatal infusions of AMPH increased and haloperidol decreased SNC neuronal activity. Since these results were inconsistent with a striato-nigral negative feedback loop, Groves et al (1975) suggested the existence of a positive striato-nigral feedback pathway and that the efficacy of this is masked by the effect of the above drugs on dopaminergic transmission within the SN; specifically, their action on autoreceptors on DA-containing neurons in the SNC. These results were consistent with the subsequent demonstrations
by Garcia-Munoz et al (1977) that lesions of the striato-nigral tract do not alter the ability of haloperidol to augment DA metabolism in the striatum.

Apart from the conclusion by Groves et al (1975) of the site of action of haloperidol and AMPH, their suggestion of autoreceptors in the SN was in agreement with earlier proposals of this by Aghajanian and Bunney (1973, 1974a, 1974b) who found that intranigral administration of DA and apomorphine caused a haloperidol reversible inhibition of SNC neurons. In addition, the concept of nigral autoreceptors was strengthened by demonstrations that 1) the dendritic processes of DA neurons contain DA and vesicles (Hajdu et al, 1973; Bjorlund and Lindvall, 1975; Paizek et al, 1971), 2) cells in the SN exhibit calcium-dependent DA release (Geffen et al, 1976; Korf et al, 1976; Nieoullon et al, 1977), and 3) nigral DA neurons engage in dendro-dendritic synaptic contacts (Wilson et al, 1977a).

Another line of investigation that has added to the saga of the DA autoreceptor involves a species of adenylate cyclase whose activity is stimulated by dopamine. When Kebabian et al (1972) demonstrated a DA-sensitive adenylate cyclase (DAC) in the striatum they suggested that the DA receptor is an integral part of the cyclizing enzyme. The desire by many investigators to interpret experimental results in accordance with this suggestion marshalled the concept of a single DA receptor-adenylate cyclase entity into dogma. This invariably influenced views regarding the DA autoreceptor. Following the initial demonstrations of DAC in the SN (Traficonte et al, 1976; Phillipson and Horn, 1976; Spano et al, 1976), it was quickly determined that DAC is not located on DA-containing nigral neurons (Kebabian and Saavedra, 1976; Premont et al, 1976) but possibly on nigral afferent terminals from the striatum (Premont et al, 1976; Gale et al, 1977a; Phillipson et al, 1977), and more specifically, on nigral afferent terminals from striatal neurons located posterior to the anterior pole of the GP (Spano et al, 1977). Although nigral DA autoreceptors had gained support from other lines of evidence, these re-
sults cast some doubt as to their existence.

Further support for the localization of DAC on nigral afferents was provided by Reubi et al. (1977) who reported that both DA and AMPH induce a flu-phenazine or haloperidol reversible release of $^3$H-GABA from nigral slices. $^3$H-GABA release was also elicited by dibutyryl-cyclic AMP. These findings dealt a particularly damaging blow to the concept of nigral DA autoreceptors since much of the earlier electrophysiological support for their existence could be reinterpreted as being due to the action of DA and apomorphine on terminals causing GABA release which in turn inhibits dopaminergic neurons. The action of haloperidol and AMPH could also be appropriately reinterpreted. At press time of this thesis, however, Bunney and Aghajanian (1978) have the latest word. These workers observed that GABA antagonists block and intra-striatal injections of kainic acid partially block the ability of AMPH to inhibit SNC neuronal activity. Since kainic acid lesions of the striatum only partially abolish the effect of AMPH and since this partial inhibition was not reversed by GABA blockers, Bunney and Aghajanian refuted some of their earlier work and suggested that some of the effects of AMPH may occur directly in the SN as originally proposed by Groves et al. (1975). In addition, they argued strongly against a GABA mediated inhibition of SNC cells through the release of GABA by DA, since both DA and apomorphine still caused a potent, haloperi-dol reversible inhibition of nigral neuronal activity which was not affected by striatal lesions or GABA blockers.

Developments regarding DA autoreceptors on DA-containing terminals in the striatum have paralleled roughly those in the SN. Following the initial demon-stration by Kehr et al. (1972) of the effects of dopaminergic drugs on DA metabolism on severed axons, evidence for the existence of these autoreceptors was provided by Christiansen and Squires (1974a, b). They found that apomorphine causes the inhibition of DA synthesis in synaptosomes and that this is re-versed by a host of DA antagonists. Striatal DA autoreceptors gained addi-
ional acceptance with the in vitro demonstrations that cAMP, through possibly an adenosine cyclic 3', 5' monophosphate (cAMP)-dependent protein kinase, alters the kinetic state of TH (Lovenberg et al, 1975a, b; Morgenroth et al, 1975). This was consistent with the presence of DAC in the striatum and suggested that DA exerted its presynaptic effects through DAC. These views stood until it was demonstrated that kainic acid striatal lesions virtually abolish DAC in the striatum suggesting an exclusively postsynaptic localization of this enzyme (McGeer et al, 1976; Schwarcz and Coyle, 1977).

The most recent data bearing on striatal DA autoreceptors have been obtained by further utilization of intrastriatal injections of kainic acid. As mentioned earlier, dendro-axonic transmission in the striatum could not be ruled out as a possible alternative to autoreceptors. In this regard, it is noteworthy that ACh, for example, has been shown to influence in vitro the release of DA from striatal tissue (Westfall, 1974). What the work with kainic acid has demonstrated is that after striatal lesions with this compound, apomorphine still decreases and haloperidol still increases DA metabolism in the striatum (Fadda et al, 1977; DiChiara et al, 1977; Argiolas et al, 1978).

These lesions largely eliminated striatal postsynaptic elements and nigral afferents from the striatum and therefore would have left the DA neurons in the SNC isolated both at their dendrites and terminals from possible transsynaptic influence. Notwithstanding the possibility of incomplete lesions, these results supported the existence of autoreceptors either on DA terminals or dendrites or both.

While it became apparent that DAC was not present on nigral DA neurons or their terminals in the striatum, such was the atmosphere surrounding DA autoreceptors that in order to avoid dispelling the existence of these altogether, it was suggested by numerous workers (McGeer et al, 1976; Gale et al, 1977; Phillipson et al, 1977; Premont et al, 1976) that, if DA autoreceptors exist, they may not be coupled with adenylate cyclase. This, of course, re-
quired a reworking of the ideas concerning the mechanisms of dopaminergic transmission. In view of this and since DA autoreceptors were "inferred" to explain data described above and in some cases subsequently relied on to interpret otherwise inexplicable findings, critical experiments of a more direct nature were required to test the hypothesis of DA autoreceptors and these awaited the merger between the production of appropriate lesions and measurements utilizing receptor binding techniques.

III The Present Investigation

a) Methodological considerations

The present studies have utilized some of the major neuroanatomical techniques currently in use to elucidate brain circuitry. Some of these techniques together with their advantages and disadvantages are discussed in this section.

More than three decades ago Weiss and Hiscoe (1948) discovered that materials are transported from the cell body of a neuron along its axon to the axon terminal. Some time later, Taylor and Weiss (1965) and Droz and Leblond (1963) demonstrated, using autoradiographic techniques, that neuronal perikarya could accumulate $^3$H-leucine injected in their vicinity, incorporate it into protein and transport the labeled $^3$H-protein along the axon processes of the cell to the nerve terminal. Subsequent investigations showed the potential of this technique in the tracing of neuroanatomical pathways (Hendrickson, 1969; Lasek et al, 1968; Schonbach et al, 1971). It was shown that the silver grains seen in autoradiographs enabled fiber systems to be traced from the cell body (in this case, the dorsal root ganglion) to terminal arborizations (Lasek et al, 1968). It was not until 1972 that Cowan et al (1972) developed and applied the method to tracing pathways in the CNS. This technique offers several advantages over other methods: 1) It is based on a physical property of nerve cells and thus does not involve perturbation of normal function
which may lead to misinterpretations. 2) There is no detectable retrograde transport of label (\(^3\)H-protein) following the injection of tritiated amino acid into the region of termination of a neuronal pathway (Cowan et al., 1972; Crossland et al., 1973; Schonbach and Guénod, 1971). 3) Axons passing through an area into which \(^3\)H-amino acids have been injected do not synthesize the label into protein or transport it. Thus, there is no "fiber of passage" problem (Cowan et al., 1972; Gottlieb and Cowan, 1973; Heuser and Miledi, 1970; Swanson et al., 1974). 4) At the EM level grains can be associated with either fibers of passage or terminals, and in a termination area the morphology of the terminals of a projection can be established. 5) The technique is very sensitive, enabling the tracing of fine or sparse fiber systems (Hendrickson et al., 1972; Meier, 1973).

There are several limitations of autoradiographic tracing techniques. 1) At the LM level since only silver grains are visualized, no information concerning the morphology of the axons or terminals can be gained. In addition, although the density and pattern of distribution of grains is generally a good indication either of fibers-of-passage or of a terminal field, the ultimate proof of this must sometimes rest with investigations at the EM level. 2) An injection of label into neuronal structures results in a gradient of labeled neurons away from the highest density which occurs at the focus of the injection site. An evaluation of the extent of spread of the label from the intended injection site to undesired adjacent areas is therefore essential in order to determine whether contamination by these areas of the fiber projection under investigation has occurred. However, it is uncertain how heavily labeled a cell must be in order for label to be detected in its axon or axon terminal.

As well as the anterograde transport of material, it is now well established that retrograde transport from the nerve terminal to the neuronal cell body occurs (Jeffrey and Austin, 1973; LaVail and LaVail, 1974). It has been
found that certain large exogenous macromolecules can be taken up by the terminals of axons and transported to the neuronal perikarya (Kristensson and Olsson, 1971, 1974). Through the retrograde transport of exogenously applied horseradish peroxidase (HRP) and appropriate histochemical techniques the cells of origin of neural pathways can be determined (LaVail and LaVail, 1972, 1974; LaVail et al, 1973). As effective as this technique has become in replacing retrograde cell degeneration techniques, there are certain limitations.

1) In common with anterograde tracing using $^3$H-amino acids, there is the problem of diffusion of HRP and of definition of the injection site. Thus, it is necessary to interpret results with consideration of the extent to which distant cells have been labeled due to diffusion and subsequent transport of HRP.

2) The amount of HRP found in the soma is partially dependent on the size, number and extent of arborization of the axon terminals in the injected area (Jones, 1975). The technique in this regard is not standardized to all anatomical systems but may vary with different types of systems.

3) It appears that for the most part fibers-of-passage do not accumulate and transport HRP. However, there is evidence that axons severed or injured by the injection procedure take up and retrogradely transport HRP (Kristensson and Olsson, 1974; Kuypers and Maisky, 1975; Nauta et al, 1974).

4) Although HRP is transported preferentially in the retrograde direction from the injection site, transport can also occur anterogradely (Lynch et al, 1973; Sherlock and Raisman, 1975).

A further set of techniques which aid in the delineation of brain structure can be subsumed under the heading of biochemical neuroanatomy. The simplest of these is the measurement in discrete brain areas of levels of substances that are established or suspected neurotransmitters and additionally the measurement of the activity of their related metabolic enzymes. Although this method offers information concerning only the gross anatomical localization of a substance or enzyme, it is an important first step since it provides the impetus for the further localization of a substance.
This is achieved in part by the production of lesions at sites which are suspected to contribute to the afferent projections of the structure of interest. Through the process of anterograde degeneration, metabolic systems associated with nerve terminals whose axons have been interrupted will be reduced. This method simultaneously provides information as to the existence of a pathway as well as the neurotransmitter involved. In addition, an estimate can be made of the relative contribution of a neurotransmitter to an area from various afferent sources.

The most recent addition to biochemical neuroanatomical studies is the measurement of neurotransmitter receptor levels. This is a valuable addition to the arsenal of techniques available to the investigator of brain function since it is an excellent complement to neuroanatomical and neurochemical studies. Thus, by producing appropriate lesions, receptors of specific neurotransmitters can be localized to distinct neuron types and to neuronal elements. These studies aid in the determination of 1) connections at the synaptic level, 2) the direction of transmitter release and, 3) the potential site of action of drugs.

Biochemical neuroanatomy suffers two major disadvantages. First, in studies involving lesions there is the fiber-of-passage problem. This, however, can be avoided by employing selective neurotoxins. Second, it is becoming apparent that the CNS is a highly plastic structure and therefore certain lesions may produce changes secondary to the initial lesions such as diaschisis, transneuronal degeneration, supersensitivity or axon sprouting (Schoenfeld and Hamilton, 1977). These phenomena may lead to misinterpretation of results, particularly since they occur over widely varying periods of time.

b) Objectives

The present experiments were undertaken to provide answers to specific questions regarding the neuroanatomy and neurochemistry of the basal ganglia. These questions vary somewhat in their depth of focus on the assorted nuclei
of the basal ganglia. In anatomical and biochemical neuroanatomical studies several nuclei were involved. In other studies, concerted effort was directed toward a single nucleus, such as the SN.

In a third series of investigations, attention was given to a specific neuronal element of the SN; the DA-containing neurons of the SNC. What follows is a more detailed account of the goals of these various experiments.

Neuroanatomical studies were aimed exclusively at the efferent projections of the striatum. Specifically, a more lucid description, as afforded by the autoradiographic and HRP anatomical methods, was sought in the rat. The striatal efferent to the EP was of particular interest as this had been only poorly described in the rodent.

Biochemical neuroanatomical studies concentrated on the striatal efferents to the GP, EP and SN and the GP efferents to the SN. These experiments were designed to determine the extent to which these pathways contain GAD and CAT and the origin of the neurons of these fiber systems within the striatum and GP. Although earlier work had been conducted on exactly this topic, the dearth of knowledge in this area and the timely nature of the present investigations is emphasized by the fact that not less than six reports on this subject appeared in the literature roughly concomitant with the published results of the present work.

Some attempts were also made to define biochemically the efferent projections of the EP and SN to the thalamus and habenula.

Two key observations prompted a detailed investigation of the SN. The first was the discovery that kainic acid appears to be toxic to neuronal perikarya and not to axons or their terminals. The second was the suggestion that axon terminals in the SN bear DAC. In experiments on the SN described here, the former was exploited to test the latter.
With regard to the DA-containing neurons of the SN, the advent of neurotransmitter receptor measurement techniques provided a means to test the hypothesis of the existence of autoreceptors on DA-containing neurons and terminals. These experiments involved the specific destruction of DA-containing neurons with 6-OHDA or the destruction of the striatal projection area of these neurons with kainic acid. This was followed by the assessment of the effect of these lesions on neurotransmitter receptor levels in the SN and striatum.

METHODS AND MATERIALS

I Animal Surgery

Male Wistar rats obtained from Woodlyn laboratories (Ontario) were employed in all the experiments. Rats were kept on a 12 hr dark/light cycle, fed ad libitum and group housed except after surgery at which time they were singly housed. During surgery animals weighing about 300 g were placed under pentobarbital anesthesia (50 mg/kg, i.p.) and immobilized in a Kopf stereotaxic apparatus. All stereotaxic co-ordinates are according to the atlas of König and Klippel (1963). Following surgery wounds were cleaned with Zepharin and closed by sewing or with wound clips. Survival times were usually from 9 to 14 days except where otherwise indicated. These procedures were common to all of the following surgical operations.

a) 6-Hydroxydopamine lesions of the nigro-striatal pathway

The neurotoxin 6-OHDA when correctly administered is known to be very effective at causing the degeneration of neurons which accumulate catecholamines. To achieve destruction of the DA-containing NSP 6-OHDA was injected into the axons of this system at about the level of the lateral hypothalamus. These lesions are known to cause near-complete anterograde and retrograde degeneration of the terminals and cell bodies of the NSP (Clavier and Fibiger, 1977). To increase further the specificity of 6-OHDA to only DA-containing neurons,
animals received desipramine HCl (25 mg/kg) 30 min before the 6-OHDA injection. Desipramine has been shown to be effective in preventing the uptake of 6-OHDA into noradrenergic axons and terminals (Evetts and Iversen, 1970). In the present experiments animals received unilateral injections of 4.0 μg of 6-OHDA contained in 2.0 μl. The injection rate was 0.2 μl/min. The stereotaxic co-ordinates were AP + 4.4, ML + 1.8, DV + 2.5. The 6-OHDA was dissolved in 0.15 M NaCl which contained 0.2 mg/ml ascorbic acid as an antioxidant.

b) Kainic acid lesions

Stereotaxic injections of kainic acid - a neurotoxic analogue of glutamic acid - into discrete brain areas has been shown to produce localized lesions (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976). Sufficient doses of kainic acid cause complete degeneration of neuronal cell bodies and the proliferation of glia at the injection site. However, the extent of damage, if any, by this compound to fibers-of-passage and to nerve terminals is not certain. The bulk of the information available to date (Schwarcz and Coyle, 1977a, 1977b; Schwarcz et al, 1977; McGeer et al, 1976; Meibach et al, 1978; Butcher and Rogers, 1978) suggests that damage to fibers and axon terminals is minimal and in part dependent on the dose and thus size of the lesioned area and whether or not cavitation occurs.

In the present experiments kainic acid lesions were employed not only as an alternative and adjunct to other lesion methods, but also to take advantage of its relative specificity for neuronal perikarya as compared with axon terminals. Four areas of the rat brain were unilaterally injected with kainic acid. These were the entire striatum, the head of the striatum, the GP and the SN. Numerous attempts were made in every brain region to achieve the desired circumscribed lesion size while maximizing the lesion specificity. Kainic acid was dissolved in sodium phosphate buffered sterile physiological saline, pH 6.9. For the large striatal lesions 10 nmoles of kainic acid was injected in 1.0 μl over a period of 5 min at the co-ordinates AP + 8.4, ML + 2.8, DV + 4.5.
For lesions of the head of the striatum 5 nmoles of kainic acid in 1.0 μl was delivered over a period of 5 min at the co-ordinates AP + 8.4, ML + 2.4, DV + 0.8. For GP lesions 2.0 nmoles of kainic acid in 0.2 μl was injected over a period of 7 min at the co-ordinates AP + 7.8, ML + 2.6, DV + 3.6. For the nigral lesions 5 nmoles of kainic acid in 1.0 μl was injected over a period of 5 min at the co-ordinates AP + 2.2, ML + 2.3, DV + 2.3.

c) Hemitransections

Hemitransections, as the term implies, involve coronal transections of the brain at some anterior-posterior level. The lesions start at midline and sever as ventral and lateral as is possible all cortical and subcortical structures. Two types of unilateral hemitransections were produced. These were at the level of the anterior commissure just rostral to the GP (AP + 7.5) and posterior to the GP at about the level of the EP. These were achieved by lowering a thin blade at the midline to the ventral surface of the brain and moving it laterally about 4 mm.

d) Electrolytic lesions

Electrolytic lesions were achieved by lowering a metal electrode (28-30 gauge) into the desired area of the brain. A thermal lesion was produced by passing current through the electrode with the current being completed by grounding the animal at some point. The size of the lesion was determined by the size of the uninsulated area of the tip of the electrode, the amplitude of the current and the duration for which it was applied. Initially, nichrome resin insulated wire electrodes were employed. Subsequently, much superior electrodes were fabricated by using stainless steel wire. To insulate these electrodes the wire was placed inside fine hematocrit capillary tubes and the tubes were pulled in a flame. Electrolytic lesions were produced unilaterally in four brain regions; the EP, the head of the striatum, the GP and the tail of the striatum. Lesions of the EP were achieved with a current of 2 mA for 30 sec at the co-ordinates AP + 6.3, ML + 2.8, DV + 1.8. Similar current and
duration parameters were employed for lesions of the head of the striatum at the co-ordinates AP + 8.5, ML + 2.4, DV + 0.8. For the GP a current of 2 mA was applied for 20 sec at the co-ordinates AP + 8.1, ML + 2.6, DV + 3.6. For lesions of the tail of the striatum a larger portion of the electrode tip was deinsulated to increase the dorsal-ventral dimension of the lesion. In addition two lesions were produced in the tail of the striatum, both with a current of 3 mA for 20 sec. The first was at the co-ordinates AP + 8.6, ML + 4.5, DV + 3.5, the second at the co-ordinates AP + 7.6, ML + 5.0, DV + 3.0.

II Anatomical Methods

a) Autoradiographic studies

The autoradiographic anterograde transport technique for the tracing of neural connections was utilized to study two anatomical systems. These were the projections from the head and the tail of the striatum to the GP, entopeduncular nucleus and substantia nigra. In each group 4 to 7 animals were injected in the appropriate areas with $^3$H-leucine (6.5 μC/μl) in phosphate buffered physiological saline. The head of the striatum received 0.1 to 0.4 μl and the tail of the striatum received 0.1 μl of the $^3$H-leucine solution. After 24 to 36 hrs the rats were given an overdose of pentobarbital and perfused with normal saline followed by 10% formalin-saline. The brains were removed and immersed in 10% formalin-saline for 2 to 3 days, then in sucrose-formalin for another two to three days. Frozen serial sections (40 μm) were taken in the frontal or sagittal plane and immersed in 5% formalin-saline for 1 to 3 days. The sections were then mounted from a gelatin-ethanol solution onto glass slides and allowed to dry. After 2 to 3 days the slides were dipped into Kodak NTB 3 emulsion, left to dry, then placed into light tight boxes and stored at 4°C. After 3 weeks the slides were developed in Kodak D-19 and counter stained for Nissl with cresyl violet.

b) Retrograde transport of horseradish peroxidase
For the investigation of anatomical pathways by the retrograde transport of horseradish peroxidase (HRP), the brain nuclei were injected unilaterally with HRP. 0.1 μl of a 25% saline solution of HRP (Sigma, Type VI) was delivered into the GP or EP by pressure through a 10 μl Hamilton microsyringe over a period of about 20 min. In some experiments a 12% solution of HRP was delivered into the EP and GP by microelectrophoresis using micropipettes with tip diameters of 30 to 60 μm and a current of 1.8 μA for 10 min. For retrograde transport of HRP from the SN, 0.1 μl of a 30% solution of HRP was injected bilaterally. After a 24 hr survival period the animals were perfused with normal saline followed by 400 ml of a mixture of 30 g paraformaldehyde, 25 g dextrose and 20 ml of 50% glutaraldehyde in 1.0 liter of 0.05 M phosphate buffer pH 7.4. The brains were then removed and immersed overnight in the same fixative minus the glutaraldehyde. A few hrs before sectioning the brains were immersed in a phosphate buffer solution containing 2.5% dextrose. Frozen sections (60 μm) were taken in the frontal plane and treated to reveal HRP activity. Two substrates for the HRP reaction were employed; diaminobenzidine according to the method of Nauta et al (1974) and dichlorobenzidine according to the method of Mesalum and Rosene (1977).

c) Dissections

Discrete brain regions were dissected either freehand from fresh brains as in the case of the whole striatum or from sections obtained on a freezing microtome. All dissections were conducted while maintaining the tissue cold on ice. Areas obtained from sections included the tail of the striatum, GP, EP, SN and habenula. These areas were dissected freehand from the sections except the GP which was obtained by using a punch 1.8 mm in internal diameter. In one experiment involving the anterior hemitranscetions three sections were obtained; one containing the anterior half of the GP, another the adjacent posterior half of the GP and the third posterior to the GP containing the caudal part of the tail of the striatum. The GP was punched from each of the
first two sections followed by dissection of the tail of the striatum (imme-
diately lateral to the GP) from within the same sections, as well as from the
third section.

d) Histology

Histological examination of brains was necessary in order to evaluate
the extent of damage produced by the various lesions. In particular, large
numbers of brains with GP kainic acid lesions and electrolytic striatal tail
lesions were examined in order to gain some idea of the variability of the
lesions. Brains were removed and placed in 10% formalin-saline for 7 to 10
days. Sections (50 μm) were placed in 5% formalin for 2 to 3 days, rinsed
in distilled water and mounted on slides. The slides were dipped in 40%
ethanol – 15% gelatin, allowed to dry in air and stained with cresyl violet.

III Biochemical Methods

Enzyme assays were employed either to determine the effect of a lesion
on various enzyme activities or to confirm the efficacy of a lesion. All en-
zyme activities were shown to be linear with time of incubation and protein
concentration and these parameters were adjusted to allow measurement of the
activities in each of the assays on the linear portion of the curves. For
CAT, GAD and TH brain tissue was homogenized in 100 to 50 volumes of 50 mM
Tris-Acetate buffer pH 6.3 containing 0.2% Triton X-100 (v/v).

a) Glutamic acid decarboxylase

GAD was assayed by a modification of the method of Albers and Brady (1959)
as previously described (Chalmers et al, 1970). The incubation mixture con-
tained 50 μl of a mixture containing (final concentration) 2.0 mM L[1-14C]
glutamate (specific activity 0.1 to 0.2 mCi/mmol, Radiochemical Centre, Amer-
sham, England), 0.02% bovine serum albumin (Sigma), 0.1 mM pyridoxal phosphate
(Calbiochem), 28 mM potassium phosphate buffer pH 7.4 and 20 µl of tissue homogenate. This mixture was incubated for 30 min at 37°C in small tubes placed into scintillation vials containing a gelatin capsule (Parke-Davis) loaded with Whatman filter paper soaked with 0.1 ml of hyamine hydroxide. The reaction was stopped by adding 0.2 ml of 2 M H₂SO₄ to the mixture through a rubber washer in the cap of the scintillation vial with a 5 ml syringe adapted with a 19 gauge needle. After 2 hrs the reaction vessel was removed from the vial, scintillation fluid added and the sample counted.

b) Tyrosine hydroxylase

TH was measured as previously described (McGeer et al, 1967). The incubation mixture contained 80 µl of a mixture containing (final concentration 1.0 mM 2-amino-5-hydroxy-6,7-dimethyl tetrahydropteridine (DMPH₄, Sigma or Calbiochem), 0.1 mM L-[¹⁴C(U)] tyrosine (specific activity 3 to 5 mCi/mmol, New England Nuclear), 0.3 mM ferric sulphate, 50 mM 2-mercapto-ethanol (Eastman) 0.2 M sodium-acetate buffer pH 6.0 and 40 µl of tissue homogenate. The reaction mixture was incubated at 37°C for 12 min after which time 2.0 ml of a solution containing 1.4% perchloric acid, 0.52% acetic acid and 0.5 µg/ml of dihydroxyphenylalanine was added. The reaction vessels were centrifuged at 2000 g for 5 min and the supernatants transferred to 25 ml beakers. The vessels were rinsed with 2.0 ml of 0.35 M KH₂PO₄ pH 6.0, centrifuged as before and the supernatants pooled in the beakers. After the addition of 2.0 ml of 0.2 M Na₂EDTA and 12 ml, of distilled water to the beakers, the samples were brought to pH 9.0 to 9.5 and poured onto columns packed with about 0.3 g of alumina (Calbiochem, acid, AG₄, 100-200 mesh). The columns were washed with 35 ml of distilled water, eluted into scintillation vials with 2.0 ml of 0.5 M acetic acid and counted.

c) Choline acetyltransferase and acetylcholinesterase

Choline acetyltransferase was assayed according to the method of McGeer and McGeer (1971). The incubation mixture consisted of 20 µl of a mixture
containing (final concentration 300 mM NaCl, 8.0 mM choline chloride, 0.2 mM eserine, 0.8 mM [L-14C] acetyl coenzyme A (specific activity 3 to 4 mCi/mmol, Radiochemical Centre, Amersham, England), 100 mM sodium phosphate buffer pH 7.4 and 20 μl of tissue homogenate. The mixture was incubated at 37°C for 5 to 30 min depending on the area measured. The reaction was terminated by adding 1.0 ml of a solution of 0.14% perchloric acid, 0.052% acetic acid and 35 μg/ml acetylcholine bromide. This was followed by the addition of 1.0 ml of 0.5 M tris-acetate buffer pH 7.0. This mixture was then poured onto 4 cm columns packed with Amberlite resin CG-50H (Type 1 BDH) which had been equilibrated with 3.0 ml of 0.5 M tris-acetate buffer pH 7.0. The columns were washed with 35 ml of water, eluted into scintillation vials with 3.0 ml of 4.1 M acetic acid, scintillation fluid was added to the vials and the samples were counted. AChE was assayed as previously described (Fonnum, 1969). Final concentrations of 15 mM sodium phosphate buffer pH 7.0 and 5.0 mM [acetyl-L-14C] acetylcholine iodide (specific activity 0.218 mCi/mmol, New England Nuclear) were employed. Two extractions with 1.5% tetraphenylboron in heptanone were used to remove the labeled substrate. Following centrifugation a portion of the aqueous phase was added to scintillation vials and counted.

d) Adenylate cyclase

Dopamine-sensitive adenylate cyclase in the SN was measured according to the method of Miller et al (1974). The tissue was homogenized in 25 to 50 volumes (w/v) of 2.0 mM tris-maleate buffer pH 7.4 containing 2.0 mM EGTA. Fifty μl aliquots of this homogenate were added to 250 μl of 80 mM tris-maleate buffer containing 2.0 mM MgSO4, 0.2 mM EGTA and the desired concentrations of DA. The incubation tubes were kept in an ice bath while ATP was added to a final concentration of 0.5 mM. The reaction mixture was incubated at 30°C for 2.5 min and then transferred to a boiling water bath for an additional 2.5 min. The tubes were centrifuged for 5 min at 2000 g and 40 μl aliquots of the supernatant were assayed for cyclic AMP content by the method of
Gilman (1970) using a linear standard curve produced with aliquots of authentic cyclic AMP between 0.2 and 8.0 pmol.

e) Protein assay and scintillation counting

Protein assays were conducted by the method of Lowry et al (1951). In general, radioactivity was counted in 5 to 15 ml of ACS (Amersham) with the exception of Millipore filters which were counted in 10 ml of a toluene based scintillation fluid. Samples were counted in a Nuclear-Chicago scintillation counter with an efficiency of about 80% for carbon-14 samples and 30% for tritium samples.

f) Tissue packaging

In collaborative work it was necessary to transport tissue samples. Brain tissue was dissected as described above, placed within 3 min into autoanalyzer cups (Trilab Industries), sealed and stored at -70°C until shipment. Samples were packaged with about 10 kg of dry ice in styrofoam boxes and shipped by air freight. The methods for the receptor binding assays employed in these experiments are described elsewhere (Seeman et al, 1976; Reisine et al, 1979a, b).

RESULTS

I Neuroanatomy

a) Anterior striatal efferents to the globus pallidus, entopeduncular nucleus and substantia nigra

Injections of [3H] leucine into the head of the striatum resulted in a localized homogeneous distribution of grains which were confined to this nucleus (Fig. 1). In the region caudal to the injection site and just rostral to the GP the grains were preferentially distributed over fiber bundles (Fig. 2A). Further caudally, within the GP, the grains were more heavily concentrated over the neuropil of the GP and to a lesser extent in the fiber bundles of this region (Fig. 2B). The anterior region of the GP appeared to contain the
greatest concentration of label.

In a similar fashion, label rostral to the EP was located over the internal capsule (Fig. 2C), while in the EP grains were observed to be preferentially distributed over the neuropil (Fig. 2D). Caudal to the EP the density of grains decreased markedly, as the labeled fibers passed within the internal capsule to terminate in the SNR (Fig. 2E-F). Of the terminal areas examined, the highest density of grains was observed over GP. Substantially less labeling was found over EP, while the activity over SNR was intermediate. In brains sectioned sagitally, these relations were especially clear. Fig. 3 shows the distribution of silver grains in a sagittal section after an injection of \(^{3}\text{H}\) leucine into the head of the striatum. Diffuse accumulation of grains could be observed over the neuropil of GP, EP and SNR. In between these nuclei the silver grains were arranged in linear arrays within the internal capsule, typical of that observed in labeled axons.

After microelectrophoretic injections of HRP into the GP, which resulted in HRP reaction product well localized within this structure (Fig. 4A), labeled cells were observed in the striatum (Fig. 4C). These cells, which were medium sized, were confined to the dorsal-central, anterior 'core' region of the CP and the cytoplasm of these cells contained the type of fine granules described by previous authors (Herkenham and Nauta, 1977; Jones and Leavitt, 1974).

Whether striatal cells outside this core region of the striatum also project to GP could not be determined with certainty from this material. Microelectrophoretic injections of HRP into EP (Fig. 4C) also resulted in the appearance of reactive perikarya in the striatum (Fig. 4D). Although clear topographic relations could not be discerned, it was apparent from examination of a large number of animals that, with the exception of the caudal tail, the entire striatum appears to project to EP. Examination of brains in which HRP had been delivered to the EP via pressure injections through a Hamilton micro-
Figure 1  Light field photomicrograph of a Nissl-stained sagittal section showing injection site of \(^3\)H-leucine in the head of the striatum. Magnification x 31.
Figure 2

A: dark-field photomicrograph of a sagittal section showing autoradiographic labeling of fiber bundles of the internal capsule caudal to the striatal injection site (Fig. 1) and just rostral to the globus pallidus (x 190). B: accumulation of silver grains in the anterior edge of globus pallidus. Note relatively sparse labeling just anterior to GP on the right side of photograph. Sagittal section (x 190). C: distribution of silver grains in the internal capsule just anterior to the entopeduncular nucleus. Note linear arrays of grains, suggesting labeling of axons. Sagittal section (x 190). D: accumulation of autoradiographic grains in the entopeduncular nucleus. Sagittal section (x 190). E: light field photomicrograph of a sagittal section of the substantia nigra. Note zona compacta in the dorsal-right quadrant (x 90). F: dark-field photomicrograph of field identical to that shown in (E). Large accumulations of silver grains can be observed in the zona reticulata, particularly in the ventral half (x 90).
Figure 3 Camera lucida drawing of a sagittal section showing distribution of autoradiographic grains after an injection of [$^3$H] leucine into head of striatum. GP, globus pallidus; AC, anterior commissure; EP, entopeduncular nucleus; OT, optic tract; SNR, substantia nigra, pars reticulata.
Figure 4  

A: injection site of microelectrophoretic application of HRP into the globus pallidus (x 19.5).  
B: dark-field photomicrograph showing HRP reactive neurons in the central core region of the striatum after HRP injection into globus pallidus (x 195).  
C: injection site of microelectrophoretic application of HRP into the entopeduncular nucleus (x 19.5).  
D and E: labeled neurons in striatum (D) and cerebral cortex (E) after HRP injection into entopeduncular nucleus (x 195).
syringe revealed a similar pattern of labeling in the striatum. Both electro­
phoretic and pressure injections of HRP into EP resulted in the occasional ap­
pearance of labeled cells in the SN, pars compacta, and in the cerebral cortex
(Fig. 4E). The appearance of these cells differed from those in the striatum
in that they contained larger, more intensely staining granules and the neuro­
nal cytoplasm had a brownish appearance. These latter results raise the pos­
sibility that some of the HRP labeling in the striatum after injections into
EP may have been due to accumulation of HRP by damaged axons in the crus cere­
bri. The extent to which this factor may have influenced striatal labeling
cannot be determined with certainty at present. However, it is relevant that
in control experiments microelectrophoretic injections of HRP into the crus cere­
bri resulted in a pattern of labeling in the striatum, SN and cortex simi­
lar to that observed after injections into EP. No labeled cells were found in
any of these areas after pressure injections of HRP into the crus cerebri.

b) Posterior striatal efferents to the globus pallidus, entopeduncular nu­
cleus and substantia nigra

The results of the \(^3\)H leucine injection into the tail of the striatum
is shown in the dark field photomicrographs in Fig. 5 and diagramatically in
Fig. 6. The injections of \(^3\)H leucine were localized dorsal and lateral to
the GP midway through this nucleus in the AP dimension (Fig. 5A). The label
diffused from the injection site to the level of the anterior extreme of the
GP (Fig. 6A). There was no spread of label from the injection site in the
tail of the striatum to the cortex or the GP. Further caudally (Figs. 5B and
6C).there was dense labeling of the ventral lateral portion of the posterior
GP. Although some grains were present in large fiber bundles traversing this
region the majority of the grains were concentrated and preferentially labeled
the neuropil of the GP. This was so pronounced that the relatively sparse la­
beling of the fiber bundles in this region resulted in their delineation by
the surrounding heavily labeled areas. Further caudally, at the level of the
Figure 5  A: dark-field photomicrograph showing the injection site of $^3$H-leucine into the tail of the striatum lateral to the GP (x 12.7).  B: accumulation of silver grains in the posterior GP (x 11.3). Note that the fiber bundles passing through the area of the GP containing silver grains are devoid of label.  C: accumulation of autoradiographic grains in the SN (x 25.6).
Figure 6  Diagramatic representation of the distribution of autoradiographic grains after injection of $^3$H-leucine into the tail of the striatum.
EP (Fig. 6D), the density of grains in the internal capsule decreased dramatically and was localized mainly over fiber bundles. Although rare, some label from the internal capsule trickled ventromedially within the neuropil towards the EP. At the level of the subthalamic nucleus (SUT), posterior to the EP (Fig. 6E), the label within the internal capsule (IC) appeared to be more clustered, relative to that observed at the level of the EP, and occupied a position in the center of the long axis of the IC and immediately lateral to the dorsal lateral tip of the SUT. No labeling of the SUT occurred. It could not be discerned whether there was a decrease in grain density at a level just posterior to the EP. At the anterior portion of the SN (Fig. 6F) the label was observed invading the extreme lateral regions of the SN pars reticulata (SNR) from the IC. Midway through the SN the accumulation of label increased compared to that observed in the IC and occupied a position in the lateral one-third of the SNR (Figs. 5C and 6B). This location of label within the SNR did not change in the most posterior extreme of the SN (Fig. 6H). No label was observed in any structures caudal to the SN.

II  Biochemical Neuroanatomy

a) The contribution of the head of the striatum to various neurotransmitter enzyme markers in the globus pallidus, entopeduncular nucleus and substantia nigra

Hemitransections at a level slightly caudal to the EP were produced in order to determine the maximal contribution to nigral glutamic acid decarboxylase (GAD) from structures rostral to these lesions. Additionally, in order to evaluate the biochemical nature of the projections from the head of the striatum to the GP, EP and SN, hemitransections, electrolytic and kainic acid lesions of the head of the striatum were produced. The hemitransections were just anterior to the GP. The effects of these lesions on GAD activity are shown in Table 1. The anterior hemitransections resulted in a significant de-
Table 1
The activity of GAD in various areas after lesions of the head of the striatum

<table>
<thead>
<tr>
<th>Area</th>
<th>Lesion technique</th>
<th>Hemitransection (11)</th>
<th>Electrolytic (8)</th>
<th>Kainic acid (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head of striatum</td>
<td></td>
<td>-</td>
<td>-</td>
<td>23.6±2.9***</td>
</tr>
<tr>
<td>GP</td>
<td>79.9±5.4*</td>
<td>74.9±13.4**</td>
<td>37.1±5.0***</td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>63.6±8.5***</td>
<td>76.7±11.4**</td>
<td>80.0±9.6*</td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td>92.4±3.9</td>
<td>101.0±7.4</td>
<td>74.5±2.08***</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as per cent of control ± S.E.M. The number of animals used is shown in parenthesis after the lesion technique employed. Expressed in terms of nmoles/mg protein/hr, the GAD activity on the unlesioned side was: striatum, 90.1±5.1; GP, 220±16; EP, 122±9.9; SN, 310±8.4.

Statistical differences compared to contralateral control side.
* p < 0.05.
** p < 0.02.
*** p < 0.01.
crease in GAD activity of about 20 and 35% in the GP and EP respectively.
Electrolytic lesions of the anterior aspect of the striatum, which did not en-
croach upon the GP, decreased GAD activity in the GP and EP by about 25 and
23% respectively. Neither of these lesions significantly altered GAD activity
in the whole SN. Injections of kainic acid into the head of the striatum re-
duced GAD activity in the injected area by 76%. These lesions reduced GAD in
the GP and EP by 63 and 20% respectively. In addition, there was a signifi-
cant 25% reduction in nigral GAD activity. It is noteworthy that the kainic
acid treated animals showed significantly greater reductions in GAD in the GP
than did either the hemitransection or electrolytic groups (p < 0.01). This
may be explained by diffusion of kainic acid to regions in the striatum and
GP caudal to the anterior pole of the GP. Indeed, histological examination of
the extent of the kainic acid lesion revealed destruction of neurons in both
the GP and the tail of the striatum. Hemitransections just caudal to the EP
reduced GAD in the SN by about 84% (data not shown).

In order to evaluate the possible cholinergic contribution of the head of
the striatum to the GP, EP and SN, a second series of experiments was conduc-
ted involving hemitransections anterior to the GP. These were placed slightly
more posterior than the previous hemitransections. The results of these ex-
periments are shown in Table 2. The decrease in pallidal GAD was greater
(58%) compared with the more rostral transection (20%). Also there was a sig-
nificant decrease of 16% in nigral GAD. Interestingly, however, the decrease
in GAD in the EP was about the same after both hemitransections. These le-
sions had no effect on choline acetyltransferase (CAT) activity in the EP or
SN. Pallidal CAT activity, however, was significantly reduced (50%).

With hemitransections close to the GP, changes in enzyme activities in
the GP may be interpreted as being due to spread of the lesion to the GP
rather than the severing of a projection emanating to the GP from regions an-
terior to the lesion. To test this the latter hemitransections were repeated
and the anterior and posterior GP, as well as the tail of the striatum immediately lateral to the GP, were each assayed separately for GAD and CAT activities. In addition, the tail of the striatum posterior to the GP was examined. The results are given in Table 3. These lesions had no effect on GAD or CAT activities in the tail of the striatum posterior to the GP. In the anterior GP GAD was decreased significantly by 57%. CAT was decreased by 38%, although this did not reach significance due to an excessive variation in the standard error. In the striatal tissue immediately lateral to the anterior GP, GAD and CAT activities were unaltered. This was true of the GAD and CAT activities in the striatal tissue lateral to the posterior GP.

b) The contribution of the tail of the striatum to various neurotransmitter enzyme markers in the globus pallidus, entopeduncular nucleus and substantia nigra

The extent of the electrolytic lesions of the tail of the striatum is shown diagramatically in Fig. 7 and in the photomicrograph in Fig. 10A. This example of the lesion is fairly representative of the eleven brains that were examined histologically. Anteriorly, the lesions started at the level of the anterior commissure and were located in the lateral one-third of the striatum (Fig. 7A). Midway through the GP, the lesions occupied the main body of the tail of the striatum and never encroached on the GP (Fig. 7B). The most medial portion of the dorsal striatum and the most ventral portions were usually spared but to various degrees. At the caudal extreme of the GP, the lesion was usually restricted to the dorsal half of the tail of the striatum while the ventral half was spared (Fig. 7C). Again there was no damage to the GP. At the rostral level of the EP (Fig. 7D), the lesions were usually quite small and restricted to the dorsal half of the striatum. Occasionally (two brains), however, some gliosis was observed in the dorsal half of the internal capsule, although no lesion could be detected.

The activities of GAD in the GP, EP and SN and of CAT in the GP and EP
<table>
<thead>
<tr>
<th>Area</th>
<th>Enzyme Activity (nmoles/mg protein / hr)</th>
<th>GAD</th>
<th>% control</th>
<th>CAT</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesion</td>
<td>243±46*</td>
<td>42</td>
<td>7.25±.68**</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>576±45</td>
<td></td>
<td>14.4±.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesion</td>
<td>240±36*</td>
<td>62</td>
<td>8.31±1.0</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>390±40</td>
<td></td>
<td>9.26±1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesion</td>
<td>884±47*</td>
<td>84</td>
<td>5.04±.49</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1047±38</td>
<td></td>
<td>4.46±.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. of seven determinations.

* $p < .025$

** $p < .001$
Table 3
The activity of GAD and CAT in various areas after hemitranssections anterior to the globus pallidus

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (nmoles/mg Protein/hr)</th>
<th>Area Relative to GP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anterior GP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GP</td>
</tr>
<tr>
<td>GAD</td>
<td></td>
<td>295±44**</td>
</tr>
<tr>
<td></td>
<td>Lesion</td>
<td>698±85</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>% of control</td>
<td>111</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td>2.96±.25</td>
</tr>
<tr>
<td></td>
<td>Lesion</td>
<td>4.81±.89</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>% of control</td>
<td>62</td>
</tr>
</tbody>
</table>

Values represent means ± S.E.M. of eleven determinations.

*p < .01

**p < .001
after electrolytic lesions of the tail of the striatum are shown in Table 4. In a separate experiment the SN was assayed for GAD while the remainder of the brain was examined histologically. Table 4 also contains enzyme values from the left and right side of unoperated animals in order to assure that any observed differences in lesioned animals were not due to dissection error. No differences were observed between the right and left side of unoperated animals. Electrolytic lesions of the tail of the striatum did not alter GAD or CAT activities in the GP. These lesions did, however, cause a significant decrease of 22 and 24% in GAD and CAT, respectively, in the EP. Small, but significant decreases of GAD were also observed in both the SN (12%) obtained from brains used solely for enzyme analysis as well as in the SN (17%) taken from brains which were utilized for histological analysis.

c) The contribution of the globus pallidus to various neurotransmitter enzyme markers in the entopeduncular nucleus and substantia nigra

In order to characterize the biochemical nature of the efferents from the GP, this structure was lesioned electrolytically and with kainic acid. The location of the electrolytic lesion of the GP is shown in Figs. 8 and 10B. Anteriorly, the lesions destroyed the majority of the GP (Fig. 8A). Midway through the GP the lesion destroyed all but the lateral one-quarter of this structure (Fig. 8B). Further caudally (Fig. 8C) the lesion spared the posterior one-quarter to one-third of the GP. The effects of these electrolytic lesions of the pallidum on GAD activity in the EP and SN and choline acetyltransferase activity in the EP are shown in Table 5. CAT activity in the EP was unaltered by the lesion. Glutamic acid decarboxylase (GAD) activity in the EP and SN was significantly reduced by 35 and 14% respectively.

In order to circumvent the drawbacks of electrolytic lesions, particularly with respect to fibers-of-passage, kainic acid lesions of the GP were employed. The lesioned area, shown in Figs. 9 and 10C, is representative of the size and location of the lesioned area in the eight brains examined. The lesion ante-
Figure 7  
Diagramatic representation of electrolytic lesions of the tail of the striatum. The extent of tissue destruction is shown by the hatched area.
Table 4
Activity of GAD and CAT in various areas after electrolytic lesions of the tail of the striatum.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>GP</th>
<th>EP</th>
<th>SN</th>
<th>SN + Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesioned</td>
<td>310±25 (17)</td>
<td>202±16* (16)</td>
<td>728±35** (15)</td>
<td>648±47* (5)</td>
</tr>
<tr>
<td>control</td>
<td>340±25 (17)</td>
<td>260±22 (16)</td>
<td>825±19 (15)</td>
<td>785±19 (5)</td>
</tr>
<tr>
<td>% of control</td>
<td>91</td>
<td>78</td>
<td>88</td>
<td>83</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesioned</td>
<td>11.7±0.8 (18)</td>
<td>5.32±.44*** (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>13.5±1.0 (18)</td>
<td>7.01±.40 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of control</td>
<td>87</td>
<td>75.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>366±27 (16)</td>
<td>276±21 (15)</td>
<td>773±21 (13)</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>314±23 (16)</td>
<td>281±19 (15)</td>
<td>764±30 (13)</td>
<td></td>
</tr>
<tr>
<td>% of control</td>
<td>117</td>
<td>98</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>12.5±1.1 (16)</td>
<td>8.50±.67 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>13.2±.96 (16)</td>
<td>8.34±.46 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of control</td>
<td>95</td>
<td>102</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enzyme activities are in terms of nmoles/mg protein/hr
Values represent means ± S.E.M., number of determinations are enclosed in parentheses.

* p < .05
** p < .025
*** p < .0.1
Figure 8  Diagramatic representation of electrolytic lesions of the GP.
Hatched area shows regions of tissue destruction.
Table 5
Activity of GAD and CAT in the entopeduncular nucleus and substantia nigra after electrolytic lesions of the globus pallidus.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (nmoles/mg Protein/hr)</th>
<th>EP</th>
<th>% of Control</th>
<th>SN</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion</td>
<td>171 ± 36** (6)</td>
<td>65</td>
<td></td>
<td>619 ± 19* (9)</td>
<td>86</td>
</tr>
<tr>
<td>Control</td>
<td>265 ± 15 (6)</td>
<td></td>
<td>724 ± 18 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion</td>
<td>6.33 ± .41 (6)</td>
<td>101</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.30 ± .23 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent means ± S.E.M., number of determinations are enclosed in parentheses.

* p < .05
** p < .001
Diagramatic representation of kainic acid lesions of the GP. Hatched areas show regions where neuron destruction and intense gliosis were observed.
rior to the anterior commissure was small and incomplete in that total neuron
destruction within this area was seldom observed (Fig. 9A). At the level of
the anterior commissure (Fig. 9B) and midway through the GP in the AP dimen-
sion (Fig. 9C) the lesion enveloped the entire GP. Neuron loss in this area
was extensive. At these levels neuron destruction in the tail of the striatum
lateral to the GP was variable. In some cases there was little damage beyond
the lateral border of the GP. In other brains neuron loss was observed in an area circumscribing one-half of the tail of the striatum immediately sur-
rounding the GP. The lesion was never observed beyond this region; thus the
peripheral half of the posterior striatum was always spared. Further caudally
(Fig. 9D) destruction was usually observed in the medial and dorsal half of
the GP but, posterior to this level where numerous pallidal neurons could still
be identified, damage was never observed. At this level (Fig. 9E) the reticu-
lar nucleus of the thalamus seemed particularly sensitive to kainic acid as
destruction of this nucleus often occurred. Of interest was the well loca-
lized and consistent destruction of the claustrum located lateral to the exter-
nal capsule (Fig. 9). No gliosis or neuron loss was ever detected between
this structure and the main body of the lesion suggesting that the claustrum
is either exquisitely sensitive to kainic acid or that it undergoes degenera-
tion for some as yet unknown reason (i.e., retrograde or transneuronal dege-
neration or perturbation of afferent input).

The activities of GAD, CAT and TH in various areas following kainic acid
GP lesions are shown in Table 6. TH was significantly increased by 25% in
the tail of the striatum on the side of the lesion. CAT activity in the tail
of the striatum was unaltered whereas it was significantly decreased by 42% in
the GP. GAD in the tail of the striatum, GP and EP were significantly de-
creased by 25, 42 and 34% respectively. GAD in the SN taken from brains
which were employed for enzyme analysis of GP, EP and tail of the striatum
showed a 10% non-significant decrease as did GAD in the SN taken from brains
Table 6
Activity of GAD, CAT and TH in various areas after kainic acid lesions of the globus pallidus.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tail of Striatum</th>
<th>GP</th>
<th>EP</th>
<th>SN</th>
<th>SN + Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesion</td>
<td>213±19*</td>
<td>183±17**</td>
<td>195±22**</td>
<td>695±36</td>
<td>701±33</td>
</tr>
<tr>
<td>control</td>
<td>283±23</td>
<td>315±27</td>
<td>295±32</td>
<td>774±23</td>
<td>783±27</td>
</tr>
<tr>
<td>% of control</td>
<td>75</td>
<td>58</td>
<td>66</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesion</td>
<td>58.3±3.5</td>
<td>5.55±.86**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>58.9±3.5</td>
<td>9.51±.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of control</td>
<td>99</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lesion</td>
<td>1.85±.11*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>1.48±.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of control</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Enzyme activities are expressed in terms of nmols/mg protein/hr
Values represent means ± S.E.M. of eight determinations.
* p < .05
** p < .025
Figure 10 Photomicrographs of electrolytic and kainic acid lesions.
A: electrolytic lesion of the tail of the striatum (x 15.4).
B: electrolytic lesion of the GP (x 15.4). C: kainic acid lesion of the GP (x 13.4). Note the needle tract and site of kainic acid is in the medial part of the GP.
which were analyzed histologically for the assessment of the kainic acid lesion.

It was mentioned earlier that, at the mid-level of the GP, kainic acid destroyed virtually all neurons. However, in cresyl violet stained sections, in the midst of the lesion a few large neurons were seen to be left intact. These were located in the medial aspect of the GP, just lateral to the internal capsule (IC) and at the ventral extreme of the GP. Acetylcholinesterase (AChE) staining of the kainic acid lesioned-GP brains was conducted to determine if the known large, intensely AChE-staining neurons were those which were left intact. Shown in Fig. 11 are the positions of the AChE staining neurons left intact by the lesion. These areas correspond closely to the positions of the neurons seen to be remaining in cresyl violet stained sections.

The area of absence of the small to medium sized, moderate AChE staining neurons in the GP and tail of the striatum in lesioned brains exactly overlapped the area of the lesion described in Fig. 9. However, large intensely AChE staining neurons were present in the medial and ventral lesioned GP and more posteriorly, they were interspersed in the internal capsule (Fig. 11C). Although it was difficult to quantitate the degree of loss, if any, of the AChE stained neurons, they did appear to have undergone some atrophy as is evident from their smaller perikarya and dendrites compared to the neurons on the control side.

The HRP retrograde transport method was employed to determine whether the large AChE staining neurons project to the SN. After the injection of HPR bilaterally into the SN, the GP was examined for HRP stained neurons. The results are shown in Figs. 12 and 13. In Fig. 12 the left is the kainic acid lesioned and the right is the control side. The numerous HRP stained neurons in the striatum are not depicted. In the anterior half of the GP on the control side, HRP stained perikarya could be seen throughout the GP (Fig. 12A). Further caudally, labeled neurons were most obvious in the lateral ventral
Figure 11  Location of intense acetylcholinesterase staining neurons in and surrounding the GP. These neurons are represented by the open circles in the diagram.
Figure 12: Diagram of the location of labeled neurons in the GP after intranigral injections of HRP. The left GP received kainic acid lesions as depicted in Figure 9. The injection of HRP into the SN was bilateral. The black dots represent HRP-labeled neurons in the GP. Note that labeled neurons on the lesions side are sparse.
Figure 13  Photomicrograph of labeled neurons in the GP after intranigral HRP injections.  A: labeled neurons in the GP on the right side of the brain.  B: labeled neurons in the same area of the GP on the left side which had received kainic acid lesions.  These few neurons are present in that part of the GP unlesioned by kainic acid (x 262.5).
half of the GP (Fig. 12C). They were scarce in the posterior half but when present were located in the ventral portion of the posterior GP. Noteworthy is the fact that there was virtually no overlap in the GP of the positions of the HRP-containing neurons after nigral HRP injections and the AChE stained neurons (compare Figs. 11 and 12).

d) Efferents of the entopeduncular nucleus

In order to characterize the biochemical nature of the efferents of the EP this structure was lesioned electrolytically. A histology of a representative lesion of the EP is given in Fig. 14. It is apparent that the lesion destroyed most of the EP at this level and extended beyond its limits both dorsally and ventrally. The lesion did not encroach upon the GP. The effects of similar lesions on habenular CAT and GAD activities are given in Table 7. EP lesions produced a significant decrease in GAD of 63% without significantly affecting CAT activity.

Another series of experiments were undertaken in order to characterize biochemically the EP and SN projections to the ventral anterior and parafascicular nuclei of the thalamus. Numerous attempts met with failure. The reason is not definitely known although lack of consistency in the production of lesions and dissections may have been a factor. In any case the project was abandoned. It might be mentioned that consistent decreases in CAT were observed in the ventral anterior nucleus of the thalamus after electrolytic lesions of the SN. This finding should be investigated further.

III Biochemical Investigations of Substantia Nigra and Striatum

a) Neurotransmitter synthetic enzyme localization in the substantia nigra

In order to characterize the nigra further from a biochemical standpoint, this structure was injected with kainic acid. The stereotaxic injection of 5 nmoles of kainic acid into the SN resulted in a virtually complete des-
Figure 14  Photomicrograph of electrolytic lesions of the EP (x 81.5).
Table 7
The activity of GAD and CAT in the habenula on the lesioned and control side after unilateral lesions of the entopeduncular nucleus.

<table>
<thead>
<tr>
<th>Side</th>
<th>n</th>
<th>GAD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmoles/mg</td>
<td>Per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>protein/hour</td>
<td>control</td>
</tr>
<tr>
<td>Lesion</td>
<td>6</td>
<td>13.6±2.40*</td>
<td>63.6</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>21.4±2.34</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Significantly different from control P < 0.05.
struction of neuronal cell bodies in both the pars compacta and the pars reti-
culata (Fig. 15), and this was accompanied by intense gliosis. The effect of
these lesions on GAD, CAT and AChE activities in the SN and on tyrosine hyroxy-
lasè (TH) in the striatum is shown in Table 8. Substantiating the histologi-
cal assessment of dopamine-containing neuron loss in the SN is the greater
than 94% reduction in striatal TH activity ipsilateral to the lesioned SN.

Following kainic acid injections, it was necessary to express the enzyme
activities of the lesioned nigra on both a per protein and a per SN basis to
control for the significant tissue shrinkage which was found to occur in the
lesioned tissue. As shown in Table 8, nigral GAD activity was decreased on
the lesioned side by 51% when expressed per mg protein, and 41% when expressed
on a per tissue basis. In marked contrast to the decrease in CAT activity
which occurs in the striatum after striatal kainic acid injections, CAT acti-
vity in the SN was unaffected by nigral kainic acid injections. There was no
difference in CAT activity between control and lesioned SN when activity was
expressed in terms of either protein or tissue. The AChE activity in the SN
on the lesioned compared to the contralateral control side was reduced by 44%
when calculated on a per protein basis, and by 64% on a per nucleus basis.
Again this difference between the extent of the reduction of AChE activity by
kainic acid when expressed in the two ways may reflect tissue shrinkage.

Data on the basal and dopamine stimulated activity of adenylate cyclase
are shown in Fig. 16. A significant dopamine stimulation was found in both
the lesioned and the control SN. This was true whether the data were calcu-
lated per mg protein, \( F(3,30) = 12.57, P < 0.001 \); (Fig. 16A), or per nucleus,
\( F(3,24) = 30.13, P < 0.001 \); (Fig. 16B). The lesion did not result in a diffe-
rential effect of the various dopamine concentrations on the adenylate cylcase
activity as compared to control [per mg protein, \( F(3,30) = 1.5, P < 0.05 \); or
per nucleus, \( F(3,24) = 0.68, P < 0.05 \)]. However, in terms of activity per mg
protein, the dopamine stimulated adenylate cyclase activity was significantly
Figure 15  Histology of normal and kainic acid-injected substantia nigra.
A: contralateral uninjected SN.  B: kainic acid injected SN.
SNC, pars compacta; SNR, pars reticulata.  The injected SN
shows complete lack of neuronal cell bodies, and an increased
density of glia.  Magnification x 250.
Table 8

Enzyme activities following intranigral kainic acid.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>(n)</th>
<th>Per mg protein</th>
<th>Per SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH (Striatum)</td>
<td>8</td>
<td>Control: 3.01±0.25</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lesion: 0.18±0.12**</td>
<td>-</td>
</tr>
<tr>
<td>GAD (SN)</td>
<td>7</td>
<td>Control: 278±10.1</td>
<td>203±32.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lesion: 142±12.9**</td>
<td>84.4±26.7**</td>
</tr>
<tr>
<td>CAT (SN)</td>
<td>5</td>
<td>Control: 24.5±2.6</td>
<td>19.5±2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lesion: 30.5±1.7</td>
<td>15.4±1.2</td>
</tr>
<tr>
<td>AChE (SN)</td>
<td>5</td>
<td>Control: 6.93±0.41</td>
<td>5.48±0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lesion: 3.88±0.27**</td>
<td>1.98±0.19**</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. of the number of determinations indicated. Enzyme activities are expressed in terms of nmoles/h, except AChE which is in μmoles/h.

* p < 0.05.

** p < 0.001, Paired t test.
Figure 16 A: The effect of intranigral kainic acid (5nmoles) on the dopamine-sensitive adenylate cyclase activity of the substantia nigra expressed as pmoles of cAMP formed per mg protein per min. Dopamine concentrations are plotted on a log scale. Each point represents the mean ± S.E.M. for 6 animals. Activity in the presence of 10 mM NaF is shown to the right of the curve.

B: The effect of intranigral kainic acid (5 nmoles) on the dopamine-sensitive adenylate cyclase activity of the substantia nigra expressed as pmoles of cAMP formed per SN per min. Dopamine concentrations are plotted on a log scale. Each point represents the mean ± S.E.M. for 5 animals. Activity in the presence of 1 mM NaF is shown to the right of the curve.
A

ADENYLATE CYCLASE ACTIVITY (pmoles/mg protein/min)

DOPAMINE CONCENTRATION (μM)

CONTROL
LESION

B

ADENYLATE CYCLASE ACTIVITY (pmoles/SN/min)

DOPAMINE CONCENTRATION (μM)

CONTROL
LESION

NaF
greater in the lesioned nigra compared to the contralateral control, $F(1,10) = 6.79, P < 0.05$. This trend towards higher dopamine stimulated activity in the lesioned SN was also seen when the activities were expressed on a per tissue basis; however, in this case the increase failed to reach significance.

The stimulation of adenylate cyclase by 10 mM sodium fluoride was not significantly different on the lesioned side compared to control per protein (Fig. 16A) or per nucleus (Fig. 16B).

b) Neurotransmitter receptor localization in the substantia nigra and striatum

Experiments were undertaken to determine the cellular localization of the receptors for the neurotransmitter DA in the SN and striatum. To this end lesions of the NSP with 6-OHDA and of the striatum with kainic acid were employed. The effect of these lesions on various enzyme activities in the SN and striatum is shown in Table 9.

The effects of 6-OHDA lesions of the NSP and kainic acid lesions of the striatum on tyrosine hydroxylase (TH), glutamic acid decarboxylase (GAD), and choline acetyltransferase (CAT) in the striatum and SN are shown in Table 9. 6-OHDA lesions of the NSP resulted in a 95% and 91% decrease in striatal and nigral TH activity, a significant increase of 19% in striatal GAD activity and no change in nigral GAD or striatal CAT activity. Striatal kainic acid lesions resulted in a significant decrease of 19% in striatal TH activity while nigral TH activity remained unaltered. These lesions decreased striatal and nigral GAD activity by 56% and 42% respectively. Striatal CAT activity was reduced by 70% on the lesioned compared to the contralateral side. Interestingly, however, CAT activity contralateral to the kainic acid lesioned striatum increased significantly by about 18% when compared to the activity in saline injected striatum (data not shown). There was no alteration in either GAD or TH on the side contralateral to the lesions relative to saline injected controls.
Table 9

The effect of 6-OHDA lesions of the NSP and kainic acid lesions of the striatum on TH, GAD and CAT activities in the striatum and SN.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Area measured</th>
<th>Enzyme Activities</th>
<th>TH</th>
<th>GAD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OHDA</td>
<td>Striatum</td>
<td>control</td>
<td>5.03±.19</td>
<td>242±12</td>
<td>65.6±3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lesioned</td>
<td>ND</td>
<td>288±14***</td>
<td>69.5±2.5</td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>control</td>
<td>3.05±.25</td>
<td>930±43</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lesioned</td>
<td>0.26±.09***</td>
<td>912±62</td>
<td>-</td>
</tr>
<tr>
<td>Kainic acid</td>
<td>Striatum</td>
<td>control</td>
<td>5.38±.54</td>
<td>242±9.1</td>
<td>76.1±4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lesioned</td>
<td>4.37±.30**</td>
<td>107±9.9*</td>
<td>23.0±2.9*</td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>control</td>
<td>2.86±.50</td>
<td>862±58</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lesioned</td>
<td>3.41±.93</td>
<td>501±23*</td>
<td>-</td>
</tr>
</tbody>
</table>

Values represent the mean ± S.E.M. of 7 or 8 determinations of the lesioned and contralateral control side. ND - not detectable.

* p < .001
** p < .05
*** p < .01
The results of \textit{in vitro} binding of $^3$H-apomorphine for DA-receptors in striatal and SN tissue after 6-OHDA lesions of the NSP is shown in Table 10. The specific binding of $^3$H-apomorphine in the SN was reduced on the lesioned side by 76%. Similarly, compared with the control side, NSP lesions reduce $^3$H-apomorphine binding in the striatum by 56%. The linear regression lines in the Scatchard plot shown in Fig. 17 further indicate that both the affinity of $^3$H-apomorphine for the DA receptor and the maximum number of binding sites (B max) in the NSP-lesioned striatum are significantly reduced compared to control striatum.

Table 11 contains the results of the binding of various ligands to DA receptors in the striatum and SN after 6-OHDA lesions of the NSP and kainic acid lesions of the striatum. In marked contrast with the reduction in striatal $^3$H-apomorphine binding, $^3$H-haloperidol binding was significantly increased by 22% after NSP 6-OHDA lesions. This was also true of $^3$H-spiroperidol binding at both the concentrations tested. Thus $^3$H-spiroperidol binding was increased by 18% at 80 pM (final concentration in the binding assay) and 19% at 500 pM. In the SN after 6-OHDA lesions of the NSP there was no significant decrease in $^3$H-spiroperidol binding at 500 pM. However, there was a significant decrease of 42% at 80 pM $^3$H-spiroperidol. Kainic acid lesions of the striatum did not significantly affect $^3$H-spiroperidol binding in the SN.
### Table 10

<table>
<thead>
<tr>
<th></th>
<th>³H-Apomorphine binding</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n fmol/mg protein</td>
<td></td>
</tr>
<tr>
<td>Striatum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>11.2 ± 1.2</td>
</tr>
<tr>
<td>Lesion</td>
<td>8</td>
<td>4.9 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>SN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>8.0 ± 0.8</td>
</tr>
<tr>
<td>Lesion</td>
<td>5</td>
<td>1.9 ± 0.8**</td>
</tr>
</tbody>
</table>

Values represent means ± S.E.M. of the number of determinations indicated.

* p < .01
** p < .001
Figure 17  Scatchard analysis of specific $^3$H-apomorphine binding to rat striatal homogenates from control unoperated side (open circles) and from contralateral 6-OHDA-lesioned side (closed circles).
Table 11
Neurotransmitter receptor binding in the striatum and SN after 6-OHDA lesions of the nigrostriatal pathway and kainic acid lesions of the striatum.

<table>
<thead>
<tr>
<th></th>
<th>6-OHDA - NSP lesions</th>
<th>Kainic acid striatal lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Striatum (fmoles/mg protein)</td>
<td>SN (fmoles/mg protein)</td>
</tr>
<tr>
<td>3H-Spiroperidol (80pM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion</td>
<td>181 ± 5.9 (5)</td>
<td>11 ± 2.3* (5)</td>
</tr>
<tr>
<td>Control</td>
<td>154 ± 7.1 (5)</td>
<td>19 ± 2.8 (5)</td>
</tr>
<tr>
<td>% of control</td>
<td>118</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>3H-Spiroperidol (500pM)</td>
<td></td>
</tr>
<tr>
<td>Lesion</td>
<td>173 ± 4.9* (5)</td>
<td>31.7±3.7 (8)</td>
</tr>
<tr>
<td>Control</td>
<td>147 ± 8.0 (5)</td>
<td>34.9±3.3 (8)</td>
</tr>
<tr>
<td>% of control</td>
<td>119</td>
<td>91</td>
</tr>
<tr>
<td>3H-Haloperidol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion</td>
<td>128 ± 7.8* (4)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>105 ± 8.0 (6)</td>
<td></td>
</tr>
<tr>
<td>% of control</td>
<td>122</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the means ± S.E.M. of the number of determinations shown in parentheses.

*p < .05
DISCUSSION

I. Anatomy of the Striato-Pallidal and Striato-Nigral Projections

As elaborated in the introduction, the striatal projections to both segments of the globus pallidus in the primate and to the SN in several species have been well established by degenerative anatomical techniques. However, the advantages of the HRP and autoradiographic tracing techniques have led, in many instances, to a reinvestigation of anatomical pathways in the CNS. The present investigations employing these techniques confirm and extend previous studies of the striatal efferent projections. In accordance with these earlier anatomical studies, injections of radiolabeled amino acid into the dorso-lateral quadrant of the striatum resulted in the transport of $^3$H-protein to the GP and hence a heavy accumulation of autoradiographic grains in this structure. Similarly, well localized injections of radiotracer into the tail of the striatum, lateral to the GP, resulted in dense labeling of the GP. From either injection site there was no diffusion of label to the cortex or GP as indicated by the lack of grain accumulations in the thalamus or SUT, respectively. That the GP represents a terminal field of striatal efferent fibers is dictated by all the criteria generally employed to draw such conclusions by the autoradiographic tracing method (see written comprehensive examinations, J.I. Nagy, 1978). The present results are also in complete agreement with the previously described topography of the striatal efferents to the GP (see Introduction). Anterior striatal $^3$H-leucine injections resulted in the highest grain densities in the anterior GP. In fact, the labeling in this region of the GP was so intense that the silver grains clearly demarcated the anterior border of the GP from the striatum immediately rostral to it. In the rat, the GP is located lateral to the internal capsule and follows the arc circumscribed by this fiber system to a considerable distance caudally. Injections of $^3$H-leucine into the tail of the striatum led to an area of unmis-
takable termination in precisely these caudal regions of the GP.

Further confirmation of the striato-GP projection was obtained in experiments utilizing HRP which demonstrated that injections into the GP resulted in the appearance of HRP reactive neurons in the striatum. These labeled neurons appeared to be confined to the central core of this structure. One interpretation of this HRP labeling pattern is that only the central core region of the striatum surrounding the GP project to the pallidum. This is clearly not the case since the autoradiographic transport data show that injections of label into the anterior and posterior extremes of the striatum labels the anterior and posterior aspects of the GP, respectively. It is noteworthy that in an attempt to keep the HRP restricted to the GP, the HRP injections were aimed at the center of this nucleus. A more plausible alternative, then, is that the HRP pattern obtained in the striatum is a further indication of the topography of the striato-GP projection. From the present material, particularly since the less sensitive diaminobenzidine HRP method was employed, it was difficult to ascertain the types of striatal neurons which project to the GP. It did appear, however, that most were of medium size.

As described in the Introduction there is a paucity of definitive studies on the striato-EP pathway. The present autoradiographic experiments involving $^3$H-leucine injections into the anterior striatum provide strong support for the existence of this pathway in the rat and further strengthen the concept that the EP in subprimates represents the homologue of the internal segment of the globus pallidus in the primate. It was somewhat more difficult to discern a posterior striatal projection to the EP. Following injections of $^3$H-leucine into the tail of the striatum autoradiographic grains could be traced descending in the internal capsule caudal to the GP. At the level of the EP label was located just dorsolateral to the EP within the internal capsule. Due to the poor definition of the dorsolateral border of the EP in the internal cap-
sule it could not be determined whether there was sparse labeling in this remote portion of the nucleus. It was apparent, however, that where the EP was distinct no label was observed and that the bulk of the descending fibers originating in the tail of the striatum avoid the EP as they forge toward the SN.

The present attempts to study the striato-EP pathway employing the HRP technique also met with some difficulties. As alluded to in the Introduction, damaged axons at an injection site can accumulate HRP and transport it in a retrograde fashion to the cell bodies. In the present study, HRP injection aimed at the EP invariably involved the adjacent internal capsule through which axons course en route to distant terminal fields. Labeled cells were occasionally observed in the cerebral cortex and SNR after microelectrophoretic injections into the EP. The fact that microelectrophoretic injections into the crus cerebri also labeled cells in the striatum, SN and cerebral cortex confirms that axons did accumulate and transport HRP. These results echo the caution which has been stressed in anatomical studies utilizing HRP. However, pressure injections of HRP into the crus cerebri did not result in the appearance of reactive cells in any of the above regions, suggesting that this method of delivery may be less likely to label cells as a result of accumulation and transport by axons in the vicinity of the injection. From experiments involving pressure injections into the EP it is apparent that, with the exception of the caudal tail, the entire striatum projects to the EP. It should be noted that the area of the striatum which appeared to be devoid of a projection to the EP, as indicated by the HRP method, is located further caudally to the placements of the $^3$H-leucine injections.

As expected from the well established striato-nigral projection, injection of label into the striatum resulted in grain accumulations in the SNR. No label was observed in the SNC. After dorsoanterior striatal injections, label was distributed primarily in the medial SNR. Injections into the dorso-
lateral tail of the striatum labeled the extreme ventral portion of the lateral SNR whereas more ventral tail injection labeled the dorsolateral SNR.

II Biochemical Neuroanatomy of Striatal and Pallidal Efferent Projections

a) Striato-pallidal projections; glutamic acid decarboxylase

The present investigations involving lesions of the anterior and posterior striatum have disclosed some information as to the biochemical organization of the striatal efferent fibers. It must be stressed, however, that these studies are by no means definitive and in certain instances conclusions drawn by the present author may require future modification in light of new data. Electrolytic lesions in the head of the striatum and hemitranssections well anterior or just anterior to the GP resulted in significant reductions in GAD in the GP. This suggests that GABA is utilized as a transmitter by at least some of the striato-GP fibers. A further observation is that hemitranssections which more closely approached the GP, although still avoiding it, resulted in greater GAD reductions (compare results in Table 2 and 3). This might be expected if the striato-GP GAD-containing fibers were organized topographically. Support for this is derived from experiments where the anterior and posterior GP was analyzed separately after anterior hemitranssections. These lesions did not cause a significant decrease in the posterior half of the GP, but this may have been due to the unusually large standard errors of these GAD values. The results, however, indicate clearly that the hemitranssections caused a greater significant GAD decrease in the anterior than the posterior GP. Thus, it may be concluded that the striatal fibers which have been repeatedly demonstrated to project topographically onto the GP (see introduction) also may be, at least in part, the fibers which contain GAD. Incidentally, because of the uncertainty of the GAD values in the posterior GP the author is not prepared to suggest that this part of the GP does not receive gaba-ergic input from the anterior striatum, but rather that this is
less dense than anterior GP regions.

Kainic acid lesions of the anterior striatum also led to a decrease in GP GAD. The results with this lesion technique, however, were equivocal in that histological examination of the extent of the lesion revealed destruction of parts of the GP and striatal areas lateral to the GP.

Consistent with the anatomical observations of a striato-EP projection, lesions of the anterior striatum caused a significant GAD reduction in the EP. This is suggestive of a GAD-containing projection from the anterior striatum to the EP. Interestingly, kainic acid lesions which destroyed the greater part of the body of the striatum failed to cause a greater reduction in EP GAD activity than did the hemitranssections. Moreover, hemitranssections which caused a greater reduction in GP GAD did not produce a simultaneous proportional decrement in EP GAD. This suggests two alternatives for the localization of the neurons within the striatum that give rise to the striato-EP GAD-containing pathway. It may be that most of these neurons are located in the anterior reaches of the striatum or that there is a discontinuity of these neurons in the rostrocaudal dimension of the striatum. The latter alternative is favored in view of the present HRP anatomical findings together with the fact that electrolytic lesions of the tail of the striatum resulted in significant reductions of GAD in the EP.

Electrolytic lesions of the tail of the striatum did not alter GAD or CAT actively in the GP. This result together with the present demonstration of a projection to the GP from the tail of the striatum suggests that the fibers comprising this projection are devoid of GAD and CAT. However, caution must be emphasized in drawing such a conclusion. As noted earlier, the tail of the striatum projects to quite posterior parts of the GP. This region of the GP is surrounded by the internal capsule medially, the amygdala ventrally and the caudal tail of the striatum laterally. In an effort to increase the accuracy of the GP dissection and to exclude striatal tissue from these samples, only
the main body of the GP was dissected. It is entirely possible that these "backwoods" regions of the GP were not included in the present analysis. If dissection difficulties could be overcome the experiments involving the projection of the tail of the striatum to these posterior areas of the GP certainly warrant reinvestigation.

The reduction of GAD in the EP after electrolytic lesions of the tail of the striatum appears to contradict the autoradiographic anatomical finding where one was hard pressed to observe a projection to the EP from striatal areas lateral to the GP. On the other hand, HRP labeled cells were present in this striatal area after EP HRP injections. It is noteworthy in this regard that the electrolytic striatal lesions were decisively larger than the \(^3\text{H}-\text{leucine} \) injection site. Thus, a sparse projection to the EP from striatal regions lateral to the GP together with a relatively restricted \(^3\text{H}-\text{leucine} \) injection in this area may have resulted in the inability to detect this projection anatomically.

b) Striato-pallidal projections; choline acetyltransferase

A hitherto unexpected result is that striatal lesions cause reductions in CAT activity in the GP and EP. These decreases were significant in the posterior GP after hemitransection anterior to the GP and in the EP after electrolytic lesions of the tail of the striatum. Although a relatively large decrease was observed in the anterior GP after hemitransections, this result was seriously compromised by the large standard error of the CAT activities in these samples. However, in cases where significance was obtained, there are several possible interpretations as to the cause of the CAT depletions. Some of the possible criticisms that may be levied against the present biochemical work in general are discussed in relation to the present observations concerning CAT. The reason is that these observations, in particular, have certain implications regarding the configuration of striatal efferents to various nuclei and the definition of the neurotransmitters involved in these striatal
The first of the possible reasons for the lower CAT activity in the GP of animals that had received striatal lesions is that the CAT losses are only apparent and merely reflect a consistent dissection error between lesioned and control samples. This is ruled out since, as shown in the results, the left and right sides of the brain regions examined can be dissected accurately from unoperated animals to yield reliable agreement of CAT and GAD between the two sides.

The second possibility is a criticism of data obtained on the GP by hemitransections closely approaching the GP. This argument suggests that enzyme changes in the GP may be due to spread of damage from the lesion site posterior to the GP thereby destroying terminals and/or interneurons in the GP containing the enzymes under investigation. There is no reason to believe that after hemitransections such a process would not be operative in the tail of the striatum immediately lateral to the GP and thus, affect enzymes contained in neural elements of this structure. This criticism is countered by the present observation that in sections containing the tail of the striatum immediately lateral to the GP and from which the anterior and posterior GP was obtained, neither CAT nor GAD activity was altered by hemitranssections.

The third possibility involves the heavily AChE-staining neurons in-and-around the GP and EP. As described in the Introduction these cells may be cholinergic, CAT-containing, projection neurons to the cortex. Indeed, some of these neurons undergo retrograde degeneration after cortical lesions (J.I. Nagy, unpublished observations). It must be emphasized that this retrograde loss is only partial. The extent to which these cells undergo retrograde degeneration after anterior hemitranssection is not known. The CAT decrease in the GP, then, may have been due to a loss of this enzyme in neurons whose corticopetal axons have been severed by hemitranssection. This explanation, although it cannot be ruled out entirely at the present time, is unlikely for
two reasons. First, retrograde degeneration requires more time to occur than
the 9 to 14 days postoperative survival period employed in the present study
(Das, 1971). Moreover, at shorter survival times, having severed the axons of
these cells, it might be expected that the deprivation of their ability to
transport CAT to distal regions of their axon would lead to an accumulation of
CAT in the perikarya rather than a depletion. Second, by fractionation tech­
niques neurotransmitter synthetic enzymes have been shown to be concentrated
in nerve terminals to a greater degree than in the cell soma. A similar argu­
ment holds for fibers-of-passage that may contain CAT and which might undergo
degeneration after having been severed. Thus, the CAT loss in the GP is more
likely to represent a depletion from terminals than nerve cell bodies. It may
be argued that the depletion is from recurrent axon collaterals of these cells.
However, if CAT in recurrent collaterals of these neurons contributed a signi­
ficant proportion of this enzyme to the GP, then these collaterals might also
be sufficient to prevent their retrograde degeneration.

Fourthly, the CAT decreases may have been due to a compensatory reaction
to the lesions of intrinsic neural systems of the GP and EP. The large alte­
rations in CAT activity after a relatively short survival period, together
with the fact that these changes occurred in both the EP and GP after two
quite different lesions, makes the occurrence of a compensatory reaction to
the lesions remote. This admittedly weak argument makes this possibility
worthy of further investigation as indeed are all the other possibilities.

The final possibility and the one favored here is the existence of a cho­
linergic projection to the GP and EP from the lesioned structures or from
other areas whose axons-of-passage were severed at the lesion site. On anato­
mical grounds the only axons projecting to the GP that would have been se­
vered, other than those from the striatum, are the accumbens-GP fibers. At
present an accumbens-GP cholinergic projection cannot be ruled out. In this
regard, it is perhaps significant that the accumbens appears not to project
to the EP (Nauta et al, 1978) and anterior hemitranssections did not alter EP CAT activity. On the other hand, CAT was reduced in the EP after lesions of the tail of the striatum which probably would not have interrupted efferent accumbens fibers.

Further support for this latter suggestion is derived from the present experiments involving kainic acid lesions of the GP. Histological examination of the kainic acid lesions revealed that neuronal degeneration had occurred in most of the GP. The only domain of the GP spared by these lesions was the most posterior extreme which, incidentally, overlaps that area spared by the electrolytic lesions of the GP. Varying degrees of kainic acid-induced neuron destruction was also observed in the medial portion of the tail of the striatum immediately lateral to the GP and in the striatum just anterior and anteriolateral to the GP. The extent to which kainic acid is toxic to axons is still controversial (see methods section). However, consistent with some views that fibers-of-passage are not disturbed by kainic acid, injections of this compound into the GP which contains ascending DA-containing fibers to the striatum did not reduce but, in fact, significantly increased TH in the tail of the striatum.

The GAD and CAT reductions in the GP, which were of equal magnitude after GP injections, probably represent destruction by kainic acid of perikarya in the GP containing those enzymes. Quantitatively, these reductions are complementary to those caused by hemitranssections anterior to the GP. The destruction of axon terminals in the GP containing these enzymes cannot at present be ruled out, although the weight of evidence is against a neurotoxic effect of kainic acid on these neuronal elements. That diffusion of kainic acid and thus neuronal destruction in the tail of the striatum occurred is indicated by the significant reduction of GAD in samples of this structure. It is both surprising and perhaps meaningful that in these same samples no reduction of CAT was observed. Three possibilities are given for this result. First, cho-
linergic neurons may be less sensitive to the toxic effects of kainic acid. This, however, is unlikely considering earlier observations involving kainic acid injections into the striatum. These injections produced equal reduction of CAT and GAD at various doses of kainic acid (McGeer and McGeer, 1976; Schwarcz and Coyle, 1977) (see, however, section on intranigral kainic acid). Second, cholinergic neurons may be located primarily in the lateral region of the tail of the striatum which, according to the histological analysis, was not affected by kainic acid. Third, cholinergic neurons in the tail of the striatum may, in fact, have been destroyed. However, if these were efferent projection neurons, their loss, by CAT analysis, may have gone undetected for arguments similar to those proposed for discounting the retrograde loss of AChE-staining neurons in the GP as being the cause of CAT decrease in the GP after anterior hemitranssections.

At present, it is difficult to decide between these alternatives. The latter possibility, however, is consistent with the present proposal of the existence of cholinergic striatal efferent fibers. Further support is derived for CAT-containing striatal efferents to both GP and EP from observations made during the developmental stages of the kainic acid GP lesion. Initially, these lesions were large but were stepwise reduced in size as correct injection volumes and kainic acid concentrations were developed. Enough animals were included in these experiments to make the following observations fairly reliable. When CAT activity in the tail of the striatum was reduced by 50%, GP CAT was reduced profoundly by 85%. A 21% CAT reduction in the tail of the striatum was accompanied by a 53% reduction in the GP, and finally when CAT was unaltered in the striatum, GP CAT was reduced by 42%. This pattern was also true of GAD in the GP, EP and notably the SN. Since the lesions of the GP in these successive experiments could not have been more complete than the one finally chosen, the parallel diminutions of CAT in the GP and striatum strongly suggest the CAT losses in the GP in the initial experiments to be
due to the destruction of CAT-containing striatal efferent projection neurons to the GP by kainic acid.

Yet another observation that bears on all the lesions that caused CAT reductions in the GP is the effect of kainic acid on the large AChE-staining cells of the GP. The location of these cells in the GP has been shown (Fig. 11). Although their numbers were not quantitated, it appeared that, apart from some atrophy, they were left intact by kainic acid. This indicates at least that CAT is present in neuronal elements in the GP other than these putative cholinergic neurons and that it is possible to obtain CAT decreases in the GP without their degeneration.

From the foregoing discussion it is proposed that ACh be added to the list of substances contained in the striatal efferents to the GP and EP. This, of course, does not preclude the existence of striatal cholinergic interneurons.

c) Striato-nigral projections

It has been previously shown that hemitransections at the level of the hypothalamus do not alter nigral CAT activity (McGeer et al, 1973; Kataoka et al, 1974). It is therefore not surprising that in the present experiments nigral CAT was not affected by hemitransections anterior to the GP. This negative finding takes on particular significance since these same hemitransections reduced CAT in the GP. Furthermore, electrolytic GP lesions did not lead to a CAT decrease in the EP and this lesion would certainly have destroyed striato-EP fibers originating in the anterior striatum. This result then is in agreement with the observation that hemitransections anterior to the GP failed to alter EP CAT activity. The significance of these findings is discussed more fully in the next section.

The present attempts to determine the location within the striatum and/or GP of the neurons giving rise to the GAD-containing afferents to the SN were relatively unsuccessful. A starting point in such an investigation is to
determine the maximal contribution to nigral GAD of afferent fibers from the striatum and GP. In agreement with previous observations (see Introduction) hemitranssections at the level of the EP resulted in a reduction of GAD in the SN of 84%. This sets the baseline to which the effects of various lesions in structures anterior to this hemitranssection should be compared. The remaining GAD in the SN may reside in nigral interneurons or in afferents to the SN from structures caudal to the level of the EP.

Of the variety of lesions placed in the striatum or GP, none resulted in a reduction of GAD activity that even approached that caused by hemitranssections at the EP level, although small significant decreases were observed. Hemitransections slightly anterior to the GP or electrolytic lesions placed in the head of the striatum did not cause a significant reduction in nigral GAD whereas hemitranssections more closely approaching the GP produced a small but significant decrease.

Kainic acid lesions of the head of the striatum also reduced nigral GAD but, as mentioned earlier, these lesions involved parts of the GP and the striatum lateral to the GP. Insofar as nigral GAD is concerned, these results indicate that there is a sensitive area in the striatum at the level of the anterior border of the GP. Electrolytic lesions of the tail of the striatum also caused small significant decreases in nigral GAD. These lesions did not extend as far anterior as the hemitranssections and therefore a separate population of GAD-containing striato-nigral fibers may have been affected than those damaged by the hemitranssections. This being the case, about 47% of nigral GAD can be accounted for. The contribution from the striatum just anterior to the GP is about 16%, the tail of the striatum contributes about 15%, and 16% is intrinsic or from sources caudal to the EP. The remaining 50% may originate from regions not enveloped by the hemitranssections or the electrolytic striatal lesions. The gross anatomy of the tail of the striatum causes considerable difficulties in producing lesions which destroy the full extent
of this structure. In the present attempts to lesion this structure, the dorsomedial and ventroposterior regions were spared. The contribution to nigral GAD from these areas is therefore unknown.

d) Pallido-nigral projections

To determine whether the GP efferent fibers to the SN contain GAD, electrolytic lesions of the GP were produced. Histological examinations showed that these lesions destroyed all but the posterior one-third of the GP. On the basis of the present autoradiographic studies these lesions would not have impinged on the descending striatal fibers originating in the tail of the striatum but would have severed some of those originating in the anterior striatum. Damage by these lesions of fibers passing through the GP compromises the interpretation of the significant reductions of GAD in the EP and SN. It is therefore impossible to conclude whether these reductions resulted from destruction of neurons in the GP or from damage to fibers-of-passage originating from regions anterior to the GP. Furthermore, because the lesions missed the lateral and posterior GP, the GAD contribution to the SN from these areas remains unknown. What is significant, however, is that the GP electrolytic lesions did not produce a reduction of GAD in either the EP or SN greater than that produced by hemi transections anterior to the GP. This suggests that the areas of the GP destroyed by the electrolytic lesions do not project GAD-containing fibers to the EP or SN.

Hemi transections anterior to the GP causing only minor reductions of nigral GAD together with the demonstration by numerous investigators of the pallido-nigral projection made this pathway a major candidate for the origin of nigral GAD. It is therefore of considerable interest that the kainic acid lesions of the GP did not significantly alter GAD activity in the SN.

Because of the significance of this negative result it may be appropriate here to digress to answer three criticisms of the present experiments. First, in drawing on histological data from a group of animals separate from those in
which biochemical data were obtained, the assumption is made that the lesions in brains taken for biochemical analysis were the same as in those analyzed histologically. Following both electrolytic lesions of the tail of the striatum and kainic acid GP lesions, in some brains only biochemical studies were conducted while in others SN GAD was measured and the remainder of the brain was taken for histology. After both types of lesions the close agreement in SN GAD values between brains taken for enzyme analysis and brains taken for histology indicates the validity of extrapolating the area covered by the lesion as assessed histologically to all animals receiving a given lesion. Furthermore, from the histological analysis of a large number of lesions, their uniformity was evident. Moreover, lesions were inspected during dissections and only those in which the desired placement was achieved were utilized for biochemical studies. Second, it may be argued that large localized losses of GAD within subregions of the SN may have occurred after any of the above lesions. Therefore, if nigral GAD originated from neurons dispersed throughout the GP and striatum, any individual lesion would cause only small or undetectable decreases of GAD in the SN as a whole. This, however, can be taken to *reductio ad absurdum* in that if areas in the SN the size of axon terminals could be assayed for GAD after given lesion in the striato-pallidal complex, many samples would yield reductions of 100%! To a first approximation, for biochemical studies the extent to which the SN should be subdivided into various components should correspond to the extent of the lesions produced in the structures giving rise to its afferents. In the present study any individual lesion encompassed a large area of tissue, thus, the SN was taken in its entirety for analysis. Thirdly, given that hemitranssections immediately anterior to the GP cause significant, albeit small decreases, in nigral GAD; that some GAD-containing nigral projection neurons are located anterior to the GP; and, that the fibers of those neurons pass through the GP en route to the SN, the failure of kainic acid injections into the GP to reduce nigral GAD is evi-
dence that under the present injection parameters kainic acid did not destroy fibers-of-passage.

There are two possible explanations for the failure of kainic acid GP lesions to effect nigral GAD. First, that pallido-nigral GAD-containing neurons exist but that kainic acid does not destroy them. To test this eventuality and determine if any pallido-nigral neurons survived kainic acid, the SN of animals which had received unilateral kainic acid GP lesions was injected bilaterally with HRP. Numerous large neurons in the GP were labeled on the un-lesioned side. Some labeled neurons were present in the posterior GP of the lesioned side and this is in agreement with the histological examinations which showed this area to be spared by the kainic acid lesions. The rest of the GP was devoid of cells containing HRP. The heavily AChE-staining cells, which appeared to be resistant to the neurotoxic effects of kainic acid, were not labeled with HRP indicating that these neurons have projection fields to areas other than the SN. In view of these results a second more likely alternative is that no pallido-nigral GAD-containing neurons exist within the confines of the kainic acid lesion.

III Striatal and Pallidal Efferents: Synthesis and Speculation

Several important aspects of the striatal and pallidal systems warrant detailed consideration insofar as they pertain to the present findings and the literature available on the striatum and pallidum. Four issues which are currently receiving considerable attention are: 1) The topography of the striato-pallidal and striato-nigral projections in relation to nigral efferents and striatal afferent projections, 2) The characterization of the types of neurons which form the striatal efferent projections, 3) The identity of the neurotransmitters contained in the striatal and pallidal efferent fibers, and 4) The location of the neurons within the striato-pallidal complex which are responsible for each of the neurotransmitters that are con-
tained in fibers emanating from these structures.

a) Topographic relations

Detailed topographic relations of the striato-GP, striato-EP and striato-nigral projections could not be determined from the limited number of injection sites for either HRP or $^3$H-leucine employed in the present study. Certain features of the striatal efferents, however, are worthy of comment. The present observations in the rat are in agreement with previous demonstrations of the organization of the striatal efferents to the globus pallidus in other species whereby all parts of the striatum, at least in the anterior-posterior and dorsal-ventral plane, project to the globus pallidus in such a way that the former is superimposed on the latter (Szabo, 1962, 1967, 1969; Nimii, 1971; Johnson and Rosvold, 1971). Whether some striatal areas project differentially to the GP and EP is unresolved. There are suggestions in the literature of a medial-lateral segregation of striatal efferents to the LGP and MGP (Cowan and Powell, 1966; Voneida, 1960; Szabo, 1962, 1967, 1969) such that progressively more medial striatal areas project preferentially to MGP. Electrophysiological work suggesting the compartmentalization of striatal efferents is derived from the observation that antidromic responses in the striatum after GP stimulation are located mainly in the central core region (Richardson et al, 1977).

Some divergence of striatal projections to the two pallidal segments may occur in the tail of the striatum. As observed here, there appears to be a paucity of fibers projecting to the EP from this area. Tulloch et al (1978) also show very little labeling in the vicinity of EP after posterior striatal $^3$H-leucine injections. In contrast, the tail of the striatum has a projection to the GP. The concept of differential striato-pallidal projections takes on added dimensions in view of certain findings regarding the striato-nigral projection. It appears that all parts of the striatum in the rat project to the SN except a central core region (Bunney and Aghajanian, 1976a). In addition,
the most dense projections to the SN from the striatum appear to arise from
the tail of the striatum (Bunney and Aghajanian, 1976) and peripheral striatal
areas (Richardson et al, 1977; Tulloch et al, 1978).

The origin within the striatum of efferent projections to the GP, EP and
SN and the extent to which these overlap has important anatomical and func­
tional implications. Each of the GP, EP and SN have some mutually exclusive
projections. This is also true on a more local scale within the SN (Faull and
Mehler, 1978). Thus, whether the striatum is a functionally heterogeneous
structure may be determined by the degree to which striatal afferent projec­
tions are congregated and striatal efferent projections segregated. Detailed
information on these points is not available since, to date, investigations
along these lines have been largely superficial. New, more sensitive, HRP
methods (Mesulam, 1978) will undoubtedly prove useful in resolving some of
these problems.

A corollary of the view of differential striatal projections to its tar­
get areas involves the collateralization of striatal efferent fibers. The oc­
currence of collateralization of these fibers has been stressed on both ana­
tomical (Fox and Rafols, 1975; Fox et al, 1975) and electrophysiological
(Yoshida et al, 1971, 1972) grounds. It is apparent from the present experi­
ments, however, that anterior striatal areas which project GAD-containing fi­
bers to the GP do not have collateral projections to the SN. If further, more
detailed studies demonstrate a considerable lack of overlap within the stria­
tum of projection neurons to the GP, EP and SN, then this would raise serious
questions as to the role of axon collaterals of the striatal efferents as a
major organizational principle.

A further point regarding the striato-nigral projection is its relation­
ship to the cortico-striatal projection. Faull and Mehler (1978) have cited
unpublished observations that the visual cortex projects to striatal areas
that have efferent projections to that region of the SN which projects to the
Similarly, the motor cortex projects to striatal areas that have efferent projections to nigral regions projecting to the thalamus. The present observations suggest that the dorsal-anterior striatum projects to nigral areas which according to Faull and Mehler (1978) and Fallon and Moore (1978), give rise to thalamic projections, whereas the tail of the striatum projects to SN areas which innervate the SC. As a detailed map of the striatal innervation from the cortex has not been published, it is not possible to determine which cortical areas innervate the striatal projection areas studied here. In any case, it should be pointed out that the conservation of cortico-nigral functional relationships through the striatum is difficult to envision in view of recent autoradiographic demonstrations of the widespread distribution of striatal afferents from the cortex (Goldman and Nauta, 1977; Yeterian and Hoeson, 1975; Kunzle, 1977; Jones et al, 1977).

In the context of the topography of the striatal innervation of the SN it may be appropriate to mention the reciprocal innervation of the striatum by the DA-containing neurons of the SNC. A direct influence of these systems on each other would naturally require a reciprocal topographical arrangement. However, a complication in establishing such reciprococity is that the DA neurons of the SNC have dendrites which radiate into the SNR. The determination of the particular DA neurons whose dendrites in the SNR receive particular striatal input requires more elaborate techniques than simply observing the topography of these inputs. In the absence of hard data the present author is given to the following speculations. In the dorsal-ventral dimension the SN appears to be arranged into three tiers. These tiers contain neurons of either the nigro-striatal, nigro-thalamic or nigro-tectal systems (Faull and Mehler, 1978; Fallon and Moore, 1978). The dendrites of the DA nigral neurons may permeate all three of these tiers or seek an appropriate one. At the same time as nigral afferent input is received to any individual tier to be subsequently relayed on to the striatum, thalamus or SC, the DA dendrites in this
tier may also receive this same input and transmit perhaps topographically to the striatal areas from which the original afferent input arose. That is, the DA neuron may monitor afferent input to a nigral tier through its dendrite and regulate this through its axon. Alternatively, the DA neurons may monitor striatal and pallidal input to all three tiers simultaneously, integrate this and relay appropriate feedback to the striatum. These two alternatives have different implications with regard to nigro-striatal topography. The latter alternative would allow this to be less stringent, in that, if the DA neurons do indeed serve as a feedback mechanism, sampling and regulating basal ganglia output, then such feedback in this case would involve diverse areas of the striatum, perhaps affecting simultaneously areas that receive, for example, visual and motor cortical input.

b) Striatal projection neurons

The issue as to which of the various classes of neurons in the striatum form the efferents of this structure is not settled. Nor for that matter, is the problem of the types of neurons that exist in the striatum. To be brief, it appears that there are three sizes of striatal neurons; small, medium and large, or a size to suit everybody. Spiny neurons seem to be exclusively of the medium size. Aspiny neurons come in all three sizes. The reader is directed to section Ia-3 of the Introduction for arguments as to why only medium spiny striatal neurons project to the SN and why Fox and coworkers (1971-1972) originally assumed that only large aspiny neurons issue striatal efferent projections. With regard to the conclusion that medium spiny neurons give rise to the striato-nigral fibers, there is no evidence to the contrary. That these neurons also project to the globus pallidus is suggested by the observation that medium sized spiny striato-nigral neurons issue collaterals to the globus pallidus (Yoshida et al, 1971, 1972) and by the demonstration that these neurons are labeled after pallidal HRP injections (Graybiel, 1979). Whether other types of striatal neurons project to the globus pallidus is de-
batable. In view of evidence presented by Fox et al (1971, 1972, 1975) it would appear that large aspiny neurons have efferents to this structure. Graybiel et al (1979) observed that some large striatal cells contained label after HRP injections into the GP and EP. However, they hedged as to whether these were neurons or glial cells which had incorporated HRP reaction product. If these were indeed neurons, and in particular large aspiny neurons, this would in part vindicate Fox et al (1971, 1972, 1975) and have certain implications with regard to the present suggestion of a striatal cholinergic efferent projection. As mentioned earlier, the large aspiny neurons of the striatum stain heavily for AChE (Butcher et al, 1975; Poirier et al, 1977). This implies, although not necessarily (see Lehmann et al, 1979 and Lehmann and Fibiger, 1978), that these may be cholinergic neurons. Thus, although highly tentative, the large aspiny AChE-staining striatal neurons may give rise to the cholinergic innervation of the GP and EP. This, of course, does not exclude the possibility that the medium sized neurons are responsible for containing Ach in this projection, particularly since there appears to be at least three subclasses of neurons of this size.

C) Neurotransmitters in striatal and pallidal efferents

As detailed in the Introduction, the neurotransmitter candidates contained in striatal efferent projections are GABA and enkephalin to the GP and GABA and substance P to both the EP and SN. Neither a substance P-containing striato-GP pathway nor a striato-EP or -SN enkephalin pathway has been demonstrated. The present results of a striato-nigral GABA-containing pathway is in accord with previous demonstrations of this projection (Kim et al, 1971; Hattori et al, 1973b; Fonnum et al, 1974). In addition, the presence of GABA-containing fibers in the striato-GP pathway as shown here substantiates earlier suggestions of this (Hattori et al, 1973b) and is in agreement with work conducted simultaneously by others (Jessel et al, 1977; Fonnum et al, 1978a). Finally, the present finding of a striato-EP GABA-containing pathway is in
essential agreement with the independent findings of Fonnum et al (1978a).
The lack of GABA-containing GP-nigral pathways as interpreted from the results obtained here is contrary to the suggestions of Brownstein et al (1977) and Jessel et al (1978). This is more fully discussed in the next section.

The present results also indicate the possible existence of ACh in striato-GP and -EP fibers. In this context it is perhaps noteworthy that biochemical neuroanatomy enjoys a healthy reputation since in numerous instances the use of this technique has resulted in the first suggestions of previously unknown pathways. Nevertheless, as pointed out earlier, the demonstration by combined lesion and biochemical methods of anatomical pathways containing specific neurotransmitters suffers from certain limitations and much additional support is required before the present suggestions of an ACh-containing striato-pallidal pathway can be taken as proven. Such studies could include: 1) the correlation of the time course of CAT depletion in the GP with terminal degeneration in this area following striatal lesions, 2) the uptake and release of ACh in the GP and the effect of striatal lesions on these, 3) the effect of injections of axoplasmic transport inhibitors such as colchicine on CAT in the GP, 4) immunoprecipitation of $^3$H-CAT obtained from the GP after $^3$H-leucine injections in the striatum, 5) immunohistochemical staining of CAT in the GP and the effect on this of striatal lesions, 6) retrograde degeneration of unequivocally identified cholinergic neurons in the striatum following lesions of the GP, 7) the electrophysiological identity of action of ACh and striatal stimulation on GP neurons, 8) the effect of cholinergic drugs on striatal evoked GP responses.

The possibility of a cholinergic striato-pallidal pathway may add yet another site of action in the brain of cholinergic drugs. The following is the rationale for such a site of action. DA antagonists are known to increase ACh metabolism in the striatum (Marco et al, 1976; Stradler et al, 1973; McGeer et al, 1974b) but decrease it in the globus pallidus (Marco et al,
Cholinergic antagonists are known to reverse the increased output of DA caused by DA antagonists (Andén and Bedard, 1971). Pallidal neurons are known to directly innervate DA-containing neurons in the SN (Hattori et al, 1975). It may be assumed that the decreased ACh output in the GP may, in part, give rise to the increased DA output in the striatum. In addition, the increased DA output in the striatum may partially overcome the DA blockade by DA-receptor antagonists. Thus, in patients suffering from schizophrenia or Huntington's disease, where DA antagonists are the treatment of choice, one possible site of action of the beneficial effects of cholinomimetics (Davis and Berger, 1978) is the GP where decreased ACh activity would be reversed resulting in reduced DA output in the striatum by way of the SN which would add to the DA blocking effects of antipsychotic drugs. By similar reasoning, anticholinergics may have a beneficial effect in Parkinson's disease (Klawans, 1968) by decreasing cholinergic activity in the GP thereby augmenting DA output in the striatum.

As the above suggests there is considerable pharmacological and some anatomical (Hattori et al, 1976) evidence for an interaction between the nigrostriatal DA system and cholinergic neurons in the striatum. It is suggested here that some of the interdependence of these systems is manifested at the cholinergic end by the striato-pallidal cholinergic neurons. The "some" in this last statement should be emphasized since, as indicated above striatal and pallidal ACh metabolism can simultaneously undergo changes in opposite direction (Marco et al, 1976). This suggests that at least two functionally distinct cholinergic neuron populations exist in the striatum; those projecting to the GP and perhaps a separate population of interneurons.

A further consideration is the electrophysiological nature of the striato-pallidal pathway with regard to cholinergic mechanisms. Excitatory responses to stimulation of the striatum have been observed in the GP and EP, although whether these were monosynaptic was not dealt with in detail
(Malliani and Purpura, 1967; Levine et al, 1974). Richardson et al (1977) have also suggested, by inference, an excitatory input to the GP from the striatum. There are presently no neurotransmitter candidates which may mediate the excitatory responses in this system. If the action of ACh on GP neurons is excitatory as it is in many other CNS neurons, this at least would be consistent with the proposal of a cholinergic striato-pallidal pathway and would suggest the excitation in the pallidum to be mediated by ACh.

d) Biochemical neuroanatomy of striatal and pallidal efferents

The determination of the origin and distribution within the striatum and GP of the efferent projection neurons containing each of the neurotransmitters that have been identified as originating from these nuclei would be of considerable value to both psychological and pharmacological studies in attempting to define striatal function and to the eventual elucidation of striatal architecture. Although, to date, such studies have given only gross localizations, it should be remembered that some of the pathways concerned and the neurotransmitter substances contained therein have only recently been demonstrated.

From the results of the present study it is unlikely that the SN receives a significant GABA-containing projection from the GP. This is in contrast to the conclusion drawn by Jessel et al (1978) which was based on the failure of lesions completely separating the GP from the striatum to reduce nigral GAD to those levels achieved by hemitranssections posterior to the GP. Brownstein et al (1977), by a similar line of reasoning, assumed that the nigral GAD that they could not account for, originated not from the GP but the nucleus accumbens. This assumption, however, is probably incorrect since Dray and Oakely (1977) observed no reduction in nigral GAD following accumbens lesions. From the present autoradiographic observations of the trajectory of the posterior striato-nigral fibers together with the fact that some of these are GAD-containing, it is possible that both Brownstein et al (1977) and Jessel et al
(1978) did not sever these fibers with their semicircular lesions between the GP and striatum. The nigral reduction obtained after their lesions together with the decrease obtained in the present study after lesions in the posterior striatum accounts for all but about 10 to 15% of the nigral GAD originating from outside the SN. Thus, the results of the work of these groups, as interpreted here, and the present results place the neurons contributing GAD-containing efferents to the SN outside the GP.

The majority of the work conducted to date shows that the contribution to nigral GAD from the entire striatum anterior to the GP does not amount to more than 10 to 15% (Brownstein et al, 1977; Hattori et al, 1973b; Fonnum et al, 1978a; Spano et al, 1977; Jessel et al, 1978). This limits the location of the majority of striato-nigral GAD-containing neurons to striatal areas posterior to the level of the rostral pole of the GP. Posteriorly, since lesions separating the GP from the striatum result in only small nigral GAD reductions, (Brownstein et al, 1977) any significant proportion of nigral GAD-containing afferents originating from this region, must do so from areas immediately lateral to the GP. That this may indeed be the case is suggested by the finding that lesions involving the lateral border between the GP and striatum cause large reductions in nigral GAD (Brownstein et al, unpublished data).

From the above results and the present work the location of the GABA-ergic projection neurons to the SN is necessarily decided by the process of elimination. The electrolytic lesions of the tail of the striatum together with the anterior hemitranssections were to some extent complementary with the kainic acid GP lesions. Kainic acid GP lesions only reduced nigral GAD when the lesions extended beyond the GP. Hemitransections only reduced nigral GAD when these approached the GP. Electrolytic lesions of the striatum lateral to the GP reduced GAD only slightly. Therefore, the GAD-containing striato-pallidal nigral neurons must be sandwiched between the GP and striatum, along their mutual borders, both anteriorly and laterally and, although no informa-
tion has been obtained in the present studies, perhaps dorsally. Such an arrangement would include the posterior GP which was not lesioned by any of the above procedures.

Since nigral injections of HRP result in a heavy density of labeled neurons in the caudal and peripheral shell of the striatum (Bunney and Aghajanian, 1976a; Tulloch et al, 1978; Richardson et al, 1977) and since the majority of the substance P in the SN originates from neurons in the anterior striatum, these, together with the above conclusions concerning GAD, suggest a third neurotransmitter in the posterior striato-nigral fibers. Since the GP-nigral pathway appears not to contain GAD, this brings into the question the nature of the transmitter of this fiber system. However, substance P may be a candidate since neurons in the GP stain immunohistochemically for this peptide. Jessel et al (1978) suggest that a significant proportion of the nigral substance P originates from the GP whereas Brownstein et al (1977) have concluded that no GP-nigral substance P pathway exists. GP kainic acid lesions of the kind employed in the present study may resolve this point.

There is little information in the literature regarding the precise location of the GAD-containing striato-pallidal neurons. Jessel et al (1978) found that hemitranssections considerably more rostral to the GP than those employed here did not cause a significant reduction in GP GAD. Since their separations of the GP from the striatum resulted in large depletions, Jessel et al (1978) concluded that the majority of GP GAD-containing afferents originate from neurons in the caudal striatum. This result is in agreement with the observation of Fonnum et al (1978a) who found that progressively more caudal lesions involving the striatum anterior to the GP resulted in progressively larger reductions in GP GAD. The present results suggest that about 50% of the GAD in the GP originates in striatal neurons located anterior to the GP. This estimate and the observation that the anterior GP regions suffer greater losses of GAD than posterior regions following anterior striatal lesions are
in close agreement with the findings of Fonnum et al (1978a). No conclusions can be drawn from the present work regarding the contribution of the tail of the striatum to GP GAD.

With regard to the source of GAD in the EP, the anterior striatum contributes about 40% and the tail of the striatum about 20%. This is in rough agreement with the report of Fonnum et al (1978a) who presented reductions of EP GAD in individual animals following oblique hemitranssections at the mid-GP level ranging from 46 to 88%. All the results to date indicate that the neurons from which the GAD-containing GP and EP afferent arise are distributed uniformly and discontinuously, respectively, in the striatum at least in the anterior-posterior dimension. Earlier discussion of the topography of these projections suggests that there may be a medial-lateral differential projection of these neurons to EP and GP.

The location of the neurons in the striatum that may provide a cholinergic projection to the GP and EP is uncertain as are the existence of these pathways. Assuming tentatively that cholinergic striatal efferent fibers exist, the anterior striatum appears to send such fibers to the GP but not to the EP. Although no conclusion can be drawn as to whether the tail of the striatum has cholinergic efferents to the GP, such efferents may exist to the EP. If the large aspiny AChE-staining neurons are the source of striatal cholinergic efferent fibers, then it may be concluded that these efferents arise from widespread areas of the striatum since these AChE-staining neurons are rather uniformly distributed throughout this structure.

IV Preliminary Observations of Nigral and Entopeduncular Efferents

It was the intention of the present set of experiments to characterize biochemically the efferents of the EP to the habenula and the efferents of both the EP and SN to the thalamus. This was undertaken since these pathways have recently been unequivocally demonstrated (Nauta, 1974; Kim et al, 1976;
Herkenham and Nauta, 1977; Carter and Fibiger, 1978; Carpenter et al, 1976; Clavier et al, 1976; Faull and Mehler, 1978; Rinvik, 1975). and since it was in keeping with the present scheme of the identification of neurotransmitters in the connections of the basal ganglia. These investigations, however, are incomplete and only briefly described here in order to aid possible future work along these lines.

The fact that lesions of the EP resulted in a reduction in the habenula of GAD but not CAT indicates the existence of GABA-containing fibers in the EP-habenular pathway. These results are in partial agreement with those of Gottesfeld et al (1977). These authors observed that electrolytic lesions of the stria medularis, GP or GP and EP significantly decreased GAD in the lateral habenula. They suggested that some GABA-containing neurons in the GP project to the lateral habenula, with a further contribution from the EP. However, that damage to the EP and not the GP is the cause of reduced habenular GAD is suggested by 1) the lack of a projection to the habenula from the GP (Carter and Fibiger, 1978) and 2) the observation that the present EP lesions decrease GAD activity in the habenula as much as the combined EP and GP lesions employed by Gottesfeld et al (1977). The results obtained by the latter workers may have been due to damage of EP-habenular fibers passing in the vicinity of the GP. These workers have more recently demonstrated a reduction of GABA uptake in the lateral habenula after lesions of the stria medullaris but make no mention of the origin of the neurons which have axons travelling in this fiber bundle to the habenula (Gottesfeld and Jacobowitz, 1978).

The experiments involving the EP and SN efferent projections to the thalamus met with little success. Among the reasons for this are the geometrically compounding difficulties of the production of successful lesions, the dissection of appropriate nuclei, and the interpretation of negative data. One fairly consistent observation which should be noted is that after SN lesions there occurred a reduction in CAT in the ventral-lateral aspect of the
ventral-lateral, ventral-anterior thalamic complex (the projection area of nigro-thalamic fibers in the rat (Carter and Fibiger, 1978).

The scarcity of knowledge of the neurotransmitters involved in the pallidal and nigral efferents to the thalamus warrants a major thrust of investigation in this direction.

V The Localization of Enzymes in the Substantia Nigra

It was the object of the present experiments to use kainic acid as a research tool rather than to test its selectivity of action. However, some comments on its selectivity are warranted as there are still doubts concerning the extent to which certain neuronal elements suffer the lethal effects of kainic acid. The observed destruction of neurons in the SN following the present intranigral injections of kainic acid is in accord with earlier work which showed this compound to be a neurotoxic agent for neuronal perikarya and dendrites (Olney et al, 1974; Coyle and Schwarcz, 1976; Schwarcz and Coyle, 1977a, 1977b; Schwarcz et al, 1977; McGeer and McGeer, 1977). To date, histochemical and biochemical analysis of neuronal tissue which has been exposed to kainic acid suggests that minimal damage to terminals and axons of passage occurs. Kainic acid injections into the striatum have been found to affect only those neurochemical parameters which are thought to be located in neuronal elements having cell bodies within this nucleus. The functional integrity of terminals at the site of a kainic acid injection has not yet been fully assessed, and it is possible that removal of the target neurons of an axon terminal will produce functional changes in that terminal, as in the case of the decreased $K_m$ of TH for its cofactor observed in dopaminergic terminals in the striatum following kainic acid lesions of this nucleus (Schwarcz and Coyle, 1977a).

With regard to the degree of neuron loss in the SN after kainic acid injections into this structure, both histological analysis and TH activity mea-
surements indicated that this was virtually complete. It is noteworthy that the present decrease of 94% of TH activity in the striatum was substantially greater than that obtained in previous investigations (McGeer and McGeer, 1976; Schwarcz and Coyle, 1977b). This may be due to differences in the exact location of the kainic acid injection. In preliminary investigations while conducting simultaneous histological and biochemical assessment of various stereotaxic coordinates, large variations were found in the extent of the kainic acid lesion. The most extensive decreases in striatal TH were observed after injections directly into the SNC. These were the coordinates utilized in the present experiments.

Keeping the above considerations in mind, some conclusions can be drawn as to the cellular localization of the neurotransmitter-related enzymes investigated here. From the present experiment involving hemitranssections between the SN and striatum, it appears that 84% of nigral GAD originates in the striatum. If the remaining 16% is contained in nigral interneurons, then the 40 to 50% reduction in GAD seen in the SN after intranigral kainic acid injections suggests a maximum damage to GAD-containing terminals in the SN of 25 to 35%. Alternatively, long term compensatory changes in activity due to removal of postsynaptic elements may be a contributory factor in the decrease in nigral GAD. This is supported by the observations that at 2-3 days (McGeer and McGeer, 1976) and at 5 days (Schwarcz and Coyle, 1977b) after intranigral kainic acid injections there was no change in nigral GAD, albeit these studies showed a smaller decrease in TH activity in the SN and striatum than that observed here. A still more speculative alternative for the large kainic acid-induced reductions in GAD is as follows. The hemitranssections between the SN and striatum and the kainic acid nigral lesions were both precariously close to the SUT which is known to project to the SN. If this projection is GAD-containing and both lesions did encroach on the SUT, then the striatal contribution to nigral GAD would be lower than suspected and the larger kainic acid-
induced nigral GAD reductions would be partly explained. This possibility is currently under investigation. Incidentally, if this speculation were to be correct, this would alter the previous discussions of the nigral GAD-containing afferents from the striatum only quantitatively and not qualitatively.

The CAT activity in the SN was unaffected by kainic acid. This suggests that CAT is not contained in neuronal cell bodies which send efferent projections from the SN or in interneurons of the SN since in either case kainic acid would have reduced this activity. Rather, it appears that CAT is contained in terminals which have their origin outside the SN. At present the sources of CAT-containing afferents to the SN are not known.

Injections of kainic acid into the SN caused a reduction in AChE activity of 64% in this nucleus. This suggests that a little over half the AChE activity in the SN is located in neurons whose cell bodies lie within the SN. Destruction of the dopaminergic neurons of the SN by injections of 6-hydroxydopamine into the NSP has been shown to reduce AChE activity in the nigra by 40% (Lehmann and Fibiger, 1978). Taken together, these data suggest that about 40% of the AChE in the SN is present in dopaminergic neurons, about 20% in other types of neurons originating in the SN, and about 40% in terminals afferent to the SN which were spared by kainic acid.

Finally, a note of caution regarding the intracerebral injections of kainic acid. The results of the present study indicate that tissue shrinkage may be an important factor at the injection locus as the SN showed visible shrinkage following intranigral injections of kainic acid. This could alter the specific activity of enzymes located on terminals not affected by this compound.

VI The Localization of Dopamine Activated Receptors and Adenylate Cyclase

a) Dopamine-sensitive adenylate cyclase in the substantia nigra

The absence of DAC on DA-containing nigral neurons (Kebabian and Saavedra,
1976; Premont et al, 1976), the reservations this caused regarding the existence of DA autoreceptors together with the novel suggestions of the presence of DAC on nigral axon terminals (Gale et al, 1977a; Spano et al, 1977; Philipson et al, 1977), and the dendro-axonic DA transmission this implied warranted further investigations of the cellular localization of DAC in the SN. For example, a decrease in nigral DAC resulting from retrograde or transneuronal changes in nigral neurons after lesions anterior to the SN could not be ruled out. Kainic acid lesions of the SN circumvent possible interpretational problems caused by these processes.

The effects of these lesions on various enzyme systems in the SN was described in the previous section. The present data obtained on DAC in kainic acid lesioned SN are consistent with previous findings that this enzyme activity is not localized on dopaminergic cell bodies (Kebabian and Saavedra, 1976; Premont et al, 1976), but rather on terminal afferents to the SN (Gale et al, 1977a; Spano et al, 1977). With the nearly complete loss of cell bodies in the SN obtained in the present study it can be further concluded that no neuronal perikarya or dendrites in the SN possess a dopamine-sensitive adenylate cyclase. DA was able to stimulate adenylate cyclase in the lesioned SN to the same extent as in control SN. In fact, when expressed on the per protein basis, the stimulation of adenylate cyclase by dopamine was significantly greater in the lesioned SN compared to the contralateral control. Although it did not reach significance in the present study, this trend toward greater dopamine stimulation in the lesioned side is also evident if the data are presented on a per SN basis, indicating that tissue shrinkage may not fully explain this increase. This may indicate a supersensitive response of adenylate cyclase to dopamine transmission as has previously been observed in the striatum (Mishra et al, 1976; Krueger et al, 1976). It is unlikely that DAC is present on glial elements within the nigra since the DAC in the striatum is dramatically reduced (McGeer et al, 1976; Schwarcz and Coyle, 1977a) after
kainic acid striatal lesions despite massive gliosis in this structure. Thus, the observation that DAC did not decrease following destruction of nigral perikarya is consistent with previous suggestions that dendritically-located dopamine may contribute to the regulation of cyclic AMP synthesis in afferent terminals in the SN (Reubi et al, 1977). The implications these findings have on DA autoreceptors in the SN are discussed in section VI-c. of the Discussion.

b) Pre- and post-synaptic dopamine receptors in the substantia nigra and striatum

The two types of lesions employed in the present study to determine the localization of neurotransmitter receptors were 6-OHDA lesions of the NSP and kainic acid lesions of the striatum. The effect of these lesions on various enzyme systems are as follows. In the striatum, 6-OHDA lesions of the NSP caused a greater than 90% loss of TH, no change in CAT activity and a significant increase in striatal GAD. The increase in striatal GAD after these lesions confirms previous reports of this (Vincent et al, 1978; Saavedra et al, 1978). The activities of GAD and CAT in the SN and striatum following striatal kainic acid injections are for the most part similar to previous reports (Schwarcz and Coyle, 1977a, 1977b; McGeer and McGeer, 1976; Coyle and Schwarcz, 1976). The significant 18% increase in CAT in the striatum contralateral to the kainic acid lesioned striatum compared to saline injected controls indicated that compensatory mechanisms may be operative in this structure. While these alterations in CAT activity may be related to the changes in muscarinic cholinergic receptors which have been demonstrated in the striatum contralateral to 6-OHDA lesions of the SN (Kato et al, 1978), the mechanisms underlying these changes remain undefined. With regard to TH, there have been previous observations of significantly increased activities of this enzyme 2 days after striatal kainic acid lesions (Coyle and Schwarcz, 1976; McGeer and McGeer, 1976) and normal activities after 21 days (Schwarcz and Coyle, 1977a). In the present study, however, after a survival time of 30 days, there was a 19% de-
crease in striatal TH activity. This observation is consistent with the decrease in DA levels (Friedle et al, 1978) and reduction of DA histofluorescence (Meiback et al, 1978) which occurs at about 2 weeks after these lesions. After kainic acid lesions of the striatum it has been shown that TH is activated having decreased $K_m$ for its cofactor (Schwarcz and Coyle, 1977a). The present TH assays were conducted at saturating cofactor and substrate concentrations. The concentrations of cofactor and substrate employed in the TH assays of the above reports could not be ascertained. In any case, the loss in DA-containing structures in the kainic acid lesioned striatum represented by TH is quite modest relative to the loss of neurons represented by GAD and CAT. In summary, the 6-OHDA lesions resulted in a profound loss of dopaminergic elements in the SN and striatum while the kainic acid striatal lesions eliminated postsynaptic neurons in the striatum and some of the terminals of these projections in the SN.

Apomorphine has been well characterized as a DA agonist and haloperidol and spiroperidol as DA antagonists. The validity of employing these compounds in the assay of the DA receptor has been previously demonstrated (Seeman et al, 1976; Leysen et al, 1978; Thal et al, 1978). The observation that selective and extensive retrograde loss of DA neurons in the SN reduced $^3$H-apomorphine binding in this structure by 76% is a direct demonstration of the existence of DA receptors on these perikarya. The fact that nigral TH activity was reduced by greater than 90% whereas nigral $^3$H-apomorphine binding was reduced by only 76% suggests that there are also DA receptors on other than dopaminergic neurons. The reduction by 56% of $^3$H-apomorphine binding after virtually complete anterograde degeneration of dopaminergic elements in the striatum provides direct support for the presence of DA receptors on DA-containing terminals. The Scatchard analysis of striatal $^3$H-apomorphine binding further indicates that both the affinity and maximal number of receptors is reduced after NSP lesions. The reduction in the affinity of $^3$H-apomorphine
for the DA receptor is consistent with behavioral evidence for a presynaptic action of low doses of apomorphine (Carlsson, 1975; Strombom, 1975). For example, it is apparent from the present data that both pre- and post-synaptic binding of apomorphine occurs in the striatum. It is also probable that striatal apomorphine receptor affinity is a composite of binding to the two types of receptor. Thus, elimination of a higher affinity presynaptic receptor would result in a greater relative representation of the lower affinity postsynaptic receptor and hence a higher Kd for apomorphine after the NSP lesions.

In marked contrast with the reduction in $^3$H-apomorphine binding in the striatum, NSP lesions caused a significant increase in $^3$H-haloperidol and $^3$H-spiroperidol binding. This suggests that the binding of these ligands occurs primarily to postsynaptic elements in the striatum. This observation may have some relevance to the reports of enhanced behavioral effects of directly-acting DA agonists such as apomorphine after lesions of the NSP or after chronic treatment with neuroleptics (Ungerstedt, 1971b; Price and Fibiger, 1974; Christensen et al, 1976). It is known (Creese et al, 1977; Burt et al, 1977; Muller and Seeman, 1977) that these treatments produce an increase in the number of striatal DA receptors assayed with $^3$H-haloperidol. The present results confirm the increase in striatal $^3$H-haloperidol and $^3$H-spiroperidol binding after NSP lesions, and support the possibility that this increase may be related to the enhanced behavioral effects of DA agonists after NSP lesions.

The present observations regarding $^3$H-spiroperidol binding in the SN pose some interpretational difficulties. At the higher concentration of $^3$H-spiroperidol examined, NSP lesions had no effect on the binding of this ligand. However, at the lower concentration $^3$H-spiroperidol binding was significantly reduced by 42%. The simplest explanation of these results is that $^3$H-spiroperidol binds preferentially to DA receptors located on non-dopaminergic elements in the SN and that the NSP lesions caused an affinity change in these
receptors. Rather than invoke some of the other possible explanations at this time, it would be more suitable to pursue this first possibility by conducting Scatchard analysis of $^3$H-spiroperidol binding in the SN after NSP lesions. In view of these results in the SN and the fact that the binding of DA antagonists increased in the striatum after NSP lesions, the present study provides no information as to the extent DA antagonists bind to dopaminergic neurons in either of these structures. However, that such receptors may be demonstrated with more rigorous investigations is suggested by the observation that kainic acid lesions of the striatum do not diminish the ability of haloperidol to influence DA metabolism in the striatum (Di Chiara et al, 1977).

A further potential site of $^3$H-spiroperidol binding is to DA receptors thought to be located on terminals of striatal afferents to the SN. However, in the present study, kainic acid striatal lesions had no effect on this binding. This result is inconsistent with the observation that DA antagonists block the DA evoked release of GABA from nigral slices (Reubi et al, 1977). Thus, it is possible that striatal kainic acid lesions induced affinity changes of DA-receptors on nigral terminals. Alternatively, the kainic acid lesions may not have destroyed those neurons in the striatum which send DA-bearing receptors to the SN. It is noteworthy in this regard that these lesions tend to be located anteriorly in the striatum and reduce nigral GAD by 42%, whereas it is lesions involving the posterior striatum that decrease DAC activity in the SN (Spano et al, 1977). Further studies are required to determine whether nigral afferents originating from the posterior regions of the striatum contain $^3$H-spiroperidol binding sites. It is interesting that the results of the attempts to localize the source within the striatum of nigral afferents containing DA-receptors and DAC parallel remarkably the studies of the origin of nigral GAD. It is tempting to speculate that the striatal-nigral neurons whose terminals in the SN contain GAD, DAC and DA receptors are one and the same.
c) Dopamine transmission in the substantia nigra and striatum: synthesis and speculation

The present observations of the differential effects of NSP lesions on the binding to striatal membranes of \(^3\)H-apomorphine, on the one hand, and \(^3\)H-haloperidol and \(^3\)H-spiroperidol, on the other, have certain implications regarding the nature of DA receptors. Creese et al (1975) have shown that in studies with receptors that have bound DA antagonists, DA agonists compete less effectively for binding than DA antagonists. Conversely, DA antagonists compete less effectively than DA agonists for receptors that have bound DA agonists. Rather than postulate the existence of two types of DA receptor, Creese et al (1975) suggested that a single receptor could exist in two forms, an agonist and an antagonist state. The present experiments cast doubt on this interpretation, as \(^3\)H-apomorphine and \(^3\)H-neuroleptic binding in the striatum would have been expected to show the same relative changes after the NSP lesions. However, this was not the case; in fact, striatal binding of the two ligands changed in opposite directions. It remains possible, of course, that the lesions locked the receptors into an antagonistic state. However, this alternative would be difficult to reconcile with the known potentiation of the behavioral effects of apomorphine after NSP lesions (Ungerstedt, 1971b; Price and Fibiger, 1974). Another possibility is that there are two types of DA receptor, both located postsynaptically. In this case, NSP lesions might change the relative numbers of each type of receptor. Again, however, an increase in \(^3\)H-haloperidol and \(^3\)H-spiroperidol receptors accompanied by a decrease in \(^3\)H-apomorphine receptors, together with the lower affinity of apomorphine for the antagonist receptor, would be difficult to reconcile with the above behavioral observations.

An alternative interpretation of the findings of Creese et al (1975) can be proposed in light of the present data. It appears that there may be two types of DA receptors; those which interact preferentially with apomorphine
and those which interact preferentially with neuroleptics. It may also be true that each of these ligands can bind to the unfavored form of the receptor. In the SN and striatum apomorphine-type receptors exist on dopaminergic neurons and terminals, respectively. In addition, these receptors may also be present on postsynaptic neurons in the striatum and axon terminals in the SN. Since neither dopaminergic neurons or terminals possess DAC (Kebabian and Saavedra, 1976; Premont et al, 1976), the apomorphine-type DA receptors on these structures appear to function independently of this enzyme and thus, cAMP. In contrast, neuroleptic receptors, at least in the striatum and possibly in the SN, are located on nondopaminergic elements, as is true of DAC in these brain regions (McGeer et al, 1976; Schwarcz and Coyle, 1977a; the present study). Thus, neuroleptic-type DA receptors in the SN and striatum may be coupled to DAC. These conclusions do not exclude the possibility that there may be adenylate cyclases on dopaminergic structures that function independently of dopamine and that some DA receptors on nondopaminergic elements may function independently of adenylate cyclase.

It is probably a safe estimate that the function(s) served by the SN-VTA DA system has received generous consideration in armchair speculations. Unfortunately, a unified theory of the function of these systems in the brain has not emerged. On the basis of available information any such theories would be just short of sheer fabrication. Suffice it to say that an hypothesis of DA function would have as a major consideration the stringent and multilevel control to which DA neurons are subject.

CONCLUSIONS

The theme of the present thesis was the study of the anatomical and biochemical interactions within and between various nuclei of the basal ganglia. The goal, to determine, at various levels, some of the circuitry of this part of the brain, was met with varying degrees of success. The results of some of
of the experiments conducted were fairly conclusive while others leave open several interpretations and therefore require further investigations. It should be added that the results of numerous experiments, some of which were rather novel but all of which were miserable failures, were not included. In short, the basal ganglia has been a highly complex and challenging proving ground.

Since its inception about a decade ago, biochemical neuroanatomy has served well in adding to the understanding of the neurotransmitters in and connections of the basal ganglia. It is clear, however, that in order to continue to be powerful in prying loose information about the brain, the family of techniques under this heading must undergo major refinements, since, relatively speaking, only a glimpse of the total picture has been gained to date. The present investigations, then, together with similar ones conducted simultaneously by others, marks the end of an era and the limit to which the current state of the methodology involved can be taken to afford answers concerning the biochemical connectivity of the basal ganglia. It will be interesting to observe what new innovations necessity will demand.

It is somewhat dissatisfying that, after more than four Bic pens, no conclusions were and can be drawn regarding functions of the basal ganglia nuclei and the systems investigated here; at least none that this author would want to be cited for, which may or may not be a reasonable criterion for deciding such matters. In any case, this outcome suggests a direction for future research. On the one hand, no amount of knowledge regarding brain circuitry at the biochemical or anatomical level is too much. On the other, the question becomes how much information and what degree of detail is needed before precise computer-like functions can be ascribed to specific cell groups of the brain? The nigro-striatal DA system may be used as an example here. Is it a system that matches real time with time required for computation? Does it
deal with frequency-response limitations in neural networks? Does it interface convergent information; for example, cortical and thalamic input? Does it regulate signal to noise ratio? Hopefully, these types of questions will be testable in the not too distant future.
REFERENCES


Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the


Feltz, P. and DeChamplain, J. (1972) Persistence of caudate unitary responses to nigral stimulation after destruction and function impairment of the striatal dopaminergic terminals. Brain Res. 43, 595-600.


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


Gilman, A.G. (1970) A protein binding assay for adenosine 3',5'-cyclic mono-


Fallon, J.H., Riley, J.N. and Moore, R.Y. (1978b) Substantia nigra dopamine


Kelly, P.H. and Moore, K.E. (1978) Decrease of neocortical choline acetyltrans-
ferase after lesion of the globus pallidus in the rat. Exp. Neurol. 61, 479-484.


Koob, G.F., Balcom, G.J. and Meyerhoff, J.L. (1975) Dopamine and norepinephrine levels in the nucleus accumbens, olfactory tubercle and corpus striatum following lesions in the ventral tegmental area. Brain Res. 94, 45-55.


Mensah, P. (1977) The internal organization of the mouse caudate nucleus:


Muller, P. and Seeman, P. (1977) Brain neurotransmitter receptors after long-term haloperidol: Dopamine, acetylcholine, serotonin, α-noradrenergic and naloxone receptors. Life Sci. 21, 1751-1758.


Parizek, J., Hassler, R. and Bak, I.J. (1971) Light and electron microscopic autoradiography of substantia nigra of rat after intraventricular administra-
tion of tritium labelled norepinephrine, dopamine, serotonin and the precur-
sors. Z. Zelforsch. 115, 137-148.

raphe, substantia nigra and locus coeruleus: Interconnections with each

Paxinos, G., Emson, P.C. and Cuello, A.C. (1978) Substance P projections to
the entopeduncular nucleus, the medial preoptic area and the lateral septum.

Phillipson, O.T. (1978) Afferent projections to A10 dopaminergic neurons in
the rat as shown by the retrograde transport of horseradish peroxidase.
Neurosci. Lett. 9, 353-359.

Phillipson, O.T., Emson, P.C., Horn, A.S. and Jessell, T. (1977) Evidence con-
cerning the anatomical location of the dopamine stimulated adenylate cyclase
in the substantia nigra. Brain Res. 136, 45-58.

Phillipson, O.T. and Horn, A.S. (1976) Substantia nigra of the rat contains a

Phillis, J.W. and Limacher, J.J. (1974) Substance P excitation of cerebral cor-
tical Betz cells. Brain Res. 69, 158-163.

characteristics of the acetylcholinesterase-containing neurons in the CNS of

Brain Res. 105, 389-403.

Psychiatry 30, 140-153.

Premont, J., Thierry, A.M., Tassin, J.P., Glowinski, J., Blanc, G. and
Bockaert, J. (1976) Is the dopamine sensitive adenylate cyclase in the rat

29, 249-252.

I. Synaptic potentials and discharge characteristics of caudate
neurons activated by thalamic stimulation. Brain Res. 6, 325-340.

Rafols, J.A. and Fox, C.A. (1976) The neurons in the primate subthalamic nu-
cleus: A golgi and electron microscopic study. J. Comp. Neurol. 168,
75-112.

Ranson, S.W. and Ranson, S.W. (1941) Efferent fibers of the corpus striatum.

Reis, D.J., Gilad, G., Pickel, V.M. and Joh, T.H. (1978) Reversible changes in
the activities and amounts of tyrosine hydroxylase in dopamine neurons of
the substantia nigra in response to axonal injury as studied by immunochemi-


Royce, G.J. (1978) Autoradiographic evidence for a discontinuous projection to the caudate nucleus from the centromedian nucleus in the cat. Brain Res. 146, 145-150.


Simke, J.P. and Saelens, J.K. (1977) Evidence for a cholinergic fiber tract connecting the thalamus with the head of the striatum of the rat. Brain Res. 126, 487-495.


Spencer, H.J. (1976) Antagonism of cortical excitation of striatal neurons by
glutamic acid diethyl ester: Evidence for glutamic acid as an excitatory transmitter in the rat striatum. Brain Res. 102, 91-101.


Westfall, T.C. (1974) Effect of muscarinic agonists on the release of $^3$H-noradrenaline and $^3$H-dopamine by potassium and electrical stimulation from rat


