ELECTROPHYSIOLOGICAL AND NEUROPHARMACOLOGICAL STUDIES IN THE STRIATUM OF THE RAT

by

John Robert Brown

Thesis presented for the degree of
Doctor of Philosophy in the
University of Edinburgh

Department of Pharmacology
University of Edinburgh
Medical School

August 1981

Dedicated

to

Mum and Dad

Neuronal Ambiguity

It used to be that one could tell
A neurone from another cell,
By shape, and other traits as well,
For messengers would ring its bell;
No other cells had that response,
Not for a milliseconds nonce.

But now the situation's hectic
Other cells turn on, electric,
And in neurones, dialectic
Processes are now eclectic;
Dendrites with axonal lapses,
Shamelessly commit synapses.

Neurones eager for a feat
No longer happy to deplete
Their vesicles at junctions, cheat
And, into cleft or vein, secrete
Their products, showing no decorum
With regard to where they pour 'em.

In misnamed "cultures", progeny
Of dubious phylogeny
Parade their heterogeny.
Of cellular androgeny
As moral eyes with horror see a
Range of hybrids, nerves cum glia.

Oh for a Becket or a More
To draw the line at and deplore
Perversions classicists abhor
And make all as it was before
When form and function let one tell
A neurone from another cell.

Theordore Melnechuk

Statement in terms of Ph.D. Regulation 2.4.15
of the Postgraduate Regulations of the University of Edinburgh

I declare that this thesis was totally composed by myself, and that all the experimental work described herein was performed by myself with the following exception.

The dopamine assay in Chapter VI was performed by Mrs. I. Ritchie.

John R. Brown

August 1981

Statement in terms of Ph.D. Regulation 2.4.11
of the Postgraduate Study Programme of the University of Edinburgh

Some of the results presented in this thesis have been published as follows:

Brown, J.R. and Arbuthnott, G.W.

Neuroscience Letters, Supp. 5 (1980) p. 334. Effects of dopamine on the cortico-striatal pathway.

Brown, J.R., Mayer, M.L. and Arbuthnott, G.W.

Journal of Neuroscience Methods (1980) 3, p. 203-204. The use of ultra-violet setting glue for microelectrode fabrication.

Brown, J.R. and Arbuthnott, G.W.

Abstract accepted for ENA Congress 1981. D_2 receptors and cortico-striatal transmission.

Brown, J.R. and Arbuthnott, G.W.

Poster presentation at BRA Easter Conference 1981. The after-effects of activity in two groups of neurones in the basal ganglia.

ACKNOWLEDGEMENTS

I would like to thank the Medical Research Council for their support, and for the facilities I was provided with in the Department of Pharmacology and in the MRC Brain Metabolism Unit.

Particular thanks to Dr. Dan McQueen who made it possible for me to study for a PhD, and to Dr. Gordon Arbuthnott for his constant help, encouragement and friendship throughout the last four years.

My thanks to all the staff in the Unit for much help and friendship; especially to Anne, Marianella, Margot, Issie, Susan, Gail and Imre Laszlo.

I remain grateful to Mrs. Jean Hunter and all the animal house staff for supplying and caring for all my animals.

My thanks to Mrs. Frances Anderson for her careful and speedy typing of this thesis.

Finally, special thanks to Carol for her love and everything that we have shared.

ABBREVIATIONS

GLU	-	Glutamate
ASP	-	Aspartate
DA	-	Dopamine
GABA	-	Gamma-amino-butyric acid
ACh	-	Acetylcholine
mV	-	millivolt
nA	-	nano amperes
msecs	-	milliseconds
secs	-	seconds
mins	-	minutes
6-OHDA	-	6-hydroxydopamine
μ	-	micron

ABSTRACT OF THESIS

Extracellular electrical recordings have been obtained from single neurones in the striatum of the rat, to investigate the interaction of the cortico-striatal and nigro-striatal projection systems. The study is in two main parts:

1. An electrophysiological characterization of the responses of striatal neurones to cortical stimulation and of the neurotransmitter involved.
2. The effects of dopamine (DA) agonist and antagonist drugs on the spontaneous, glutamate (GLU)-evoked, and cortically-evoked, activity of striatal neurones, with particular reference to the pre-synaptic DA receptors reported to be present on the terminals of the cortical efferents.

The electrophysiological characteristics of both spontaneous and cortically evoked firing of striatal neurones in the halothane anaesthetized rat are similar to those reported previously from studies in which other anaesthetics were used. Striatal output neurones were identified by antidromic stimulation from the crus cerebri and exhibited the phenomenon of supernormality. The excitant amino-acids, Glutamate (GLU) and Aspartate (ASP), have been proposed as candidates for the cortico-striatal transmitter. This hypothesis was tested by examining the effects of iontophoretically applied amino acid antagonists on the amino acid and cortically evoked excitations of striatal neurones. While the results are in support of an amino acid being the transmitter it was not possible to distinguish GLU or ASP.

Iontophoretically applied DA was found to have a depressant action on spontaneous glutamate-evoked, and cortically evoked activity on the majority of striatal neurones. No excitations were observed in response to DA. However, it was not possible to distinguish between the pre- and post-synaptic effects of DA with the agonists. The DA antagonist sulpiride was found to facilitate cortico-striatal transmission but had no effect on the spontaneous activity of striatal neurones. It is proposed that there is a tonic inhibition of cortical transmitter release by DA. The characteristics of cortico-striatal transmission were tested in rats with a 6-OH-DA lesion of their nigro-striatal pathway. In lesioned rats there was an increase in the number of spontaneously active cells on the lesioned side and the threshold stimulation current for cortically evoked activity was reduced.

CONTENTS

		Page
CHAPTER I:	GENERAL INTRODUCTION	
	Introduction	1
	Literature Review	
1a	The cortico-striatal pathway	2
1b	The nigro-striatal pathway	9
1c	The raphe-striatal pathway	13
1d	The thalamo-striatal pathway	15
1e	Other striatal afferents	18
2	Striatal neurone types	22
3	Striatal efferents	28
4	Aim of the investigation	34
CHAPTER II:	GENERAL METHODS	35
CHAPTER III:	THE ELECTROPHYSIOLOGY OF THE CORTICO-STRIATAL PATHWAY	
	Introduction	52
	Results	55
	Discussion	80
	Summary	86
CHAPTER IV:	NEUROPHARMACOLOGY OF THE CORTICO-STRIATAL PATHWAY	
	Introduction	87
	Methods	93
	Results	95
	Discussion	101
	Summary	105

Page

CHAPTER V: THE EFFECTS OF DOPAMINE IN THE RAT STRIATUM

Introduction 106
Methods 119
Results 120
Discussion 135
Summary 143

CHAPTER VI: THE EFFECTS OF 6-OHDA LESIONS OF THE NIGRO-STRIATAL PATHWAY ON CORTICO-STRIATAL TRANSMISSION

Introduction 144
Methods 145
Results 148
Discussion 153
Summary 154

CONCLUSION 155

BIBLIOGRAPHY 157

LIST OF FIGURES

Fig.		Page
1	Afferent fibres to the striatum.	21
2	Spiny striatal neurones.	24
3	Aspiny striatal neurones.	25
4	Striatal efferent pathways.	33
5	The recording area.	37
6	Location of cortical stimulating electrode.	38
7	Location of crus cerebri stimulating electrode.	39
8	The iontophoretic electrode.	41
9	Diagram of recording equipment.	45
10	Photo of stained recording area.	49
11	High power photo of recording site.	50
12	Photo of crus electrode site.	51
13	Bursting activity in striatal neurones.	57
14	Interval histogram.	58
15	Cortically evoked action potential from a striatal neurone.	60
16	Cortically evoked action potentials from a striatal neurone.	61
17	The distribution of PSTH modal values.	62
18	Oscilloscope trace of PSTH.	63
19	PSTH of a striatal neurone.	64
20	Double shock stimulation of a striatal neurone.	65
21	Antidromic spike from a cortical neurone.	67
22	Antidromic spike from a striatal neurone.	68
23	The collision test.	69
24	Distribution of antidromic response latencies.	71
25	Depth distribution of antidromically driven cells.	72
26	A pair of cells responding to cortical stimulation.	73

Fig.		Page
27	The supernormal effect.	75
28	Antidromic stimulation at 100 Hz.	76
29	Interaction of cortical stimulation with crus cerebri stimulation.	77
30	Time course of supernormality.	78
31	Crus cerebri stimulation and glutamate induced firing.	79
32	Chemical structures of new amino acid antagonists.	91
33	Blockade of GLU and ASP excitations by APV.	98
34	Differential blockade of GLU and ASP by APV.	99
35	Antagonism of the cortically evoked excitation of a striatal neurone by γ DGG.	100
36	Selective antagonism of the ASP response by PDA.	102
37	The effect of iontophoretically applied DA on spontaneous neuronal firing.	121
38	Depression of firing by different iontophoretic ejection currents of DA.	122
39	The effect of iontophoretically applied DA on GLU-induced firing.	124
40	The effect of iontophoretically applied DA on the response to cortical stimulation.	127
41	The effect of DA on GLU and ACh excitations.	131
42	The effect of sulpiride on the response to cortical stimulation.	133
43	Summary of the effect of sulpiride on cortical stimulation.	134
44	A 6-OHDA lesioned rat in a rotometer.	147
45	Comparison of INTs from 6-OHDA lesioned and control striata.	150

LIST OF TABLES

Table		Page
1	Striatal neurone firing.	56
2	Summary of results with amino-acid antagonists.	96
3	The effects of nigral stimulation on striatal neurones.	109
4	The effects of iontophoretically applied DA on striatal neurone firing.	113
5	DA inhibitions of striatal neuronal firing.	125
6	Correlation of the effects of iontophoretically applied DA on striatal neuronal activity.	128
7	The effect of iontophoretically applied DA on antidromically identified striatal output neurones.	129
8	Summary of the types of DA inhibitions of striatal neuronal activity.	136
9	Summary of the control of neurotransmitter release in the striatum.	141
10	Comparison of data from 6-OHDA lesioned and unlesioned striata.	149
11	Comparison of cortical stimulation currents for spontaneously active and silent neurones in 6-OHDA lesioned and control striata.	152

CHAPTER I

General Introduction

INTRODUCTION

The striatum is by far the largest sub-cortical cell mass in the mammalian brain. It has been studied extensively for over a century, but particular attention has been focussed on it in recent years following the description of its involvement in Parkinson's Disease (Ehringer and Hornykiewycy, 1960). The loss of striatal dopamine in this disease causes severe impairment of motor function. The experiments in this thesis have been designed to investigate electrophysiologically the actions of dopamine on: a) neuronal activity in the striatum, and b) the transmission of information from the cortex to the striatum.

The following literature describes the development of the current anatomical and biochemical knowledge which is necessary for the interpretation of electrophysiological experiments.

Definition:

The term striatum does not exist in the offical Nomina Anatomica (Kopsch, 1957). Instead, the approved term is corpus striatum, which includes the caudate nucleus, the putamen, and the globus pallidus. In most non-primates the caudate and the putamen are not clearly separated, and the cellular homogeneity of these two nuclei has led to them being classified as one, even in animals where the internal capsule forms a clear division between them. Thus the term striatum has come to describe the caudate and putamen collectively, and will be used in this context throughout this thesis.

LITERATURE REVIEW

This review is divided into three main parts: 1) afferents to the striatum, 2) striatal cell types, and 3) striatal efferent fibres.

1a. The cortico-striatal pathway

A cortico-striatal pathway composed of axon collaterals from the cortico-fugal fibres in the internal capsule was first demonstrated by Cajal (1891). Since this initial description there has been considerable debate about the existence of this pathway. Many of the contradictory reports came from the use of the Marchi method (1886) for visualizing the degeneration of myelinated axons, and from the variety of experimental animals used. The existence of the pathway was supported by evidence obtained in cats (Bianchi, 1914; Poljak, 1927), rabbits (Coenen, 1929) and rhesus monkeys (Mettler, 1935; Mettler, 1942). On the contrary, investigations with the Marchi method in the rat (Le Gros Clark, 1933; Kneg, 1947) and monkey (Levin, 1936; Verhaart and Kennard, 1940; Krieg, 1954) all refuted the existence of a direct cortico-striatal connection.

Wilson (1914), studying monkeys, found no evidence of Cajal's collaterals, and, suggested that as the cortico-fugal fibres passed through the striatum, all the observed cortical degeneration was due to the interruption of fibres of passage. Until the advent of other neuroanatomical techniques, this view could not be disproved. Furthermore, it was also impossible to decide whether the axons shown running perpendicularly from the internal capsule were efferent from the striatum, afferents from the cortex, or afferent collaterals of the ascending thalamo-cortical system.

From the review by Mettler (1942) it appears that by 1942 there was a consensus of opinion in favour of a cortico-striatal pathway projecting from the cortex via the subcallosal fasiculus (Poljak, 1927; Mettler, 1942).

Electrophysiological experiments by Dusser de Barenne and co-workers (Dusser de Barrene, Garol and McCulloch, 1942) added to the evidence in favour of the pathway. This work was based on the detection of electrical activity in sub-cortical structures after local stimulation of the cortex by the application of pieces of filter paper soaked in strychnine solution. The authors named this technique "physiological neuronography" and claimed that it was useful for "delimiting the axonal distribution of nerve cells in any grey mass of the central nervous system". During the mapping of cortico-cortical connections Dusser de Barenne *et al* discovered a small strip of cortex which when stimulated electrically, mechanically or chemically caused a transient diminution of electrical activity, initially in area 4 of Brodman and subsequently in the rest of the cortex. This area was named 4S - S for suppression - and was found to correspond to the strip area previously reported by Hines (1937), to cause a relaxation of existing muscular contractions.

The suppression of electrical activity occurred with a latency of approximately four minutes and could be prevented by undercutting the cortex. These findings led to the prediction that the effects were mediated by a cortico-thalamo-cortical pathway. From lesion experiments it was found that the cortical output from area 4S proceeded via the caudate nucleus rather than the thalamus. A systematic investigation of the cortico-striatal projection led to the discovery of three other suppression areas - 8S, 2S and 19S - which projected

to the caudate nucleus, and also the finding that areas 4 and 6 of motor cortex projected to the putamen. Similar projections were found in the cat (Garol, 1942). The inability of workers in Dusser de Barenne's laboratory to find degeneration in the caudate after a cortical lesion of areas 4, 6 and 4S (Verhaart and Kennard, 1940), was interpreted as meaning that the cortico-fugal axons lose their myelin sheath on entering the striatum. This was confirmed by the use of a modified silver stain in material from cats and rabbits (Glees, 1944). Degenerating axons could be demonstrated leaving the internal capsule after lesions of the suppressor areas, and Glees also found no evidence for a projection from the motor areas 4 and 6 to the caudate.

Dusser de Barenne *et al's* experiments were scrutinized in 1952 by Druckman (1952) who made several criticism's of their experimental technique. He comments on the variability of the monkey cortical surface, and the small number of lesions which were performed. He claimed that it was impossible to localize the projection into strips. The work of Gellhorn (1947) and Leao (1944, 1947) cast further doubt on the validity of Dusser de Barenne's work.

Gellhorn showed that suppression could be elicited from a single cortical point at varying times but not at others. He also found that suppression effects were more easily demonstrated eighteen or more hours after the initial surgery of the experiment. Some of Dusser de Barenne's animals were maintained alive for eighty-four hours.

Leao reported a phenomen which he named "spreading depression". This referred to a diminution of electrical activity in the cortex which could be elicited from, and spread to, any area of cortex. The

time-course of spreading depression was very similar to the effects reported by Dusser de Barenne.

Further evidence for Druckman's thesis came from the work of Sloan and Jasper (1950), who showed that the effects of strychnine on the electro-encephalogram were identical whether it was applied to suppressor or non-suppressor areas. They also showed that the strychnine effects on the EEG were present after undercutting the cortex, thus making the postulation of sub-cortical pathways unnecessary for the phenomenon of suppression.

Despite the acceptance of the criticisms the concept of a cortico-striatal projection arising from the strip areas and motor areas 4 and 6 was widely held (Jung and Hassler, 1960) until the work of Webster (1961).

Webster employed a variety of histological methods for staining degenerating fibres in albino rats. Using a modified Marchi method (Swank and Davenport, 1935) he was unable to show any degeneration within the striatum after large lesions of the frontal cortex. However, using the Nauta method (Nauta and Gygax, 1954) he was able to show degenerating axons passing through the white matter into the internal capsule and terminating in complex pericellular nests in the striatum. The projection appeared to be organized topographically both in the antero-posterior and medio-lateral planes.

Further work with rabbits (Carman, Cowan and Powell, 1963) and cats (Webster, 1965) confirmed the results from the rat and contradicted the conclusions of Dusser de Barenne (1942) and Garol (1940, 1942) that there was a differential projection from the cortex to the caudate and the putamen. The striatum receives projections from motor and primary sensory cortical areas. The densest

projection arises from the sensorimotor cortex and the smallest from visual cortex (Kemp and Powell, 1965). The cortical axons approach the striatum via the internal capsule and the subcallosal fasiculus.

Bilateral projection

Both Carman *et al* (1963) and Webster (1965) reported that in some, but not all of their preparations, degeneration was observed in the contralateral striatum. On closer examination they found that contralateral degeneration occurred only after lesions of the sensorimotor cortex (Carman *et al*, 1965). This projection has been found in the rat, rabbit and cat, and crosses the midline in the corpus callosum. In the opposum it arises from frontal, orbital and parietal regions and crosses in the anterior commisure (Martin and Hamel, 1967). In monkeys the projection was initially thought to be restricted to the sensorimotor cortex (Jones *et al*, 1977), but subsequent experiments by Goldman and Nauta (1977) and Fallon (1979) have shown a prefrontal component of the projection. The question of whether two populations of cells are involved or whether the contralateral projection arises from collaterals of the ipsilaterally projecting cells remains to be resolved.

Topography

As mentioned in the previous section, the degeneration studies all established that there was a distinct topographical organization of the cortico-striatal pathway in all planes, despite there being a considerable amount of overlap. More recent experiments have revealed an intricately patterned topography with a mosaic-like pattern of terminations in the striatum. The results have been obtained by injecting tritiated amino acids - usually leucine or proline - into

the cortex. The amino acids are incorporated into proteins by the neurones and some are transported anterogradely to the axon terminals. Here, they can be visualized by autoradiography.

Injections made into the rat somato-sensory cortex (Beckstead, 1979), rat medio-dorsal projection cortex (Beckstead, 1979), monkey sensorimotor cortex (Jones *et al*, 1977b) and monkey prefrontal cortex (Goldman and Nauta, 1977; Kunzle, 1978) all showed clusters of silver grains on the autoradiographs of the striatum. The patch-like appearance of the terminal fields are separated by areas in which the grain density does not exceed background. These patterns are most clearly seen in results from the newborn monkey (Goldman and Nauta, 1977) in which spherical and bell-shaped clusters of terminals are seen surrounding clear areas. With increasing age some of the hollow areas become filled and the clusters become less dense and less sharply defined. Injections of smaller volumes of tritiated amino-acid label only one or two clusters, implying that the terminal ramifications of fibres end in a restricted manner and that fibres arising from one cortical area may alternate with those of others. Goldman and Nauta (1977) have therefore proposed that the striatum is organised more as a functional mosaic rather than a homogeneous structure, as previously considered.

Cells of origin of the cortico-striatal pathway

The introduction of retrograde axonal tracing using the enzyme Horseradish Peroxidase (HRP) (Nauta *et al*, 1974) made it possible to visualize the cells afferent to a particular area. The method relies on the transport of the HRP molecule from nerve terminals at the injection site to their cell bodies. The enzyme

retains its activity in fixed tissue and its presence can be visualized by incubating the tissue with a suitable substrate which produces a dark brown reaction product.

Nauta *et al* (1974) were unable to demonstrate the cortico-striatal pathway in their study. This failure has since been attributed to the low intensity of the reaction product obtained by these workers and the lack of use of a dark field optical system (Jones *et al*, 1977b). Subsequently, successful HRP studies have been made in the rat (Wise and Jones, 1977; Hedreen, 1977), cat (Kitai *et al*, 1976b; Oka, 1980) and monkey (Jones *et al*, 1977b; Jones and Wise, 1979).

In the rat and monkey the cortical cells are the small and medium-sized cells (dia. 14-16μ) localized in layer V. In the cat Kitai (1976b) found the projection to be mainly from layer III with only slight labelling in layer V. Kitai's work in the cat has recently been extended by Oka (1980). His results suggest that two cortico-striatal projections exist, one to a direct projection from layer III and the other from layer V consisting of collaterals of cells projecting to the lower brainstem or spinal cord.

The question of whether a component of the projection is from collaterals of cortico-fugal fibres is still open. From electrophysiological experiments, Kitai (1976b) concluded that the cortico-striatal and cortico-spinal pathways were completely independent. Jinnai and Matsuda (1979), however, found two populations of cells: one responding only to caudate stimulation and the other to stimulation of both caudate and/or medullary pyramid. The distribution of the cells which they recorded is in good agreement with the results of Oka (1980). In the rat (Wise and Jones, 1977), and monkey (Jones

et al, 1977b), the cortico-striatal system appears to be independent of the cortico-fugal system. This view is based on the relative cell sizes and the distributions of the two identified populations of cells. Thus, the present anatomical evidence is contrary to the original work of Cajal (1895) and Webster (1961). The use of double retrograde labelling techniques (Kuypers *et al*, 1977) may resolve this problem.

Pharmacology and physiology

The transmitter of the cortico-striatal pathway is thought to be an amino acid, possibly glutamate or aspartate. The electrophysiology and pharmacology of this pathway are considered fully in the following chapters.

1b. Nigro-striatal pathway

A nigro-striatal pathway was first suggested from the results of retrograde degeneration studies, initially in the dog (Holmes, 1901), and later in the monkey (Mettler, 1943) and cat (Rosegay, 1944). Contrary to these findings, investigations with the Marchi technique in the cat (Kimmel, 1944; Rosegay, 1944), and monkey (Ramson and Ramson, 1942), found no evidence for a nigro-striatal projection, but suggested a nigro-pallidal pathway. Similarly, results obtained with the Nauta technique in monkeys (Carpenter and McMasters, 1964; Carpenter and Strominger, 1967), cats (Afifi and Kaelberg, 1965), and rats (Faull and Carman, 1968) showed no evidence for a nigro-striatal projection. On the contrary, the results of biochemical and histochemical studies performed during the same period provided the following evidence for the presence of a nigro-striatal pathway using dopamine as a transmitter:

1. The loss of cells in the zona compacta of the substantia nigra of humans who had died from Parkinson's Disease was correlated with a decreased level of DA in the striatum. This finding was complemented by experiments which demonstrated a similar loss of striatal DA in monkeys (Poirier *et al*, 1965) and cats (Bédard *et al*, 1969) with lesions of the zona compacta.

2. Using the Falck-Hillarp technique (Falck *et al*, 1962) for the visualisation of catecholamines by fluorescence histochemistry, Scandinavian workers (Anden *et al*, 1964; Anden *et al*, 1965) demonstrated a decreased fluorescence in the substantia nigra (cell groups A8 and A9 of Dahlstrom and Fuxe, 1964) and an increased fluorescence in surviving internal capsule fibres following striatal ablation. The latter finding was attributed to a "pile-up" of DA in the cut axons.

The loss of striatal DA following SN lesions was confirmed by fluorescence and electron microscopy (Hökfelt and Ungerstedt, 1968). These studies culminated in a comprehensive mapping of the nigro-striatal dopaminergic projection by Ungerstedt (1971), using the fluorescence method.

Despite the mounting evidence from fluorescence microscopy and biochemical studies, "classical" neuroanatomists remained unconvinced about a nigro-striatal projection and favoured a nigro-pallidal projection alone. The biochemical and histo-fluorescence changes were attributed to transneuronal effects (Mettler, 1970). General acceptance of the projection came only after the introduction of the more sensitive Fink-Heimer (1967) and Wutanen (1969) silver impregnation techniques for demonstrating anterograde degeneration. Electrolytic lesions of the SN in cat (Szabo, 1971; Moore *et al*, 1971; Ibata *et al*, 1973; Usunoff, 1976), monkey (Carpenter and Peter, 1972) and rat (Ibata *et al*, 1973) all

demonstrated a nigro-striatal projection. Lesions of the ascending dopamine projection, produced by 6-OH-DA, also demonstrated a nigro-striatal pathway in rats when used in conjunction with the Fink-Heimer method (Hedreen and Chalmers, 1972; Maler *et al*, 1973). The previous failures to demonstrate the pathway were attributed to the fine diameter of the unmyelinated fibres (Fuxe *et al*, 1964; Mori, 1966; Faul and Carman, 1968).

Subsequently, the projection has been demonstrated by the anterograde transport of tritiated amino acids in the rat (Beckstead *et al*, 1979), and monkey (Carpenter *et al*, 1976). Retrograde transport of HRP, injected into the striatum, has also demonstrated the projection in the rat (Nauta *et al*, 1974; Kuypers *et al*, 1974; Carter and Fibiger, 1977; Fallon and Moore, 1977; Faul and Mehler, 1978), cat (Kocsis and Vandermaelen, 1977; Szabo, 1977) and monkey (Szabo, 1977). Initial reports stated that the projection was exclusively ipsilateral in rats, cats and monkeys, but recently a small sparsely ordered contralateral projection has been described in cats (Royce, 1978b) and rats (Fass and Butcher, 1981).

Taken together these studies have shown that the pathway leaving the zona compacta runs medially before turning sharply rostral into a well-defined bundle that ascends in the H_2 field of Forel dorsolateral to the medial forebrain bundle. Fibres begin to leave the bundle at the level of the subthalamic nucleus to run laterally and rostrally through the internal capsule to the caudal parts of the striatum. The remaining fibres run rostrolaterally into the medial edge of the internal capsule. Some then turn dorsolaterally into the central parts of the striatum. The remaining fibres run more rostrally before turning laterally through globus pallidus to the head of the striatum. The projection is topo-

graphically arranged (Carpenter and Peter, 1972; Domesick, 1977; Fallon and Moore, 1978; Guynet and Aghajanian, 1978). The rostro-caudal and medio-lateral innervation of the striatum reflects the position of the cells in the substantia nigra, e.g. lateral areas of striatum are innervated from lateral areas of SN. In contrast, the dorso-ventral innervation is reversed, with cells in the dorsal nigra projecting to ventral areas of striatum. Retrograde transport studies using two different tracers in one animal, have supported the topographical organisation suggested by the HRP tracing (Van der Kooy, 1979) and have also shown that the dopamine-containing cells in the zona compacta do not have collateral axons to other brain areas. Conversely, some cells in the zona reticulata which project to the striatum also project to the thalamus (Bentivoglio *et al*, 1979; Steindler and Deniau, 1980) and the superior colliculus (Bentivoglio *et al*, 1979).

As mentioned above, retrograde tracing studies have demonstrated that while the pathway originates mainly from the zona compacta, some neurones in the pars reticulata of SN and para-nigral cell groups in the ventral and ventro-medial tegmental areas also contribute to it. While some of the cells lying outside the zona compacta may be displaced dopamine cells, the nigro-striatal pathway does not appear to originate exclusively from the dopamine-containing cells. Following 6-OHDA lesions which destroyed >93% of striatal tyrosine hydroxylase (TOH) there was still an appreciable transport of radiolabelled protein from the substantia nigra to the striatum after intra-nigral injection of radio-labelled amino acids (Fibiger *et al*, 1972). Retrograde transport of HRP combined with histofluorescence procedures have shown that although the majority of cells labelled with HRP also stain for TOH (Ljungdahl *et al*, 1975) or DA (Berger *et al*, 1978), a small population does not.

Similar findings from the retrograde transport of fluorescent dyes combined with catecholamine fluorescence (Van der Kooy and Wise, 1980) have added more weight to the earlier results, because both the tracer and catecholamine could be visualized on the same slide, whereas the HRP technique required a comparison of serial sections. Recently it has been proposed that results from 6-OHDA lesioned animals provide an overestimate of the non-DA projection (Van der Kooy *et al*, 1981). Non-DA containing cells in the zona reticulata and ventral tegmental area may compose less than 5% of the nigro-striatal projection. At present their transmitter is unknown.

1c. Raphe-striatal pathway

A raphe-striatal projection was proposed from the evidence of histochemical and biochemical experiments, several years before it was demonstrated by anatomical tracing methods. Ascending fibres from the raphe nuclei to the mesencephalon had been shown by degeneration techniques (Nauta and Kuypers, 1958; Brodal *et al*, 1960) but had not demonstrated a raphe-striatal projection. However, when the cell bodies of the raphe nuclei were shown to contain 5-hydroxy-tryptamine (5HT) (Dahlstrom and Fuxe, 1964, 1965) the previous biochemical descriptions of decreased levels of 5HT in forebrain structures (Harvey *et al*, 1963; Moore *et al*, 1965) after medial forebrain bundle lesions, led to the conclusion that there was an ascending pathway from the raphe nuclei to the forebrain. Tryptophan-hydroxylase, the enzyme required for 5HT synthesis, was then shown to be present in the striatum and its levels could be decreased by lesions of the ventral tegmental area (Pourier *et al*, 1968) and the raphe nuclei (Kuhar *et al*, 1972). Kuhar *et al* (1972) were also able to demonstrate a loss of 5HT fluorescence in certain areas after lesions of the raphe, but the high intensity

of the DA fluorescence in the striatum prevented the visualization of 5HT terminals there. More discrete lesions of the raphe suggested that the projection to the striatum was mainly from the dorsal raphe nucleus (Lores and Guldberg, 1974), with a minor projection from the median raphe nucleus (Jacobs *et al*, 1974).

The first anatomical demonstration of a raphe-striatal pathway came from a study of striatal afferents using HRP in the rat (Nauta Pritz and Lasek, 1974) and this has been confirmed in the cat using the same method (Pasquier *et al*, 1977). Although the first anterograde autoradiographic tracing study of the raphe efferents failed to show a projection to the striatum (Conrad *et al*, 1974), subsequent experiments in the cat (Bobillier *et al*, 1975) and rat (Miller *et al*, 1975; Azmitia and Segal, 1978) have all demonstrated one.

The striatum is innervated by the dorsal-raphe-cortical tract (Azmitia and Segal, 1978), which is located ventrolaterally to the medial longitudinal fasciculus. The fibres ascend rostrally through the fields of Forel and move laterally through the zona incerta to join the internal capsule from which they ramify to their striatal terminations.

The uptake of radiolabelled 5HT into slices of striatum suggests that the 5HT terminals are localised in the ventrocaudal striatum of the cat and rat (Ternaux *et al*, 1977). Similarly results from experiments with HRP suggest that the striatal projection arises only in rostral regions of the dorsal raphe (Jacobs *et al*, 1978). The topography of the raphe efferents has been further revealed by the use of double retrograde labelling with fluorescent dyes.

These studies have confirmed the results of Jacobs *et al* (1978) and have shown that many cells in the dorsal raphe project to more than one brain area (Van der Kooy and Kuypers, 1979). Van der Kooy and

Hatton (1980) have also shown that while many dorsal raphe cells were not labelled by a nigral injection of the tracer, the vast majority of those that were, were also labelled by the tracer injected into the striatum. This result suggests that the raphe nigral system may consist of collaterals of the raphe-striatal pathway.

The electrophysiological results with 5HT are contradictory. Extracellular recording has shown both excitatory (Bevan *et al*, 1968) and inhibitory effects (Davies and Tongroach, 1978; Herz and Zieglgänsberger, 1968). Stimulation of the dorsal raphe has been found to have inhibitory effects in extracellular experiments (Miller *et al*, 1975; Olpe and Koella, 1977) and excitatory effects when intracellular recording was used (Vandermaelen *et al*, 1979). However, none of the stimulation studies attempted to show that the effects recorded on striatal cells could be reduced by the application of 5HT antagonists.

1d. Thalamo-striatal pathway

Using degeneration techniques, Le Gros Clark and Russel (1939) and Drooglever-Fortuyn and Stevens (1951) tentatively suggested the existence of a projection from the centre median nucleus of the thalamus to the ipsilateral striatum. This was confirmed by Powell and Cowan (1954) who showed that all of the intralaminar nuclei projected to the striatum via the inferior thalamic radiations. In a more detailed study with rabbits (Cowan and Powell, 1955), they demonstrated pathways from the intralaminar nuclei - centro-median, paracentral and centrolateral - to the head of the caudate and from the parafasicular nucleus to the putamen. The early studies of the cat showed that the centromedian nucleus projected mainly to the putamen with only a small projection to the caudate. The main projection to the caudate appeared to come

from the dorsomedial nucleus (Johnson, 1961). The anterograde and retrograde degeneration methods cannot, however, give detailed information about the precise origin of thalamo-striatal fibres because lesions of the thalamus inevitably interrupt axons emanating from several thalamic nuclei.

Anterograde tracing studies with tritiated amino acids have confirmed the ipsilateral path of the projection (Kalil, 1978; Royce, 1978). With data from monkeys, Kalil supported the view that the centromedian nucleus projects primarily to the putamen, and suggested the pathway might have a latero-ventral, medio-dorsal topography. This was contrary to the antero-posterior distribution suggested by Powell and Cowan (1956). Kalil also described a clustered distribution of terminal labelling similar to the pattern found after injections of radiolabelled amino acid into the motor (Kunzle, 1975) and somato-sensory cortex (Jones *et al*, 1974) of monkeys.

Clearly there are differences in the pathway, between different species. Royce working with cats (1978a) and using an identical technique of injection into the centro-median nucleus also described a patch-like distribution, but found the major projection was to the caudate, with only a sparse labelling of the putamen. Examination of serial sections of this material revealed that the patches did not represent continuous longitudinal columns. The projection is not exclusively to the patches, as the areas between patches contained label at levels well above background. The topography in this preparation is dorso-dorsal, ventro-ventral. It remains to be shown whether the terminal clusters found after cortical injections are superimposed on those of the thalamo-striatal projection or interdigitate with them.

Retrograde tracing studies with HRP in the rat, and monkey (Jones and Leavitt, 1974), and cat (Royce, 1978b; Sato *et al*, 1979) have essentially confirmed the results of autoradiographic studies. Injection of HRP into the striatum of rats and cats labels cells in all the intra laminar nuclei, but in the monkey, labelling is found mainly in the centro-median and parafasicular nuclei (Jones and Leavitt, 1974).

Royce (1978b) confirmed the findings of his previous study (Royce, 1978a) which found only a small projection from the cat parafasicular nucleus to the striatum. He also confirmed Johnson's (1961) suggestion that there is a projection from the dorso-medial nucleus. Royce also demonstrated a projection from the ventral anterior nucleus to the caudate. However, in a recent double labelling study, Van der Kooy (1979) suggested that label found in the ventral anterior nucleus was due to spread of the tracer back up the needle tract to the cingulate cortex.

By making discrete injections of different fluorescent tracers in the medial and lateral striatum of the rat, Van der Kooy (1979) was able to show that the thalamic projection does not branch within the striatum. The label is found in a diagonal band across the striatum, indicating a medio-dorsal, latero-ventral topography. The fasiculus retroflexus cuts across the bands but does not disturb the ordered bands. In one animal with widely separated medial and lateral striatal injection sites, a clear diagonal band containing no label was seen between the labelled dorsal and ventral bands. Electrophysiological experiments have suggested that cells in the centro-lateral and centro-median nuclei of the cat project to both pericruciate cortex and either to or through the putamen (Rasminsky *et al*, 1973).

Both Jones and Leavitt (1974) and Sato *et al* (1979) commented on the similarity of the distribution of cortico-thalamic and cortico-striatal cell bodies and suggested that the same cells might project to both areas. A double retrograde labelling study will be required to confirm this possibility.

The transmitter used by this pathway is not yet known. Acetylcholine (ACh) has been proposed as the transmitter on the basis of lesion experiments (Simke and Saelers, 1977) but other groups have not been able to confirm the observations (McGeer *et al*, 1971; Butcher and Butcher, 1974; Fonnum and Walaas, 1979).

Electrical stimulation of the thalamus evokes excitatory responses in striatal neurons which can be observed with both extracellular and intracellular recording (Albe-Fessard *et al*, 1960a,b; Malliani and Purpura, 1967; Buchwald *et al*, 1973; Kocsis and Kitai, 1977).

1e. Other striatal afferents

The following pathways have been studied to a lesser extent than those in preceding sections and, therefore, will only be considered briefly.

Reticulo-striatal pathway

Studies employing anterograde degeneration (Nauta and Kuypers, 1958; Lynch *et al*, 1973) led to the proposal of a pathway from the brain stem to the striatum which was independent of the nigro-striatal system. This pathway has been further demonstrated by retrograde transport of HRP (Robertson and Travers, 1975). The projection appears minor, as only a few labelled cells were found, scattered through the pontine reticular formation.

Short latency field potentials have been recorded in the striatum following electrical stimulation of the pontine reticular formation, but the involvement of fibres of passage in producing these effects cannot be discounted. There have been no studies to determine the transmitter of this pathway.

Pallido-striatal pathway

The development of a new technique to limit the diffusion of HRP from the injection site (Staines *et al*, 1980) has allowed the description of a pallido-striatal pathway in the rat (Staines *et al*, 1981). Prior to the use of this technique, the spread of HRP from striatal injection sites had prevented the visualization of labelled cells in the globus pallidus.

The pathway arises from the body of the globus pallidus and its cells of origin lie in a diagonal band. In rostral regions the cells lie medially, but are found in progressively more lateral positions at caudal levels.

Because it has only recently been described, there are as yet no reports on the biochemistry and electrophysiology of this pathway. It has not yet been demonstrated in other species.

Amygdala-striatal pathway

With results from Golgi and Nisal stained material, Gurdjian (1928) noted, that in caudal regions of the rat brain, the amygdala appeared continuous with the striatum, and proposed that the two regions were connected. Subsequently, results from degeneration studies in the bat (Williams, 1953), cat (Lammers and Lohman, 1957), and monkey (Nauta, 1961) have all demonstrated fibres coursing from the amygdala to the striatum. The retrograde transport of HRP has

demonstrated that the pathway arises from the magnocellular portion of the basal amygdaloid nucleus (Royce, 1978b).

Electrophysiological studies using extracellular recording have confirmed the anatomical findings in the cat (Gloor, 1955; Adey, 1959; Adey and Dunlop, 1960; Russell *et al*, 1968), and rat (Dafry *et al*, 1975). Short latency excitations have been reported, suggesting a monosynaptic pathway. There are no reports of studies using intracellular recording.

This pathway may provide a connection between the limbic system and the extrapyramidal motor system.

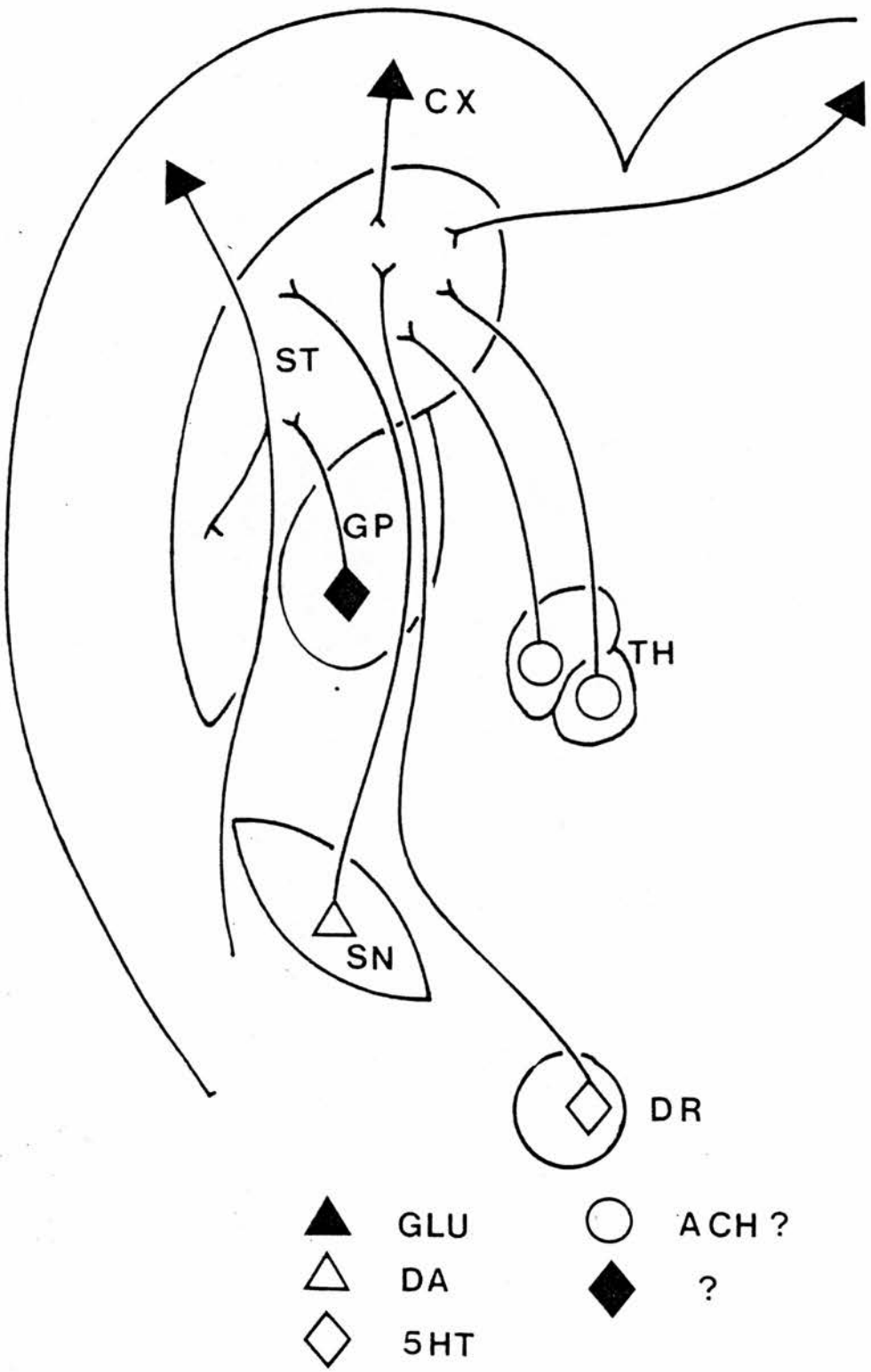
Locus coeruleus-striatal pathway

A bilateral projection from the locus coeruleus to the striatum of the rat has been reported in one HRP tracing study (Couch and Goldstein, 1977).

Anterograde transport of [^{3}H]-labelled proline and also [^{3}H]-3-4-dihydroxyphenylalanine has demonstrated a diffuse pathway from the locus coeruleus to the striatum (Jones *et al*, 1977) although no pathway was found in an earlier autoradiographic study (Pickel *et al*, 1974). In support of the existence of this pathway, nerve terminals containing noradrenaline have been demonstrated in the striatum (Lindval and Bjorklund, 1974).

This pathway has not yet been demonstrated electrophysiologically.

The afferents to the striatum and their proposed transmitters are summarised in Figure 1.


CX
ST
GP
TH
SN
DR
GLU
DA
5HT
ACH ?
?

2. Striatal neurone types

Using the Golgi method, the neuronal elements of the striatum have been described in man (Cajal, 1911), monkey (Fox *et al*, 1971a,b; Pasik *et al*, 1976; Di Figlia *et al*, 1976), cat (Adinolphi and Pappas, 1968; Kemp and Powell, 1971) and rat (Mensah and Deadwyler, 1974; Dimova *et al*, 1980). These authors have classified the observed neurons according to their cell body size and shape, number and structure of dendrites and length of axon. Despite the variations in species studied, and differences between authors in interpretation, there are clear similarities in the reports, which suggest a good basis for comparing the results of physiological experiments on different animals. A most comprehensive review by Pasik *et al* (1979) gives a detailed description of the various cell types. The following section will describe the characteristics which have permitted a differentiation of the cell types.

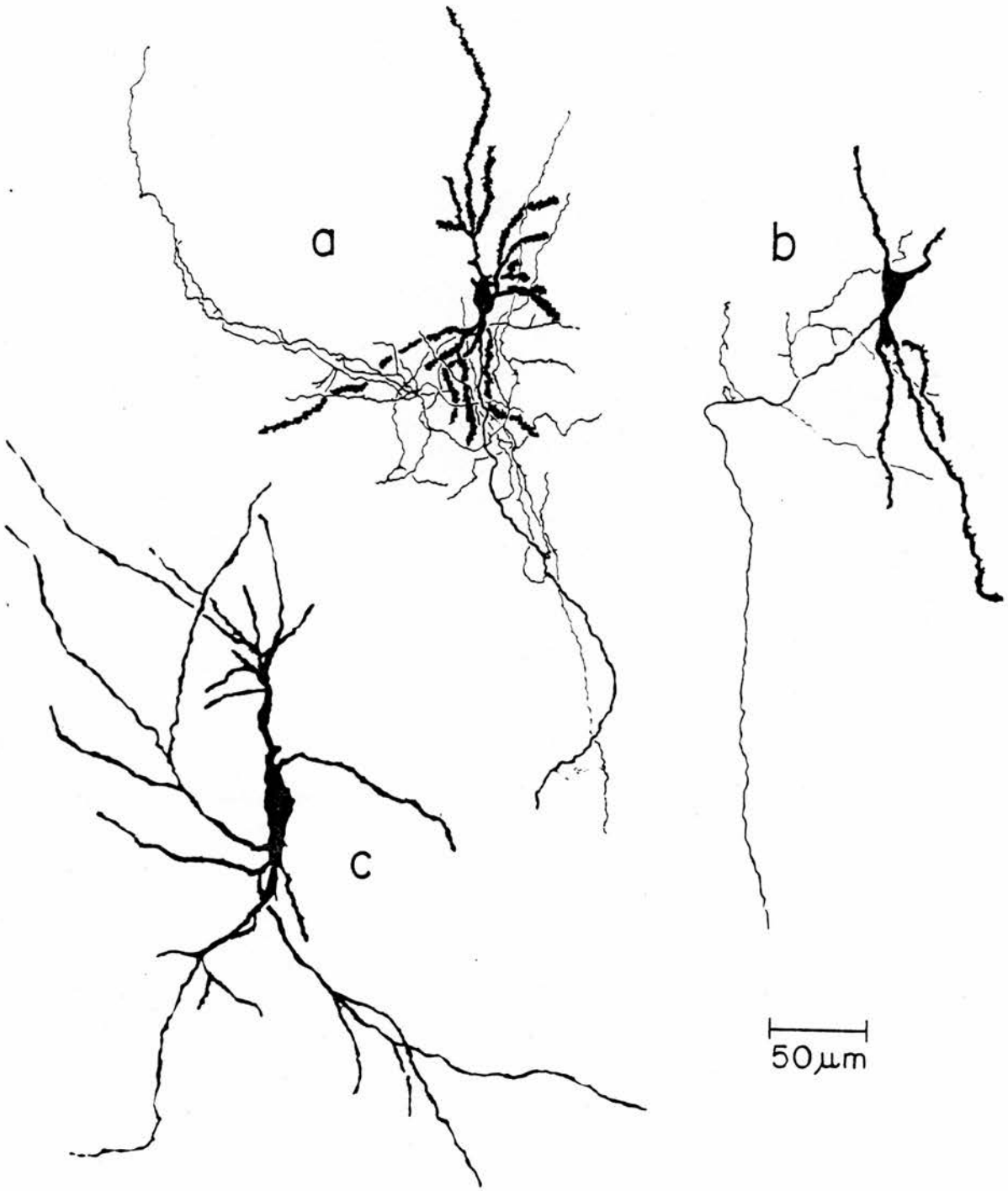
All the investigators using the Golgi method have reported a medium sized neuron, whose dendrites are heavily covered with spines, to be by far the most prevalent cell type. The earlier studies claimed that more than 90% of the neurons were of this type. However, because of the indiscriminate nature of Golgi impregnation it is not valid to make quantitative measurements of the relative numbers of cell types, as it is possible that the medium spiny neuron is preferentially labelled by this process. Similarly, these reports considered the medium spiny neuron to have a short axon which terminated either within or close to the limits of the parent cell's dendritic apparatus (diameter $\simeq$ 300-400μm), and these cells were therefore considered as interneurons rather than output neurons. This concept caused the striatum to be seen as an integrative structure having less than 5% of its cells as outputs.

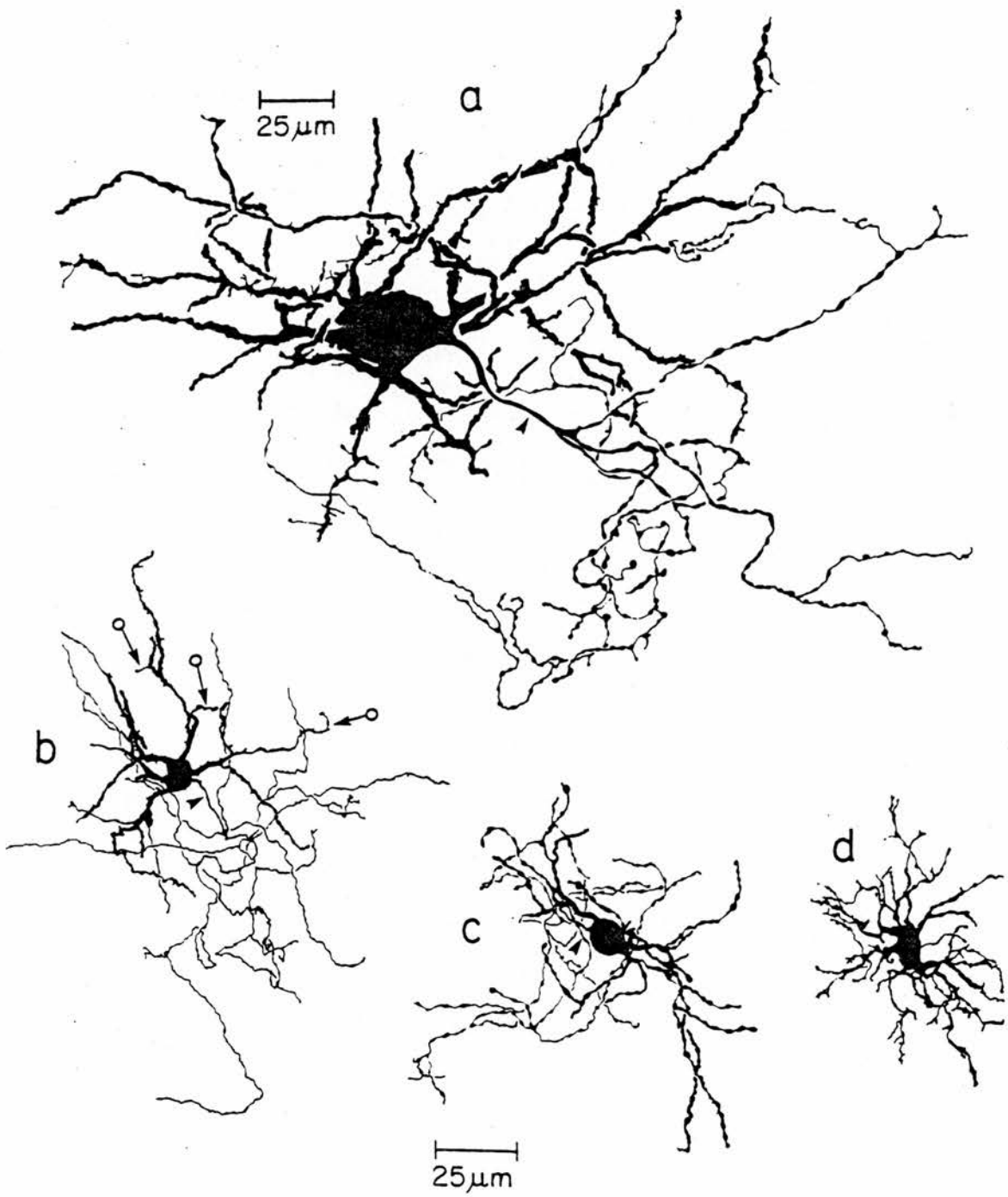
This view was first challenged by the HRP study of striatal efferents by Grofova (1975) who found numerous striatal cells filled with HRP from a nigral injection. The HRP filled cells were of medium size although two sub-types were present. Further retrograde transport experiments have shown that up to 50% of striatal neurons are "output cells" (Bunney and Aghajanian, 1976; and see Chapter III; this volume). Morphological evidence in support of this argument came from the Golgi study of Di Figilia *et al* (1976) who demonstrated two types of spiny neuron with long axons. The axons could be traced for up to 500µm without any indication of terminal arborization. Occasionally the axons could be followed into fibre bundles coursing towards the globus pallidus, suggesting that they originated from the same cell types as those described by Grofova (1975).

These spiny cells were classified as type I and type II (Fig. 2). The type II cell, although having a considerable variation in size is slightly larger than the type I and is distinguished by having less extensive axon collaterals (Fig. 2). Some of the type I cells were observed to have axon collaterals terminating within their dendritic fields.

The remaining neurones have spine-free dendrites (Fig. 3) and are classified as follows:

a) The largest aspiny neurone (diameter > 30µm) has been designated AII; it has long, smooth sparsely branching dendrites and a myelinated axon with collateral branches. This type was originally considered as an output neuron on the basis of its long axon. Nevertheless, none of the retrograde transport experiments of striatal efferents has found any of these cells labelled. Since the HRP method initially failed to demonstrate the well-established cortico-striatal


a
b
c
50µm


25μm
a
b
c
d
25μm

pathway, the large neurons may yet be identified as output cells.

b) Two medium-sized aspiny neurones have been identified and both have short axons. The aspiny I has been described as "spidery" (Fox *et al*, 1971) and is distinguished by its varicose dendrites. The aspiny III cell is similar in size to the type I but has some somatic spines and smooth dendrites.

c) The last category are the small, dwarf or neurogliform cells first described by Cajal (1911). These were considered as to be neurones by most authors because of their dendritic characteristics, although none of the investigations has demonstrated an axon. However, a recent study in which light and electron microscopy were correlated could not demonstrate any synapses on these cells, and concluded they were not neurones (Dimova *et al*, 1980).

Electrophysiological experiments in which the investigations have succeeded in labelling the recorded cells by an intracellular injection of HRP (Kitai *et al*, 1976b) have only labelled medium spiny neurones. Because of its technical difficulty this technique has produced only a small number of results and a larger sample may find other cell types labelled. Whether the different cell types can be separated electrophysiologically by their inputs and outputs demands further investigation.

Similarly, little is known about the transmitters used by the various cell types. As yet a suitable technique does not exist to visualize the transmitter of Golgi or HRP filled cells. The medium spiny output cells may use GABA, Substance P or enkephalin as a transmitter (see Section 3, this chapter). The striatum contains a high concentration of ACh (Hebb, 1957) and hence ACh has been proposed as a transmitter of

striatal interneurones. Lesions of the known afferents to the striatum have failed in the majority of cases, to modify the concentration of ACh (Butcher and Butcher, 1974), or its synthetic enzyme choline acetyl transferase, (CAT) (McGeer *et al*, 1971) in the striatum. On the contrary, a more recent study (Simke and Saelens, 1977) has claimed that thalamic lesions decrease striatal ACh.

The presence of CAT in striatal cell bodies (McGeer *et al*, 1974; Peng *et al*, 1981) does, however, make it probable that some striatal neurons use acetylcholine as a transmitter. Acetylcholine esterase has also been demonstrated in the striatum (McGeer *et al*, 1971; Lynch *et al*, 1972; Butcher *et al*, 1975), but this enzyme has also been shown to hydrolyse Substance P (Chubb *et al*, 1981) and its function in the striatum may not be exclusively cholinergic.

Electrophysiological studies of the effect of acetylcholine have provided a mixture of results. *In vivo* experiments using an iontophoretic application of ACh have all reported a mixture of excitatory and inhibitory effects (Bloom *et al*, 1965; Herz and Zieglgänsberger, 1968; Zarzecki *et al*, 1976; Spencer, 1976; Davies and Tongroach, 1978), and also a large proportion of cells which are unaffected by ACh. The ACh-induced excitations which have been observed appear to be of the muscarinic type, with a slow onset and offset.

In vitro experiments with slices of isolated striatum have been proposed as the best method of investigating the physiology of striatal interneurones (Misgeld *et al*, 1979). Investigations by different groups have revealed conflicting results from this type of preparation. Local stimulation has been claimed to cause excitation alone (Misgeld and Bak, 1979; Misgeld *et al*, 1980), or excitation and inhibition (Lightall *et al*, 1981). Both the excitatory and inhibitory effects have been attributed

to ACh. Neither is it clear which type of ACh receptor mediates the responses. A muscarinic inhibition (Takagi and Yamamoto, 1978) and a mixed muscarinic and nicotinic excitation (Misgeld *et al*, 1980) have been reported. There is no obvious methodological difference to explain the conflicting results.

Gamma-amino-butyric acid (GABA) has also been proposed as a transmitter of striatal interneurons (McGeer and McGeer, 1975). This suggestion arose from the results of experiments to investigate the origin of a GABA-containing-pathway to the substantia nigra. The authors showed that isolating the striatum from the globus pallidus had no effect on the levels of GABA in the striatum and with more caudal knife cuts suggested that nigral GABA originated exclusively in the globus pallidus. GABA-containing striatal cells have since been conclusively shown to project outwith the striatum (Nagy *et al*, 1978; Staines *et al*, 1980), so further evidence is required to establish the existence of gaba-ergic interneurones.

3. The striatal efferents

Studies of retrograde degeneration in the dog (Holmes, 1901) and from human post-mortem material (Edinger, 1911) described a bundle of fibres descending from the basal ganglia, with terminations in the substantia nigra. The fibres emanating from the striatum first unite to form pencil-like bundles (Wilson, 1914) which then converge in the globus pallidus (Papez, 1938; Papez, 1942) before continuing in a caudal direction in the internal capsule. It was noted that the volume of the fibre bundles decreased as they passed through the globus pallidus and it was concluded (Papez, 1938) that there was a large striato-pallidal pathway. The fine diameter of the thinly myelinated fibres leaving the

globus pallidus proved to be beyond the resolution of the Marchi technique. Investigations using this method (Ranson, Ranson and Ranson, 1941; Mettler, 1942), while supporting the presence of a striatal projection to the globus pallidus, denied the existence of a striatonigral pathway, and suggested that other authors had misinterpreted the polarity of the fibres (Mettler, 1943). Support for the striatonigral pathway came from a further degeneration study in the cat (Rosegay, 1944) and from fibre tracing in unlesioned monkeys (Verhaart, 1950). The striatonigral pathway was further demonstrated in the cat (Vonevda, 1960) and monkey (Szabo, 1962) by the Nauta method. It was shown to terminate in the zona reticulata of substantia nigra. This finding was later confirmed by electron microscopic studies of degenerating boutons (Kemp, 1970; Grovofa and Rinvik, 1970; Hadju, Hassler and Bak, 1973).

Despite this evidence, degeneration studies can be questioned on the grounds that fibres of passage may be interrupted. While discrete lesions of the caudate and putamen are possible in the cat and monkey, in which the cortico-fugal fibres are contained in a clearly defined internal capsule, this is not possible in the rat. In the rat striatum the cortico-fugal axons run in many separate bundles rather than an identifiable internal capsule. Therefore, the pathway in the rat could not be confirmed before the advent of more modern tracing methods.

Anterograde tracing with the autoradiographic technique has confirmed the pathway in the rat (Hattori *et al*, 1975; Domesick, 1977; Tulloch *et al*, 1978). The pathway passes radially through the globus pallidus and the fibres enter the internal capsule. They continue caudally and travel in the ventro-medial part of the crus cerebri before turning dorsally through the cerebral peduncle in the comb system of

Edinger, and entering the substantia nigra. Evidence from EM studies suggests that the striatal projection is exclusively to the zona reticulata and that fibres terminating in the zona compacta emanate from the globus pallidus (Hatton *et al*, 1975).

The striatal projection to the globus pallidus is to both the internal and external segments in the primate (Voneida, 1960; Nauta and Mehler, 1966; Kemp, 1970; Szabo, 1970). In the rat and cat the entopeduncular nucleus is homologous with the primate internal segment. A striatal projection to this nucleus has been reported using the Nauta method in the cat (Niimi *et al*, 1970) and autoradiographically in the rat (Nagy *et al*, 1978; Van der Kooy *et al*, 1981).

Retrograde transport of HRP injected into the substantia nigra has further confirmed the existence of the striato-nigral pathway in the rat (Bunney and Aghajanian, 1976; Kanazawa *et al*, 1976; Tulloch *et al*, 1978), cat (Grofova, 1975; Szabo, 1977) and monkey (Szabo, 1977).

A topographic representation of the pathway was initially suggested from preparations stained with the Nauta method (Voneida, 1960; Szabo, 1962, 1967, 1970, 1972) and this has subsequently been confirmed by the autoradiographic and HRP experiments. Anterior regions of striatum project to medial substantia nigra and globus pallidus and posterior regions to more lateral areas. Dorsal striatum projects to the entopeduncular nucleus (or internal segment) and to ventral substantia nigra. Ventral striatum projects to the globus pallidus (or external segment) and more dorsal areas of SN. It has been suggested that some striatal cells may project to both segments of globus pallidus (Cowan and Powell, 1966; Fox and Rafols, 1975a,b) and that some striato pallidal fibres are collaterals from striato-nigral cells (Fox and Rafols, 1976). The solution to this question may come from the use of fluorescent tracers in a double labelling study.

Biochemical and histochemical studies indicate that the striatal efferents may use three different transmitters: GABA, Substance P and enkephalin.

GABA loss in the substantia nigra following a hemisection at the level of the subthalamic nucleus, was first reported by Kim *et al* (1971). Subsequent investigators have measured the activity of the GABA synthesizing enzyme L-glutamic acid decarboxylase (GAD), as a marker for GABA containing nerve terminals. GAD has been shown to decrease in the SN of the monkey (Kataoka *et al*, 1974), rat (Nagy *et al*, 1978) and cat (Fonnum *et al*, 1974) following striatal lesions. Similar decreases in GAD have been shown in the GP and the EPN of rats (Fonnum *et al*, 1978) suggesting the striato-pallidal pathways also contain GABA.

The precise origin of the GABA containing terminals has been the subject of some controversy. It was initially proposed that the nigral GAD was contained exclusively in terminals of the pallido-nigral pathway (Hattori *et al*, 1973; McGeer *et al*, 1974). This conclusion was first questioned by Fonnum *et al* (1974) who suggested that the lesions of globus pallidus were interrupting striato nigral GABA-containing fibres. However, GP lesions made with the neurotoxin Kainic acid, which destroys cells while sparing fibres of passage, did produce a decrease in nigral GAD (Garcia-Munoz, 1978). Further experiments with kainic acid (Nagy *et al*, 1978) and precise knife cuts (Gale *et al*, 1977; Brownstein *et al*, 1977) have shown that the cells producing the nigral GAD are located in the posterior region of the striatum and in the globus pallidus. The lesions made by Hattori *et al* (1973) may have spared this posterior region of the striatum.

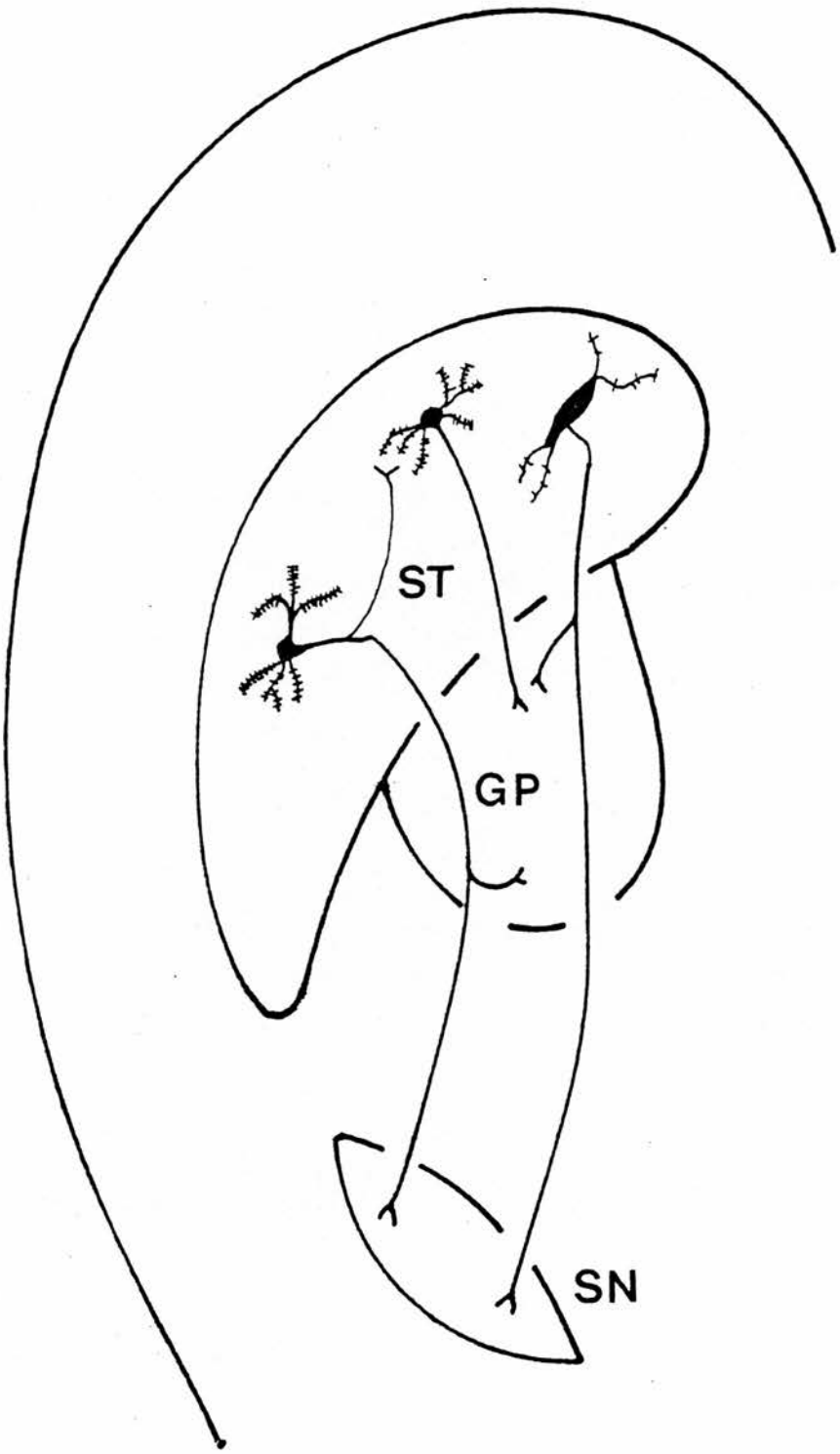
Electrophysiological experiments have also provided evidence for a transmitter role of GABA. Iontophoretic GABA mimics the depressant

effects of caudate stimulation, and the effects of both can be blocked by the GABA antagonists picrotoxin (Precht and Yoshida, 1971; Crossman *et al*, 1973) and bicuculline (Dray *et al*, 1976). The substantia nigra also contains the highest concentration of substance P in the brain (Kanazawa and Jessel, 1976). Both striatal and pallidal lesions can reduce the nigral content of substance P (Gale *et al*, 1977; Mroz *et al*, 1977; Brownstein *et al*, 1977). The involvement of fibres of passage in the results from lesions of globus pallidus does not appear to have been tested with kainic acid, but a small number of substance P-containing cells have been visualized in the globus pallidus using the immunofluorescence technique (Kanazawa *et al*, 1977). In contrast to the GABA-containing cells, the substance P-containing efferents are localised in the anterior part of the striatum (Gale *et al*, 1977).

Release of substance P has been shown from nigral slices (Jessel, 1978) but not from pallidal slices. There is no evidence to suggest a substance P innervation of GP from striatum.

In electrophysiological experiments, excitations have been recorded in SN in response to striatal stimulation (Dray *et al*, 1976; Kanazawa and Yoshida, 1980). While substance P has been shown to have a mainly excitatory effect on nigral neurons (Davies and Dray, 1976; Dray and Straughan, 1976; Walker *et al*, 1976), proof that it is a transmitter will require the discovery of a specific antagonist.

The high concentration of enkephalin in the globus pallidus (Hong *et al*, 1977) coupled with the visualization of enkephalin-containing cell bodies in the striatum (Hokfelt *et al*, 1977), suggested that some striato-pallidal efferents use enkephalin as a transmitter. This has been supported by the immunofluorescence studies following selective knife cuts between the striatum and globus pallidus (Cuello and Paxinos, 1978).


ST
GP
SN

The enkephalin-containing cell bodies are located predominantly in the dorso-medial areas of the striatum (Walmsley *et al*, 1980). Potassium stimulated release of enkephalin has been demonstrated from striatal tissue (Iversen *et al*, 1978). However, due to the scarcity of specific antibodies it is not certain whether both met- and leu-enkephalin are involved in the pathway. The immunofluorescence study of Cuello and Paxinos (1978) demonstrated a leu-enkephalin pathway, but high concentrations of met-enkephalin have also been found in the globus pallidus.

4. Aim of the investigation

The preceding literature review has described some of the large body of knowledge concerning the anatomy and physiology of the striatum. Whilst this structure has been the object of intense study during the last decade, several important questions remain to be answered. This thesis is directed towards clarifying the nature of cortico-striatal and nigro-striatal transmission in the rat and to examining the interaction between these two major striatal inputs.

Electrophysiological experiments have been performed to investigate the effects of cortical stimulation on both the intrinsic and the output cells of the striatum, and the effects of dopamine on neuronal activity.

CHAPTER II

General Methods

Experimental animals

Male albino Wistar rats weighing 210-250 g were used for all recording experiments.

Surgical procedure for recording experiments

Anaesthesia was induced by placing the rat in a plastic box through which a 3% halothane/air mixture was circulated by a Vapor-Halothan vapouriser. Once the flexor reflexes were lost, anaesthetic was maintained via a rubber nose cuff, and a tracheotomy was performed. Anaesthesia was then continued at 1.5% Halothane via a tracheal cannula at an air flow rate of 200-300 mls/min. The tracheal cannula was attached to a Y-piece which allowed the expired Halothane to be drawn off by suction, and absorbed into liquid paraffin. The use of the Y-piece also allowed the introduction of a small bore cannula to clear blockages, by suction, without affecting the flow of anaesthetic.

When the neck wound was closed, the rat was placed on a homeothermic electric blanket (Epil : 240). The animal's temperature was monitored by a rectal probe and thermostatically maintained between 36° and 38° Centigrade. The animal was then fixed in a David Kopf stereotaxic frame using blunt ear bars with a 45° tip. The tooth bar of the frame was fixed at -2.4 mm below the ear bar zero.

The skull was opened by a midline incision and the skin flaps were pulled to the side. In all preparations the junction between the bregma suture and the midline was taken as the stereotaxic zero reference point.

In order to see this clearly, the overlying periosteum was scraped away.

The stereotaxic co-ordinates for recording striatal cells were:

Anterior/posterior	(AP)	+0.8 - +1.3 mm
Lateral	(L)	2.4 - 2.8 mm
Vertical	(V)	3.0 - 6.0 mm
		(measured from the cortical surface)

These co-ordinates defined a block of striatal tissue which could be reproducibly located in different animals (Fig. 5).

Under microscopic guidance a 2 mm diameter hole was bored in the skull using a dental trephine. The dural membrane was then ruptured with a small needle and pulled to the side using fine forceps. The exposed cortex was bathed with warm liquid paraffin to prevent drying.

Implantation of stimulating electrodes

Electrodes were implanted for stimulation of a) cortex and b) crus cerebri at the following co-ordinates:

a) Cortical stimulating electrode (Fig. 6)

AP	+3.00 mm
L	2.5 - 2.7 mm
V	1.5 - 2.0 mm (measured from the cortical surface)

b) Crus cerebri stimulating electrode (Fig. 7)

AP	-2.8 mm
L	7.4 mm
V	7.0 mm (measured from the cortical surface)

When a crus cerebri stimulating electrode was used the temporalis muscle was pushed away from the skull because the crus was approached at an angle of 45° to increase the chance of successful stimulation.

The stimulating electrodes were of the concentric bipolar type. In initial experiments the stimulating electrodes were fabricated from 25 gauge needles with an inner core of insulated stainless steel wire.

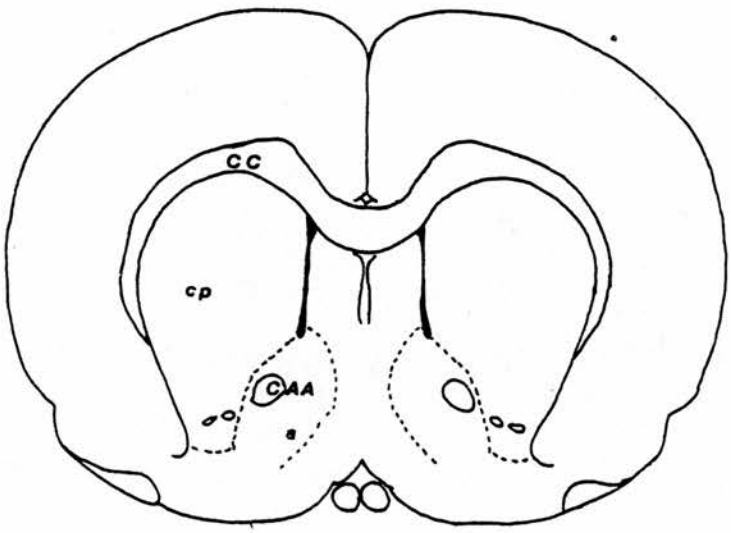


FIGURE 5: The recording site. This section of a rat brain shows the part of the striatum at which the recording electrode was aimed. The diagram is adapted from the atlas of Koenig and Klippel (1963).

Abbreviations: CC - corpus callossum; CAA - anterior commisure; a - nucleus accumbens; cp - striatum.

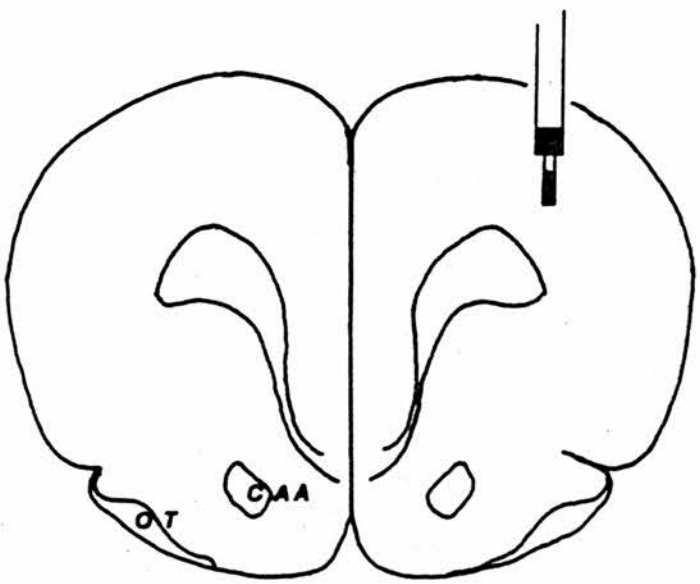
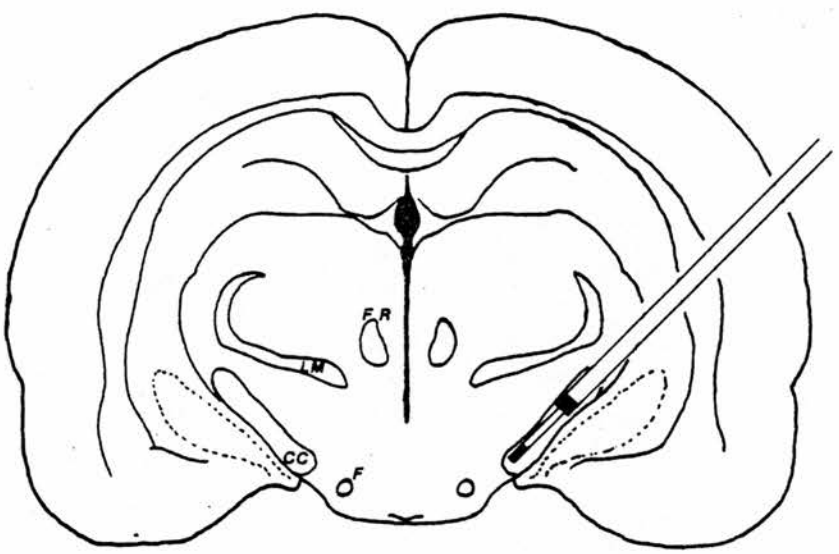


FIGURE 6: The location of the cortical stimulating electrode in the rat brain. The diagram is adapted from the atlas of Koenig and Klippel (1963).

Abbreviations: CAA - anterior commisure; OT - olfactory tract.



A 3180

FIGURE 7: The location in the rat brain of the crus cerebri stimulating electrode. The diagram is adapted from the atlas of Koenig and Klippel (1963).

Abbreviations: FR - fasciculus retroflexis; LM - medial lemniscus; F - fornix; CC - crus cerebri.

In later experiments commercially prepared electrodes (Rhodes Medical Instruments) were used. The electrodes had an outer diameter of 0.25 mm. The centre contact protruded 1.0 mm from the shaft.

Micro-electrodes

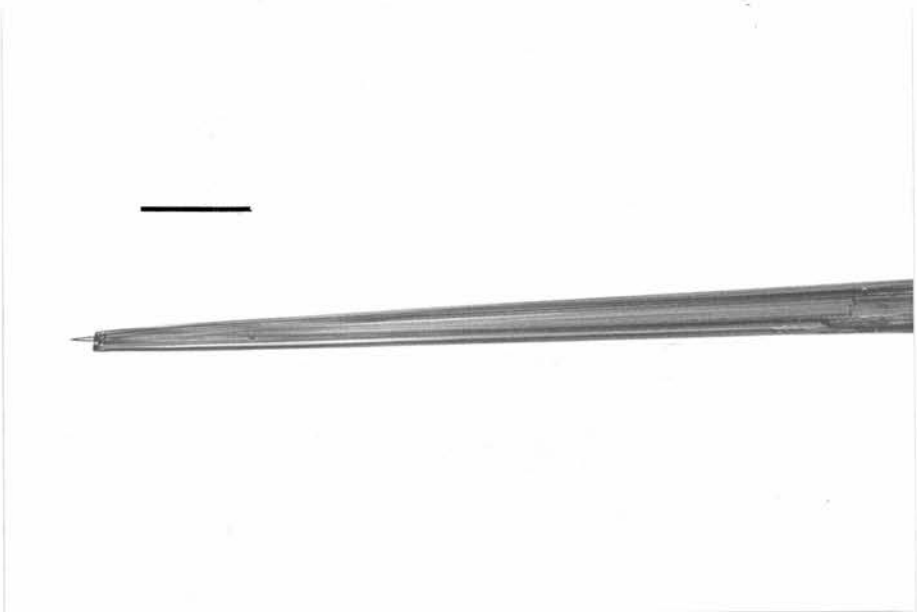
Glass-micro electrodes for recording were made from 'theta-style' glass capillary tubing (TGC 150, Clark Electromedical Instruments). The glass was pulled with a micro-electrode puller (Forth Instruments Ltd). Using a 30 gauge exploring needle, the micro-electrodes were filled with pontamine sky blue (Gurr Ltd) made up as a 2% solution in 0.5M sodium acetate. The pontamine blue solution was filtered through a medium fast paper filter (Qualitative No. 1, Whatman) and the pH adjusted to 7.7.

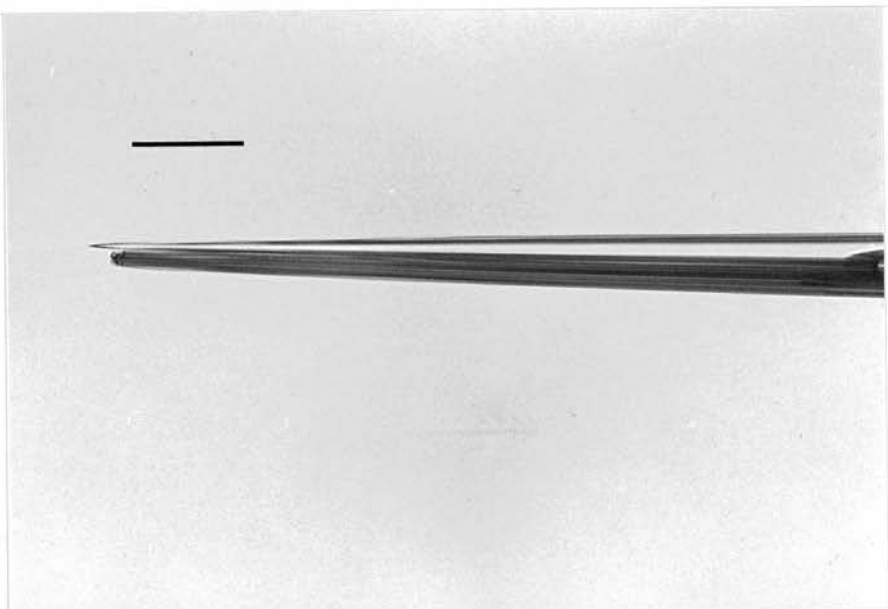
The electrode resistance was determined using the ohmeter function of a WPI volt-ohmeter (Model F-29, W.P. Instruments Ltd).

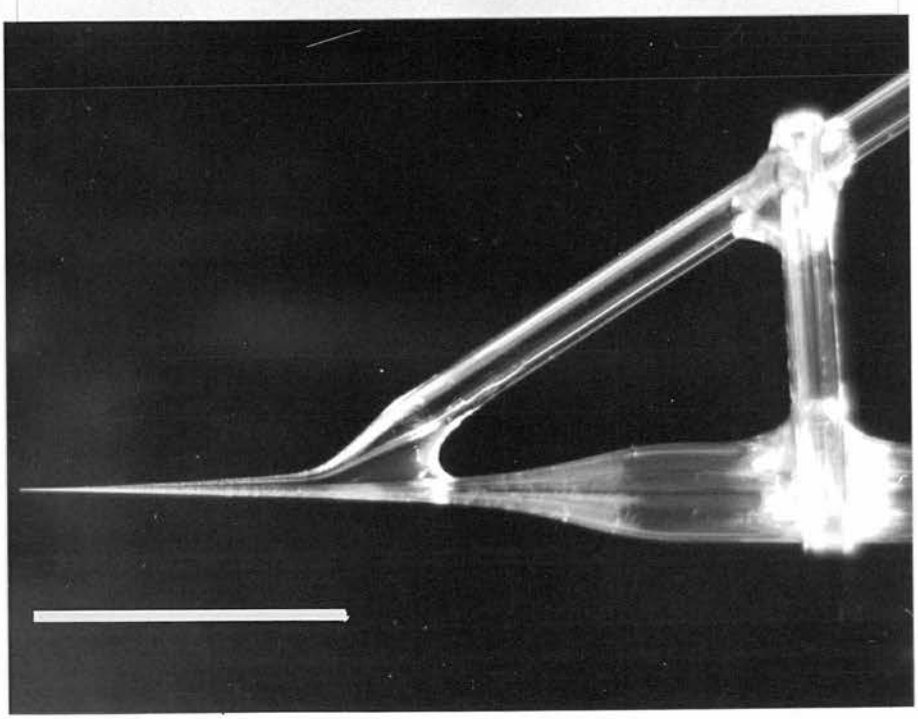
Successful recordings were made from electrodes which had a resistance of 45-60 MΩ in one compartment of the capillary glass.

Iontophoresis electrodes

During initial experiments using the iontophoretic technique electrical recordings were made from the central barrel of an electrode pulled from a blank produced from seven glass capillaries fused together (7GC-100F, Clark Electromedical Instruments). The tip of the electrode was broken back to approximately 3-4μm by bumping it against a piece of broken glass under microscopic control. However, despite the use of current balancing during iontophoretic ejection (see following section for details), several problems were encountered with this type of electrode: 1) poor signal to noise ratio for electrical recording using tip diameters which gave usable resistances for the iontophoretic barrels; 2) production







of large artefacts during the passage and switching of iontophoretic currents; 3) effects of iontophoretic current alone on cell firing.

To try to overcome these problems, electrodes were fabricated with separate micro-electrodes for electrical recording and iontophoresis. The theta-glass micro-electrodes were pulled as previously described but immediately after pulling, while the glass was still hot, the tips were bent to an angle of approximately 40° using a blunt seeker. The multibarrel blanks were pulled on a separate electrode puller (Narashige Instrument Co.) because different settings for the heating coil and solenoid were required to pull the different types of glass. The multibarrel electrodes then had their tips broken back to between 10 and 15μm by bumping them against a glass rod under microscopic control.

The electrodes were then carefully aligned under microscopic control with the recording electrode protruding 25-30μm past the iontophoresis electrode (Fig. 8).

Several types of glue were tested for their ability to bond the electrodes together. Loctite 358 proved superior to epoxy resins or cyanoacrylic glues. The Loctite 358 was applied near the shoulder of the multibarrel electrode, and flowed easily down to the tips. It has the advantage that it will not set in air without ultra-violet light. This property allows repositioning of the electrodes - if they have moved during application of the glue - before the curing process begins. An ultra-violet light with a long wavelength and a spectral energy distribution concentrated around 365 nanometres (Phillips HPR 125w) is used to cure the glue. Handling strength is achieved after irradiation for approximately 1 minute. There is no appreciable shrinkage of the glue during curing. To increase the strength of the assembly a cross member is added between the two electrodes. This method of construction produces

not only a very strong electrode but also a slim profile which is essential when the recording electrode is positioned close to the stimulating electrode, as is required for cortical stimulation of striatal cells. These electrodes could be stored for several weeks in an air-tight container and still produce excellent recordings.

Before filling, the butt end of the electrode was lightly smeared with sillicone stopcock grease to prevent wicking of fluid between adjacent electrodes and crystallization of drug solutions round the barrel tops. The drug barrels were filled with solutions using 30 gauge exploring needles (Needle Industries). Care was taken never to fill the top 15 mm of the barrels in order to allow room for fluid displacement by the connecting wires. The barrels were always filled immediately before use and filled electrodes were never stored overnight.

No more than five barrels of the seven-barrel electrode were filled with drug solutions and the central barrel was always filled with 1M NaCl for current balancing.

The filled electrodes were mounted in a Trent Wells hydraulic microdrive for penetration into the brain. Once the electrode had penetrated the cortical surface, the skull of the animal was covered with a layer of 4% agar in saline to prevent cooling and drying.

Iontophoretic ejection of drugs was effected by a Neurophore Unit (Medical Systems Corporation). This unit incorporated five current ejection pumps and an automatic current balancing facility. This allowed current flow to ground, and thus current artefact, to be minimized. The drug barrels were normally only subjected to a retaining current (5-10 nA) when a cell was being recorded. It was found that if retaining currents were used continuously a substantial "warm-up" period was required before reproducible responses could be elicited from identical ejection periods from the same barrel.

Electrode resistance could be monitored "in situ" by the Neurophore and successful ejection was achieved from barrels with resistances varying between 20-50 MΩ. The Neurophore was also able to indicate if blocking of the electrode occurred during drug ejection.

An analogue voltage was taken from the Neurophore to a Grass Polygraph to allow correlation of neuronal events with iontophoretic ejections.

Electrical recording

The recording electrode was connected by a silver chloride wire to the gate of a field-effect transistor (FET, No. 2N3819, Radio Spares Components Ltd) used as a voltage follower. The voltage for the FET came from two 6 volt batteries connected in series. The output from the source was connected to a differential amplifier (5A22N; Tektronix) of a dual beam storage oscilloscope (D.13 Tektronix). The reference point for the differential amplifier was provided by a silver wire inserted into a neck muscle. The bandwidth of the amplifier was set at 1.0-10.0 KHz.

An earth lead was connected to the rat's skin by a crocodile clip. The earth of all the recording equipment was referred to a copper water pipe.

With these recording conditions the noise level was approximately 100 μV with a signal to noise ratio varying between 5 : 1 and 25 : 1.

The output of the differential amplifier was also connected to two window discriminators. The first set of windows (D130 - Spike Processor, Digitimer Ltd) were displayed on a separate oscilloscope (Devices 312 - Digitimer Ltd).

The D130 was set to count the number of spikes crossing the window every 1.25 secs and its output was connected to the driver

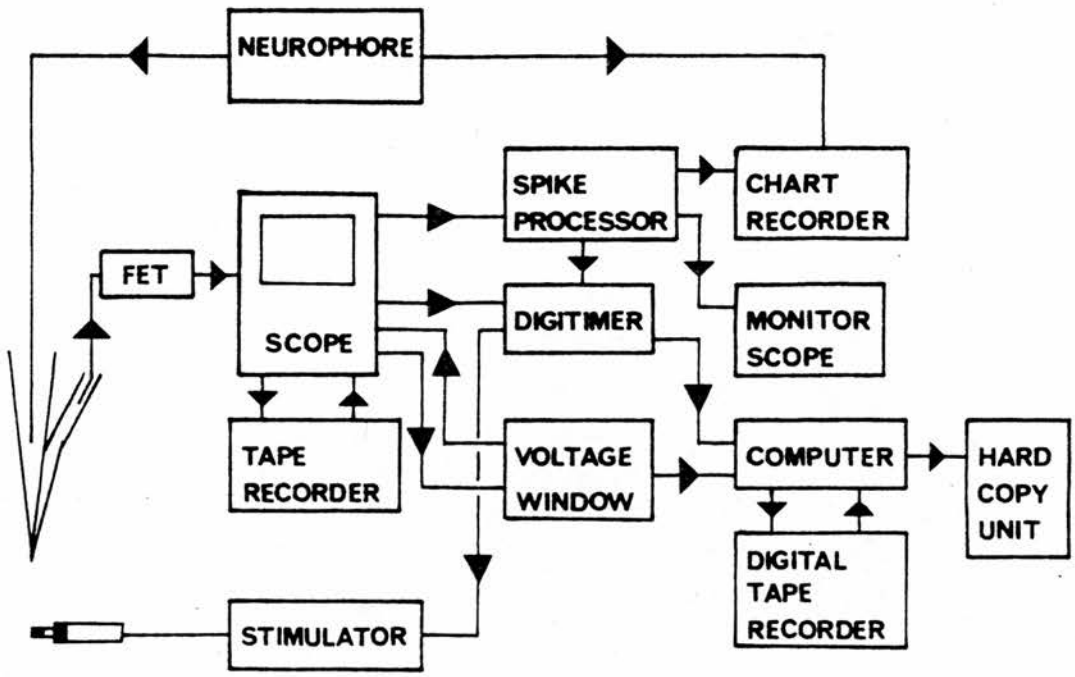


FIGURE 9: Schematic diagram of the electrical recording equipment.

amplifier of a Grass Polygraph Recorder. The D130 also provided an audio output of the recorded signals.

The second window discriminator was connected to a GEC 2050 computer terminal. The windows from this system were displayed on the Tektronix oscilloscope along with a monitor pulse for any action potentials counted.

In some experiments, the signal was tape recorded (TEAC, A-1340 four-channel tape recorder) for subsequent analysis. The system is shown diagrammatically in Fig. 9.

Data analysis

The computer could be instructed to compile post stimulus histograms (PSTH) or interval histograms of varying duration (1000 events, 5 minutes, or continuously) and bin width (1 or 5 msec. bins).

On completion of the histogram, a monitor oscilloscope provided a visual display of the histogram, and the following information for each type of histogram.

Interval histogram:

number of events
value of the mean interval
value of the histogram mode.

Post stimulus histogram:

number of stimuli
number of responses
mean response latency.

The information in the histogram was then transferred to a digital tape recorder (Tandberg TDC 3000) and stored on a tape cartridge (Scotch DC 300A).

At the end of each experiment a printout of the histograms was obtained, showing in numerical form the contents of each bin. In initial experiments this service was provided by the Central Computer Unit (Western General Hospital, Edinburgh). In later experiments the procedure was carried out in our laboratory using a Ferranti F100-L micro-computer system.

Histograms were then plotted manually from the information in the printout.

Stimulation

Electrical stimulation was applied from an isolated stimulator (DS2, Digitimer Ltd) controlled by a Digitimer (Model 3290, Devices Ltd). Stimulation pulses were normally of 0.2 msec. duration and stimulation current was measured by observing the voltage drop across a 1 K Ω resistor placed in series with the stimulating electrode. Stimulation currents varied between 0.1-1.4 mA.

Histology

At the end of each experiment, the position of the recording electrode was marked by passing current (10 μA for 5 min, electrode negative) through the electrode. This produced a discrete spot of pontamine blue at the tip of the electrode (Figs 10 and 11). The stimulating electrode sites were marked by connecting the electrodes to a 6 volt battery for 10 secs. This procedure produced a small lesion at the tip of the electrode (Fig. 12).

The animal was then killed by an overdose of anaesthetic and the brain removed.

The block of tissue for histological analysis containing the electrode sites was stored in a deep freeze in a glass beaker containing

ice and covered by "cling film". The tissue was frozen onto a cork disc and mounted on a cryostat chuck. Sections were cut at 40 µm thickness and every second section was collected in the region of the electrode tract. The sections were melted onto glass slides in preparation for staining. The sections containing the pontamine blue spot were left in the vapour of formaldehyde solution (Analar BDH Chemicals Ltd) for 24 hours. Then all the tissue was stained by a modified version of the Kluver and Barrera technique for staining myelinated fibres and cell bodies.

The sections were treated as follows:

a) Dehydrated in 70% alcohol for 5 mins.

b) Dehydrated in 95% alcohol for 5 mins.

c) Stained for 30 minutes in a 95% alcohol solution containing 1 mg/ml of Luxol Fast Blue (BDH Chemicals Ltd) and 5 µl/ml of 10% acetic acid solution.

d) Rinsed in 95% alcohol to remove excess stain.

e) Rinsed in distilled water.

f) Differentiated by immersion in 0.05% lithium carbonate for 10 secs followed by immersion in 70% alcohol for 30 secs. The differentiation steps were repeated four or five times until a clear distinction was seen between the blue stained white matter and the colourless grey matter.

g) When differentiation was complete the sections were washed in distilled water and then transferred to a solution containing 0.1% cresyl fast violet (Fluka, AG, Switzerland) in distilled water and left for 5 mins.

h) Rinsed in distilled water.

i) Differentiated in changes of 70%, 95% and 100% alcohol, 5 mins each.

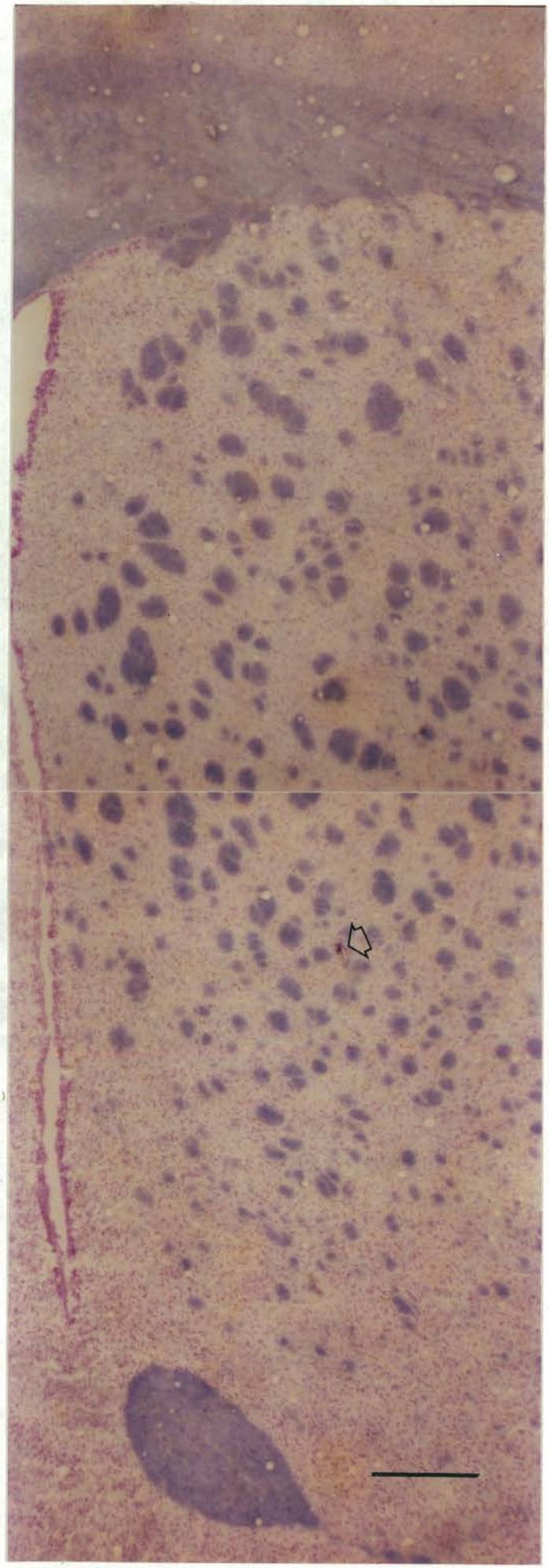
j) Cleared in xylene for 10 mins.

k) Coverslipped using DPX (BDH Chemicals Ltd).

The slides were studied under a light microscope.

CHAPTER III

The Electrophysiology of the Cortico-Striatal Pathway



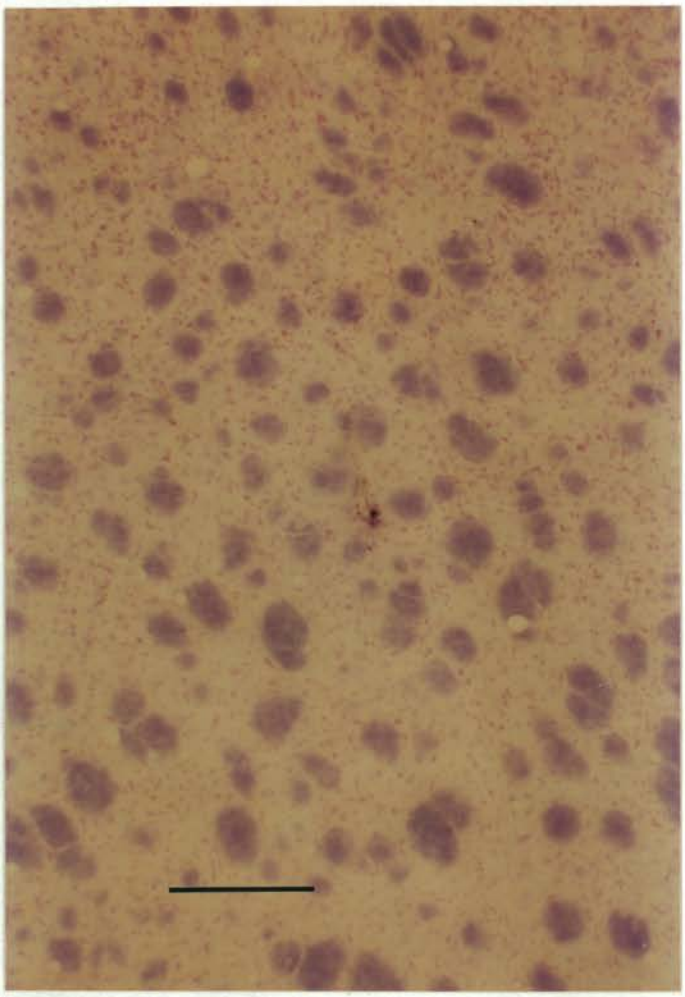


FIGURE 11: High power photograph of the section shown in Fig. 10. The dye spot is clearly seen lying in a group of cells between the axon bundles.

Scale bar - 50μ

FIGURE 12: Photograph of the crus cerebri electrode site in a 40μ brain slice stained by the method of Kluver and Barrera (1953). The lesion produced by passing 6 volts DC through the electrode for 10 secs is clearly seen at the base of the crus cerebri.

Scale bar - 100μ

Introduction

Electrical activity in the striatum evoked from the cerebral cortex was first shown by the technique of strychnine neuronography (Dusser de Barenne *et al*, 1942). Several years elapsed before responses were recorded following peripheral sensory stimulation and also electrical stimulation of the motor cortex of the cat (Albè-Fessard *et al*, 1960a). These authors recorded only gross evoked potentials and did not find any somatotopic relationship between stimulus and response. Subsequently, Albè-Fessard *et al* (1960b) were able to record from single units in response to stimulation of several cortical sites, and also peripheral sensory nerves. These findings were confirmed by Laursen (1961) in a careful study using a multi-lead electrode which allowed precise definition of the limits of stimulation current spread. A more detailed analysis of caudate single unit activity in both anaesthetised (chloralose and also nembutal) and unanaesthetised (cerveau isolé) preparations (Rocha-Miranda, 1965) showed that the predominant response to single shock stimulation was a single spike. Only rarely was a two or three spike response seen.

While the majority of responses were excitatory some inhibition of spontaneously active neurones was seen after stimulation of anterior cortical regions. Because so few striatal cells fire spontaneously, the small proportion of inhibitory responses reported (Rocha-Miranda, 1965; Sedgewick and Williams, 1967), were difficult to interpret using extracellular recording techniques.

Intracellular studies (Purpura and Malliani, 1967; Hull *et al*, 1970; Buchwald *et al*, 1973; Hull *et al*, 1973) showed the predominant response of striatal neurones to stimulation of afferent pathways as an e.p.s.p/-

i.p.s.p. sequence. When cortical, thalamic and substantia nigra stimulations were assessed on the same cell, the initial response was an e.p.s.p. in 94% of the tests. In 81% this was followed by an i.p.s.p. Initial i.p.s.p.'s were recorded in only 6% (Levine *et al*, 1978). Similar responses were evoked using stimulating electrodes placed locally in the striatum (Marco *et al*, 1973).

Considered alone, cortical stimulation produced e.p.s.p.-i.p.s.p. sequences in 88% of tests. Pure e.p.s.p.'s were never seen after cortical stimulation and were seen most often following stimulation of the centromedian/parafascicular complex of the thalamus (Buchwald *et al*, 1973). These results led to the hypothesis that all inputs to the striatum were excitatory and that the observed inhibitions were due to the simultaneous activation of neighbouring neurones which in turn inhibited each other. At that time it was thought the majority of striatal neurones had axons which terminated within the striatum (Kemp and Powell, 1971). The observations that the majority of striatal neurones had very little spontaneous activity fitted well with the concept of many inhibitory interneurones. This hypothesis cannot, however, explain the occurrence of e.p.s.p.'s alone, and there is no anatomical evidence to suggest that the thalamic centromedian/parafasicular complex projects exclusively to neurones which do not have axonal terminations within the striatum. Indeed, recent electrophysiological evidence has suggested that the output neurones have an axon collateral which terminates within the striatum (Park *et al*, 1980). In only one of the previously mentioned studies (Hull *et al*, 1970) was an attempt made to identify the striatal output neurons; unfortunately, it was unsuccessful.

The topographical organization of the cortico-striatal pathway described in anatomical studies (Kemp and Powell, 1970; Webster, 1961;

Webster, 1965) has been confirmed electrophysiologically by Liles (1973). In contrast, Blake *et al* (1976), also using evoked potential recording, could only demonstrate an antero-posterior organization. Despite this reported topography, the convergence of several different cortical inputs onto single neurons has been seen to occur in both extracellular (Spencer, 1976), and intracellular recording experiments (Kitai, Kocsis and Wood, 1976).

Striatal output neurones can be identified electrophysiologically by antidromic invasion after stimulation of the entopeduncular nucleus or substantia nigra (Frigyesi and Purpura, 1967; Liles, 1974; Kitai *et al*, 1975; Matsuda and Jinnai, 1980). The results from these studies have shown that stimulation of cortex and thalamus rarely evokes action potentials in output neurones. However, e.p.s.p. responses to cortical, thalamic and nigral stimulation have been reported (Kitai *et al*, 1979; Matsuda and Jinnai, 1980).

None of the previous studies has used antidromic invasion as a means of differentiating between interneurones and output neurones during pharmacological investigations.

This section of the thesis therefore describes the electrophysiological characteristics of rat striatal neurones as determined by extracellular recording. Attention has been focused on the antidromic identification of output neurones, and on the response of striatal neurones to cortical stimulation, with the object of using these techniques to investigate some of the pharmacology of the striatum.

RESULTS

The data in this section is derived from extracellular recordings of 375 striatal neurones from 227 rats. The majority of neurones were completely silent during the experiments and fired only in response to a stimulus (Table 1). Spontaneous activity was recorded from 16% of the neurones. This was usually presented as bursts of spikes (action potentials) (Fig. 13). Only 12 cells (0.03%) were unresponsive to cortical stimulation.

Neurones which exhibited bursting activity had within-burst. firing rates ranging between 14 spikes/second (s.) and 200 spikes/s. with a mean of 42 spikes/s. and up to 20 spikes per burst. The mean firing rate of these neurones ranged between 2 and 13.5 spikes/s. The within-burst firing rate and the mean firing rate were calculated from the mode and mean values, respectively, of an interval histogram (Fig. 14). Action potentials recorded from these cells ranged from 0.2 mV to 3.0 mV in amplitude.

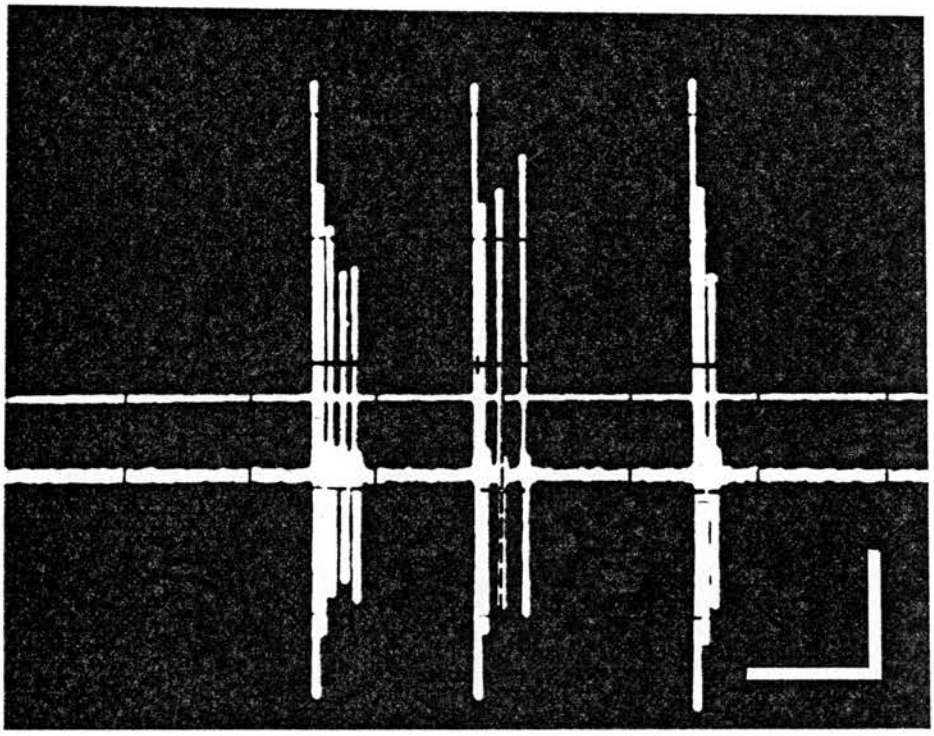
Neurones exhibiting random single spikes were also recorded. These neurones never achieved firing rates greater than 0.18 spikes/s. and it was usual to observe only 2 or possibly 3 spikes/min. (0.05 spikes/s.).

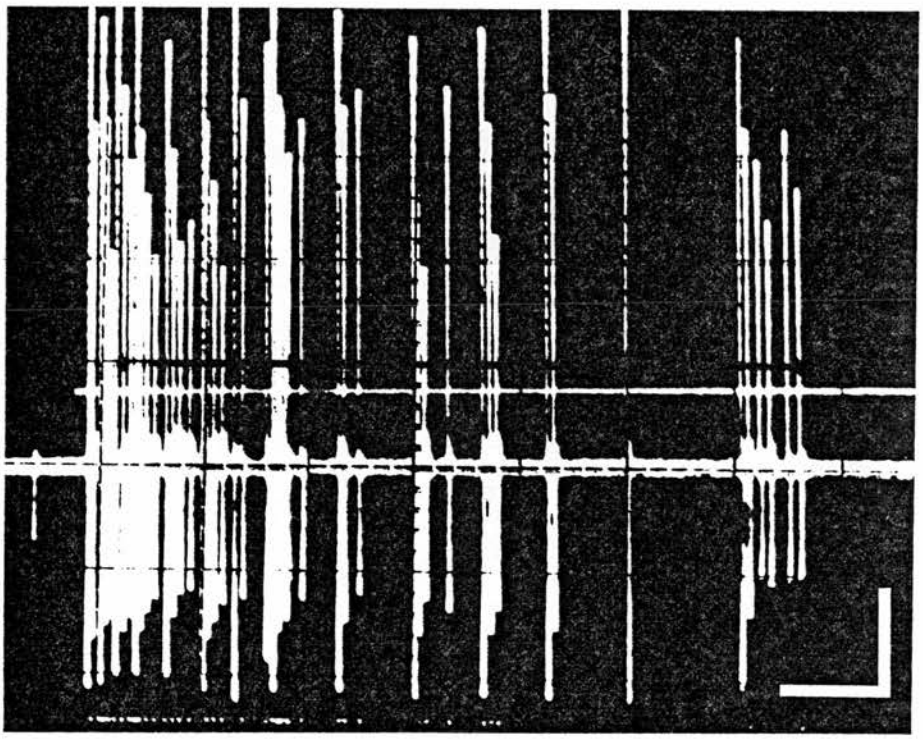
Responses to cortical stimulation

Most striatal neurones could be excited by electrical stimulation of the sensorimotor cortex (0.2-1.2 mA; pulse width 0.2 msec). The stimulation site was similar to that used by Schultz and Ungerstedt (1978) and the stereotaxic co-ordinates are described in Chapter 2. Several neurones (4-6) could usually be recorded during each successful penetration, and in exceptional experiments as many as 16 neurones were recorded during one electrode track.

TABLE 1: Striatal neurone firing.

	No. of cells	% of total
Completely silent	228	61
Occasional (rate: <0.18 Hz)	88	23
Bursting activity	59	16
	375	100





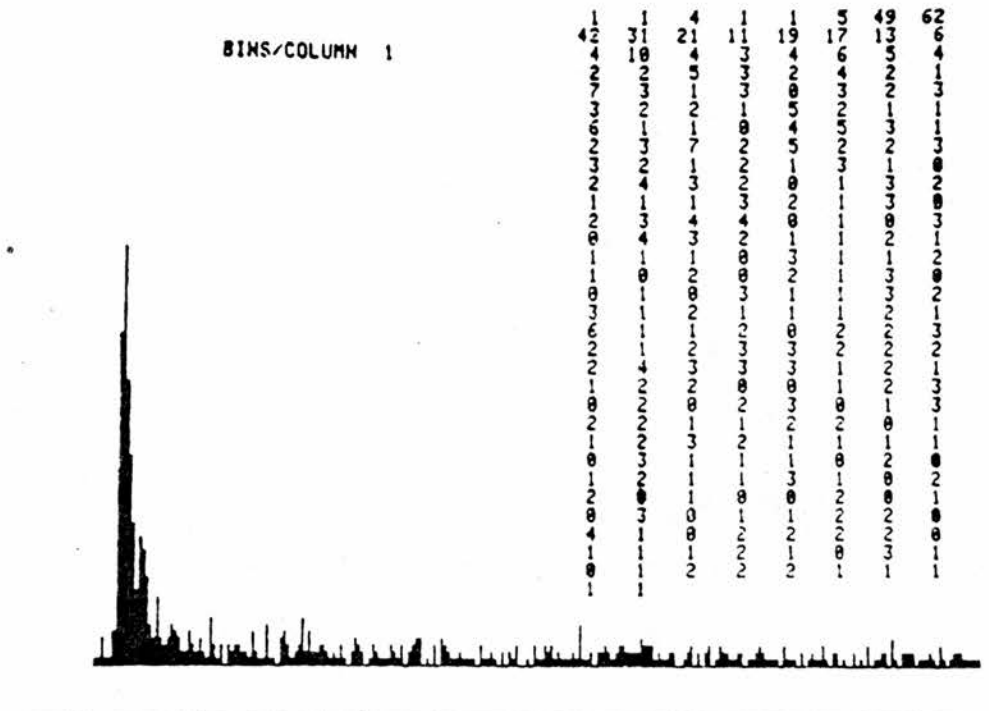


FIGURE 14: An interval histogram of a spontaneously firing cell. This histogram was constructed from a total of 716 spikes counted over a 5 min. period. The bin width in this histogram is 1 msec. A further 108 spikes which occurred at intervals greater than 256 msecs are not shown on the histogram but were used to compute the mean firing rate. This cell had a modal value of 7 msecs, indicating a within-burst firing rate of 143 spikes/sec. The mean firing rate was 2.7 spikes/sec.

The usual response to cortical stimulation was one spike per stimulus (Fig. 15), but in some cases 2 or 3 spikes per stimulus were observed from neurones which were normally silent (Fig. 16). In the case of spontaneously active neurones, 3 or 4 spikes per stimulus were often seen.

At stimulus intensities lower than those required for 1 : 1 following, considerable variation in latency was observed. With increasing stimulus strength, there was a decrease both in the mean latency of the response and in the variation of the latency. Post stimulus histograms (PSTH) were constructed for 212 cells; the response latency ranged between 4 to 19 msecs (Fig. 17). These figures include data from both spontaneously active neurones and silent neurones. Cells exhibiting spontaneous activity responded with an initial excitation which was of the same latency as that of silent cells. This initial excitation was followed by a period of inhibition, lasting for around 200 msecs, and a subsequent rebound excitation (Fig. 18). The inhibition had a range of between 160 msecs and 290 msecs with a mean duration of 220 msecs. A PSTH from a typical cell is shown in Fig. 19.

Post-tetanic potentiation

Thirteen cells were tested for an effect of tetanic stimuli before the test PSTH. Trains of stimuli at frequencies from 5-10 Hz were applied for 5-10 secs, at intervals from 100 msecs to 1 sec. before the test PSTH, but produced no change in the response to cortical stimulation.

However, silent cells which responded poorly to cortical stimulation could often be induced to fire in response to a test stimulus if a single stimulation pulse (width 0.2 msec.) was applied 10 to 55 msecs before the test pulse (Fig. 20).

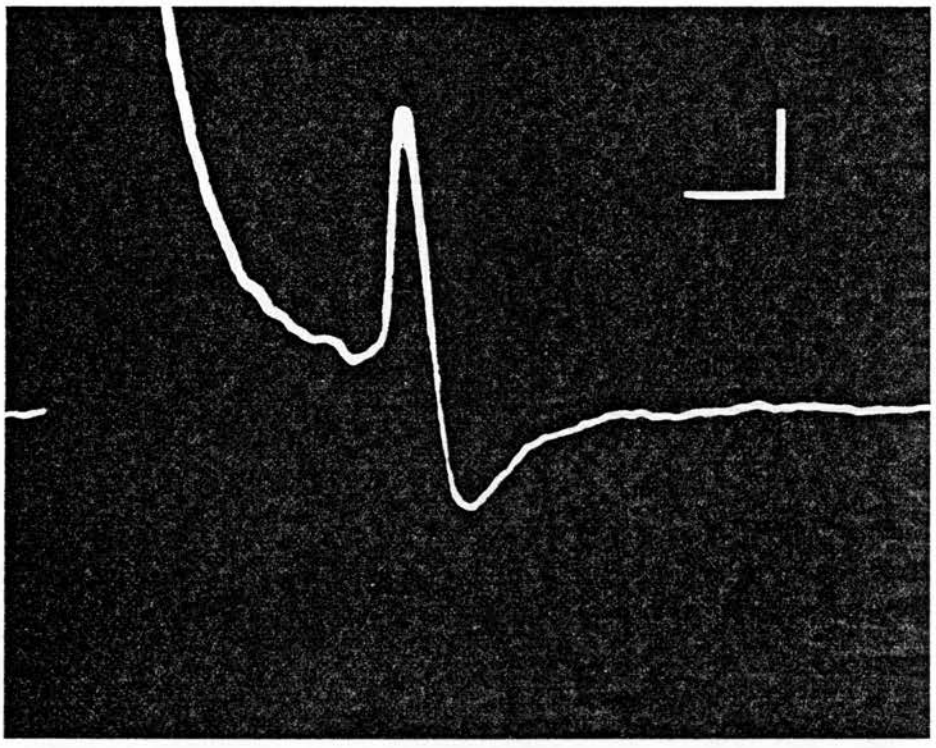


FIGURE 15: An action potential evoked from a silent striatal cell by a single stimulus applied to the cortex.

Scale - 0.5 mV 2 msecs

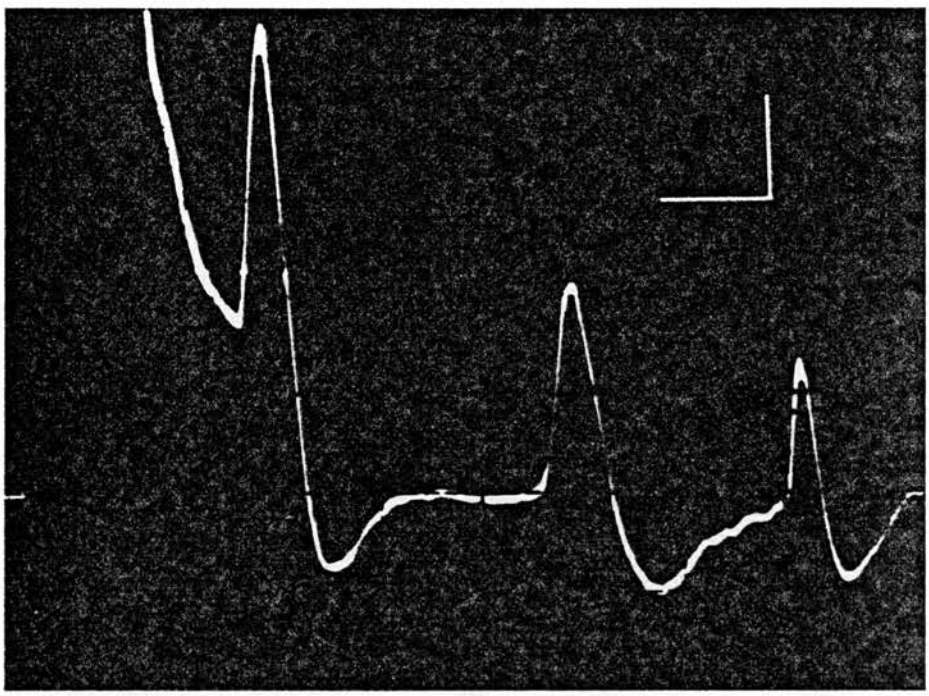


FIGURE 16: Three spikes evoked from a striatal cell by a single cortical stimulus.

Scale - 0.5 mV 2 msecs

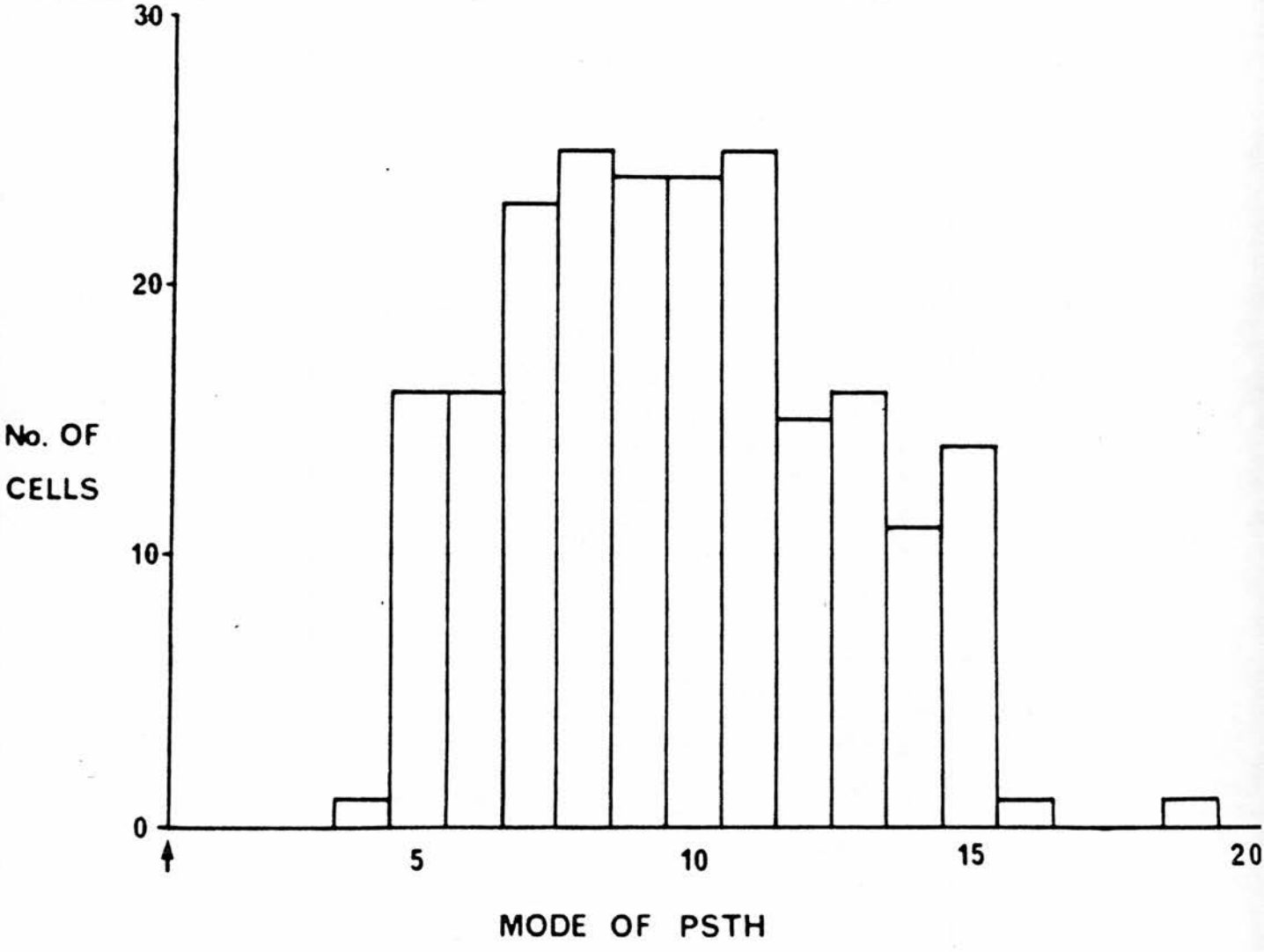


FIGURE 17: The distribution of the modal values of post-stimulus histograms constructed from 212 cells.

Bin size 1 msec.

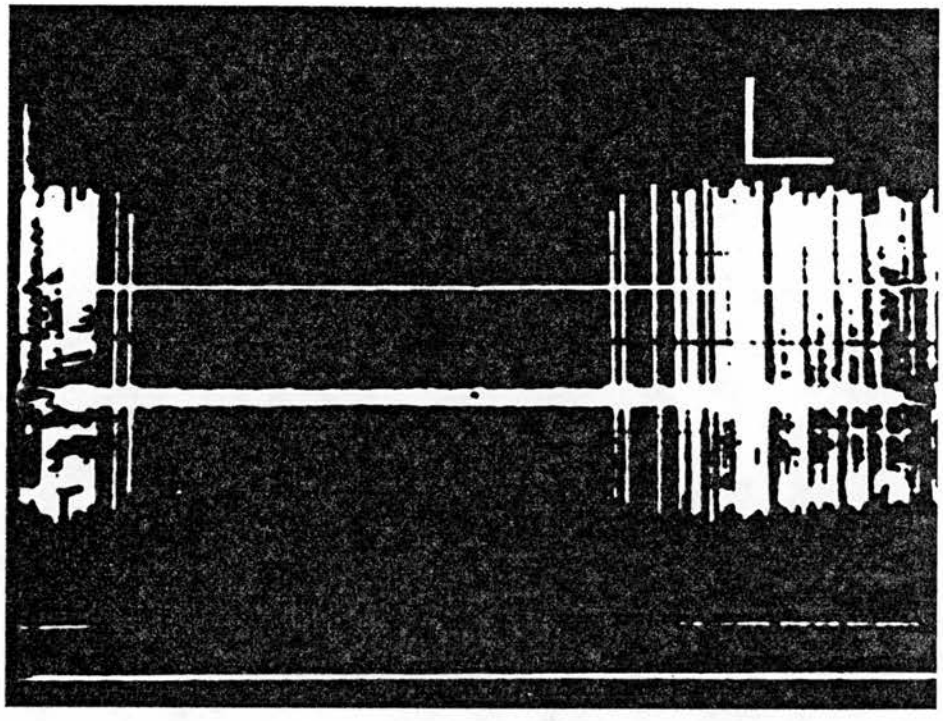


FIGURE 18: An oscilloscope trace photographed during the construction of a post-stimulus histogram from a spontaneously firing cell. The inhibition of spontaneous firing, starting approximately 30 msecs after the stimulus can be clearly seen.

Scale - 0.5 mV 50 msecs

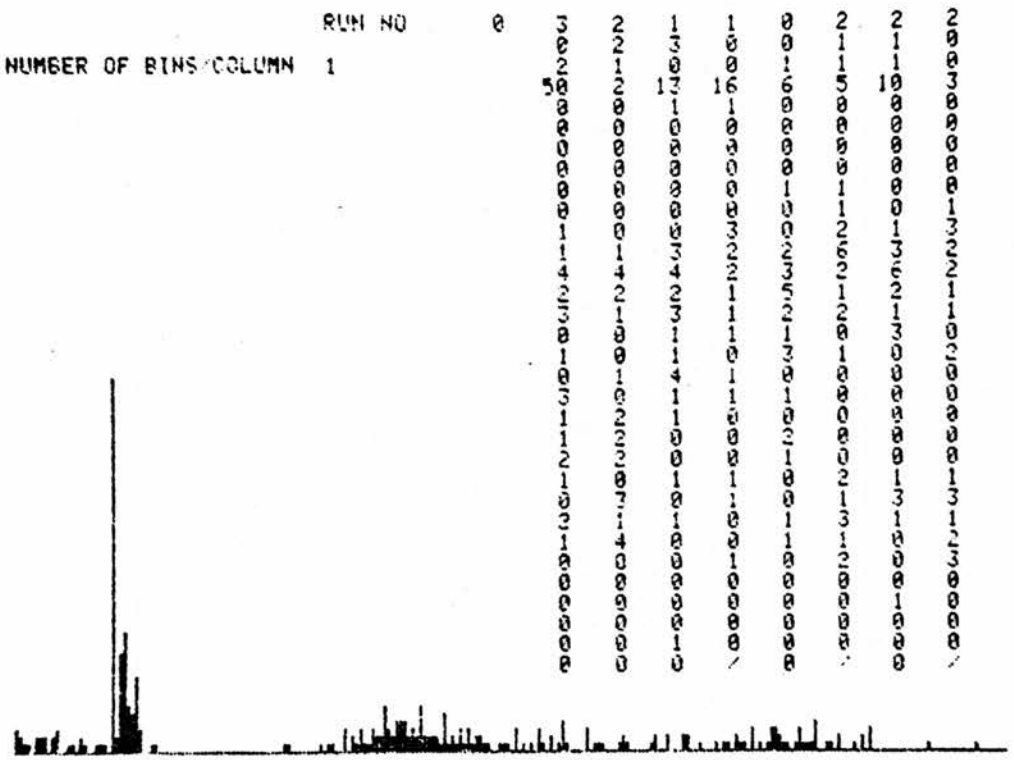


FIGURE 19: A post-stimulus histogram (PSTH) of a spontaneously firing cell. The PSTH is constructed from 50 consecutive stimuli. The number of spikes collected in each bin is shown on the right hand side of the diagram and are read from left to right, top to bottom.

Stimulus rate	1/sec.
Bin size	5 msecs

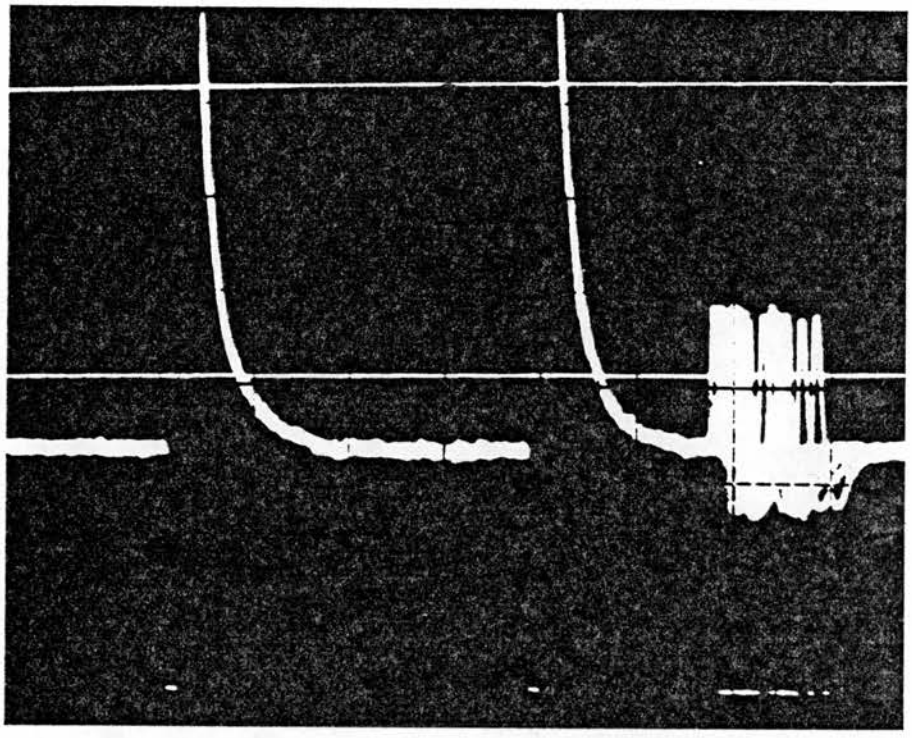


FIGURE 20: Double shock stimulation. The first stimulus evokes no responses but an identical stimulus 20 msecs later produces 1 : 1 following.

Scale - 0.5 mV 5 msecs.

Antidromic activation of striatal output neurones

In animals prepared with a stimulating electrode in the crus cerebri, 76 (31%) neurones from a population of 242 were found to be antidromically driven. Correct placement of the crus cerebri stimulating electrode was often indicated by antidromic excitation of cortical neurones, recorded during the passage of the recording electrode to the striatum (Fig. 21).

The action potentials of all the cells were biphasic with a total duration of approximately 4 msecs. Occasionally, a notch was seen on the rising phase of the action potential. At threshold stimulation this was revealed as an initial segment spike if the stimulus failed to invade the soma (Fig. 22).

Spontaneous activity was recorded from 9 (12%) of the neurones. This was always in the form of random single spikes at intervals of 30 secs to 3 mins. Bursting activity was never observed. None of the 9 neurones was located by virtue of its spontaneous firing. This only became apparent during tests to confirm antidromicity.

Three criteria were used to confirm antidromicity:

1. Constant latency of action potential at suprathreshold stimulation.
2. The ability to follow stimulation at high frequencies ($\geqslant$ 100 Hz). To test for ability to follow frequencies greater than 100 Hz, a double shock technique was employed. The striatal output cells were able to follow two stimuli, applied at constantly decreasing interstimulus intervals, down to 2 msecs.
3. Collision of an antidromic action potential with an orthodromic action potential (Fig. 23). Because of the silent nature of the output cells, the third criterion was usually met by evoking an orthodromic

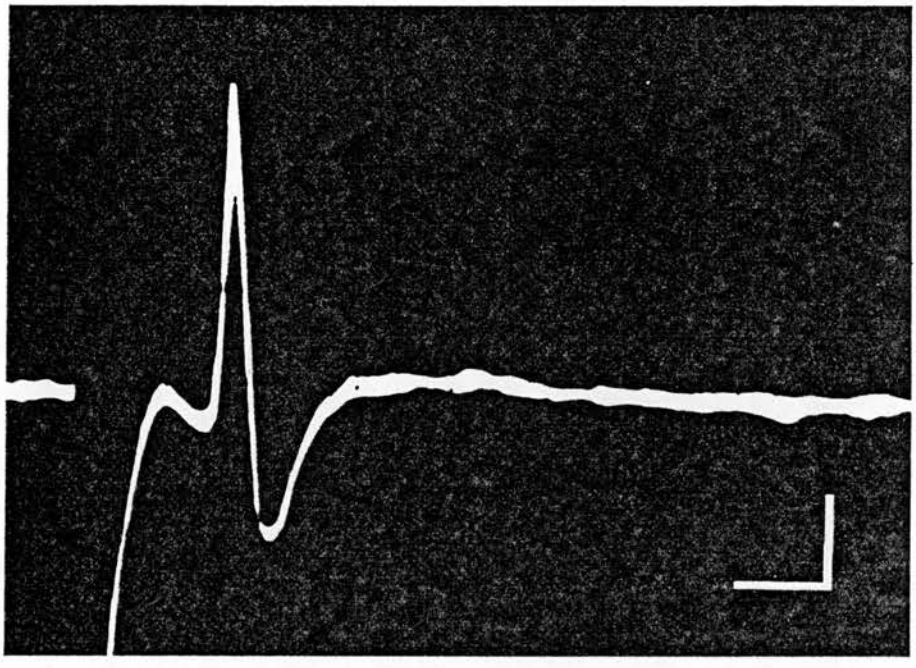


FIGURE 21: An antidromic action potential evoked from a cortical cell by crus cerebri stimulation.

Scale - 0.2 mV 2 msecs.

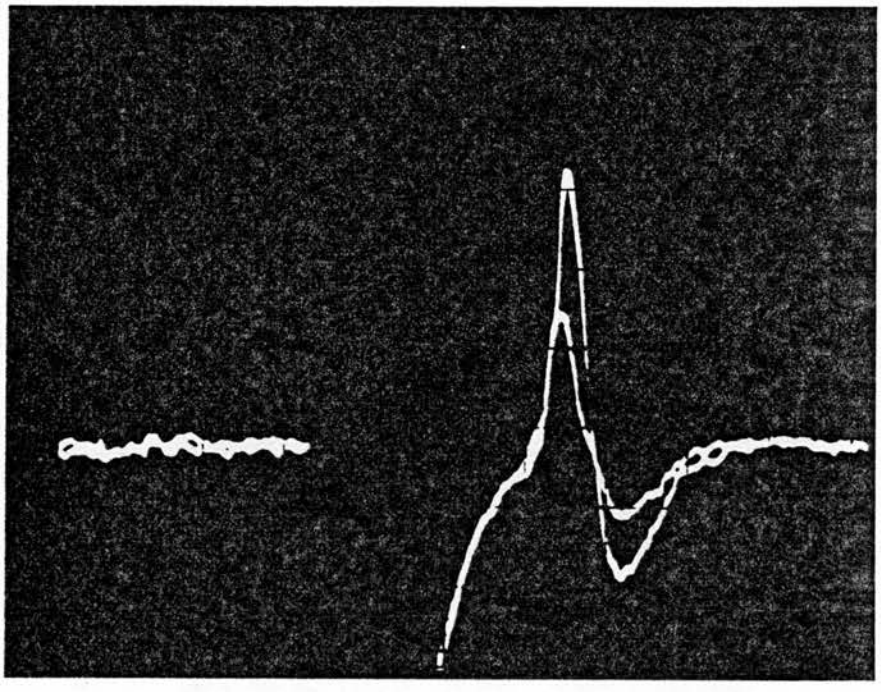


FIGURE 22: An antidromic action potential from a striatal cell. Both the initial segment (IS) and full somato-dendritic (SD) spikes are illustrated.

Scale - 0.2 mV 2 msecs.

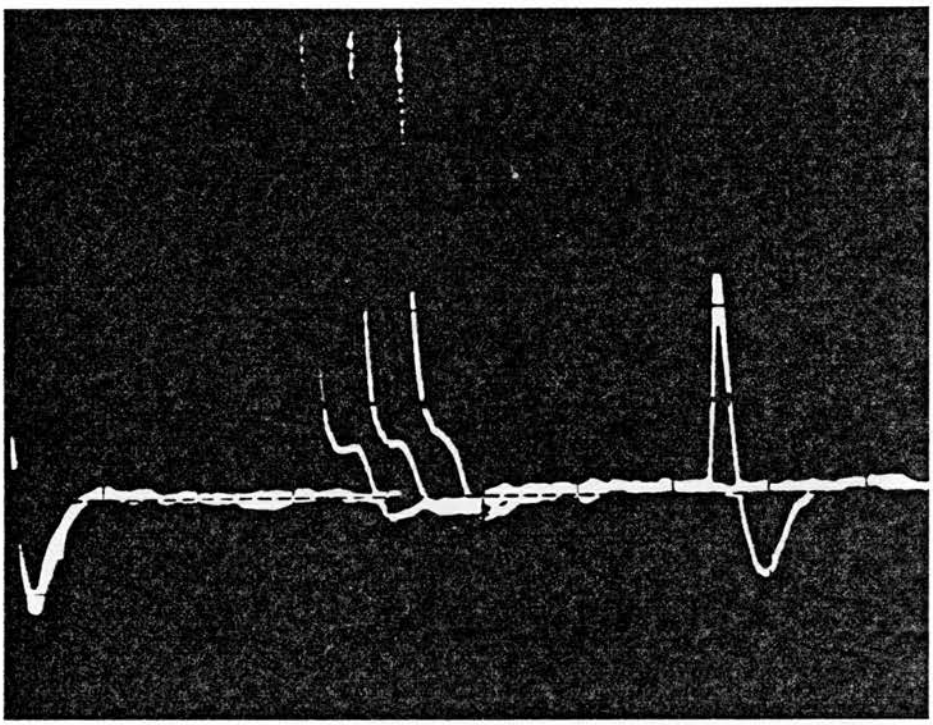


FIGURE 23: A collision test. Two unsuccessful stimuli are shown at 6 and 7 msecs after the triggering spike. An antidromic spike is evoked from a stimulus occurring 8 msecs after the triggering spike.

Scale - 0.5 mV 2 msecs.

action potential with cortical stimulation or, if this was not possible, by applying glutamate to the cell by iontophoresis at very low ejection currents. It was not always easy to perform the collision test because:

a) in the former case,the window discriminator was not always able to discriminate between the artefact of the cortical stimulation and the evoked action potential.

b) In the second case, the cells tended to produce a burst reponse to glutamate, rather than single spikes. As the interspike interval of the burst was usually less than the antidromic latency it was often impossible to perform a successful collision test. Consequently, several cells which fulfilled the first two criteria but which did not fulfil the third, were discarded.

The latency of the antidromic response varied between 4 and 16 msecs, with a mean value of 9.4 msecs (Fig. 24).

From the latency histogram only one population of cells appears to be present. However, 78% of the cells were recorded in the dorsal half of the nucleus (Fig. 25). Those which were recorded at greater depths were located in lateral areas. All the cells were tested for their response to cortical stimulation. Sixty-eight cells (89.5%) were excited by cortical stimulation and 8 cells (10.5%) were completely unresponsive. Of these 8 cells, none failed to respond to iontophoretic glutamate.

Fourteen pairs of cells were recorded in which only one cell of the pair could be driven antidromically (Fig. 26). The antidromically driven cell always had a larger amplitude than the other cell, was normally silent and showed similar characteristics to the other antidromically driven cells in this study.

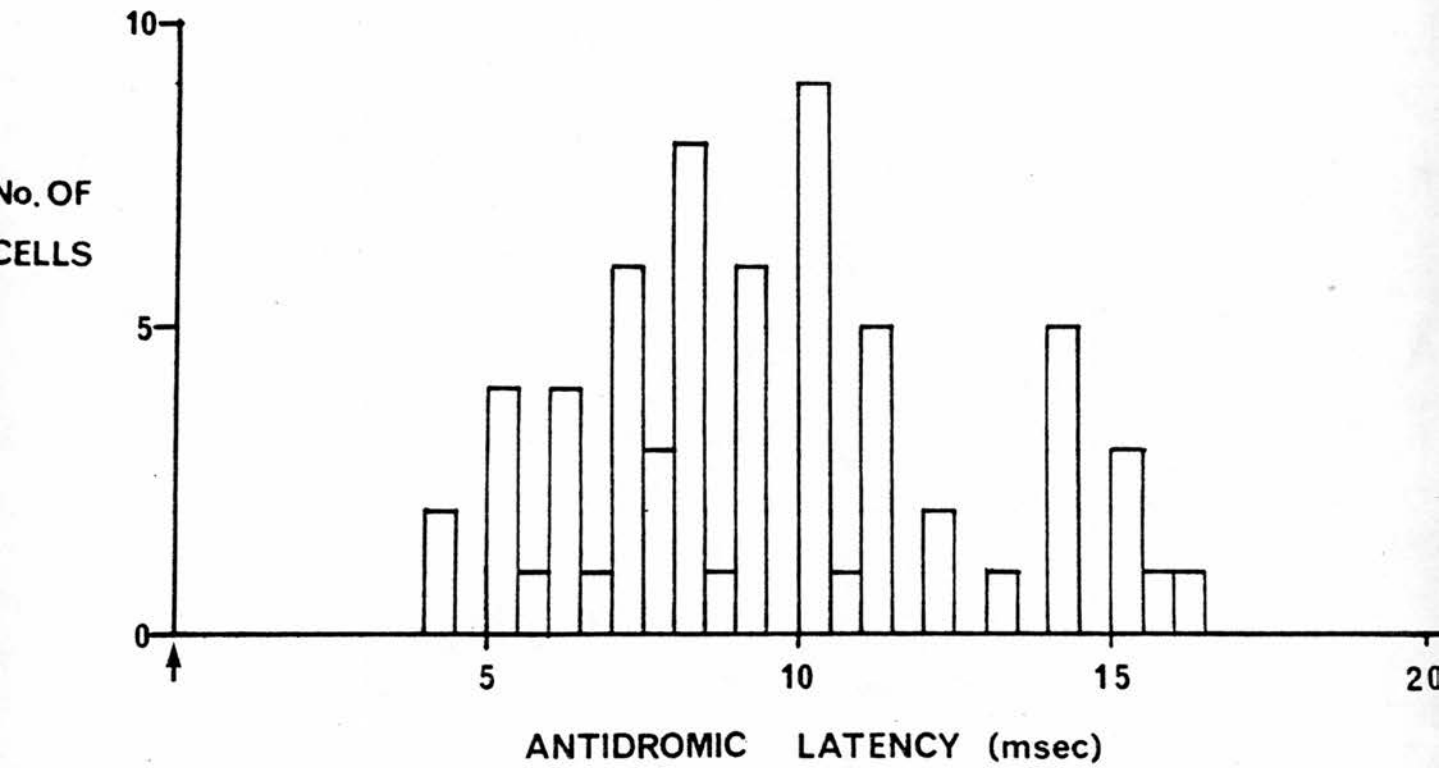


FIGURE 24: The distribution of antidromic response latencies of striatal neurones stimulated from the crus cerebri. The bin width is 0.5 msec. The arrow indicates the stimulus.

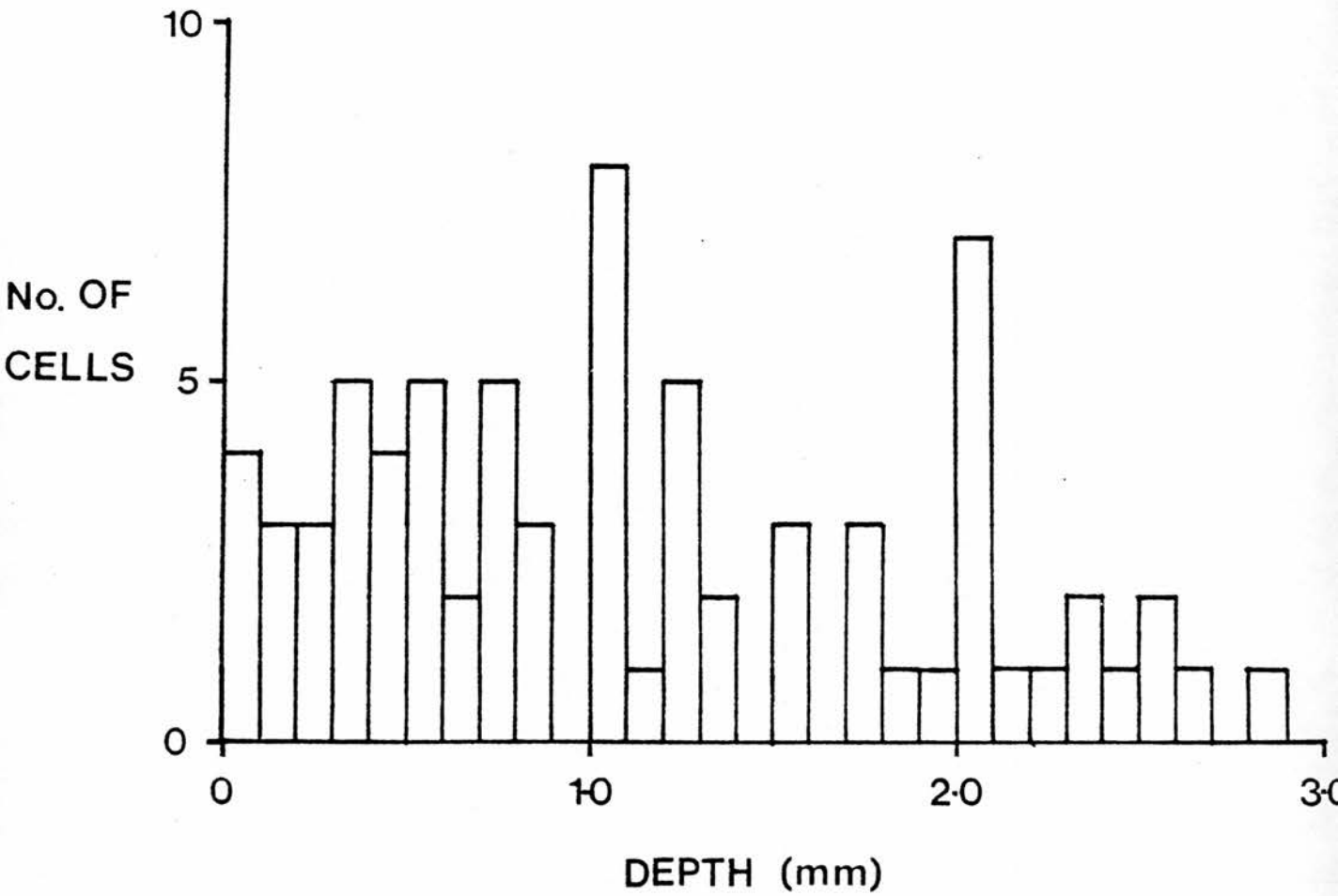


FIGURE 25: The depth distribution of antidromically driven cells.

The figure indicates the distribution of cells recorded throughout the striatum. Recording was commenced 3 mm. below the cortical surface and was continued for 3 mm through the striatum.

Bin size 0.1 mm.

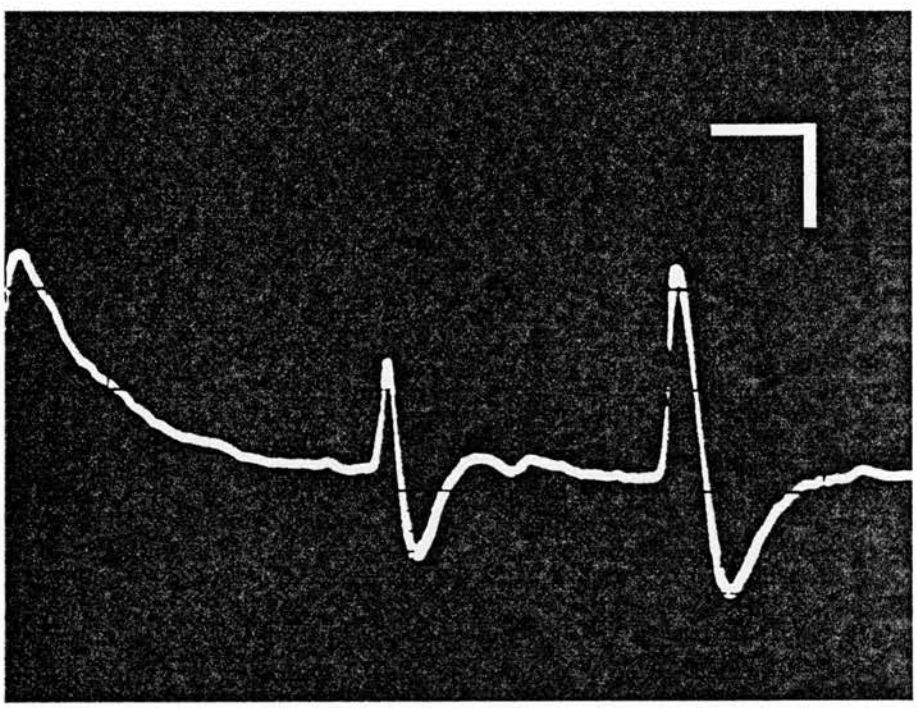


FIGURE 26: A pair of cells responding to cortical stimulation. The cell with the longer latency was antidromically driven from the crus cerebri.

Scale - 0.5 mV 2 msecs.

The other member of the pair usually had a spontaneous firing pattern which was of the "bursting" type, and often had a triphasic action potential. Both cells could be driven by cortical stimulation at similar stimulating currents and had similar latencies of response, but their firing was independent. The small cell was always more sensitive to iontophoretic glutamate than the antidromically driven cell.

Interaction between cortical stimulation and crus cerebri stimulation

Two interactions of cortical stimulation with crus cerebri stimulation were observed.

1. A cortical stimulus, sufficient to produce an action potential, if applied approximately 50 msecs to 2 msecs before the collision latency of the cell, allowed the production of an antidromic spike from a subthreshold antidromic stimulus.

2. A cortical stimulus sufficient to evoke an action potential could alter the latency of an antidromic spike. This phenomenon came to light when it was observed that the antidromic latency of a cell changed, depending on whether the stimulator was operated manually, or was triggered by a preceding orthodromic spike (Fig. 27). If there was a preceding orthodromic spike, the latency of the antidromic response decreased. The change was usually in the order of 10%, i.e. 1-1.5 msecs.

The effect was dependent on the production of an action potential preceding the antidromic stimulation. Cortical stimuli which were subthreshold for striatal cell excitation had no effect on the antidromic latency. The preceding action potential did not, however, require to be orthodromic since consecutive antidromic stimuli at appropriate intervals could also produce the effect (Fig. 28).

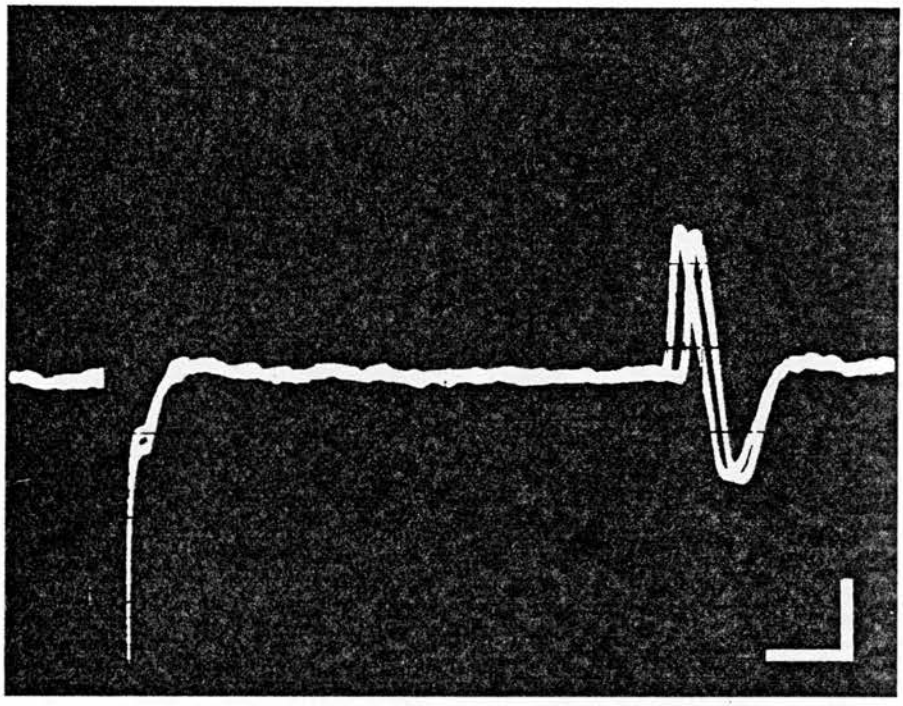


FIGURE 27: The supernormal effect. The shorter (supernormal) latency is obtained by evoking an orthodromic action potential in the striatal cell, 20 msecs before applying the antidromic stimulus.

Scale - 5 mV 2 msecs.

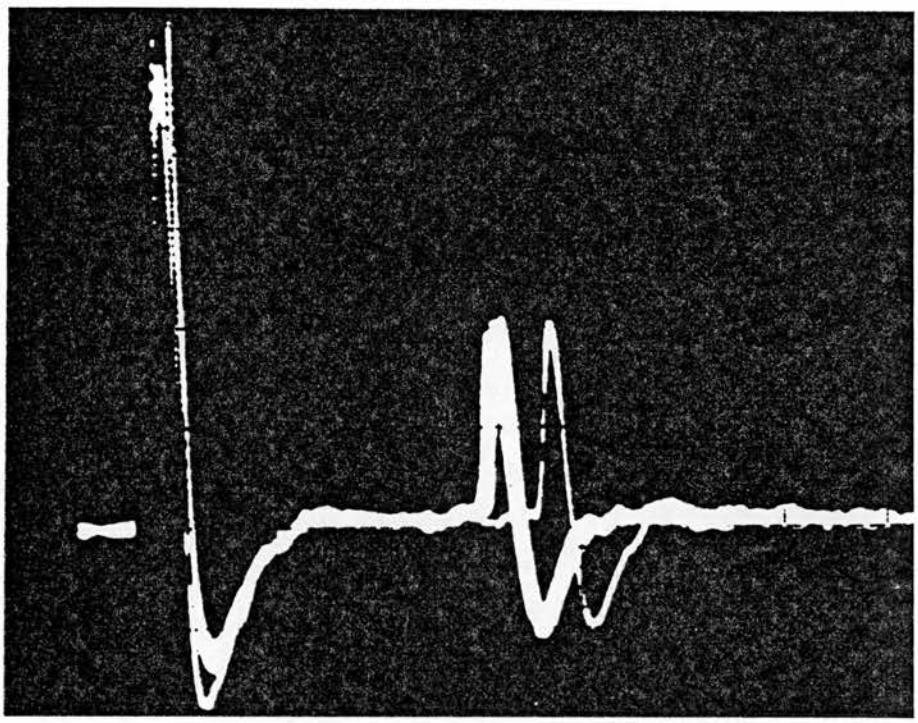


FIGURE 28: Stimulation of an antidromically identified striatal cell at 100 Hz. Only the first stimulus has a longer latency. The subsequent action potentials (24) all show the supernormal effect.

Scale - 0.5 mV 2 msecs.

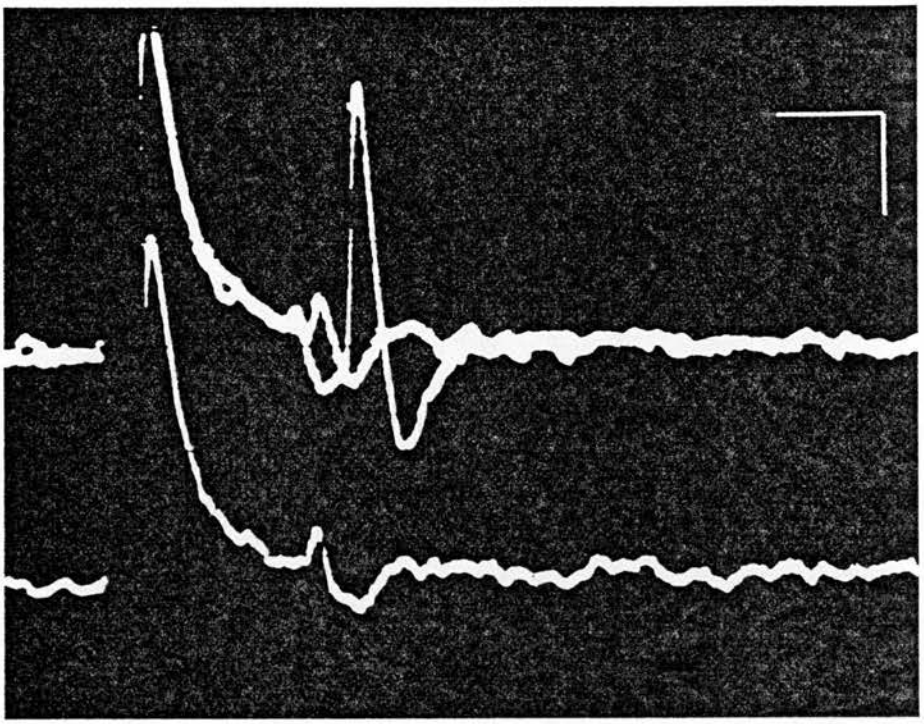


FIGURE 29: Change in IS latency with cortical stimulation. The upper trace shows an IS spike evoked by cortical stimulation and a full spike produced when a cortically evoked conditioning spike preceded the test stimulation. There is a clear shift in the latency of the IS spike when the full spike occurs. For comparison the bottom trace shows an IS spike alone.

Scale - 0.5 mV 2 msecs.

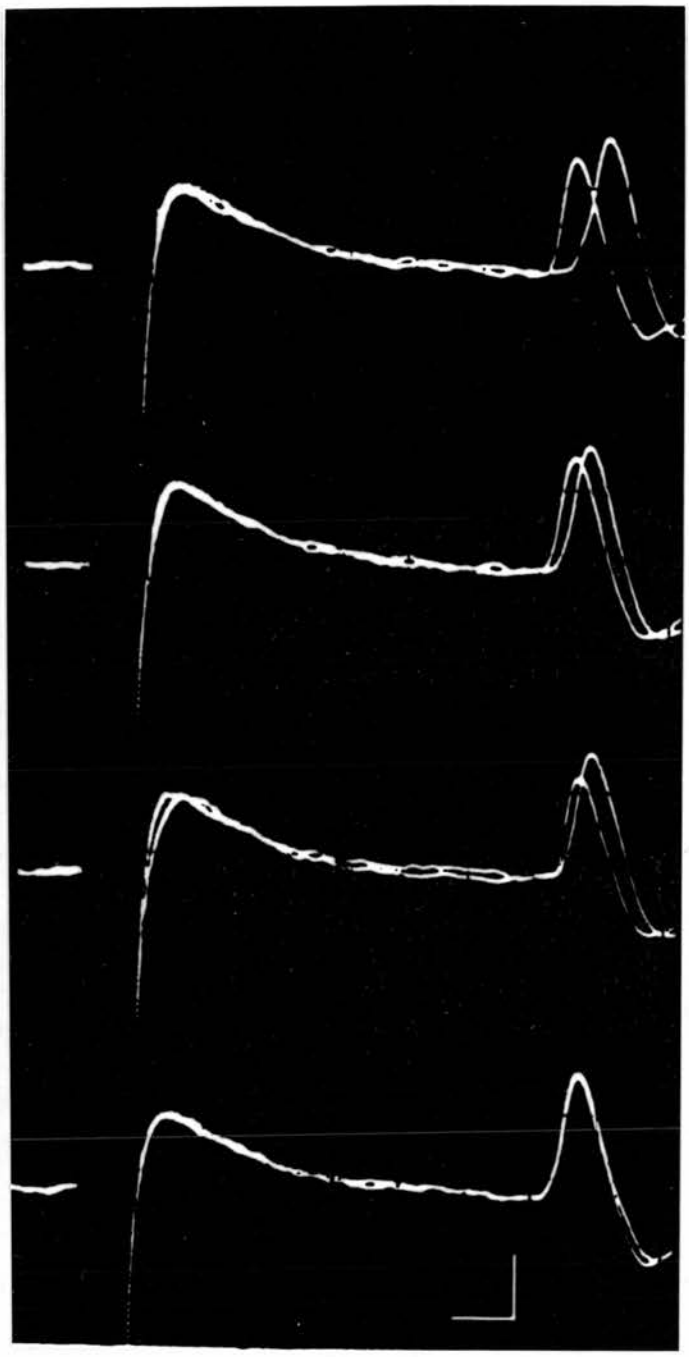


FIGURE 30: Time course of the supernormal effect. A montage showing the change in latency shift with varying intervals between the conditioning stimulus and the test stimulus. From top to bottom the intervals were: 20 msecs, 30 msecs, 40 msecs. 50 msecs.

Scale - 0.5 mV 1 msec.

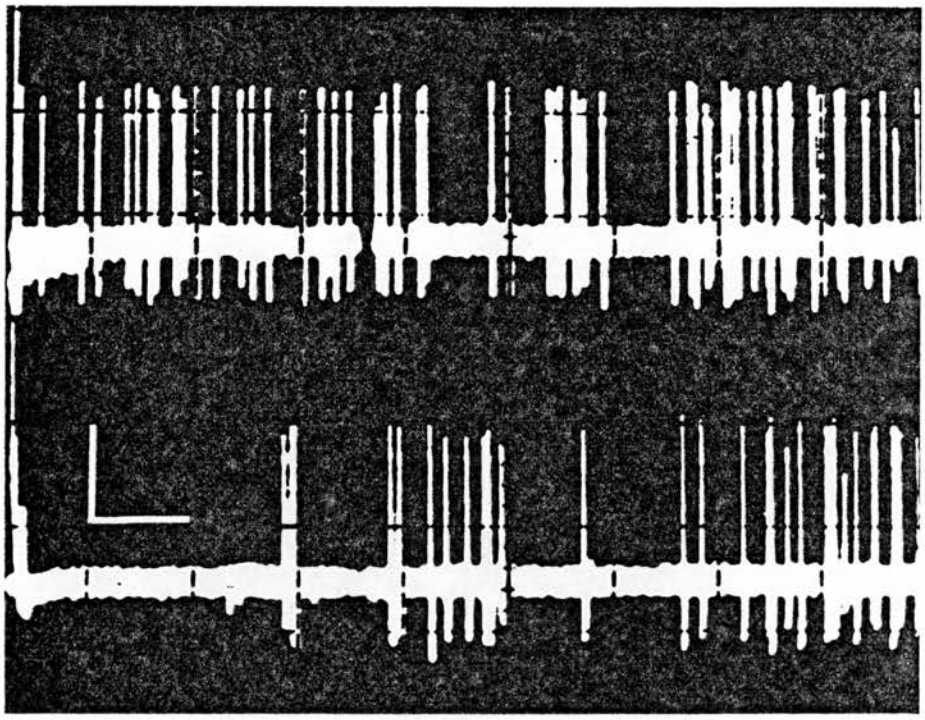


FIGURE 31: The effect of crus cerebri stimulation on the glutamate induced firing of an antidromically identified striatal output cell.

upper trace: Control - 5 sweeps with the stimulator switched off.
lower trace: Test - 5 sweeps with stimulator on.

There is an inhibition of firing lasting for over one hundred msecs after the antidromic spike.

Scale - 0.2 mV 50 msecs

If the antidromic stimulus produced only an initial segment (IS) spike, the conditioning cortical stimulus decreased the latency of the IS spike and produced a full spike (Fig. 29).

The interval between the conditioning action potential and the test antidromic stimulus affected the change in latency. The effect was maximum immediately the interval exceeded the collision time, and became progressively smaller with increasing intervals (Fig. 30). The maximum interval at which the latency change occurred varied between cells but was normally between 80 and 100 msecs.

Antidromic activation and glutamate induced firing

Three cells made to fire repetitively by the iontophoretic application of glutamate showed an inhibition of firing after antidromic stimulation. The inhibition lasted for approximately 100 msecs (Fig. 31). This suggests that recurrent inhibition may be occurring.

DISCUSSION

The characteristics of spontaneous firing patterns and responses to cortical stimuli recorded from the Halothane anaesthetised rat are very similar to those previously reported from rats anaesthetised with other anaesthetics (Spencer and Havlicek, 1974; Spencer, 1976; Davies and Tongroach, 1978; Schultz and Ungerstedt, 1978), and in an unanaesthetized preparation (Skirboll and Bunney, 1979). In particular, the low occurrence of spontaneous activity and the excitatory responses to cortical stimuli from silent and spontaneously active cells have been confirmed. During the course of the experiments it became apparent that precise positioning of the stimulating and recording electrodes was of the utmost importance for the successful recording of striatal excitations from cortical stimuli. Although no

topographical mapping was done, it was found in this study that the stimulating electrode had to be in close apposition to the recording electrode (see Methods section for co-ordinates). However, excitation of striatal cells from more remote cortical regions has been reported (Spencer, 1976), indicating that there may be considerable convergence of cortical inputs to striatal cells.

Responses of five or six spikes to single cortical stimuli reported by other authors (Stone, 1979; Spencer, 1976) were not seen here. The usual response was a single action potential, and multiple responses were usually only recorded from spontaneously active cells. In cells which responded poorly to cortical stimulation, the increased response ratio to 1 Hz stimulation caused by preceding conditioning stimuli was probably due to the presence of an e.p.s.p. when the second stimulus reached the cell. This increase in response when a preceding conditioning stimulus was applied has been reported using both extracellular (Liles, 1974) and intracellular recording techniques (Hull *et al*, 1973).

The monosynaptic nature of the excitations has been inferred from the close correlation of the results with those of other workers. It has been shown that a proportion of striatal responses may be mediated via the cortico-thalamo-striatal pathway (Cherubini *et al*, 1979). These responses are of a longer latency than any in the present study. However, as no thalamic lesions were made it remains possible that some of the responses observed could have been produced polysynaptically.

It has been proposed that post-tetanic potentiation may occur in the cortico-striatal pathway (Spencer, 1976). In the present study some unresponsive cells did increase their response ratio during stimuli at 2 or 3 Hz but there was no facilitation of response to 1 Hz stimuli after a train of conditioning stimuli.

Antidromic responses in the striatum have been recorded in cats (Frigyesi and Purpura, 1967; Liles, 1974; Kitai *et al*, 1975; Kocsis and Kitai, 1977; Kitai *et al*, 1979; Kocsis and VanderMaelen, 1979; Matsuda and Jinnai, 1980) and rats (Richardson *et al*, 1977; Davies and Tongroach, 1978). Intracellular recording of antidromic responses has been claimed in rats (Kitai *et al*, 1979) but few details are given and no records are shown.

The antidromically identified output cells reported by Richardson and co-workers (1977) are also not discussed in great detail but their results suggest that inhibition seen in cells orthodromically excited from the substantia nigra's mediated by collaterals of output cells that have been antidromically driven.

Burst responses of the type attributed by these authors to inhibitory interneurones excited by output cell collateral stimulation, were never observed in the present study.

When pairs of cells were recorded, only one cell of the pair was excited by crus cerebri stimulation and the excitation was always antidromic. The interaction of antidromic firing with the spontaneous or glutamate induced firing in the other cell of the pair was not investigated.

Recurrent inhibition of striatal medium spiny neurones has been shown in the cat (Park *et al*, 1980). Their experiments demonstrated that an action potential triggered by intracellular current injection could decrease the amplitude of subsequent e.p.s.p.'s evoked by substantia nigra stimulation, but could not affect e.p.s.p.'s evoked by cortical stimulation. Bicuculline was found to antagonise the effect, suggesting that GABA may be the transmitter involved. The authors could not exclude the presence of an inhibitory neurone mediating the

effect but concluded that it was unlikely, and suggested that autoptic synapses as well as recurrent collaterals of neighbouring medium spiny neurones were responsible for the inhibition.

Further experiments are planned to investigate whether the inhibition of glutamate-induced firing following antidromic excitation presented here is also mediated by GABA.

Another report using rats (Davies and Tongroach, 1978) contains antidromic latencies in agreement with the present study, but contrary to those of previous workers (Richardson *et al*, 1977), who showed responses of a much shorter latency (1.9-4.5 msecs). However, Davies and Tongroach found only 4% of the neurones to be antidromically activated by substantia nigra stimulation, a figure similar to that obtained in cats (Liles, 1974). This is in contrast to the figure of 31% recorded here, a figure that probably underestimates the number of output cells for two reasons, namely:

1. the cortical stimulating electrode was normally used as a "hunting" stimulus and cells stimulated from it were then tested for an antidromic response. Consequently, output neurones not excited by cortical stimulation may have been missed.
2. The striatal projection is topographically organized within the crus cerebri and it is unlikely that all the output fibres could be stimulated by the electrode.

Estimates of the relative numbers of output cells to interneurones calculated from anatomical techniques have changed dramatically in recent years. The original Golgi study (Kemp and Powell, 1971) suggested that only 5% of the striatal cells were output neurones. More recent investigators have used retrograde transport of HRP (Grofova,

1975; Bunney and Aghajanian, 1976; Tulloch *et al*, 1978) and Herpes Simplex virus (Bak *et al*, 1978) to show that as many as 30-50% of the cells in some areas of the striatum are output neurons. The distribution of cells filled retrogradely by tracer substances is in good agreement with the locations of cells recorded in the present experiments, the majority being situated in the outer shell of the striatum (Bunney and Aghajanian, 1976).

Intracellular injection of HRP has revealed that medium spiny neurones can project axons outwith the striatum (Kitai *et al*, 1979; Preston *et al*, 1980). However, none of these neurones was driven antidromically from the substantia nigra, although two axons were followed past globus pallidus into the internal capsule. Whether all striatal output neurones are of the medium spiny type, and whether all medium spiny neurones are output cells, still remains to be established. Kitai and co-workers appear to fill medium spiny neurones exclusively in their experiments with HRP and it may be that the type of electrodes required for intracellular recording introduce a sampling bias.

Inputs to striatal output neurones from cortex (Liles, 1974, Kitai *et al*, 1979) and from thalamus and substantia nigra (Kitai *et al*, 1979) have been shown in cats but the study using rats mentions only inputs from the substantia nigra. The majority of output cells recorded in the present study (89.5%) responded to cortical stimulation. It may be that the remaining 10.5% received an input from an area of cortex distant from the stimulating electrode. Studies in the cat have provided widely differing numbers of cortical responses, varying from 6% (Liles, 1974) to 100% (Kitai *et al*, 1979). However, it remains feasible that a population of striatal output neurones in the rat do not receive a cortical input.

The facilitating effect of afferent input on an antidromic spike invasion found in the present study has also been shown in cats (Matsuda and Jinnai, 1980). Conditioning shocks from cortex allowed the production of full action potentials by crus cerebri stimulation which when applied alone was only capable of producing initial segment (IS) spikes. However, these authors made no observations on latency changes after cortical stimulation because their cortical stimuli usually evoked only an e.p.s.p. rather than a full action potential.

The phenomenon of changes in antidromic latency following a previous impulse in the nerve is known as supernormality (Lucas, 1917; Graham, 1934). It is characterized by a period of increased conduction velocity and excitability in the axon membrane following the relative refractory period of an action potential. The phenomenon has been demonstrated in cerebellar parallel fibres (Gardner-Medwin, 1972), visual callosal axons (Waxman and Swadlow, 1976) and cat striatal output neurones (Kocsis and VanderMaelen, 1979). The latter experiments used intracellular current injection to alter the resting potential of the cell and to evoke action potentials. They showed that the soma polarization level does not influence the invasion time of an antidromic spike. Neither depolarizing currents which were close to threshold for an action potential, nor hyperpolarizing currents sufficient to block the SD spike had any influence on the latency of the IS spike. The change in conduction velocity reported by these authors was dependent on an antecedent action potential conducted along the axon, and agrees with changes noted in the present work.

Other authors working on lumbar motorneurones and spinocerebellar tract cells (Gustafsson and Lipoki, 1980), and facial motorneurons (Kitai *et al*, 1972) have shown that the cell resting potential can have a marked

effect on antidromic invasion latency; hyperpolarizing pulses delay invasion and depolarizing pulses decrease the latency. It remains to be shown why striatal output neurons do not exhibit this effect.

SUMMARY

1. The electrophysiological characteristics of striatal neurons in the Halothane anaesthetized rat are similar to those reported by other authors.

2. Striatal output cells were identified by antidromic stimulation of the crus cerebri.

3. Cortical stimulation applied at appropriate intervals before antidromic stimulation facilitated the production of antidromic spikes.

4. The striatal output cells exhibited the phenomenon of supernormality.

CHAPTER IV

Neuropharmacology of the Cortico-striatal Pathway

Introduction

Evidence that the cortico-striatal transmitter could be an amino-acid, i.e. glutamate (GLU) or aspartate (ASP)- was first provided from the results of electrophysiological experiments (Spencer, 1967). These showed that the excitation induced in striatal cells by cortical stimulation could be blocked by glutamic acid diethyl ester (GDEE), an established glutamate antagonist (Haldeman and McClennan, 1972; Haldeman *et al*, 1972; Haldeman and McClennan, 1974). In this preparation GDEE blocked the action of GLU, ASP and homocysteic acid concomitantly with the synaptic blockade, thus preventing a discrimination of which amino acid was the synaptic transmitter. Subsequent evidence for an amino acid transmitter has come from neurochemical studies of release, uptake and binding of amino acids in the striatum. By analogy with catecholamine systems (Iversen, 1967), the presence of a high affinity GLU uptake system has been proposed as a method for localising synapses which use GLU as a transmitter (Logan and Snyder, 1971). However, uptake measured in studies performed using synaptosomal preparations (Logan and Snyder, 1971; Divac *et al*, 1977; McGeer *et al*, 1977; Walaas, 1981) may not be mediated exclusively by presynaptic elements. Isolated glial cells, and also a "gliasome" preparation from cultured glial cells have both been shown to have a high affinity GLU uptake system similar to that demonstrated in whole brain preparations (Henn *et al*, 1974). Allowing for a glial component of the total GLU uptake system, evidence for a GLU synapse in the striatum has come from changes in GLU uptake following cortical lesions. Reductions in GLU uptake of approximately 40% are observed 7-14 days after the removal of the cortex by suction (Divac *et al*, 1977; Walaas, 1981), or by a series of knife cuts (McGeer, 1977). Cortical lesions did not alter ~~in~~ the levels of GABA or dopamine, or of the enzymes choline acetyltransferase.

glutamic acid decarboxylase and dopa decarboxylase which are found in the striatum. Lesions of the ventrolateral and intralaminar thalamic nucleii did not affect striatal GLU uptake, and when performed in conjunction with lobectomy there were no neurochemical changes additional to those produced by lobectomy alone.

The high affinity uptake systems for GLU and ASP in the central nervous system appear to have identical characteristics (Logan and Snyder, 1971; Balcar and Johnston, 1972), therefore although the uptake of GLU has been intensely studied, these experiments cannot distinguish whether GLU or ASP is the transmitter. GLU has become the preferred candidate because the results of Kim *et al* (1977) showed that following cortical ablation, the level of GLU in the striatum falls by approximately 30%, whereas the level of ASP remains unchanged. These results have been confirmed recently in a more detailed study by Fonnum *et al* (1981).

The release of GLU by striatal tissue has been studied both *in vitro* and *in vivo*. *In vitro* studies have demonstrated the Ca^{++}-dependent release of both endogenous GLU (Rowlands and Roberts, 1980) and [3H]-GLU, synthesized from [3H]-glutamine (Reubi and Cuenod, 1979; Reubi, 1980) by a high $[K^+]$ stimulation of striatal slices. A large cortical ablation performed between 2 and 3 weeks prior to the experiments caused a 30% drop in the release of endogenous GLU (Rowlands and Roberts, 1980) and a 65% drop in the release of [3H]-GLU (Reubi and Cuenod, 1979).

A similar type of experiment has also shown that [3H]-aspartate, newly synthesized from [3H]-asparazine, can be released from striatal tissue in a Ca^{++}-dependent manner. The release was decreased by 75% in tissue from rats with a unilateral ablation of the frontal cortex

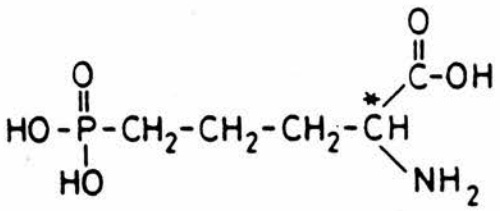
(Reubi *et al*, 1980). Bearing in mind the uncertainty over which structures take up [3H]-glutamine from incubation media, studies of the release of non-endogenous amino acid are only of value when combined with lesion experiments. This type of experiment also assumes that glutamine is the precursor of glutamate *in vivo*. While this view is well-supported (Bradford and Ward, 1976; Bradford *et al*, 1978; Reubi, 1980), glucose has been proposed as a more appropriate precursor (Costa *et al*, 1979). It has also been shown that following cortical ablation, there was no significant correlation between glutaminase activity and glutamate uptake (McGeer *et al*, 1979). These authors suggested that although the glutaminase was located in the P_2 fraction of the synaptosomal preparation, it originated from glia and GABA-ergic systems. They could not support the proposition that glutamate was directly derived from glutamine in the terminals of cortical afferents to the striatum. Similarly, the hypothesis that there is a flow of glutamine from glia to neurones (Benjamin and Quastel, 1975) has been challenged by the similarity of the glutamine uptake systems in neurones and glia and the presence of glutaminase in glia (Schousboe and Hertz, 1981).

There is a single report of GLU release *in vivo* (Godukhin *et al*, 1980). Unfortunately, the authors measured the release of [3H]-GLU which had been injected into the striatum 20 minutes before cortical stimulation. Although the release of GLU was increased during cortical stimulation, the previous criticisms of release and uptake studies also apply to this experiment, and the results would have been of more significance if endogenous GLU had been measured. Glutamate binding-sites have been demonstrated in striatal tissue by using [3H]-GLU (Biziere *et al*, 1980) and [3H]-Kainic acid (Simon *et al*, 1976; Henke and Cuenod, 1979) as ligands.

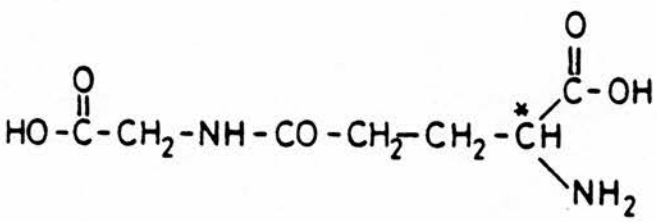
While GLU was a good competitor of kainic acid binding, kainic acid was not a good competitor of glutamate binding. This data suggests that there may be distinct binding sites for kainic acid and GLU in the striatum, something which receives further support from the failure of antagonists of iontophoretically-applied GLU, namely α-aminoadipate and GDEE, to compete for [3H]-kainic acid binding (Henke and Cuenod, 1979).

Recent work (see McClennan, 1981, and Watkins, 1981, for reviews) has suggested that there are three types of GLU receptor. These have been characterised by their selective agonists, N-methyl-D-aspartate (NMDA), quisqualic acid (QA) and kainic acid (KA). A range of antagonist drugs has also been found which can block the responses at the different receptor types in a selective manner: a) 2-amino-5-phosphonovaleric acid (APV) is a selective antagonist at NMDA receptors (Davies *et al*, 1981); b) γ-D-glutamyl glycine (γ-DGG) is an antagonist at NMDA receptors and KA receptors but not at quisqualate receptors (Davies *et al*, 1980a; Francis *et al*, 1980); Davies and Watkins, 1981); c) Cis-2-3 piperidine dicarboxylate(PDA) is a broad spectrum antagonist at all three types of receptor (Davies *et al*, 1981; Salt and Hill, 1981) (Fig. 32).

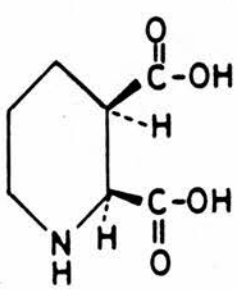
In addition, GDEE has been proposed as a selective antagonist at the quisqualate receptor (McClennan and Lodge, 1979; McClennan, 1981), but also has a significant action as an acetylcholine antagonist (Curtis *et al*, 1972; Davies and Watkins, 1979). α-Aminoadipate (Biscoe *et al*, 1978; Evans *et al*, 1978), 1-hydroxy-3-aminopyrollidone-2 (HA966) (Davies and Watkins, 1972; Curtis *et al*, 1973; Stone, 1975) and magnesium ions (Davies and Watkins, 1977; Evans *et al*, 1978) are also antagonists at NMDA receptors. 2-amino-4-phosphonobutyrate (2APB) is an antagonist at KA and NMDA receptors (Davies and Watkins, 1979).



2-AMINO-5-PHOSHONO-
VALERIC ACID (2APV)



γ-D-GLUTAMYL GLYCINE (γ DGG)



CIS-2,3-PIPERIDINE
DICARBOXYLIC ACID (PDA)

Of the three categories of receptor, the PDA/GDEE/QA receptor appears to be a GLU-preferring receptor and the APV/NMDA receptor appears to be an ASP-preferring receptor. In the present experiments, a range of antagonist drugs have been tested in order to obtain further evidence concerning an amino acid transmitter and to determine whether GLU or ASP is that transmitter.

METHODS

Extracellular recording from neurones and the iontophoretic application of drugs were performed as described in Chapter II (General Methods). The iontophoretic currents used to administer the excitant amino acids were adjusted to yield approximately equal numbers of spikes from each amino acid. Once stable control responses had been achieved the ejection cycle was controlled automatically by the Neurophore. After at least two control cycles the effect of the antagonist drug was tested and measured as the percentage change from the control response. Neurones in which a return to the control response did not occur were discarded.

To investigate the effects of the antagonist drugs on cortico-striatal transmission, post stimulus histograms (PSTH) were constructed from the responses of striatal cells to cortical stimulation. The testing procedure was similar to that used for the excitant amino acids. The stimulus intensity was adjusted to produce approximately 1 : 1 following with a reproducible total of spikes obtained during each PSTH. The PSTH's were generally constructed at 120 sec. intervals. After two control PSTH's had been constructed the antagonist drug was applied iontophoretically for between one and two minutes before the test PSTH. Any change in the PSTH was calculated as the percentage change from the number of spikes in the control PSTH. Further PSTH's were constructed at two-minute intervals until the response returned to control values.

In some experiments where a differential blockade of excitants was obtained PSTH's were constructed without the control period described above. These experiments were designed to compare the blockade of the response to excitant amino acids with that of cortical

stimulation. In these experiments control PSTH's were constructed before and after the cyclic application of the excitants.

Lack of effects of iontophoretically applied drugs

Results which show a lack of effect of an applied drug are difficult to interpret when using the technique of iontophoresis. In the present study neurones were only assigned to this category if they fulfilled the following criteria:

1. The drug ejection barrel had a resistance which was within the compliance of the Neurophore system.
2. The iontophoretic and recording barrels were subsequently shown to be still in close apposition to each other as indicated by a response to another drug, e.g. glutamate.
3. A neurone subsequently recorded using the same electrode showed a response to the drug.

Drug solutions

The following drug solutions were used to fill the barrels of the iontophoretic electrode: sodium L-glutamate (GLU) (0.2M, pH 7.0); potassium L-aspartate (Asp) (0.2M, pH 7.0); acetyl-choline chloride (ACh) (0.2M, pH 4.5); L-glutamic acid diethyl ester (GDEE) (0.2M, pH 4.0); sodium DL-α-aminoadipate (αAA) (0.1M, pH 7.0); 1-hydroxy-3-aminopyrrolid-2-one (HA 966) (0.2M in 0.2M HCl, pH 5.5); 2-amino-5-phosphonovaleric acid (APV) (0.05M, pH 7.0); γ-D-glutamyl glycine (γ-DGG) (0.2M, pH 7.0); cis-2-3-piperidine dicarboxylic acid (PDA) (0.2M, pH 7.0).

APV, PDA and γDGG were made up as 200 mM solutions dissolved in 0.2M NaOH to give a final pH of 7.0. APV was then diluted to a 0.05M solution in 165 mM NaCl.

Sources of drugs

HA-966 was a gift from Professor Bonta (Organon, Oss, Netherlands). APV, γ-DGG and PDA were gifts from Dr. J.C. Watkins (Bristol), and Dr. J. Davies (London). All other drugs were obtained from Sigma Chemical Company.

RESULTS

The results of the experiments with this range of antagonist drugs have been summarised in Table 2 and a more detailed description of the effects of each drug appears below.

GDEE

GDEE (50-130 nA for 1-3 minutes) antagonized the excitatory effects of GLU (6 out of 10 cells tested 60%) and ASP (2 out of 5 cells tested 40%). On several cells it was totally ineffective in blocking the effects of GLU (4 cells 40%) or ASP (3 cells 60%). On 2 cells where both GLU and ASP were antagonized, no differential sensitivity to GLU or ASP was observed. On 1 cell with occasional spontaneous activity, GDEE abolished the spontaneous activity during the period when the GLU response was antagonized. GDEE was ineffective in blocking the cortically evoked excitation of striatal cells (1 cell out of 8 tested [12.5%]) even at currents as high as 130 nA for up to 3 minutes.

HA 966

HA 966 (30-75 nA for 1½-2 minutes) antagonized the excitatory effects of GLU (12 cells out of 20 tested [60%]) and cortical stimulation (5 cells out of 8 tested [62.5%]) on striatal cells. Three cells with spontaneous activity, showed a reduction in firing rate during the blockade of glutamate responses.

TABLE 2:

Antagonist Ejection current nA range (mean ± SEM)		Excitant No. of cells depressed / No. of cells tested (mean ± SE of depression)		
		GLU	ASP	Cortical stimulation
APV	35 - 50 nA 41.0 ± 1.8	7/11 (71.7 ± 11.3)	9/9 (72.0 ± 5.2)	8/10 (76.0 ± 5.2)
⍺ DGG	25 - 60 NA 41.0 ± 2.8	9/9 (69.3 ± 7.7)	7/7 (86.3 ± 4.6)	7/7 (82.8 ± 5.4)
PDA	20 - 60 nA 50.5 ± 2.6	7/7 (44.0 ± 11.2)	7/7 (66.6 ± 3.2)	4/4 (90.5 ± 3.5)
GDEE	60 - 130 nA 89.0 ± 6.8	6/10 (68.0 ± 7.1)	2/5 (79.0 ± 15.5)	1/8 50
αAA	30 - 70 nA 48.1 ± 4.4	7/11 (86.4 ± 5.3)	-	8/15 (57.4 ± 9.4)
HA 966	30 - 60 nA 52.5 ± 3.3	12/20 (50.8 ± 8.2)	-	5/8 (57.2 ± 8.6)

αAA

Similar results to those obtained with HA 966 were seen with αAA (30-80 nA for 1½-2 minutes). GLU excitation was blocked (7 out of 11 cells tested [64%]) as was synaptic excitation (8 out of 15 cells tested [53%]). Spontaneous activity was reduced in 3 cells during GLU antagonism.

APV

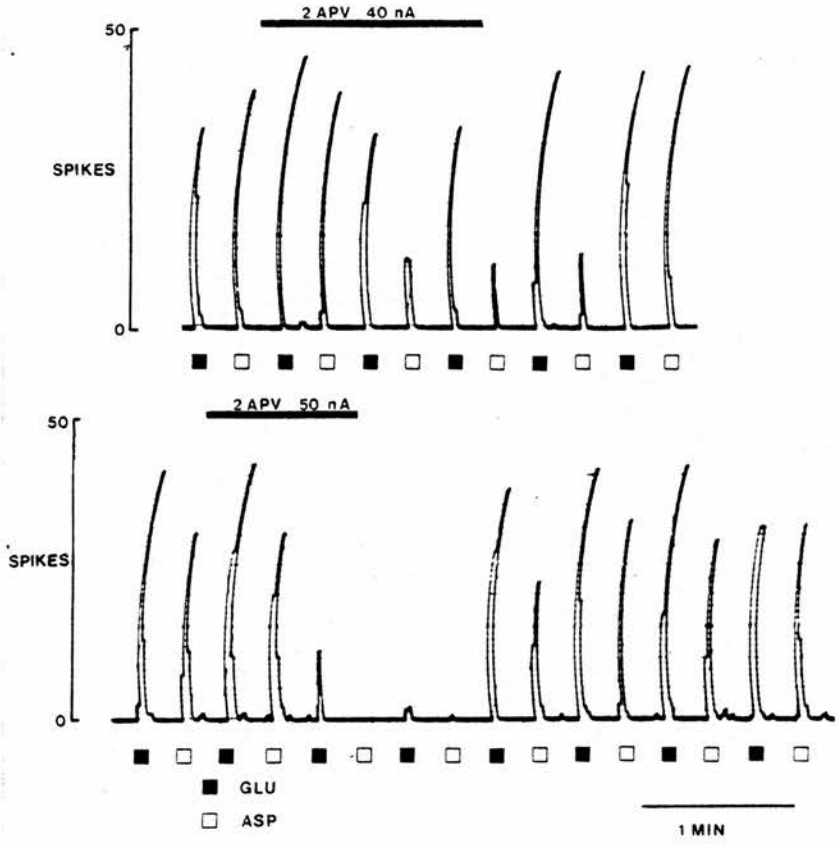
APV (20-50 nA for 1-2 minutes) was effective in blocking GLU (7 cells out of 11 tested [64%]), ASP (9 cells out of 9 tested [100%]) and cortical excitation (8 cells out of 10 tested [80%]). The 4 cells on which GLU was ~~unaffected~~ ineffective were recorded while attempting to achieve a differential blockade of GLU and ASP. If higher ejection currents had been used they would presumably also have blocked the GLU response. Differential blockade of GLU and ASP was achieved on 4 cells. Complete blockade of ASP without affecting GLU responses was not achieved.

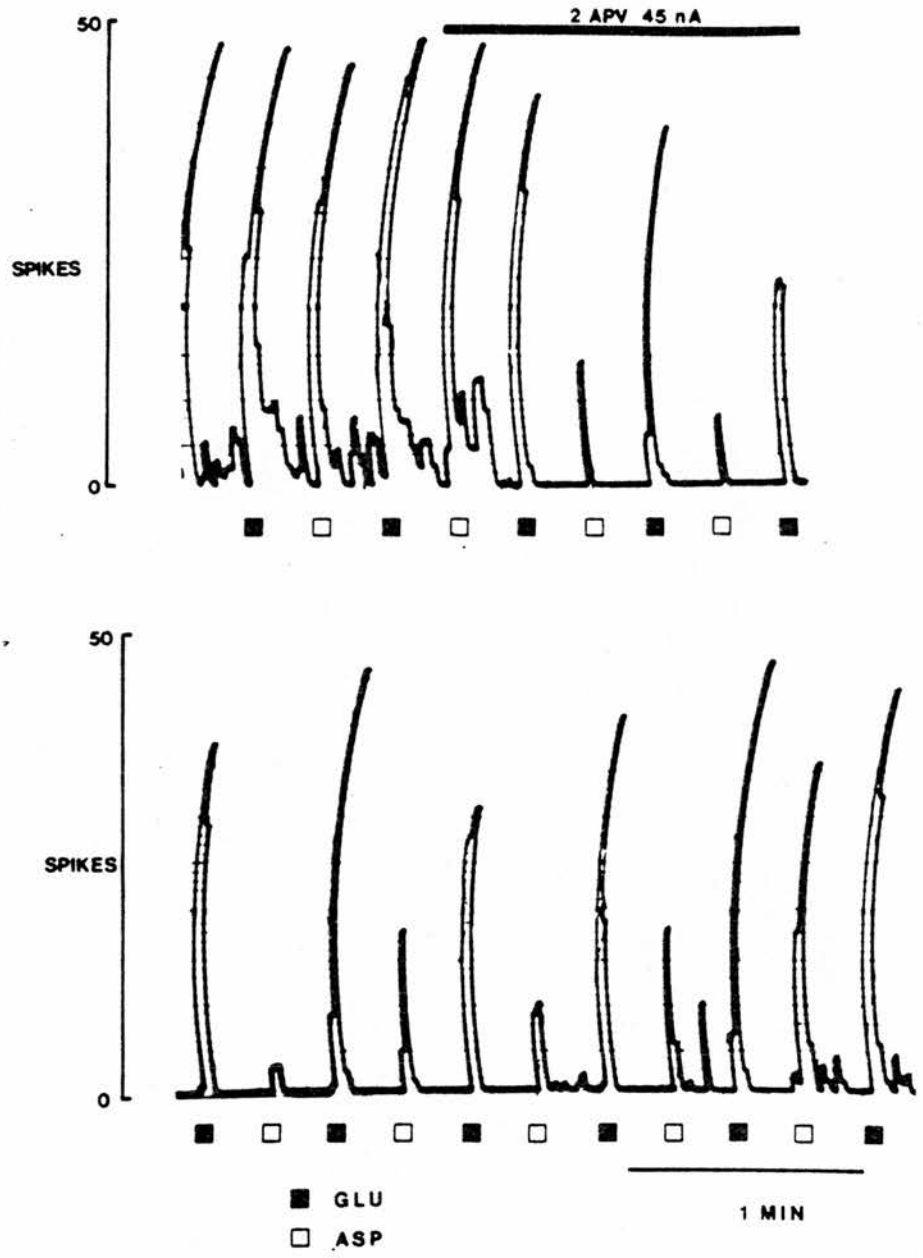
Increasing the APV ejection current in an attempt to abolish completely the ASP response resulted in an equal blockade of both responses (Fig. 33). Cortical excitation was tested on two cells when differential blockade was present. In both cases the PSTH was unaffected when the ASP response was severely reduced, but not completely abolished (Fig. 34).

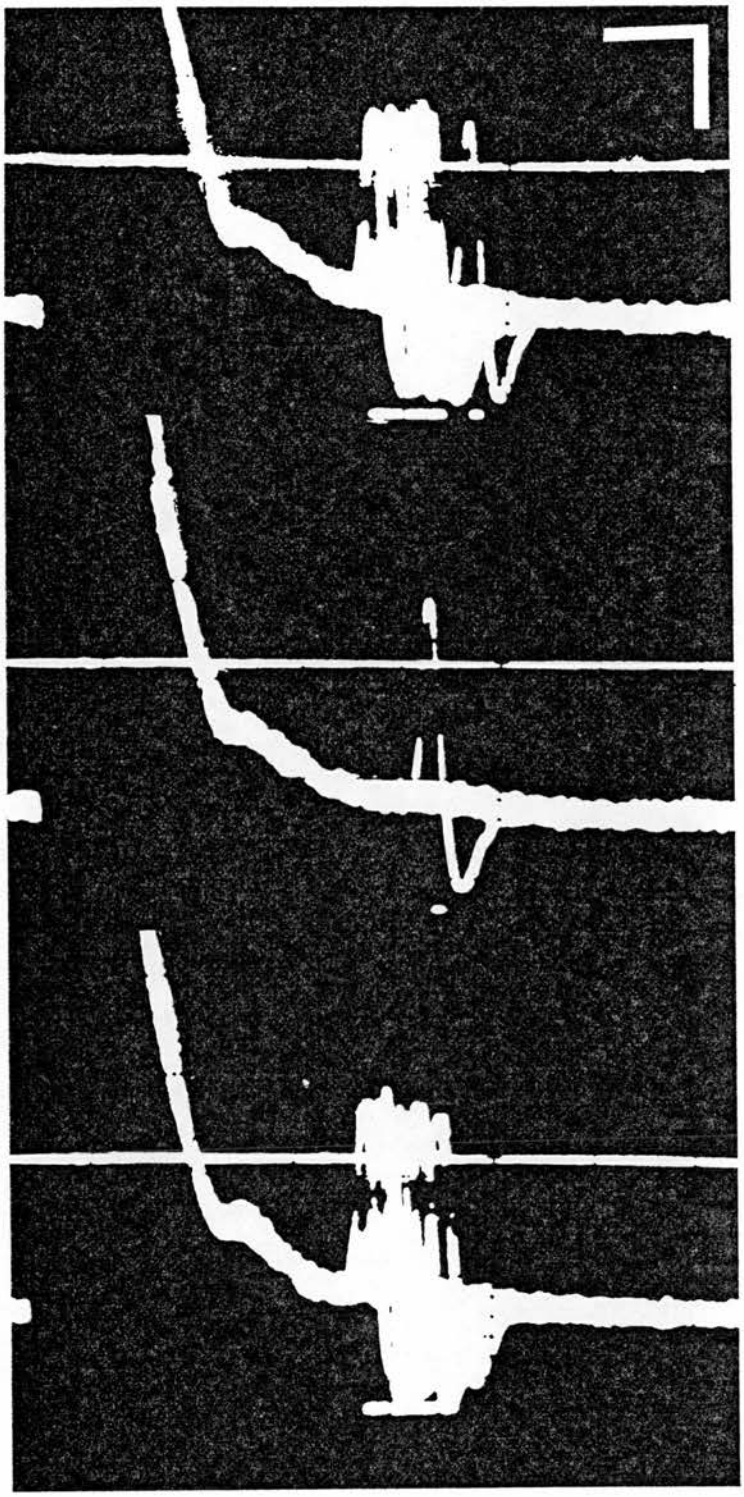
No spontaneously firing cells were tested with APV.

γ-DGG

γ-DGG (25-60 nA for 1-2 minutes) was effective in antagonizing GLU (9 cells out of 9 tested [100%]), ASP (7 cells out of 7 tested [100%]), and cortical stimulation (7 cells out of 7 tested [100%]) (Fig. 35). Differential effects on GLU and ASP were not observed. The spontaneous firing of two cells was depressed during GLU and ASP blockade.


2 APV 40 nA
50
SPIKES
0
2 APV 50 nA
50
SPIKES
0
GLU
ASP
1 MIN


2 APV 45 nA
50
SPIKES
0
50
SPIKES
0
GLU
ASP
1 MIN



PDA

PDA (45-60 nA for 1-2 minutes) antagonized the excitatory responses of GLU (7 cells out of 7 tested), ASP (7 cells out of 7 tested) and cortical stimulation (4 cells out of 4 tested). A differential effect on GLU and ASP was observed on only one cell (Fig. 36). The responses to both amino acids were reduced, but responses to ASP considerably more than those of GLU. No spontaneously active cells were tested with PDA.

Output cells

Eight of the cells on which cortical driving was antagonized by PDA (1), APV (3), γ-DGG (2), HA 966 (1) and GDEE (1) were identified as output cells by antidromic stimulation from the crus cerebri.

DISCUSSION

The ability of all the drugs tested in the present experiments to act as antagonists of the excitatory amino acids GLU and ASP has previously been demonstrated by other workers (see introduction to this section for references). However, only two of the drugs, GDEE (Spencer, 1967) and αAA (Stone, 1979) have previously been tested on the cortico-striatal pathway.

In contrast to the results of Spencer (1967) the present experiments found GDEE to be ineffective in blocking the cortically-induced excitation of striatal cells. Table 2 indicates that the new antagonists APV, PDA and γDGG were all more successful antagonists of GLU, ASP and cortical stimulation than αAA, HA 966 or GDEE. Two antagonists were rarely compared on the same cell, so no attempt has been made to compare the relative potencies of the drugs.

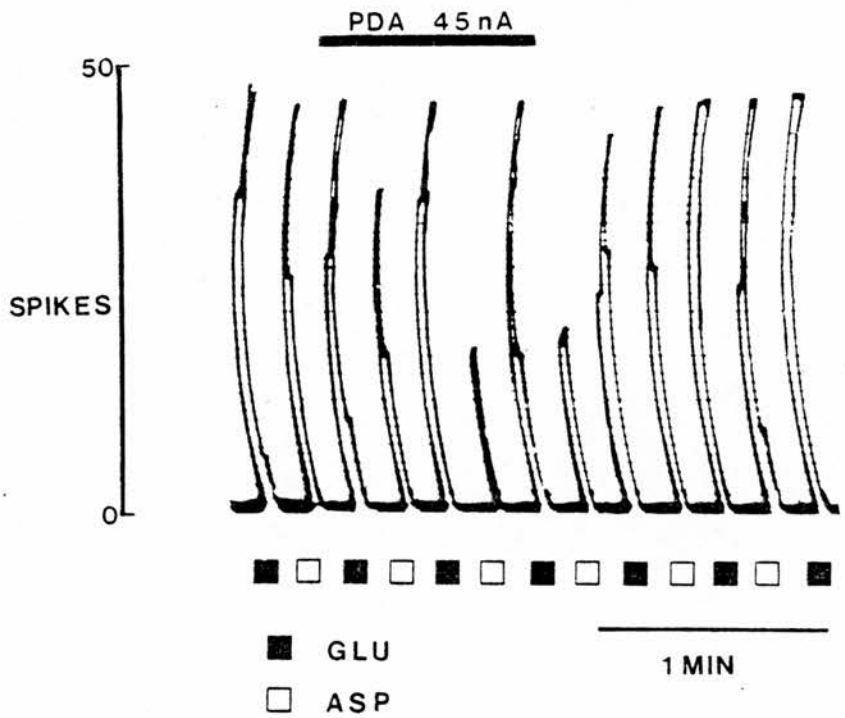


FIGURE 36: The selective antagonism of the aspartate (ASP) excitation of a striatal neurone, by cis-2-3-piperidine dicarboxylic acid (PDA). The response to glutamate (GLU) was only slightly reduced.

Ejection currents: GLU 23 nA; ASP 24 nA.

The number of cells which appeared insensitive to HA 966, αAA and GDEE might have been fewer if higher ejection currents, or longer ejection times, had been employed. The lack of success in antagonizing cortical stimulation with GDEE at the ejection currents tested may be attributed to the type of GLU receptor present on striatal cells, but this cannot explain the results with HA 966 and αAA - antagonists at the NMDA receptor (Biscoe *et al*, 1978) - when they are considered against the success of the other NMDA antagonists. The ratio of cells on which αAA was ineffective is, however, similar to that reported by Stone (1979).

It has been shown that HA 966 can block the release of dopamine (DA) in the striatum (Broxterman *et al*, 1979; Henk *et al*, 1981) and while the timecourse of the effect is uncertain, the complex interactions between DA and striatal cell firing, and between DA and GLU release from cortico-striatal slices *in vitro* (Mitchell, 1980; Rowlands and Roberts, 1980a,b; Chapter V, this thesis) make the HA 966 results more difficult to interpret.

Further experiments are clearly required to resolve the type of amino acid receptor present on striatal cells, but the success of APV in blocking the cortically evoked excitation points to the presence of an NMDA receptor. APV, γ-DGG and PDA are all antagonists at the NMDA receptor, and the blockade of cortically evoked excitation by all three supports the involvement of an NMDA receptor in synaptic transmission. In contrast, the results of Spencer (1976) would suggest a QA receptor.

The identity of the synaptic transmitter involved in the cortico-striatal pathway is not resolved by the present experiments. Spencer (1976) could not differentiate between GLU and ASP, but Stone (1979)

tentatively suggested that GLU was the probable transmitter. This is in agreement with the small number of cells reported in the present study. However, results of this type, where an increased level of blockade capable of antagonizing both GLU and ASP is required to abolish synaptic transmission, may merely reflect the "tight" nature of synaptic transmission, rather than the identity of the transmitter candidate. Results described in Chapter V demonstrate that when the response of some cells to GLU was totally depressed, they would still respond to cortical stimulation. The similarity of the block of cortical excitation achieved by a range of antagonists on both intrinsic and output cells, suggests that the same transmitter is involved.

The effects of the antagonists on spontaneous activity could be interpreted as resulting from the blockade of an excitatory input to these cells, but the absence of a control excitant other than an amino acid does not allow this interpretation. In agreement with the results of Spencer (1976), cells which were sufficiently strongly, or consistently excited, by ACh were never recorded during the course of the experiments. Therefore a direct depressant effect of the drugs on the cells studied cannot be discounted.

PDA has been reported to potentiate neuronal responses to ACh and to have partial agonist activity at GLU receptors (Davies *et al*, 1981). No excitatory effects of PDA were observed in the present experiments.

The success of HA 966, APV, PDA and γDGG in blocking the effects of ASP and GLU in the anaesthetized preparation is not supported by results from conscious animals. The unilateral injection of KA and other GLU analogues into the striatum of the conscious rat has been shown to cause a complex circling behaviour which the author's claim is dependent on an intact cortico-striatal pathway. The antagonists caused

no rotational behaviour when they were injected intrastriatally, and failed to block the circling response to several excitatory amino acids (Jenner *et al*, 1981). The exact nature of the circling response was not common to all of the amino acids tested, and whether it is mediated by the receptors involved in cortico-striatal transmission is not clear.

The results from the present study are in broad agreement with previous work (see Introduction) on the antagonists, and they have not really extended the information concerning the cortico-striatal transmitter. Further experiments, comparing the blockade of responses to NMDA and KA with synaptic transmission, are planned to resolve the questions of which amino acid receptors are present on striatal cells and whether the KA binding site is a KA receptor involved in synaptic transmission.

SUMMARY

1. The blockade of cortico-striatal transmission by the amino-acid antagonists supports the view that the cortico-striatal pathway uses an amino acid transmitter.

2. The amino acid receptor involved in cortico-striatal transmission is probably of the NMDA type.

3. It was not possible to determine whether GLU or ASP was the transmitter.

4. Further experiments to determine the transmitter are discussed.

CHAPTER V

The Effects of Dopamine in the Rat Striatum

Introduction

As described previously (see Chapter I, Section 1b), dopamine (DA) is now accepted as one of the transmitters in the nigro-striatal pathway. However, despite 15 years of investigation, there is no agreement in the literature on the question of whether its electrophysiological actions on striatal neurones are inhibitory, excitatory, or both. The diversity of results may be due to the variety of preparations and species used, or to the way in which the problem has been addressed. The action of DA on striatal neurones has been studied in three main ways:

a) Studies of the spontaneous firing rates of striatal cells following lesions of the DA containing cell bodies in the substantia nigra and of their ascending axons.

b) Studies of the effects of electrical stimulation of the substantia nigra on the membrane potential and firing rates of striatal cells.

c) Studies of the effects of the iontophoretic application of dopaminergic agonist and antagonist drugs.

Each of these approaches will now be critically reviewed.

a) Lesion studies

Electrolytic lesions of the medial forebrain bundle in cats (Ohye *et al*, 1970) were claimed to increase the firing rates of neurones in the putamen of the lesioned side, as compared to those on the contralateral side and in unoperated animals. This increase was attributed to the loss of an inhibitory dopaminergic input. However, these authors did not measure the effect of the lesion on the levels of dopamine in the striatum. In more rigorous studies electrolytic lesions

made in substantia nigra (Hull *et al*, 1974) and in the MFB (Garcia-Rill *et al*, 1980), caused 90% reductions in striatal dopamine concentrations. These authors found that cell firing rates ipsilateral to the lesion slowed 3 days post-lesion, but returned to normal by 7 days post-lesion; firing rates on the contralateral side slowed progressively post-lesion. These changes in firing rate could not be correlated with loss of dopamine. However, this approach must be considered in the light of the non-specificity of electrolytic lesions, which destroy axons of passage along with the target group of cell bodies. Therefore, damage to axons of passage may have been as significant as the unilateral dopamine loss in causing the observed effects. The existence of a non-dopaminergic nigro-striatal pathway has been claimed (Feltz and De Champlain, 1972a) and this would also have been destroyed by electrolytic lesions.

A more selective lesion of dopaminergic cells can be achieved using the neurotoxin 6-hydroxy-dopamine (6-OHDA) (Ungerstedt, 1968).

Results obtained from rats with unilateral 6-OHDA lesions of substantia nigra (Arbuthnott, 1974; Siggins *et al*, 1976) showed an increased firing rate on the side ipsilateral to the lesion. The firing rate within bursts is not changed but the interval between bursts is decreased. Using an iontophoretic application of excitant amino acids to locate otherwise silent cells, the ratio of spontaneously active cells to silent cells was shown to be increased in the striatum ipsilateral to a nigral 6-OHDA lesion (Siggins *et al*, 1976). These results point to an inhibitory role for DA, but it is still possible that the effects could be due to the loss of dopaminergic innervation to other areas which could in turn influence neuronal activity in the striatum. For example, Beckstead (1979) has shown that areas of prefrontal cortex innervated

from substantia nigra, project to the same part of striatum as that innervated from substantia nigra.

Lesion studies therefore have not given satisfactory evidence for the role of DA in the striatum.

b) Electrical stimulation of the substantia nigra

Electrical stimulation of substantia nigra should indicate the actions of the nigro-striatal dopaminergic pathway, but nigral stimulation has produced a wide variety of results (Table 3). This could be attributed to the different anaesthetics used, but a consideration of the anatomy of the substantia nigra and the neighbouring nucleii and fibre tracts may provide the explanation.

The zona compacta of substantia nigra is a thin laminar structure running obliquely in the rostro-caudal plane. In the rat it may be as thin as 0.1 mm and in the cat 0.5 mm in the horizontal plane. This presents an extremely small target for stimulating electrodes, which may have a tip diameter larger than the thickness of the nucleus itself. Nigro-striatal dopaminergic fibres are thin and unmyelinated having terminal diameters less than 0.5μ in the striatum (Fuxe, 1964; Hokfelt, 1968; Lundvall and Bjorklund, 1974).

Around the zona compacta are several large myelinated, fast-conducting fibre tracts; the medial lemniscus, the cerebral peduncles, and the dentato-rubro-thalamic tract, which certainly have lower stimulation thresholds and higher conduction velocities than the dopaminergic axons. The ventral tegmental area, the zona reticulata of substantia nigra, the subthalamic nucleus, and the red nucleus also appose the zona compacta. The close proximity of these structures therefore makes it extremely unlikely that selective activation of cells in the zona compacta can be achieved. Even if stimulation could be restricted to

TABLE 3: The effects of nigral stimulation on striatal neurones.

References		Animal	Preparation	EXTRACELLULAR RECORDS: Total No. of cells responding	% Cells excited	% Cells inhibited
Frigyesi & Purpura	(1967)	cat	encéphale isolé	47	100	-
Albè-Fessard *et al*	(1967)	cat	enchéphale isolé	94	32	68
McClennan & York	(1967)	cat	cerveau isolé	?	100	-
Connor	(1968)	cat	cerveau isolé	100	-	44
Feltz & MacKenzie	(1969)	cat	gallamine paralysis	96	100	-
Connor	(1970)	cat	cerveau isolé	-	16	46
Feltz & Albè-Fessard	(1972)	cat	gallamine paralysis	166	-	61
Gonzalez-Vegas	(1974)	rat	urethane	87	46	22
Zarzecki *et al*	(1976)	cat	decerebrate	139	17	39
Zarzecki *et al*	(1977)	cat	decerebrate	103	10	38
Richardson *et al*	(1977)	rat	urethane	?	100	-

Katayama *et al*	(1978)	cat	gallamine paralysis	136	72	28	
Norcross & Spehlman	(1978)	cat	cerveau isolé	39	100	-	
				INTRACELLULAR RECORDS:			
				Total No. of cells responding	epsp (%)	epsp-ipsp (%)	ipsp (%)
Hull *et al*	(1970)	cat	gallamine	51	16	44	0.5
Kitai *et al*	(1975)	cat	pentobarbitone	116	77	23	-
Kitai *et al*	(1976)	cat	pentobarbitone	122	100	-	-
Kocsis *et al*	(1977)	cat	α-chloralose	112	84	-	-
Morris *et al*	(1979)	cat	halothane	17	-	100	-

the substantia nigra, there are several reports of non-dopaminergic fibres ascending from the substantia nigra (Hedreen, 1971; Fibiger *et al*, 1972, Ljungdahl *et al*, 1975). Striatal output neurones can also be driven from substantia nigra (see Chapter III). These neurones have collateral axons which synapse on neighbouring striatal neurones (Sőmogyi *et al*, 1981) and may be involved in recurrent inhibition in the striatum (Park *et al*, 1979).

The short latencies of many nigrally evoked striatal responses are not compatible with those expected from the unmyelinated dopaminergic axons, particularly since there is extensive branching within the striatum (Anden *et al*, 1966; Collingridge *et al*, 1980).

Conduction velocities of peripheral c-fibres (Nishi *et al*, 1965) would suggest that the conduction velocity of the dopaminergic axons would be <1 msec. This has been supported by studies in the rat where conduction velocities of around 0.5 m/sec. have been found (Guyenet and Aghajanian, 1978; Deniau *et al*, 1978). Using this information, one would predict latencies of at least 10 msecs in the rat, taking the synaptic delay as 1 msec. This is considerably longer than the latency of epsp's recorded intracellularly after nigral stimulation (4.2 msecs) (Preston *et al*, 1980), although longer latency effects have been recorded extracellularly (Richardson *et al*, 1976; Davies and Tongroach, 1978). There is no similar conduction velocity data for properly identified DA containing neurones in the cat.

Moore and Bloom (1978) have suggested that in the cat a low conduction velocity similar to the rat would give latencies in the order of 30-100 msecs. This is also in excess of the latencies reported for epsp's in the cat (Kitai *et al*, 1976; Kocsis and Kitai, 1977).

However, a recent report from Kitai's group demonstrates that the fast excitation recorded in the striatum is due to the antidromic

excitation of the cortical efferent axons which have a collateral branch synapsing on striatal neurones (Wilson *et al*, 1980; Donoghue and Kitai, 1980).

It has been proposed that the postsynaptic DA receptor in the striatum is of the D_1 type, linked to adenylate cyclase (Kebabian *et al*, 1972; Kebabian and Colne, 1979). In this case, a considerable synaptic delay may occur during activation of the cyclase and associated protein kinase. The time-course of adenylate cyclase activation in the striatum is not known. Delays of around 25 msecs have been reported for a dopamine-stimulated adenylate cyclase in the superior cervical ganglion, but the type of synapse involved is probably different from that found in the striatum (Libet, 1967, 1979). This data implies that effects due to stimulation of dopaminergic cells would be extremely difficult to see against the background of other faster inputs stimulated from the area surrounding zona compacta.

In common with lesion studies, the interaction of polysynaptic effects from other areas innervated from zona compacta should also be considered.

c) Iontophoretic application of DA

DA has been applied iontophoretically to striatal cells by several groups of investigators (Table 4).

In contrast to results from nigral stimulation, the validity of which has been questioned above, the predominant effects of iontophoretic DA are inhibitory, although there are reports of excitations (Bevan *et al*, 1975; Kitai *et al*, 1976c).

Because of the silent nature of striatal neurones, iontophoresis of DA has been performed either on the small number of spontaneously active cells or on cells activated by iontophoresis of excitant amino acids

TABLE 4:

Reference		Animal	Anaesthetic/ immobilization	Type of neuronal activity	Total No. of cells tested	% Cells responding to dopamine	% Cells depressed
Bloom *et al*	(1965)	cat	several anaesthetics	spont	80	62.5	60
Bloom *et al*	(1965)	cat	decerebrate	spont	86	64	50
McClennan & York	(1967)	cat	decerebrate	spont + DLH	189	69	60
York	(1967)	cat	decerebrate	spont + DLH	213	73	63
Connor	(1970)	cat	decerebrate	DLH or GLU	101	80	67
Herz & Zieglgänsberger	(1968)	rabbit	gallamine paralysis	spont + DLH	100	-	70
York	(1970)		penthrane/decerebrate	DLH	120	75	31
York	(1972)	monkey	penthrane	DLH	89	-	43
Feltz & De Champlain	(1972b)	cat	dial + urethane	GLU	136	-	43
Siggins *et al*	(1974)	rat	halothane	spont + DLH	96	95	90
Spencer & Havlicek	(1974)	rat	penthrane	GLU	102	62	26
Spencer & Havlicek	(1974)	rat	dial	GLU	96	82	78
Spehlman & Stahl	(1974)	cat	cerveau isolé	spont	64	-	54
Gonzalez-Vegas	(1974)	rat	urethane	spont + DLH	120	74	63
Ben-Ari & Kelly	(1975)	cat	pentobarbitone	spont + GLU	115	92	92
Ben Ari & Kelly	(1975)	cat	halothane	spont + GLU	226	97	92
Bevan *et al*	(1975)	rat	halothane	spont	-	62	34
Spehlman	(1975)	cat	cerveau isolé	spont	64	-	52
Stone & Bailey	(1975)	rat	urethane	spont	32	78	72
Yarbrough	(1975)	rat	chloral hydrate	GLU	40	-	30
Kitai *et al*	(1976c)	cat	pentobarbitone		26	100	0
Stone	(1976)	rat	urethane	spont + GLU	32	78	73
Zarzecki *et al*	(1976)	cat	cerveau isolé	spont + GLU	157	79	74
Ewart & Williams	(1977)	rat	dial/urethane	spont	64	85	69

McCarthy *et al*	(1977)	rat	urethane	spont + DLH	44	100	100
Bernardi *et al*	(1978)	rat	succinylcholine paralysis	spont + GLU	51	41	37
Bioulac *et al*	(1978)	cat	urethane + decerebrate	spont + GLU	52	100	67
Davies & Tongroach	(1978)	rat	urethane	spont + DLH	103	73	71
Gmelin	(1978)	cat	N_2O/O_2	spont	46	100	35
Norcross & Spehlman	(1978b)	cat	cerveau isolé	caudate nucleus stimulation	42	98	14
Skirboll *et al*	(1979)	rat	gallamine paralysis	spont	-	100	100
Herrling & Hull	(1980)	cat	flaxedil paralysis	-			
Arnauld *et al*	(1981)	rat	urethane	spont + GLU	103	86	84

Abbreviations:

spont - spontaneous activity

GLU - glutamate induced activity

DLH - DL homocysteic acid induced activity

such as glutamate, aspartate or homocysteic acid. However, it can be argued that as spontaneously active cells are so uncommon, they do not provide a representative sample for testing. Similarly, observing effects on cells which are made to fire artificially may not reflect the true action of DA.

The effects of DA on orthodromic responses of striatal cells are also mixed. Dopamine has been found to inhibit excitations evoked from substantia nigra (McClennan and York, 1967; Feltz and De Champlain, 1972b [after 6 OHDA]; Connor, 1970) and thalamus (Herz and Zuglgänsberger, 1968). However, facilitation of nigrally evoked responses has also been reported (Connor, 1970; Norcross and Spehlman, 1978a). Connor (1970) showed antagonism of both the DA- and nigrally-evoked depression of cell firing by α-methyl dopa, but had no control excitant to show the specificity of the blockade.

Proponents of the view that DA is excitatory have suggested that the inhibitions may be due to the application of DA onto nearby inhibitory neurones (Kitai *et al*, 1976c). While it is possible that effects seen with extracellular recording and iontophoresis could be due to the effects of the drug on neurones other than the one being recorded, results from the large population of cells recorded by many investigators (Table 4) would have been likely to show a higher proportion of excitations if this was the case.

Inferences concerning a hyperpolarizing action of DA on striatal neurones have been made from extracellular recordings of the action of DA on the depolarization block produced by the iontophoretic application of amino acids (Feltz and De Champlain, 1972b; McClennan and York, 1967; Herz and Zieglgänsberger, 1968).

However, intracellular recording is required to show the true action of DA on membrane potential and membrane resistance compared to those using extracellular recording, studies combining intracellular recording with iontophoresis are few in number. This is not surprising because of the great technical difficulty involved in these experiments when performed *in vivo*.

Three studies have been reported. Two (Bernardi *et al*, 1978; Herrling and Hull, 1980) report slow depolarizations with a simultaneous decrease in firing rate. The other (Kitai *et al*, 1976) reports depolarizations similar to epsp's and the initiation of action potentials. The methodology used by the latter authors has been questioned on several counts, namely:

a) the short duration of their drug application;

b) the similarity of the depolarizations to epsp's;

c) the short distance (20-40μ) between recording and iontophoretic electrodes.

When electrodes with similar inter-tip distances were used in another study (Herrling and Hull, 1980), a hyperpolarization resulted and it was concluded that this was an action of dopamine on the region of the axon hillock causing the decreased firing rate. The slow depolarizations recorded were attributed to changes in the dendritic membrane. Both groups who showed slow depolarizations were also unable to see any effects from short-lasting ejection pulses of DA.

In summary, the greater part of the evidence from iontophoretic studies suggests that the action of DA on striatal neurones is inhibitory. However, the fact that most investigators found some excitation must not be overlooked. Further intracellular studies which identify the cells by their efferent projections and afferent input may reveal a subset of cells on which DA is excitatory.

The evidence reviewed so far has concentrated on the post-synaptic action of DA. Evidence for a presynaptic action was first provided by Schwartz *et al* (1978). These investigators used the activity of the DA-stimulated adenyl cyclase and [^{3}H]-haloperidol binding as markers for dopamine receptors. They found that following cortical ablation the number of [^{3}H]-haloperidol binding sites was decreased by 35% without a concomitant decrease in adenylate cyclase activity. Injections of Kainic acid, which reduced adenylate cyclase activity by 85%, only reduced the [^{3}H]-haloperidol binding by 40%. These results suggested the existence of a population of DA receptors located on the terminals of the cortical afferents and unrelated to the DA-stimulated adenyl cyclase. The two types of receptor have since been designated D_1 (cyclase linked) and D_2 (Kebabian and Calne, 1979), but there are several variations on this nomenclature (see Costall, B. and Naylor, R.J., 1981 for review). The discovery of the receptors on the terminals of the cortical afferents was the stimulus for the work described in this chapter. The aim of the investigation was twofold:

a) to duplicate the experiments of Bevan *et al* (1975) who found DA to be excitatory in the halothane anaesthetised rat, and

b) to investigate the interaction of DA with cortico-striatal transmission.

Electrophysiological evidence for an interaction between DA and the cortical input to striatum had been proposed (Olpe and Koella, 1978), but only evoked potentials rather than single units were recorded in that study, and the validity of results from experiments using electrical stimulation of substantia nigra has already been questioned.

The experiments in the present study were designed to investigate the action of DA on identified striatal cells and to differentiate pharmacologically the effects of D_1 and D_2 receptors.

METHODS

Extracellular recording of neurones and the iontophoretic application of drugs were performed as described in Chapter II (General Methods). The comparisons of the effects of drugs on glutamate-induced firing and cortically-evoked firing were performed as described in Chapter IV. The effects of drugs on spontaneous firing were expressed as the percentage change in firing rate during the test period as compared with the average firing rate during the control period.

Drug solutions for iontophoresis

Sodium-L-glutamate (0.2M, pH 7.0); acetyl-choline chloride (ACh, 0.5M, pH 4.5); dopamine (DA, 0.5M, pH 4.0); ɣ-amino-butyric acid (GABA, 0.5M, pH 4.0); α-Flupenthixol diacetate (0.2M, pH 4.0); Fluphenazine (0.1M, pH 4.0); Bromocryptine (0.001M, pH 4.0); sulpiride (0.1M, pH 5.5); D-L Lactic acid (1% adjusted to pH 4.0).

All of the drugs except bromocryptine were dissolved in distilled water and the pH adjustment was made with hydrochloric acid and sodium hydroxide.

Bromocryptine was dissolved by grinding with 2-3 drops of 1% D-L lactic acid in a small glass mortar and adjusting the resulting solution to the required pH and concentration. In experiments where bromocryptine was used, one iontophoretic barrel was filled with a 1% lactic acid solution to act as a control for the lactic acid in the bromocryptine solution.

Sources of drugs

Fluphenazine, HCl (Squibb, Liverpool); DA, GLU, ACh, GABA (Sigma Chemical Company); Haloperidol (Serenace) (G.D. Searle,

High Wycombe, England), α-Flupenthixol (Lundbeck, Sweden). Bromocryptine was a gift from Dr. P.L. Herrling (Sandoz Ltd, Basel) and -Sulpiride was a gift from Dr. P. Jenner (Institute of Psychiatry, London).

RESULTS

Dopamine was applied iontophoretically to 78 neurones in the rat striatum. Spontaneous activity was recorded from 31 neurones and 47 were normally silent. Excitation was evoked in neurones by cortical stimulation. The effects of DA on spontaneous, glutamate-induced, and cortically-evoked activity are now described.

Dopamine and spontaneous activity

DA (25-80 nA) was tested on 31 neurones which exhibited spontaneous activity. Depression of firing ranging from 50-100% was observed on 30 cells (97%) (Table 5) (Fig. 37). The onset of depression usually occurred 2-3 secs after ejection commenced. The depression persisted for several seconds after the ejecting current was terminated and normal firing resumed 15-30 secs later. However, in some cells, the depression persisted for over 60 secs after terminating the current. The depression obtained on individual neurones was dependent on the strength of the ejecting current (Fig. 38), but the current required to produce a given level of depression varied between neurones. Complete inhibition was obtained by DA currents as low as 40 nA but on other neurones 70 nA was required to produce this effect.

Facilitation of spontaneous firing was never seen with the DA ejection currents used in this study, and only one neurone (3%) was insensitive to DA.

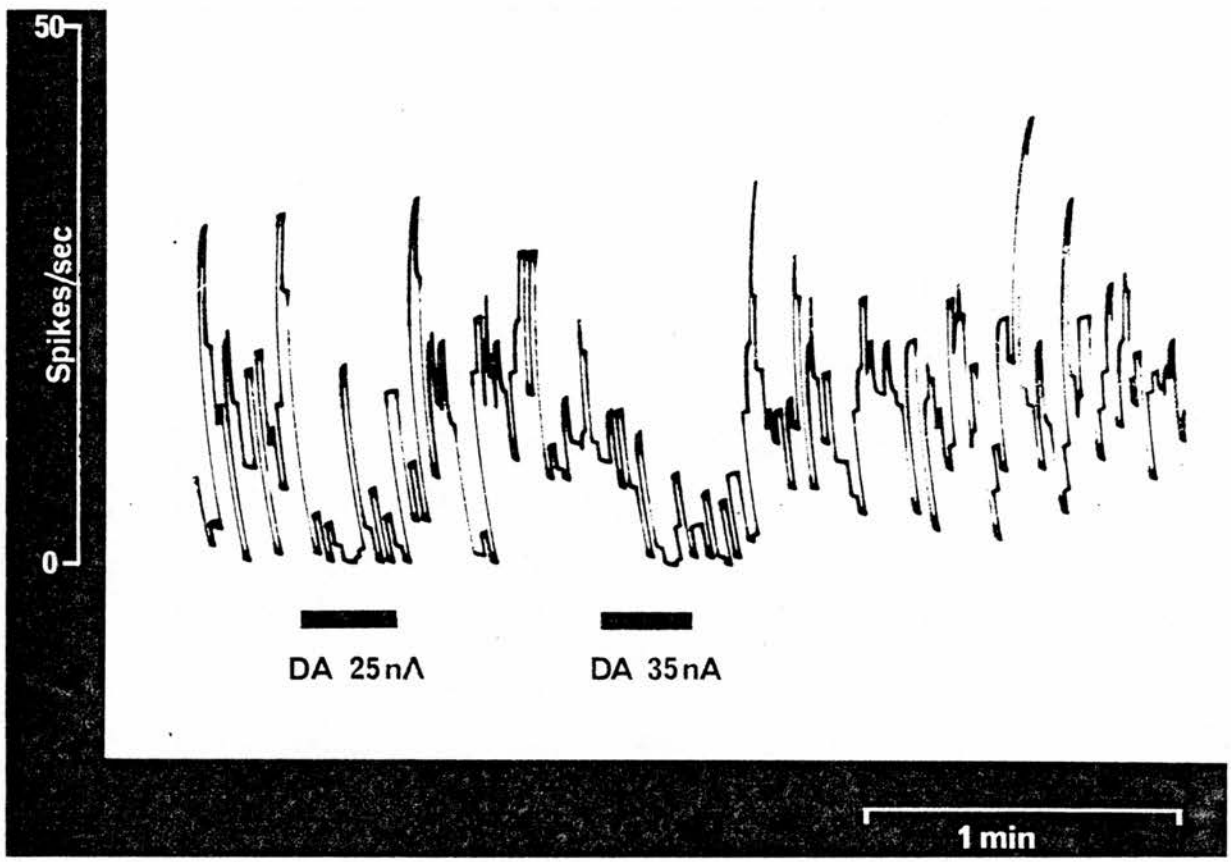


FIGURE 37: The effect of iontophoretically applied dopamine (DA) on the spontaneous firing of a striatal neurone.
Polygraph tracing of the ratemeter output. The horizontal bars indicate the periods of iontophoretic release of drugs.

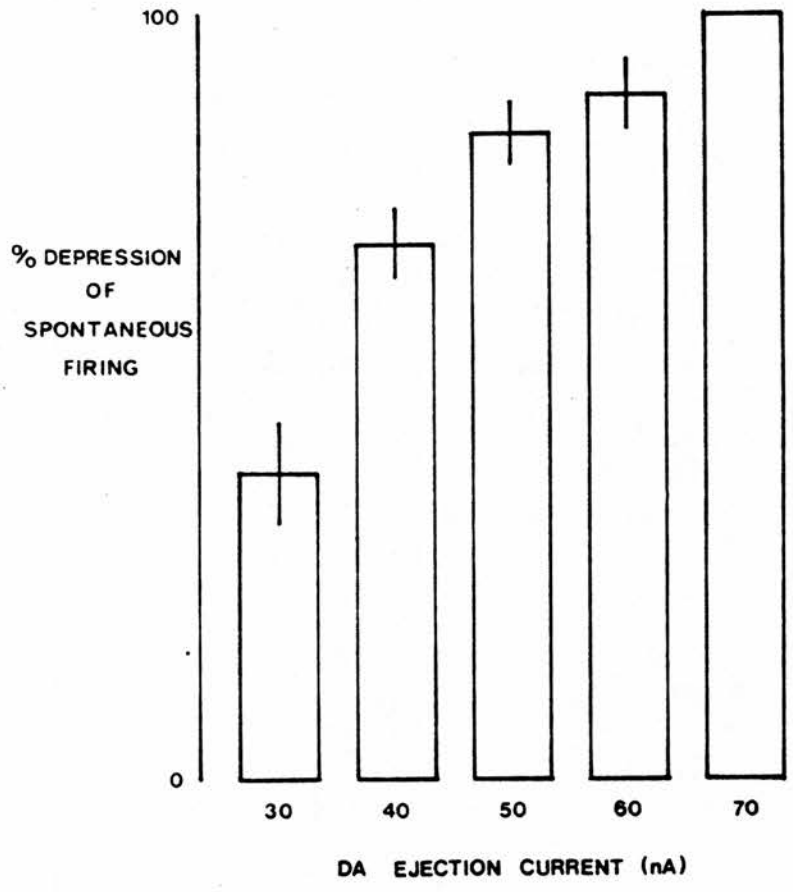


FIGURE 38: The depression of spontaneous firing rates by different iontophoretic ejection currents of DA (values are mean ± standard error).

Dopamine and amino acid induced excitation

Forty-seven neurones which exhibited no spontaneous activity became active in response to iontophoretically applied GLU (15-60 nA). GLU was applied in short pulses (1-4 secs) at regular intervals (between 10 and 30 secs apart) rather than continuously at low currents. With a continuous application it was difficult to achieve a stable firing rate at low ejection currents (5-10 nA). At slightly higher currents there was a gradual increase in firing which often continued into a depolarization block.

DA inhibited the GLU induced excitation on 43 neurones (Fig. 39) (Table 5). The relation between ejection current and response was similar to that described for spontaneously active neurones. Increasing the ejection current increased the depression obtained on individual cells, but the effect produced by the same ejection current varied between cells. Complete depression of the GLU response was obtained by currents varying from 30-90 nA.

Potentiation of the GLU excitation was never observed. The GLU excitation was unaffected by DA on four cells.

Dopamine and cortical stimulation

Cortical stimulation evoked an excitation in 51 neurones tested with DA. Stimulation currents were adjusted to produce approximately 1 spike per stimulus and PSTHs were compiled from 50 consecutive stimuli at 1/sec. Care was taken to achieve a stable response before testing with DA and two or three control histograms were always compiled depending on the stability of the response. DA was applied both before and during the test PSTH. If a change occurred during the test histogram, further test PSTHs were not performed until the control response had returned in two consecutive histograms.

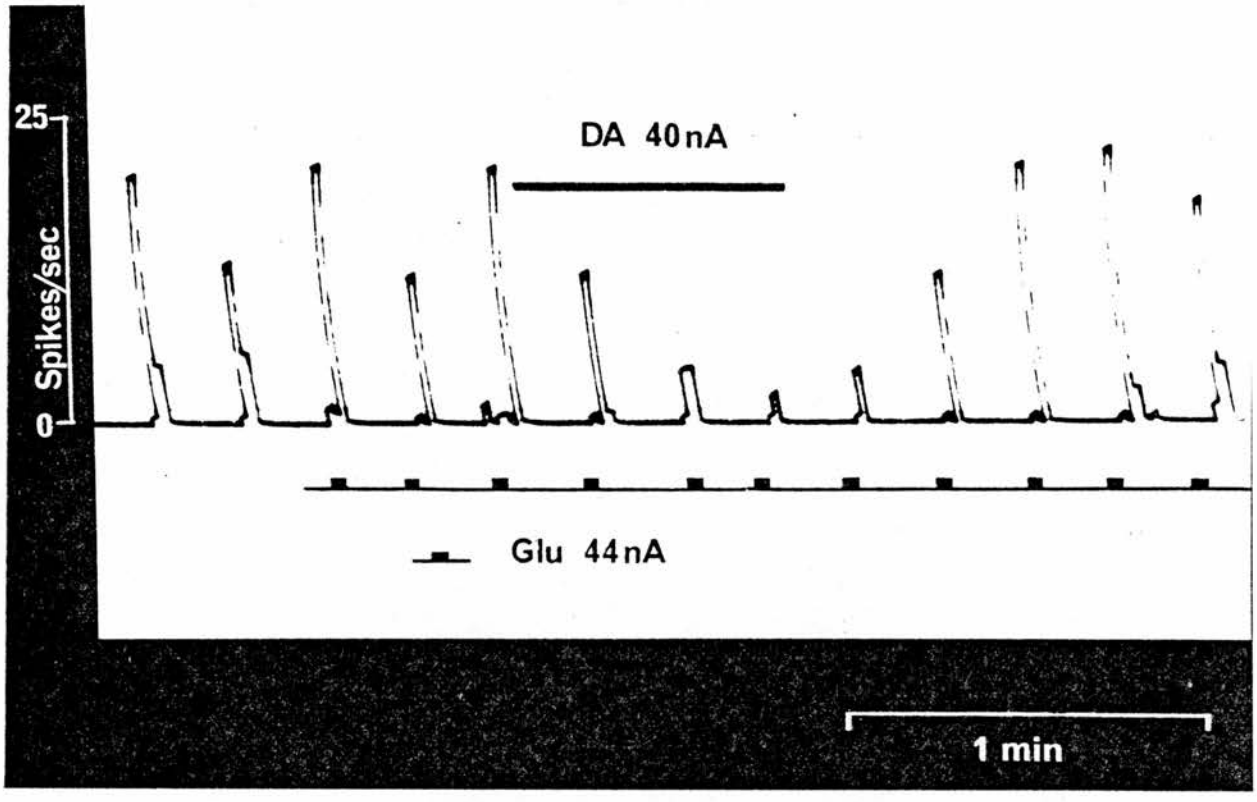


FIGURE 39: The effect of iontophoretically applied dopamine (DA) on the glutamate-induced (GLU) excitation of a silent striatal neurone. Polygraph tracing of the ratemeter output. The period of DA release is indicated by the horizontal bar and the occurrence of pulsatile GLU release is indicated below the polygraph trace.

TABLE 5: Dopamine inhibitions of striatal neurone activity.

Type of neuronal activity	No. of neurones	Range of ejection currents	Mean % depression of firing ± SE
spontaneous firing	30	30 - 70 Na	76.6 ± 4.5
glutamate-induced firing	43	25 - 90 nA	74.6 ± 3.7
cortically-induced firing	29	20 - 80 nA	59.5 ± 4.2

DA inhibited the number of cortically evoked excitations in 29 out of 51 cells (57%) tested (Fig. 40) (Table 5). Recovery of the response was usually complete after 2-4 minutes. Cortical driving alone, was tested on 7 of the 29 (24%) neurones on which DA was inhibitory. On the remaining 22 cells (76%) DA ejection currents which depressed cortical driving, also had a marked depressant effect on spontaneous (9 neurones) and GLU induced (13 neurones) firing (Table 6).

On 9 spontaneously active cells and 10 neurones activated by GLU, a DA ejection sufficient to markedly depress activity left the cortical excitation unaffected. On 5 silent neurones which were not tested with GLU, the cortical excitation was unaffected by DA.

The relationship between ejection current and response to cortical stimulation was similar to that described in the previous two sections, being current-dependent on individual cells, but varying between cells.

Facilitation of cortical driving was never observed on any of the 5 neurones tested.

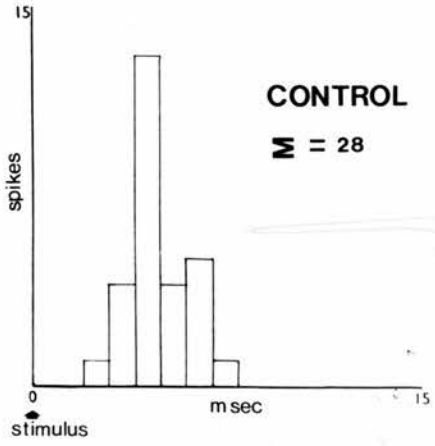
Dopamine and output cells

Dopamine was tested on 7 antidromically identified striatal output cells and it inhibited the production of an antidromic spike in all 7 cells. Glutamate excitation was inhibited on 4 cells and cortical driving was inhibited on 3 cells (Table 7).

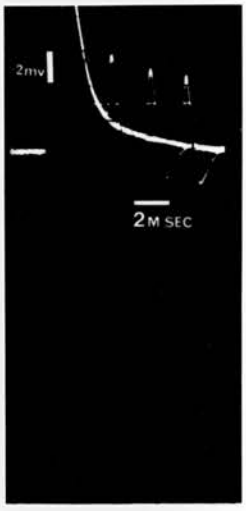
Dopamine and ACh

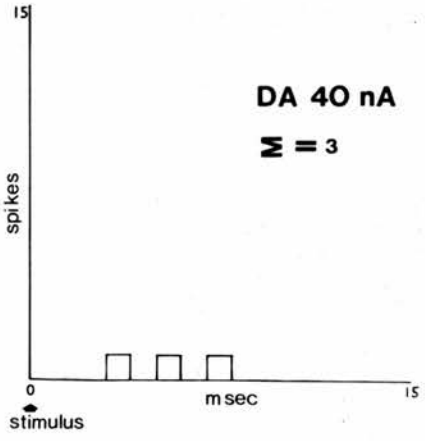
To investigate whether the effects of DA were specific to GLU, ACh was tested along with GLU on 12 cells. Excitation (6 cells)(50%) and inhibition (4 cells) (33%) was observed with ACh, 2 cells (17%) were insensitive to ACh. The ACh excitations were slow in onset and offset


2mv
2M SEC

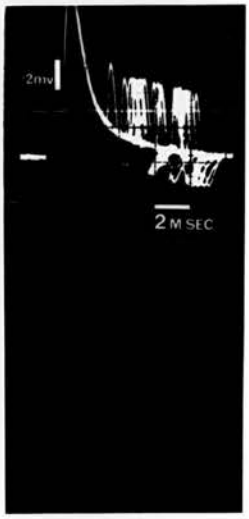

15
spikes
CONTROL
N = 28
0
msec
15
stimulus

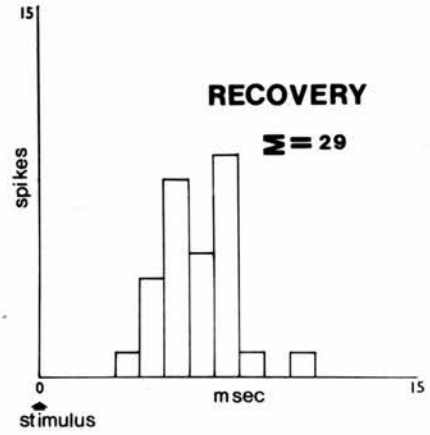
(a)


2mv
2M SEC


15
spikes
DA 40 nA
N = 3
0
msec
15
stimulus

(b)


2mv
2M SEC


15
spikes
RECOVERY
N = 29
0
msec
15
stimulus

(c)

TABLE 6: Correlation of the effects of iontophoretically applied dopamine (DA) on striatal neuronal activity.

Type of neuronal activity tested	Type of neuronal activity affected by iontophoretic dopamine: Inhibition of both types	Inhibition of cortical stimulation	Inhibition of spontaneous firing	No effect
cortical stimulation	-	7	-	3
cortical stimulation + spontaneous activity	9	-	9	1
cortical stimulation + glutamate-induced firing	13	-	10	4
total	22	7	19	8

TABLE 7: The effect of iontophoretically applied dopamine (DA) on antidromically identified striatal output neurones.

Type of neuronal activity	Effect of iontophoretic DA: Inhibition	Excitation	No effect
glutamate-induced activity	3	0	0
cortical driving	4	0	0
antidromic spike	7	0	0

in comparison to the glutamate excitations. DA inhibited the effect of both ACh and GLU on all 6 neurones (Fig. 41) on which ACh was excitatory.

Dopamine antagonists

The DA antagonists, fluphenazine, α-flupenthixol and haloperidol, were tested for their ability to antagonize the effects of DA in this preparation.

Fluphenazine (50-75 nA) iontophorised for 2-3 minutes before the ejection of DA antagonized the depressant effects of DA both cortical driving and spontaneous activity on 6 neurones, and was innefective on 2 neurones. During the period of antagonism, fluphenazine did not cause silent cells to fire, nor did it affect the firing rate of spontaneously active cells. The antagonism was reversible within 4-5 minutes.

Successful antagonism was never achieved with α-flupenthixol because of problems with its application by iontophoresis. The α-flupenthixol-barrel resistance rose to levels beyond the compliance of the apparatus within less than a minute of the start of the ejection.

Haloperidol was administered peripherally by intraperitoneal injection (i.p., 0.1 mg/kg) in 8 experiments. Only one cell was studied in each experiment after the haloperidol injection. In five experiments several injections of haloperidol were given up to a cumulative dose of 0.5 mg/kg.

Haloperidol did not induce spontaneous firing or affect the rate of spontaneous firing in any of the cells. In addition, the depressant effects of iontophoretic dopamine were not antagonized by i.p. haloperidol.

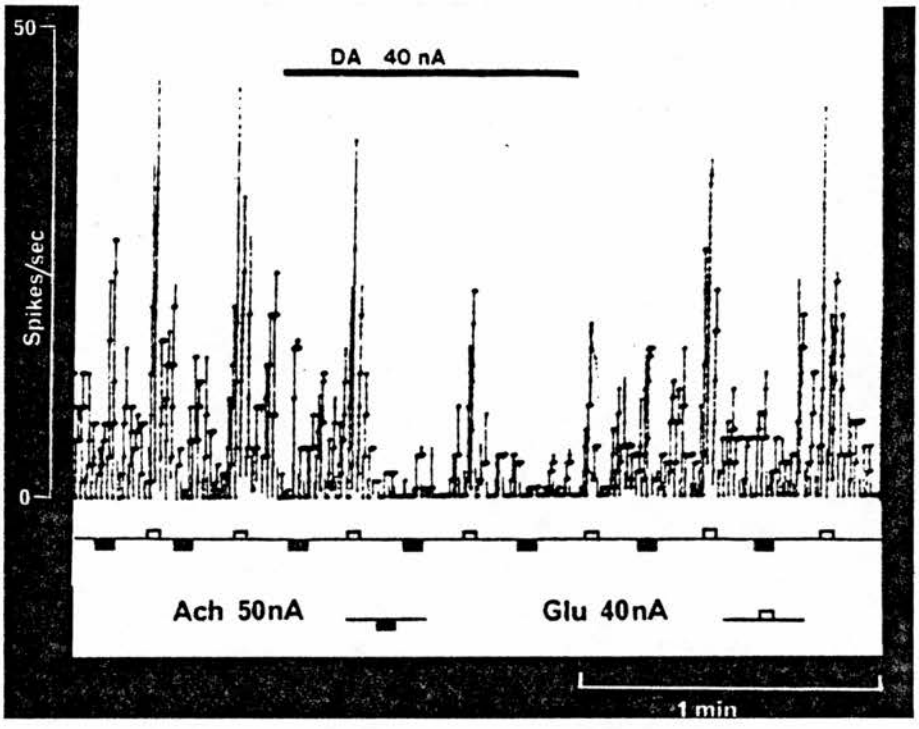


FIGURE 41: The effect of dopamine (DA) on the glutamate (GLU) and acetylcholine (ACh) induced excitations of a striatal neurone. The figure shows an original polygraph trace of a ratemeter output. Alternate pulses of ACh and GLU were applied as indicated under the trace. The upper horizontal line indicates the duration of the DA ejection.

Sulpiride

i) Spontaneous and glutamate induced firing

The selective D_2 antagonist sulpiride (Jenner and Marsden, 1979) was used in an attempt to pharmacologically dissect the effects of dopamine on pre- and post-synaptic sites. The effects of iontophoretically applied (-) sulpiride were investigated on 33 cells. Sulpiride (30-70 nA) did not induce firing from 27 silent cells, neither did it affect the excitatory response to GLU in 18 of these cells tested. Spontaneous activity was recorded from six cells, four were unaffected by sulpiride but in two there was a decrease in spike height and an increase in firing rate, suggestive of depolarization block.

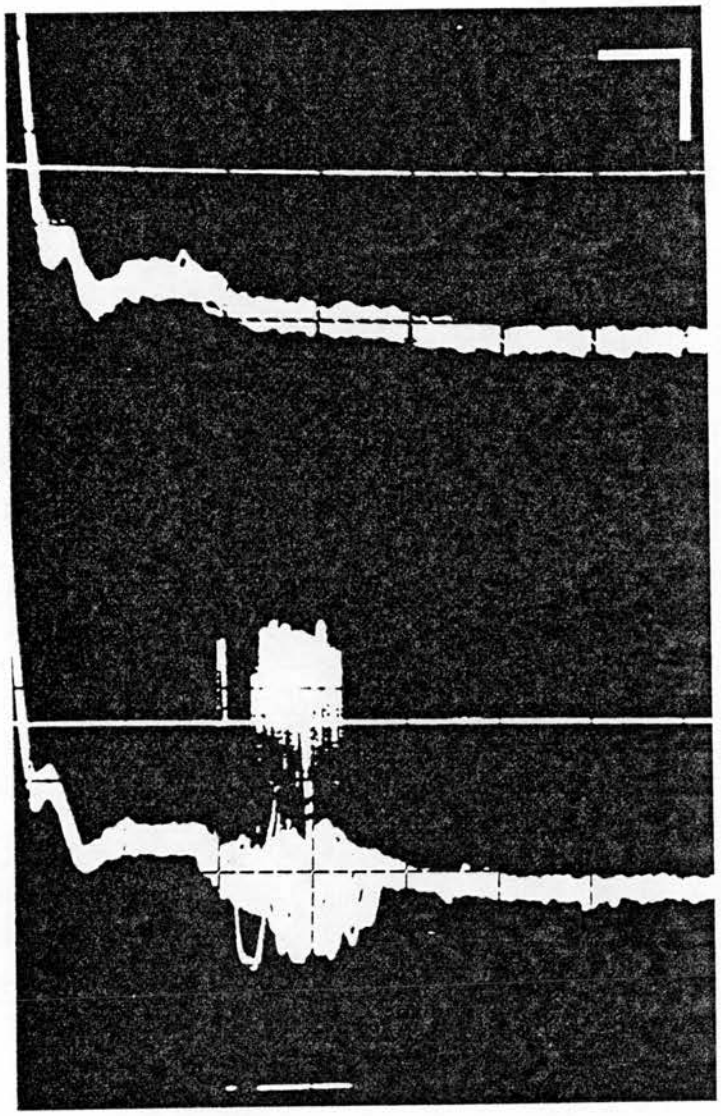
(-) Sulpiride failed to antagonize the depressant effects of iontophoretically applied DA on 15 neurones tested.

ii) Sulpiride and cortical stimulation

The response of striatal neurones to cortical stimulation was tested during the iontophoresis of (-) sulpiride. At stimulation currents which were just threshold for excitation, (-) sulpiride (30-70 nA) decreased the number of stimuli which failed to produce a spike (Fig. 42). This effect was observed from 18 cells with 1 cell being insensitive.

Sulpiride applied for between 30 secs and 2 mins before the test PSTH reduced the number of stimuli which failed to produce a spike to 22% (SE 6.1) of the control value. The mean number of spikes produced by 50 cortical stimuli was increased from 8.4 ± 2.5 (SE) to 29.9 ± 2.8 (SE) (Fig. 43). The increase in response was larger at higher currents but the response rate never exceeded 1 spike/stimulus.

The facilitation of cortical stimulation was blocked on 6 neurones to which DA was applied by iontophoresis simultaneously with the sulpiride.



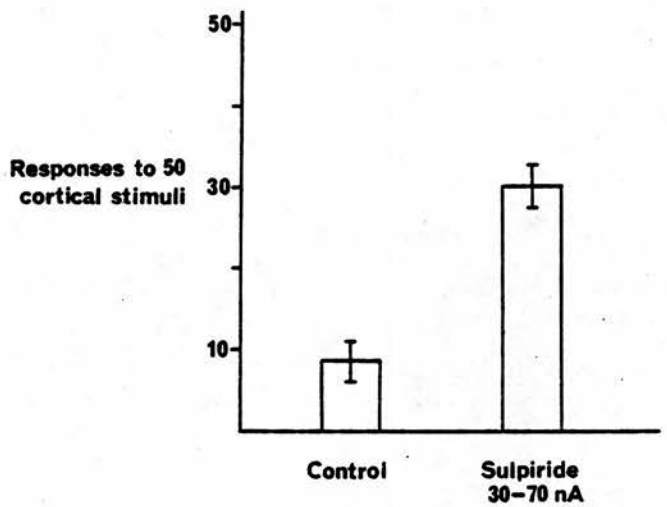


FIGURE 43: The effect of iontophoretically applied sulpiride on the response of striatal neurones to cortical stimulation. The Figure pools data from 18 neurones. (Bars represent standard errors).

However, this antagonism was only accomplished using ejection currents of dopamine which also depressed spontaneous firing in a similar manner to that described previously for cortical stimulation.

Two cells were insensitive to sulpiride.

Bromocryptine

The D_2 agonist bromocryptine was used in an attempt to avoid the involvement of postsynaptic D_1 dopamine receptors in the experiment. During the application of bromocryptine (30-100 nA for up to 5 mins) no effects were seen on the response to glutamate (5 neurones) spontaneous firing (4 neurones) or on the response to cortical stimulation (9 neurones). Bromocryptine could not be dissolved in water and had to be dissolved initially in 1% lactic acid. No effects were observed when similar ejection currents to those used for bromocryptine were passed through a barrel containing only lactic acid.

GABA

GABA has been reported to increase the release of GLU from striatal tissue *in vitro* (Mitchell, 1980). GABA was applied iontophoretically in a similar type of experiment to that performed for sulpiride. Iontophoretic GABA (30-50 nA) was tested on 6 neurones. On all 6 it caused an immediate depression of both spontaneous firing (2 neurones) and GLU induced firing (4 neurones). Cortical excitation was completely blocked in all 6 neurones.

DISCUSSION

The results described in the preceding section support the hypothesis of DA as an inhibitory transmitter in the striatum. Iontophoretic DA had a depressant action on spontaneous

TABLE 8: The effects of iontophoretically applied dopamine (DA) on neuronal activity

Type of neuronal activity	Effect of DA: Inhibition	Excitation	No effect
spontaneous firing	18	0	1
glutamate-induced firing	43	0	4
firing induced by cortical stimulation	29	0	22

and cortically driven firing (Table 8), and on GLU and ACh induced excitations, as previously described (Herz and Zieglgänsberger, 1968; Bloom *et al*, 1965).

In agreement with two other studies (Feltz and De Champlain, 1972a; Herrling and Hull, 1980) no excitatory responses to iontophoretic DA were observed. This contrasts with many of the previous reports which, although having a predominance of inhibitions, also reported a small proportion of excitations (Table 4). The methods used in this study are almost identical to those of Bevan *et al* (1975) who reported a predominance (50%) of pure excitations with DA. The only difference between the study of Bevan *et al* and the work presented in this thesis is that for the present study different electrodes were used for electrical recording and for iontophoresis; Bevan *et al* used the same multibarrel electrode for both purposes. However, as current balancing techniques were employed in both sets of experiments, this small methodological difference should not have affected the results obtained.

The results of the present study are also at variance with those of an earlier study which reported a predominance of facilitatory effects on cortical driving by iontophoretically applied DA (Norcross and Spehlman, 1978a). Norcross and Spehlman also reported that DA facilitated excitations evoked from stimulation of the substantia nigra, thalamus and from within the caudate nucleus itself. A minority of cells showed depression of the caudate (20%) and cortical driving (11%). Facilitation of GLU-induced and spontaneous firing was also reported. These authors proposed the existence of distinct excitatory and inhibitory DA receptors on the striatal neurones as an explanation for their results. This was on the basis that the DA ejection currents

required for 50% inhibition (50 nA) were five times greater than those required for a 50% facilitation (9 nA). In the present study, no excitatory effects of DA were observed over the whole range of ejection currents tested (10-100 nA).

There are, however, several methodological differences which may explain the disparity between the results of these two studies. Norcross and Spehlman used an encephalé isolé cat preparation as opposed to the anaesthetised rat of the present study. Neither did they use current balancing during iontophoretic ejections. In addition, Norcross (1978) describes that electrodes were filled with drug solutions the day before use and it may be questionable whether the DA had remained unoxidized over this period. DA contained in an iontophoretic electrode under similar conditions to those used in the present study has been shown to retain its activity in an adenylate cyclase assay over the timecourse of a typical experiment in the present study (Herrling and Hull, 1980).

Despite the wide range used, a DA ejection current was never found which could affect the cortical driving without also affecting the cell postsynaptically. Therefore any presynaptic component of the depression of cortical driving was masked. The glutamate-induced and spontaneous firing of several cells (34%) was depressed by DA without any effect on the response to cortical driving. A similar difference in the efficacy of iontophoretic DA on synaptic and spontaneous activity has been observed in conscious monkeys (S.J. Thorpe, personal communication). The significance of these observations is not yet clear. Thorpe has suggested that DA may act to alter the signal to noise ratio of striatal cells. By lowering the spontaneous activity the incoming cortical impulse would be "seen" more clearly. Alternatively, the results may be indicative of a particularly high safety margin in cortico-

striatal driving or to the position of the iontophoretic pipette relative to the synapses of the cortical terminals with the striatal neurones.

The successful antagonism of the effects iontophoretic DA by the iontophoretically applied antagonist fluphenazine is in contrast to the lack of effect of peripherally applied haloperidol. Other authors (Zarzecki *et al*, 1977; Ben Ari and Kelly, 1975; Skerboll and Bunney, 1979) have reported a similar failure of peripherally-applied antagonist on iontophoretic DA. The dose of haloperidol used initially (0.1 mg/kg) is enough to cause a large increase in the firing of dopamine containing cells in the substantia nigra (Garcia-Munoz, 1979), yet doses considerably in excess of this were still ineffective. The difficulty in iontophoresing α-flupenthixol has been reported previously (Ben Ari and Kelly, 1975), although α-flupenthixol has been iontophoresed successfully (Davies and Tongroach, 1978).

In an attempt to investigate the presynaptic receptor more selectively, the D_2 agonist bromocryptine and the D_2 antagonist (-) sulpiride were used. Bromocryptine was insoluble at the concentrations described in the single paper where its iontophoretic ejection is described (Bioulac *et al*, 1978). It was barely soluble in lactic acid at 0.001M (Markstein *et al*, 1978; P.L. Herrling, personal communication), and this concentration was used in the present study. The results obtained with bromocryptine are in accord with those of Bioulac *et al*, who found no effect on spontaneous or glutamate firing of caudate neurones. However, it was never certain that bromocryptine was being ejected iontophoretically. Pressure ejection of bromocryptine from a microelectrode (Gmelin, 1978) may provide a better method of application, although the results from this study are a mixture of excitatory and inhibitory effects.

The facilitation of the cortical driving by sulpiride may reflect the blockade of a tonic action of DA on the cortical terminals. DA has been shown to inhibit the release of [3H] glutamate from prisms of striatal tissue (Mitchell 1980; Mitchell and Dogget, 1980) and to inhibit the release of endogenous GLU from brain slices prepared from striatal tissue (Roberts and Rowlands, 1980).

The facilitation of cortical driving shown in the present study supports the notion of an increased amount of transmitter release during blockade of the DA receptor. Sulpiride was ineffective in altering the action of DA on striatal cells, suggesting that the DA receptor on these cells is indeed of the D_1 type. Sulpiride has been shown to block the action of DA on DA-containing cells in the zona compacta of substantia nigra (Pinnock *et al*, 1979) which have the D_2 receptor located post-synaptically and the adenyl cyclase linked D_1 receptor presynaptically on the terminals of afferent fibres from the striatum (Spano *et al*, 1976).

Despite the lack of effect of sulpiride on the spontaneous activity of striatal cells, or on their response to glutamate, the extracellular recording used in the present experiments would not have detected any change on the membrane potential of the cell. Therefore intracellular studies need to be performed on striatal cells during sulpiride application before a presynaptic role can be ascribed to sulpiride.

The control of transmitter release by presynaptic mechanisms has been reported for several putative transmitters in the striatum (Table 9). The picture provided by these results suggests that the control of transmitter release in the striatum is extremely complicated. If, as these results suggest, all the transmitters can have presynaptic effects on the paramaters of each other's release, the action of sulpiride is

TABLE 9:

References		Transmitter	Release increased by:	Release decreased by:
Starr	(1979)	dopamine	GABA	
Giorgiueff-Thesslet *et al*	(1979)	"	GABA	
Stoof *et al*	(1979a)	"	GABA	
Bartholini & Stadler	(1976)	"		GABA
Westfall	(1974)	"		ACh (Musc)
Westfall *et al*	(1976)		flupenthixol	dopamine
De Belleroche & Bradford	(1978)	"	ACh (Nic)	ACh (Musc)
De Belleroche *et al*	(1979)			
Giorgiueff *et al*	(1977)	"	ACh (Nic)	
De Belleroche & Bradford	(1980)	"	5HT	
Harms *et al*	(1978)	"		Adenosine
Roberts & Sharif	(1978)	"	glutamate	
Roberts & Anderson	(1979)	"	glutamate	
Starke *et al*	(1978)	"	[chlorpromazine haloperidol]	[apomorphine bromocryptine]
De Belleroche & Bradford	(1981)	"		haloperidol
Van der Heyden *et al*	(1980)	GABA		dopamine
Stoof *et al*	(1979a)	ACh		GABA
Stoof *et al*	(1979b)	"		dopamine
Mitchell & Doggett	(1980)	glutamate		dopamine
Rowlands & Roberts	(1980)	glutamate		dopamine
Mitchell	(1980)	glutamate	GABA	
Ennis *et al*	(1981)	dopamine		5HT

unlikely to be solely on the cortical terminals. A detailed description of the precise location of axo-dendritic and axo-axonic synapses in the striatum, and the transmitters used in each, is required before any of the presynaptic mechanisms is assigned a physiological role. This type of identification might be accomplished by using the electron microscopical analysis of the anterograde transport of neurotransmitters labelled by the selective application of radiolabelled precursors: the retrograde transport of labelled transmitters has recently been demonstrated. If sufficient specificity could be obtained from anterograde transport, a combination of this technique with the anatomical techniques of Somogyi *et al* (1979) could provide valuable data about synaptic contacts in the striatum.

Methods for directly measuring presynaptic inhibition electrophysiologically have been reported for primary afferent fibres (Wall, 1958; Madrid *et al*, 1979) and vestibulospinal and rubrospinal afferent fibres (Rudomin *et al*, 1980). The excitability of the terminals is monitored by the stimulating current required to produce a pre-determined level of antidromic response in the afferent cell when its terminals are stimulated. Under computer control, the stimulator can be automatically adjusted to provide a continuous measurement of excitability. Changes in excitability can then be measured during drug application, or stimulation of other afferents to the area being investigated. (Rudomin *et al*, 1980).

Unfortunately, the rat striatum is not suitable for this type of experiment. The large number of myelinated cortical efferent fibres passing through the striatum make it unlikely that cortico-striatal cells could be driven antidromically without first activating other cortical output cells. Intra-cortical events could also then affect the invasion

of an antidromic impulse if a cortico-striatal cell was recorded (Lipski, 1981). This type of experiment could be performed in an animal in which the internal capsule remains in a single bundle, for example, the cat.

SUMMARY

1. DA was shown to inhibit glutamate-induced and spontaneous firing of both intrinsic and output cells in the striatum.

2. DA inhibited cortical driving of striatal neurones but this effect could not be attributed directly to a presynaptic effect, as the spontaneous or GLU-induced firing was depressed by similar dopamine ejection currents.

3. The D_2 antagonist sulpiride facilitated cortical driving without affecting spontaneous or GLU-induced firing.

4. Further approaches to the investigation of presynaptic control of cortico-striatal terminals are discussed.

CHAPTER VI

The Effects of 6-OHDA Lesions of the Nigro-Striatal Pathway on Cortico-Striatal Transmission

Introduction

As described in the previous chapter, a lesion of the dopaminergic nigro-striatal pathway produces an alteration in the firing rate of striatal neurones on the lesioned side (Steg, 1969; Ohye *et al*, 1970; Arbuthnott, 1974; Siggins *et al*, 1976). More spontaneously active cells are recorded on the lesioned side, and the mean firing rate of cells on the lesioned side is increased in comparison to the intact side.

These results have been interpreted as indicating a disinhibition of striatal neurones following the destruction of an inhibitory DA input. This explanation has assumed a purely post-synaptic action of DA. In the light of the demonstration of dopamine receptors on the terminals of the cortical afferents to the striatum (Schwarz *et al*, 1978) it seemed possible that the changes observed in the firing patterns of striatal neurones in the 6-OHDA lesioned animals could be due to an alteration of the cortical input to the striatum.

The following section describes the results of electrophysiological experiments to investigate the characteristics of cortical stimulation in the 6-OHDA lesioned rat.

METHODS

Male albino wistar rats weighing 195-205 g at operation were used. Anaesthesia was induced by placing the rat in a plastic box through which a halothane/air mixture (3% halothane) was circulated. When the flexor reflexes were lost the rat was transferred to a David Kopf stereotaxic frame where halothane anaesthesia was continued through a mask fitted over the nose and mouth. The skull was exposed by a midline incision and the scalp retracted. The periosteum was scraped off and a hole (3 mm dia.) was bored in the skull at the appropriate co-ordinates.

6-OHDA injections

Fifteen minutes before commencing the operation the animals were given an injection of Pargyline (50 mg/kg: Abbott) and Desmethyl Imipramine (25 mg/kg: Cibi Geigy). 6-OHDA (2 mg/ml: Hassle, Biotec) was dissolved in sterile saline (0.9% w/v) containing 1 mg/ml ascorbic acid, and the pH adjusted to 7.4.

A stainless steel needle for cartridge syringes (30 gauge dia. 0.46 mm) was lowered into the brain aimed at the following co-ordinates (taken from the atlas of Konig and Klippel, 1953).

Antero-posterior	-	4.2 mm
Lateral	-	1.2 mm
Vertical	-	8.5 mm (from skull surface)

Using a micrometer syringe, 4 µl of the solution was injected over a period of 5 minutes. The needle was left in place for a further 5 minutes after the injection was completed. The wound was then closed and the animal allowed to recover.

Turning behaviour

One week after the operation, all of the animals were tested for their behavioural response to a dose of apomorphine (0.3 mg/kg intraperitoneally: MacFarlan Smith Ltd).

In rats with a unilateral lesion of the dopaminergic nigro-striatal pathway, the administration of a dopaminergic agonist causes the rat to turn in circles away from the lesioned side (Ungerstedt and Arbuthnott, 1970; Uretsky and Schoenfeld, 1971). The turning behaviour is present only if 90% or more of the nigro-striatal dopamine neurones are destroyed (Hefti *et al*, 1980).

To measure turning behaviour, the rats were placed in a hemispherically shaped plastic bowl and connected to an automatic counter (Cowan Consultant Electronics, Humberside) by a waist strap (Fig. 44). The counting started 1 minute after the injection of apomorphine.

Animals were selected for electrophysiology only if they turned more than 200 times in 30 minutes.

DA in substantia nigra

To verify the amount of DA depletion in the substantia nigra the DA content was measured by the radioenzymatic assay of Palkovits *et al* (1974).

FIGURE 44: A rat with a 6-OHDA lesion of the left dopaminergic nigro-striatal pathway, exhibiting turning behaviour. The rat has been injected with apomorphine (0.3 mg/kg i.p.) and turns towards the side contralateral to the lesion. The rat is connected via the spring cable to an automatic counter housed above hemispherical bowl.

RESULTS

Turning behaviour

The six rats used in this study all turned more than 200 times (mean 305 turns/30 mins, range 265-359).

Dopamine content of substantia nigra

The quantity of tissue used proved a critical factor on this assay. Only two of the assays provided results. The dopamine content was reduced to 8.2% and 6.5% of the control side respectively.

Electrophysiology

The electrophysiological experiments were performed on two groups of rats: a) 10 days after the lesion, and b) 3 months after the lesion. Recordings were made from both sides of the brain. The results obtained from the striatum on the unlesioned side were taken as control values.

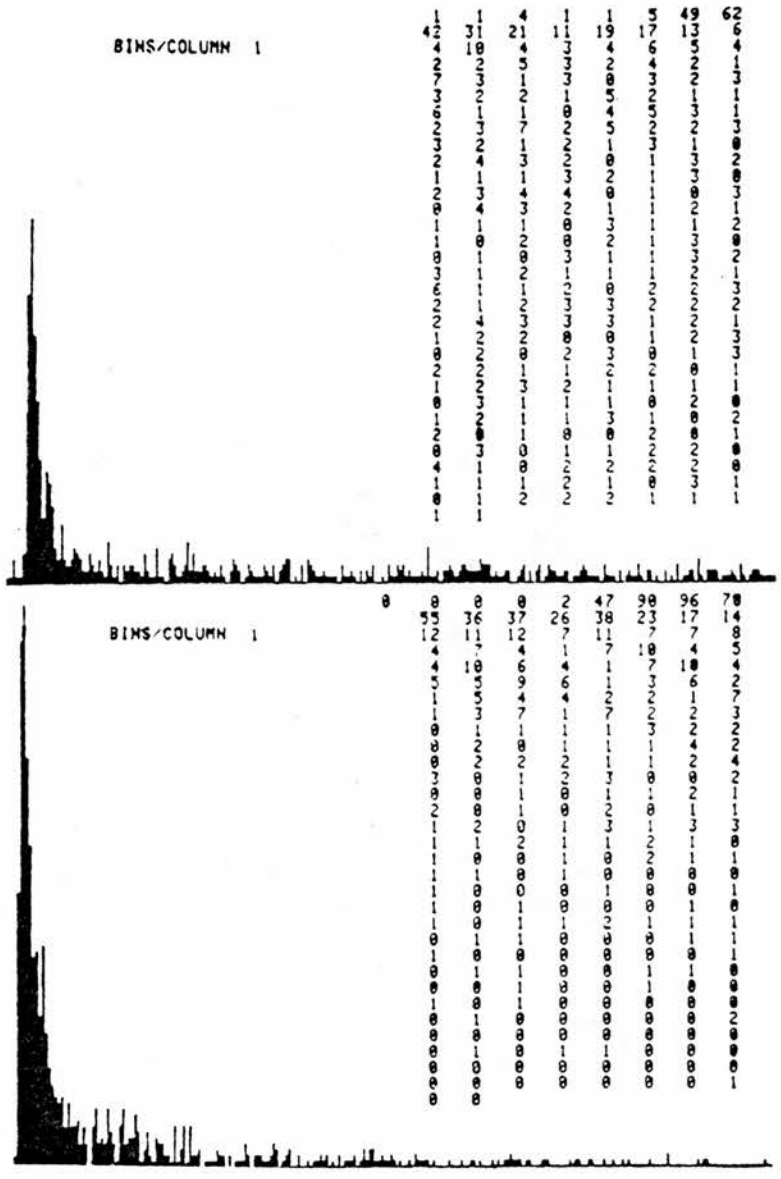
a) *Recordings made 8-10 days after the lesion (acute lesions):*

The most striking difference between the lesioned and unlesioned sides, was the number of spontaneously active cells encountered. On the lesioned side, 73% of the cells had some spontaneous activity in comparison with 26% on the unlesioned side (Table 10). The increase in the number of spontaneously active cells was accompanied by a higher mean firing frequency of the cells on the lesioned side. The increased mean firing rate was a reflection of a change in the pattern of firing. As shown in Chapter III, the normal discharge pattern of striatal cells is small bursts of firing interspersed between periods of silence. On the lesioned side, the intervals between the bursts are much shorter than those on the control side, but the firing rates during the bursts remain similar (Fig. 45, Table 10).

TABLE 10: Comparison of the data from 6-OHDA lesioned and unlesioned striata

Treatment	Total cells	% cells with spontaneous activity	Mean stimulation current mA (± SE)	Mean interburst frequency (Hz) (± SE)	Mean firing frequency (Hz) (± SE)	Spontaneously active cells/cm	Latency of cortical stimulation (msecs)	Range (msecs)
Acute lesion	37	73	0.467* ±0.052	117.5 ± 14.5	8.79 ±1.33	4.50	6.19 ±1.70	5-10.0
Acute unlesioned	19	26	0.866 ±0.004	96.9 ± 51.8	0.64 ±0.22	0.66	6.58 ±1.40	5- 9.0
Chronic lesioned	48	25	0.933 ±0.045	110.8 ± 35.0	4.75 ±1.75	0.85	9.05 ±2.60	4-13.0
Chronic unlesioned	17	17.6	1.23 ±0.071	174.0 ± 80.9	7.53 ±3.90	0.50	8.10 ±2.70	5-13.5

* significantly different, $P < 0.01$ (Mann Whitney Test)


BINS/COLUMN 1
BINS/COLUMN 1

The stimulating currents required for the cortical driving of striatal cells were significantly less ($P < 0.01$, Mann Whitney Test) on the lesioned side (0.467 mA, SE 0.052) than on the unlesioned side (0.866 mA, SE 0.004), but no differences were observed in the way the striatal cells responded to the stimulus. The stimulus response ratio remained at approximately 1 : 1 and there was no significant difference in the latency of the excitation. On the lesioned side, the stimulation current required for spontaneously active cells was significantly lower than that required for silent cells ($P < 0.01$, Mann Whitney Test) (Table 11).

b) *Three months post-lesion (chronic lesion):*

In these older animals, the percentage of spontaneously active cells recorded from both the lesioned and unlesioned ~~animals~~ sides was similar to that described for the unlesioned side of the acutely lesioned animal. Similarly, the within burst and mean firing rates on the lesioned side were not significantly different from the control side of the acute animals. The mean firing rate is higher on the control side of the chronic animal than the acute animal. However, this figure may be biased by the small sample (3 spontaneously active cells) and requires a fuller study.

The stimulating currents required on both sides of the chronically lesioned animal were not significantly different from each other or from the unlesioned side of the acute animal. They were significantly higher than those required on the lesioned side of the acute animals (Mann Whitney Test, $P < 0.01$).

The stimulation currents required to excite silent cells on the control side of the chronic animals were significantly higher ($P < 0.01$,

TABLE 11: Comparison of stimulation currents required for the cortical excitation of silent and spontaneously active cells.

Neurone type	Stimulation current (mA ± SE): Spontaneously active	Silent
acute unlesioned	0.84 ± 0.1	0.88 ± 0.05
acute lesioned	0.32 ± 0.03*	0.86 ± 0.09
chronic unlesioned	0.85 ± 0.13	*1.30 ± 0.07
chronic lesioned	0.99 ± 0.05	0.97 ± 0.1

* significantly different, $P < 0.01$ (Mann Whitney Test)

Mann Whitney Test) than those required on the lesioned side (Table 11) of the chronic animal and on both sides of the acutely lesioned animal.

The response patterns of neurones on both sides of the chronically lesioned animal to cortical stimulation were similar to those recorded in younger normal animals (Chapter III).

DISCUSSION

The changes found in the firing rates of neurones in the striatum of rats acutely lesioned by 6-OHDA in this study were similar to those previously described by other workers (Siggins *et al*, 1976; Schultz and Ungerstedt, 1978b). The alteration in spontaneous activity on the lesioned side appears to return to normal within 3 months but the exact time course of the change has not yet been revealed.

The lower stimulation currents required for cortically evoked excitation of neurones in the acutely lesioned striatum could be viewed as evidence for a decreased presynaptic inhibition. However, this must be considered against the observed changes in firing rates of the neurones. The population of neurones which have become spontaneously active or increased their firing rates may have done so because of the loss of a direct inhibitory DA input. Alternatively, the changes may be due to an increased cortical input. Spontaneously active neurones in the acutely lesioned striatum required significantly lower stimulating currents than silent neurones (Table 11). This is in contrast to the chronically lesioned animal and normal control animals. Whether this is totally attributable to the loss of an inhibitory post synaptic influence, remains to be shown.

Electrophysiologically, a post synaptic change would be best investigated by intracellular recording.

A presynaptic effect could be investigated by the use of push-pull cannulae (Cheramy *et al*, 1981) or dialysis tubing (Tossman and Ungerstedt, 1981) to monitor changes in the basal levels of amino-acid release in the striatum. The high stimulating currents required for cortical stimulation of silent cells in the unlesioned side were an unexpected finding. It is possible that the recovery process which restored normal firing on the lesioned side has overcompensated for any deficit in the striatum contralateral to the lesion. Further experiments are required in the light of this result.

It is not yet known whether D_2 receptors will exhibit the same increase in numbers in response to the DA denervation as that shown by D_1 receptors (Coyle *et al*, 1977; Creese and Snyder, 1979). If this does occur it may be possible to show a supersensitive response to a selective D_2 agonist applied iontophoretically. It would also be interesting to compare the effects of DA on amino acid release from lesioned and unlesioned striata both *in vivo* and *in vitro*.

SUMMARY

1. In the acutely lesioned striatum there was an increase in the number of spontaneously active cells and in their firing pattern.

2. The stimulation currents required for cortical excitation of striatal neurones were significantly lower in the acutely lesioned striatum.

3. The significance of the low cortical stimulation currents in the lesioned animal is discussed along with further experimental approaches.

CONCLUSION

In conclusion I wish to focus on the areas which require development from the work presented in this thesis.

The identity of the cortico-striatal transmitter remains unresolved. The results obtained with the amino-acid antagonists in this study indicate the presence of an NMDA receptor involved in cortico-striatal transmission. Previous studies with amino-acid antagonists in the spinal cord have suggested that the transmitter using an NMDA receptor is ASP rather than GLU (Watkins *et al*, 1981). Thus these electrophysiological results are incompatable with the biochemical studies which are strongly in favour of GLU rather than ASP as the cortico-striatal transmitter (Fonnum *et al*, 1981). However, it has recently been reported that (±)-2-amino-7-phosphono-heptanoic acid (APHEPT) can distinguish between ASP and NMDA on cortical neurones (Perkins *et al*, 1981). This raises the possibility that ASP is not the natural ligand at NMDA receptors, and that the amino acid receptors present on central neurones may have different characteristics to those in the spinal cord. Further characterization of the striatal neurone amino-acid receptor is necessary and the use of selective antagonists like APHEPT may reveal whether GLU or ASP has a role in cortico-striatal transmission.

The evidence for a functional role for D_2 receptors on the terminals of the cortical afferents rests on the results obtained with (-) sulpiride. As previously stated, further electrophysiological experiments are required to prove this a purely presynaptic effect. Recent biochemical studies have, however, shown that (-) sulpiride increases the release of [^{3}H]-GLU from striatal slices *in vitro* (P.R. Mitchell, personal communication) and of endogenous GLU from the rat striatum *in vivo* (U. Ungerstedt, personal communication). This biochemical data supports the suggestion that there is a tonic inhibition of GLU release mediated via D_2 receptors

and is in accord with the electrophysiological results. The synthesis of new selective D_2 agonists has now been reported (Goodale *et al*, 1980; Hjorth *et al*, 1981; Tsuruta *et al*, 1981) and these drugs must be tested for an effect on cortico-striatal transmission. The integration of the pre- and post-synaptic effects of DA also requires further investigation.

A similar differential effect on spontaneous activity and synaptically evoked activity to that of DA on some striatal neurones has been reported for the action of noradrenaline on cerebellar Purkinje cells (Freedman *et al*, 1976). It remains to be shown whether this is indeed a mechanism for altering the signal to noise ratio of striatal neurones.

BIBLIOGRAPHY

ADEY, W.R. (1959). Recent studies of the rhinencephalon in relation to temporal lobe epilepsy and behaviour disorders. Int. Rev. Neurobiol. 1, 1-46.

ADEY, W.R. and DUNLOP, C.W. (1960). Amygdaloid and peripheral influences on caudate and pallidal units in the cat, and effects of chlorpromazine. Exp. Neurol. 2, p. 348-363.

ADINOLPHI, A.M. and PAPPAS, G.D. (1968). The fine structure of the caudate nucleus in the cat. J. Comp. Neurol. 133, p. 167-184.

AFIFI, A. and KOELBERG, W.W. (1965). Efferent connections of the substantia nigra in the cat. Exp. Neurol. 11, p. 474-482.

ALBÈ-FESSARD, D., OSWALDO-CRUZ, E. and ROCHA-MIRANDA, C. (1960a). Activités évoquees dans le noyau caudé du chat en response a des types divers d'afferences. 1 Étude macrophysiologique. Electroenceph. Clin. Neurophysiol. 12, p. 405-420.

ALBÈ-FESSARD, D., ROCHA-MIRANDA, C. and OSWALDO-CRUZ, E. (1960b). Activités évoquees dans le noyau caudé du chat en response a des types divers d'afferences. II Étude microphysiologique. Electroenceph. Clin. Neurophysiol. 12, p. 649-661.

ALBÈ-FESSARD, D., RAUEVA, S. and SANTIAGO, W. (1967). Sur les relations entre substance noire et noyau caudé. J. Physiol.(Paris). 59, p. 324-325.

ANDÉN, N-E., CARLSSON, A., DAHLSTRÖM, A., FUXE, K., HILLARP, N.A. and LARSSON, K. (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurones. Life Sciences.3, p. 523-530.

ANDÉN, N-E., DAHLSTRÖM, A., FUXE, K. and LARSSON, K. (1965). Further evidence for the presence of nigro-neostriatal dopamine neurones in the rat. Amer. J. Anat. 116, p. 329-333.

ANDÉN, N-E., FUXE, K., HAMBERGER, B. and HOKFELT (1966). A quantitative study on the nigro-neostriatal dopamine neurone system in the rat. Acta Phys. Scand. 67, p. 306-312.

ARBUTHNOTT, G.W. (1974). Spontaneous activity of single units in the striatum after unilateral destruction of the dopamine input. J. Physiol. 239, p. 121-122.

AZMITIA, E.C. and SEGAL, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nucleii in the rat. J. Comp. Neurol. 179, p. 641-668.

BALCAR, V.J. and JOHNSON, G.A.R. (1972). The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. J. Neurochem. 19, p. 2657-2666.

BARTHOLINI, G. and STADLER, H. (1977). Evidence for an intra striatal gaba-ergic influence on dopamine neurones of the cat. Neuropharmacology. 16, p. 343-347.

BECKSTEAD, R.M. (1979a). Convergent prefrontal and nigral projections to the striatum of the rat. Neuroscience Letters. 12, p. 59-64.

BECKSTEAD, R.M. (1979b). An autoradiographic examination of cortico-cortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. J. Comp. Neurol. 184, p. 43-62.

BECKSTEAD, R.M., DOMESICK, V.B. and NAUTA, W.J.H. (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat.

BÉDARD, P., LAROCHELLE, L., PARENT, A. and POIRIER, L.J. (1969). The nigrostriatal pathway - a correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey. Exp. Neurol. 25, p. 365-377.

BEN-ARI, Y. and KELLY, J.S. (1975). Dopamine evoked inhibition of single cells of the feline putamen and basolateral amygdala. J. Physiol. 256, p. 1-22.

BENJAMIN, A.M. and QUASTEL, J.H. (1975). Metabolism of amino acids and ammonia in rat brain cortex slices *in vitro*: a possible role of ammonia in brain function. J. Neurochem. 25, p. 197-206.

BENTAVOGLIO, M., VAN DER KOOY, D. and KUYPERS, H.G.J.M. (1979). The organization of the efferent projections of the substantia nigra in the rat. A retrograde fluorescent double labelling study. Brain Res. 174. p. 1-17.

BERGER, B., NGUYEN-LEGROS, J. and THIERRY, A.M. (1978). Demonstration of horseradish peroxidase and fluorescent catecholamines in the same neurone. Neuroscience Letters. 9, p. 297-302.

BERNARDI, G., MARCIANI, M.G. MOROCUTTI, L., PARONE, F. and STANTIONE, P. (1978). The action of dopamine on rat caudate nucleus neurones intracellularly recorded. Neuroscience Letters. 8 (1978), p. 235-240.

BEVAN, P., BRADSHAW, C.M. and SZABADI, E. (1975). Effects of desipramine on neuronal responses to dopamine noradrenaline 5-hydroxy-tryptamine and acetylcholine in the caudate nucleus of the rat. Br. J. Pharmac. 54, p. 285-293.

BIOULAC, B., BOULARD, G., VINCENT, J-D. and PUIL, E. (1978). Effects of dopamine agonists on neurones in the caudate nucleus and cerebral nortex of cats chronically treated with neuroleptics. Neuro-pharmacology. 17, p. 965-996.

BIOULAC, B., FUMOUX, F., FERRON, A. and VINCENT, J.D. (1978). Responses of central neurones to piribedil and 2-bromo- -ergocryptine: comparison with dopamine and apomorphine. Europ. J. Pharmacol. 51, p. 29-37.

BISCOE, T.J., DAVIES, J., DRAY, A., EVANS, R.H., MARTIN, M.R. and WATKINS, J.C. (1978). D-α-aminoadipate, α-ε,-diaminopimelic acid and HA 966 as antagonists of amino acid-induced and synaptic excitation of mammalian spinal neurones *in vivo*. Brain Res. 148, p. 543-548.

BIZIERE, K., THOMPSON, H., COYLE, J.T. (1980). Characterization of specific, high affinity binding sites for L-[^{3}H] glutamic acid in rat brain membranes. Brain Research. 183, p. 421-433.

BLAKE, D.J., ZARZECKI, P. and SOMJEN, G.G. (1976). Electrophysiological study of corticocaudate projections in cats. J. Neurobiol. 7, p. 143-156 .

BLOOM, F.E., COSTA, R., SALMORAGHI, G.C. (1965). Anesthesia and the responsiveness of individual neurones of the caudate nucleus of the cat to acetylcholine norepinephrine and dopamine administered by microelectrophoresis. J. Pharmacol. and Exp. Therapeutics.

BOBILLIER, P., PETITJEAN, F., SALVERT, D., LIGIER, M. and SEGUIN, S. (1975). Differential projections of the nucleus raphe dorsalis and nucleus raphe centralis as revealed by autoradiography. Brain Res. 85, p. 205-210.

BRADFORD, H.F. and WARD, H.K. (1976). On glutaminase activity in mammalian synaptosomes. Brain Res. 110, p. 115-125.

BRADFORD, H.F., WARD, H.K. and THOMAS, A.J. (1978). Glutamine - a major substrate for nerve endings. J. Neurochem. 30, p. 1453-1459.

BRODAL, A., TABER, E. and WALBERG, F. (1960). The raphe nucleii of the brain stem. II. Efferent connections. J. Comp. Neurol. 114, p. 239-259.

BROWNSTEIN, M.J., MROZ, E.A., TAPPAZ, M.L. and LEEMAN, S.E. (1977). On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. Brain Res. 135, p. 315-323.

BROXTERMAN, H.J., NOACH, E.L. and VAN VOLKENBURG, C.F.M. (1979). Differential effects of acute and subacute HA 966 treatment on storage and release of striatal dopamine. Europ. J. Pharmacol. 60, p. 153-161.

BUCHWALD, N.A., PRICE, D.D., VERNON, L. and HULL, C.D. (1973). Caudate intracellular response to thalamic and cortical inputs. Exp. Neurol. 38, p. 311-323.

BUNNEY, B.S. and AGHAJANIAN, G.K. (1976). The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res. 117, p. 423-435.

BUTCHER, S.G. and BUTCHER, L.L. (1974). Origin and modulation of acetylcholine activity in the neostriatum. Brain Res. 71, p. 167-171.

BUTCHER, L.L., TALBOT, K. and BILEZIKIAN, L. (1975). Localization of acetylcholinesterase within dopamine containing neurones in the zona compacta of the substantia nigra. J. Neural. Trans. 37, p. 127-153.

CAJAL, R.Y.S. (1891). Structure de l'écorce cérébrale de quelques mammifères. Cellule. 7, p. 125.

CAJAL, R.Y.S. (1911). Histologie du système nerveux central. p. 504-518, Maloine, Paris.

CARMAN, J.B., COWAN, W.M. and POWELL, T.P.S. (1963). The organization of cortico-striate connections in the rabbit. Brain. 85, p. 525-562.

CARMAN, J.B., COWAN, W.M., POWELL, T.P.S. and WEBSTER, K.E. (1965). A bilateral cortico-striate projection. J. Neurol. Neurosurg. Psychiat.

CARMEL, P.W. (1970). Efferent projections of the ventral anterior nucleus of the monkey. Am. J. Anat. 128, p. 159-184.

CARPENTER, M.B. and McMASTERS, R.E. (1964). Lesions of the substantia nigra in the rhesus monkey. Efferent fiber degeneration and behavioural observations. Amer. J. Anat. 114, p. 293-319.

CARPENTER, M.B., NAKANO, K. and KIM, R. (1976). Nigrothalamic projections in the monkey demonstrated by autoradiographic technics. J. Comp. Neurol. 165, p. 401-416.

CARPENTER, M.B. and PETER, P. (1972). Nigrostriatal and nigro-thalamic fibers in the rhesus monkey. J. Comp. Neurol. 144, p. 93-116.

CARPENTER, M.B. and STOMINGER, N.L. (1967). Efferent fibres of the subthalamic nucleus in the monkey. A comparison of the efferent projections of the subthalamic nucleus, substantia nigra and globus pallidus. Amer. J. Anat. 121, p. 41-72.

CARTER, D.A. and FIBIGER, H.C. (1977). Ascending projections of presumed dopamine-containing neurones in the ventral tegmentum of the rat as demonstrated by horseradish peroxidase. Neuroscience. 2, p. 569-576.

CHUBB, I.W., HODGSON, A.J. and WHITE, G.H. (1980). Acetyl-cholinesterase hydrolyzes substance P. Neuroscience. 5, p. 2065-2072.

CHERUBINI, E., NOVACK, G., HULL, C.D., BUCHWALD, N.A. and LEVINE, M.S. (1979). Caudate neuronal responses evoked by cortical stimulation: contribution of an indirect corticothalamic pathway. Brain Res. 173, p. 331-336.

CLARK, W.E. Le Gros (1933). An experimental study of thalamic connections in the rat. Phil. Trans. B. 222.

COLLINGRIDGE, G.L., JAMES, T.A., MacLEOD, N.K. (1980). Antidromic latency variations of nigral compacta neurones. Experientia. 36, 970-971.

CONNOR, J.D. (1968). Caudate unit responses to nigral stimuli: evidence for a possible nigro-neostriatal pathway. Science. 160, p. 899-900.

CONNOR, J.D. (1970). Caudate nucleus neurones: correlation of the effects of substantia nigra stimulation with iontophoretic dopamine. J. Physiology. 208, p. 691-703.

CONRAD, L.C.A., LEONARD, C.M. and PFAFF, D.W. (1974). Connections of the median and dorsal raphe nucleii in the rat: an autoradiographic and degeneration study. J. Comp. Neurol. 156, p. 179-205.

COSTA, E., GIUDOTTI, A., MORONI, F. and PENALTA, E. (1979). in Glutamic Acid: advances in biochemistry and physiology. Eds. Filer *et al*. Raven Press.

COSTALL, B. and NAYLOR, R.Y. (1981). Mini review - The hypothesis of different dopamine receptor mechanisms. Life Sciences. 28, p. 215-229.

COUCH, J.R. and GOLDSTEIN, G.D. (1977). Projections of locus coeruleus and raphe nucleii to neostriatum. Neuroscience Abs. 3, p. 36.

COWAN, W.M. and POWELL, T.P.S. (1955). The projection nucleii of the midline and intralaminar nucleii of the thalamus of the rabbit. J. Neurol. Neurosurg. Psychiat. 18, p. 266.

COWAN, W.M. and POWELL, T.P.S. (1966). Strio-pallidal projection in the monkey. J. Neurol. Neurosurg. Psychiat. 29, p. 426-439.

CREESE, I., BURT, D.R. and SNYDER, S.H. (1977). Dopamine receptor binding enhancement accompanies lesion-induced behavioural super-sensitivity. Science. 197, p. 596-597.

CREESE, I. and SNYDER, S.H. (1979). Nigrostriatal lesions enhance striatal ^{3}H-apomorphine and ^{3}H-spiroperidol binding. Europ. J. Pharmacol. 56, p. 277-281.

CROSSMAN, A.R., WALKER, R.J. and WOODRUFF, G.N. (1973). Picrotoxin antagonism of GABA inhibitory responses and synaptic inhibition in the rat substantia nigra. Br. J. Pharmac. 49, p. 696-698.

CUELLO, A.C. and PAXIMOS, G. (1978). Evidence for a long leu-enkephalin striopallidal pathway in rat brain. Nature. 271, p. 178-180.

CURTIS, D.R., DUGGAN, A.W., FELIX, D., JOHNSTON, G.A.R., TEBECIS, A.K. and WATKINS, J.C. (1972). Excitation of mammalian central neurones by acidic amino acids. Brain Res. 41, p. 283-307.

CURTIS, D.R., JOHNSTON, G.A.R., GAME, C.J.A. and McCULLOCH, R.M. (1973). Antagonism of neuronal excitation by 1-hydroxy-3-amino-pyrrolidone-2. Brain Res. 49, p. 467-470.

DAFNY, N., DAUTH, G. and GILMAN, S. (1975). A direct input from amygdaloid complex to caudate nucleus of the rat. Exp. Brain Res. 23, p. 203-210.

DAHLSTRÖM, A. and FUXE, K. (1964). Evidence for the existence of monoamine-containing neurones in the central nervous system. Demonstration of monoamines in the cell bodies of brainstem neurones. Acta. Phys. Scand. 62, Sup. 232.

DAHLSTRÖM, A. and FUXE, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. Distribution of monoamine nerve terminals in the central nervous system. Acta. Phys. Scand. 64, Supp. 247.

DAVIES, J. and DRAY, A. (1976). Substance P in the substantia nigra. Brain Res. 107, p. 623-627.

DAVIES, J., EVANS, R.H., FRANCIS, A.A., JONES, A.W. and WATKINS, J.C. (1981). Antagonism of excitatory amino acid-induced and synaptic excitation of spinal neurones by cis-2,3-piperidine dicarboxylate. J. Neurochem. 36, p. 1305-1307.

DAVIES, J., FRANCIS, A.A., JONES, A.W. and WATKINS, J.C. (1980a). Dipeptide antagonists of amino acid-induced and synaptic excitation in the vertebrate central nervous system. J. Physiol. 306, p. 21-22P.

DAVIES, J., FRANCIS, A.A., JONES, A.W. and WATKINS, J.C. (1980b). 2-amino-5-phosphonovalerate (2APV) a highly potent and specific antagonist at spinal NMDA receptors. Brit. J. Pharmacol. 70, p. 52-53P.

DAVIES, J. and TONGROACH, P. (1978). Neuropharmacological studies on the nigro-striatal and raphe striatal system in the rat. Europ. J. Pharmacol. 51, p. 91-100.

DAVIES, J. and WATKINS, J.C. (1972). Is 1-hydroxy-3-aminopyrrolidone-2 (HA 966) a selective excitatory amino acid antagonist? Nature New Biol. 238, p. 61-63.

DAVIES, J. and WATKINS, J.C. (1979). Selective antagonism of amino acid-induced and synaptic excitation in the cat spinal cord. J. Physiol. 297, p. 621-635.

DAVIES, J. and WATKINS, J.C. (1981). Differentiation of kainate and quisqualate receptors in the cat spinal cord by selective antagonism with -(andL)-glutamylglycine. Brain Res. 206, p. 172-177.

DENIAU, J.M., HAMMOND, C., RISZK, A. and FEGER, J. (1978). Electrophysiological properties of identified output neurones of the rat substantia nigra (pars compacta and pars reticulata). Evidence for the existence of branched neurones. Exp. Brain Res. 32, p. 409-422.

DE BELLEROCHE, J. and BRADFORD, H.F. (1978). Biochemical evidence for the presence of presynaptic receptors on dopaminergic nerve terminals. Brain Res. 142, p. 53-68.

DE BELLEROCHE, J.S. and BRADFORD, H.F. (1980). Presynaptic control of the synthesis and release of dopamine from striatal synaptosomes: a comparison between the effects of 5-hydroxytryptamine, acetylcholine and glutamate. J. Neurochem. 35, p. 1227-1234.

DE BELLEROCHE, J.S. and BRADFORD, H.F. (1981). Evidence for an inhibitory presynaptic component of neuroleptic drug action. Brit. J. Pharmacol. 72, p. 427-433.

DE VITO, J.L. and SMITH, O.A. (1964). Subcortical projections of the prefrontal lobe of the monkey. J. Comp. Neurol. 123, p. 413-424.

DIFIGLIA, M., PASIK, P. and PASIK, T. (1976). A Golgi study of neuronal types in the neostriatum of monkeys. Brain Res. 114, p. 245-256.

DIMOVA, R., VUILLET, J. and SEITE, R. (1980). Study of the rat neostriatum using a combined golgi-electron microscope technique and serial sections. Neuroscience. 5, p. 1581-1596.

DIVAC, I., FONNUM, F. and STORM-MATHISEN, J. (1977). High affinity uptake of glutamate in terminals of corticostriatal axons. Nature. 266, p. 377-378.

DOMESICK, V.B. (1977). The topographical organization of the striato-nigral connection in the rat. Anat. Rec. 187, p. 567.

DONOGHUE, J.P. and KITAI, S.T. (1980). Collateral input to the neo-striatum from descending axons of the rat somatic-sensory motor cortex. Neuroscience Abstracts. 6, p. 187.

DRAY, A., GOYNE, T.J. and OAKLEY, N.R. (1976). Caudate stimulation and substantia nigra activity in the rat. J. Physiol. 259, p. 825-849.

DRAY, A. and STRAUGHAN, D.W. (1976). Synaptic mechanisms in the substantia nigra. J. Pharm. Pharmac. 28, p. 400-405.

DROOGLEEVER-FORTUYN, J. and STEFENS, R. (1951). On the anatomical relations of the intralaminar and midline cells of the thalamus. EEG Clin. Neurophys. 3, p. 393-400.

DRUCKMANN, R. (1952). A critique of "suppression", with additional observations in the cat. Brain. 75, p. 226-243.

DUSSER DE BARENNE, J.G. and McCULLOCH, W.S. (1938). The direct functional interrelation of sensory cortex and optic thalamus. J. Neuro-physiol. 1, p. 69-85.

DUSSER DE BARENNE, J.G., GAROL, H.W. and McCULLOCH, W.S. (1942). Diseases of the basal ganglia. Res. Pubs. Ass. Nerv. Ment. Dis. 21. p. 246-266.

EDINGER, L. (1911). Vorlesungen uber den Bau der nervozen Zentralorgane. Vol. 1. Vogel. Leipzig.

EHRINGER, H. and HORNYKIEWICZ, O. (1960). Verteilung von noradrenalin und Dopamin (3-hydroxy-tyramin) im Geherin des menschen und ihr verhalten bei erkankungen des extrapyramidalen systems. Klin. Wdchr. 38, p. 1236-1239.

ENNIS, C., KEMP, J.D. and COX, B. (1981). Characterisation of inhibitory 5-hydroxytryptamine receptors that modulate dopamine release in the striatum. J. Neurochem. 36, p. 1515-1520.

EVANS, R.H., FRANCIS, A.A. and WATKINS, J.C. (1978). Mg^{2+}-like selective antagonism of excitatory amino acid induced responses by α, ε-diaminopimelic acid, D-α-aminoadipate and HA 966 in isolated spinal cord of frog and immature rat. Brain Res. 148, p. 536-542.

EWART, W.R. and WILLIAMS, T.D. (1977). Effect of dopamine on firing patterns of striatal units in adult and 21-day old rats. Exper. Neurology. 57, p. 434-439.

FALCK, B., HILLARP, N-Å., THIEME, G. and TORP, A. (1962). Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. 10, p. 348-354.

FALLON, J.H. and MOORE, R.Y. (1977). Topography of mesencephalic dopamine cell projections to the telencephalon. Anat. Rec. 187, p. 764-.

FALLON, J.H. and MOORE, R.Y. (1978). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J. Comp. Neurol. 180, p. 545-580.

FALLON, J.H. and ZIEGLER, B.T.S. (1979). The crossed cortico-caudate projection in the rhesus monkey. Neuroscience Letters. 15, p. 29-32.

FASS, B. and BUTCHER, L.L. (1981). Evidence for a crossed nigro-striatal pathway in rats. Neuroscience Letters. 22, p. 109-113.

FAULL, R.L.M. and CARMAN, J.B. (1968). Ascending projections of the substantia nigra in the rat. J. Comp. Neurol. 132, p. 73-92.

FAULL, R.L.M. and MEHLER, W.R. (1978). The cells of origin of nigro-tectal, nigrothalamic and nigrostriatal projections in the rat. Neuro-Science. 3.

FELTZ, P. and ALBE-FESSARD, D. (1972). A study of an ascending nigro-caudate pathway. Electroenceph. Clin. Neurophysiol. 33, p. 179-193.

FELTZ, P. and DE CHAMPLAIN, J. (1972a). Persistence of caudate unitary responses to nigral stimulation after destruction and functional impairment of the striatal dopaminergic terminals. Brain Res. 43, p. 595-600.

FELTZ, P. and DE CHAMPLAIN, J. (1972b). Enhanced sensitivity of caudate neurones to microiontophoretic injections of dopamine in 6-hydroxydopamine treated cats. Brain Res. 43, p. 601-605.

FELTZ, P. and MacKENZIE, J.S. (1969). Properties of caudate unitary responses to repetitive nigral stimulation. Brain Res. 13, p. 612-616.

FIBIGER, H.C., PUDRITZ, R.E., McGEER, P.L. and McGEER, E.G. (1972). Axonal transport in nigro-striatal and nigro-thalamic neurones: effects of medial forebrain bundle lesions and 6-hydroxydopamine.

FINK, R.P. and HEIMER, L. (1967). Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4, p. 369-374.

FONNUM, F., GOTTESFELD, Z. and GROFOVA, I. (1978). Distribution of glutamate decarboxylase choline acetyltransferase and aromatic amino acid decarboxylase in the basal ganglia of normal and operated rats. Evidence for a striatopallidal, striatoentopeduncular and striatonigral GABAergic fibres. Brain Res. 143, p. 125-138.

FONNUM, F., GROFOVA, I., RINVIK, E., STORM-MATHIESEN, J. and WALBERG, F. (1974). Origin and distribution of glutamate decarboxylase in substantia nigra of the cat. Brain Res. 71, p. 77-92.

FONNUM, F., STORM-MATHIESEN, J. and DIVAC, I. (1981). Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. Neuroscience. 6, p. 863-873.

FONNUM, F. and WALAAS, I. (1979). In The Neostriatum. Localization of neurotransmitter candidates in neostriatum. Eds Divac and Oberg. Pergamon Press.

FOX, C.A., ANDRADE, A.N., HILLMAN, D.E., SCHWYN, R. (1971a). The spiny neurones in the primate striatum: a Golgi and electron microscopic study. J. Hirnforsch. 13, p. 181-201.

FOX, C.A., ANDRADE, A.N., HILLMAN, D.E. and SCHWYN, R.C. (1971b). The spiny neurones and the glia in the primate striatum: a Golgi and electron microscopic study. J. Hirnforsch. 13, p. 341-362.

FOX, C.A. and RAFOLS, J.A. (1975a). The radial fibers in the globus pallidus. J. Comp. Neurol. 159, p. 177-200.

FOX, C.A., RAFOLS, J.A. and COWAN, W.M. (1975b). Computer measurements of axis cylinder diameters of radial fibers and comb bundle fibres. J. Comp. Neurol. 159, p. 201-224.

FOX, C.A. and RAFOLS, J.A. (1976). In The Basal Ganglia. The striatal efferents in the globus pallidus and in the substantia nigra. Ed. M.D. Yahr. Raven Press, New York.

FOX, C.A. and SCHMITZ, J.T. (1944). The substantia nigra and the entopeduncular nucleus in the cat. J. Comp. Neurol. 80, p. 323-334.

FRANCIS, A.A., JONES, A.W. and WATKINS, J.C. (1980). Dipeptide antagonists of amino acid-induced and synaptic excitation in the frog spinal cord. J. Neurochem. 35, p. 1458-1460.

FREEDMAN, R., HOFFER, B.J., PURO, D. and WOODWARD, D.J. (1976). Noradrenaline modulation of the responses of the cerebellar Purkinje cell to afferent synaptic activity. Brit. J. Pharmacol. 57, p. 603-606.

FRIGYESI, T.L. and PURPURA, D.P. (1967). Electrophysiological analysis of reciprocal caudato-nigral relations. Brain Res. 6, p. 440-456.

FUXE, K., HOKFELT, T. and NILSSON, O. (1964). Observations on the cellular localization of DA in the caudate nucleus of the rat. Z. Zellforsch. 63, p. 701-706.

GALE, K., HONG, J.S. and GUIDOTTI, A. (1977). Presence of substance P and GABA in separate striatonigral neurones. Brain Res. 136, p. 371-375.

GALLAGHER, J.P., INOKUCHI, H. and SHINNICK-GALLAGHER, P. (1980). Dopamine depolarization of mammalian primary afferent neurones. Nature. 283, p. 770-772.

GARCIA-MUNOZ, M. (1978). The striato-nigral pathway: its role in the regulation of dopamine metabolism. PhD Thesis, University of Edinburgh.

GARCIA-RILL, E., HULL, C.D., CHERUBINI, E., LEVINE, M.S. and BUCHWALD, N.A. (1979). The spontaneous firing patterns of forebrain neurones. IV. Effects of bilateral and unilateral frontal cortical ablations on firing of caudate, globus pallidus and thalamic neurones. Brain Res. 165, p. 23-36.

GARCIA-RILL, E., HULL, C.D., CHERUBINI, E., LEVINE, M.S. and BUCHWALD, N.A. (1980). The spontaneous firing patterns of forebrain neurones. V. Time course of changes in caudate unit activity following dopamine depleting lesions. Brain Res. 190, p. 415-424.

GAROL, H.W. (1940). Some observations on suppression of electrical activity of areas 4 and 6. Am. J. Physiol. 129, p. 361.

GAROL, H.W. (1942). Cortical origin and distribution of corpus callosum and anterior commisure in the cat. J. Neuropath. and Exp. Neurol. 1, p. 422-429.

GELLHORN, E. (1947). Effect of afferent impulses on cortical suppressor areas. J. Neurophysiol. 10, p. 125-132.

GIORGUEFF, M.F., LE FLOC'H, M.L., GLOWINSKI, J. and BESSON, M.J. (1976). Involvement of cholinergic presynaptic receptors of nicotinic and muscarinic types in the control of the spontaneous release of DA from striatal dopaminergic terminals in the rat. J. Pharmacol. Exp. Therap. 200, p. 534-544.

GIORGUEFF-CHESSELET, M.F., KEMEL, M.L., WANDSCHEER, D. and GLOWINSKI, J. (1979). Attempts to localize the GABA receptors involved in the GABA-induced release of newly synthesized [3H] dopamine in rat striatal slices. Brain Res. 175, p. 383-386.

GLEES, P. (1944). The anatomical basis of corticostriate connections. J. Anat. 78, p. 47-51.

GLOOR, P., (1955). Electrophysiological studies on the connections of the amygdaloid nucleus in the cat. 1. The neuronal organization of the amygdaloid projection system. Electroenceph. Clin. Neurophysiol. 7, p. 223-242.

GMELIN, G. (1978). Microelectrophoretic studies with 2-bromo-α-ergocryptine on dopaminergic neurones in the feline caudate nucleus. Life Science. 23, p. 485-488.

GODUKHIN, O.V., ZHARIKOVA, A.D. and NOVOSELOV, V.I. (1980). The release of labelled L-glutamic acid from rat neostriatum *in vivo* following stimulation of frontal cortex. Neuroscience. 5, p. 2151-2154.

GOLDMAN, P.S. (1978). Neuronal plasticity in the primate telencephalon: anomolous projections induced by prenatal removal of frontal cortex. Science. 202, p. 768-776.

GOLDMAN, P.S. and NAUTA, W.J.H. (1977). An intricately patterned prefrontocaudate projection in the rhesus monkey. J. Comp. Neurol. 171, p. 369-386.

GONZALEZ-VEGAS, J.A. (1974). Antagonism of dopamine mediated inhibition in the nigro-striatal pathway: a mode of action of some catatonia-inducing drugs. Brain Res. 80, p. 219-228.

GOODALE, D.B., RUSTERHOLZ, D.B., LONG, J.P., FLYM, J.R., WALSH, B., CANNON, J.G. and LEE, T. (1980). Neurochemical and behavioural evidence for a selective presynaptic dopamine receptor agonist. Science. 210, p. 1141.

GREENGARD, P. (1976). Possible role for cyclic nucleotides and phosphorylated membrane proteins in post synaptic actions of neurotransmitters. Nature. 260, p. 101-108.

GROFOVA, I. (1975). The identification of striatal and pallidal neurones projecting to substantia nigra. An experimental study by means of retrograde axonal transport. Brain Res. 91, p. 286-291.

GROFOVA, I. and RINVIK, E. (1970). An experimental electron microscopical study on the striatonigral projection in the cat. Exp. Brain Res. 11, p. 249-262.

GURDJIAN, E.S. (1928). The corpus striatum of the rat. Studies on the brain of the rat. No. 3. J. Comp. Neurol. 45, p. 249-281.

GUSTAFSSON, B. and LIPSKI, J. (1980). Effect of membrane polarization and synaptic activity on the timing of antidromic invasion. Brain Res. 181, p. 61-74.

GUYNET, P.G. and AGHAJANIAN, G.K. (1978). Antidromic identification of dopaminergic and other output neurones of the rat substantia nigra. Brain Res. 150, p. 69-84.

HADJU, F., HASSLER, R. and BAK, I.J. (1973). Electron microscopic study of the substantia nigra and the strio-nigral projection in the rat. Z. Zellforsch. 146, p. 207-221.

HALDEMAN, S., HUFFMAN, R.D., MARSHALL, K.C. and McCLENNAN, H. (1972). The antagonism of the glutamate-induced and synaptic excitation of thalamic neurones. Brain Res. 45, p. 393-400.

HALDEMAN, S. and McCLENNAN, H. (1972). The antagonistic action of glutamic acid diethyl ester towards amino acid induced and synaptic excitations of central neurones. Brain Res. 39, p. 419-425.

HALL, R.D. and LINDHOLM, E.P. (1974). Organization of motor somatosensory neocortex in the albino rat. Brain Res. 66, p. 23-38.

HARMS, H.H., WARDEH, G. and MULDER, A.H. (1978). Effects of adenosine on depolarization-induced release of various radiolabelled neurotransmitters from slices of rat corpus striatum. Neuropharmacol. 18, p. 577-580.

HARVEY, J.A., HELLER, A. and MOORE, R.Y. (1963). The effect of unilateral and bilateral medial forebrain bundle lesions on brain serotonin. J. Pharmac. Exp. Ther. 140, p. 103-110.

HATTORI, T., FIBIGER, H.C. and McGEER, P.L. (1975). Demonstration of a pallido-nigral projection innervating dopaminergic neurones. J. Comp. Neurol. 162, p. 487-504.

HATTORI, T., McGEER, P.L., FIBIGER, H.C. and McGEER, E.G. (1973). On the source of GABA-containing terminals in the substantia nigra. Electron microscopic, autoradiographic and biochemical studies. Brain Res. 54, p. 103-114.

HATTORI, T., McGEER, E.G. and McGEER, P.L. (1979). Fine structural analysis of the corticostriatal pathway. J. Comp. Neurol. 185, p. 347-354.

HEBB, C.O. (1957). Biochemical evidence for the neuro-function of acetylcholine. Physiol. Rev. 37, p. 196-220.

HEDREEN, J.C. (1971). Separate demonstration of dopaminergic and non-dopaminergic projections of substantia nigra in the rat. Anat. Rec. 169, p. 338.

HEDREEN, J.C. (1977). Cortico-striatal cells identified by the peroxidase method. Neuroscience Letters. 4, p. 1-7.

HEDREEN, J.C. (1978). Non-dopaminergic and dopaminergic nigrostriatal pathways in rats. Neuroscience Abs. 4, p. 45.

HEDREEN, J.C. and CHALMERS, J.P. (1972). Neuronal degeneration in rat brain induced by 6-hydroxy-dopamine; a histological and biochemical study. Brain Res. 47, p. 1-36.

HEFTI, F., MELAMED, E. and WURTMAN, R.J. (1980). Partial lesions of the dopaminergic nigrostriatal system in rat brain: biochemical characterisation. Brain Res. 195, p. 123-137.

HENK, J.M., BROXTERMAN, J. and VAN BENNEBOM, W.P. (1981). *In vivo* voltammetric investigations into the action of HA 966 on central dopaminergic neurones. Brain Res. 207, p. 465-470.

HENKE, H. and CUÉNOD, M. (1979). L-glutamate specific [^{3}H] Kainic acid binding in the rat neostriatum after degeneration of the cortico-striatal pathway. Neuroscience Letters. 11, p. 341-345.

HENN, F.A., GOLDSTEIN, M.N. and HAMBERGER, A. (1974). Uptake of the neurotransmitter candidate glutamate by glia. Nature. 249, p. 663-664.

HERRLING, P.L. and HULL, C.D. (1980). Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurones. Brain Res. 192, p. 441-462.

HERZ, A. and ZIEGLGÄNSBERGER (1968). The influence of micro-electrophoretically applied biogenic amines, cholinomimetics and procaine on synaptic excitation in the corpus striatum. Int. J. Neuropharm. 7, p. 221-230.

HINES, M. (1937). The motor cortex. Bulletin of the John's Hopkins Hospital. 60, p. 313-336.

HJORTH, S., CARLSSON, A., WIKSTROM, H., LINDBERG, P., SANCHEZ, D., HACKSELL, U., ARVIDSSON, L-E., SVENSSON, U. and NILSSON, J.L.G. (1981). 3-PPP a new centrally acting DA-receptor agonist with selectivity for autoreceptors. Life Sciences. 28, p. 1225-1238.

HÖKFELT, T. (1968). *In vitro* studies on central and peripheral monoamine neurones at the ultrastructural level. Z. Zellforsch. Mikrosk. Anat. 91, p. 1-74.

HÖKFELT, T., ELDE, R., JOHANSSON, O., TERENIUS, L. and STEIN, L. (1977). Distribution of enkephalin-like immunoreactivity in the rat central nervous system. Neuroscience Letters. 5, p. 25-31.

HÖKFELT, T. and UNGERSTEDT, U. (1969). Electron and fluorescence microscopical studies on the nucleus caudatus putamen of the rat after unilateral lesions of ascending nigroneostriatal dopamine neurones. Acta. Phys. Scand. 76, p. 415-426.

HOLMES, G. (1901). The physiology of the dog without a forebrain. J. Physiol. 27, p. 1-25.

HONG, J.S., YOUNG, H-Y. T., FRATTA, W. and COSTA, E. (1977). Determination of methionine enkephalin in discrete regions of rat brain. Brain Res. 134, p. 383-386.

HORNYKIEWICZ, O. (1966). Dopamine (3-hydroxytyramine) and brain function. Pharmac. Rev. 18, p. 925-964.

HOLMAN, R.B. and VOGT, M. (1972). Release of 5-hydroxytryptamine from caudate nucleus and septum. J. Physiol. 223, p. 243-254.

HULL, C.D., BERNARDI, G. and BUCHWALD, N.A. (1970). Intracellular responses of caudate neurones to brain stem stimulation. Brain Res. 22, p. 163-179.

HULL, C.D., BERNARDI, G., PRICE, D.D. and BUCHWALD, N.A. (1973). Intracellular responses of caudate neurones to temporally and spatially combined stimuli. Experimental Neurology. 38, p. 324-336.

HULL, C.D., LEVINE, M.S., BUCHWALD, N.A., HELLER, A. and BROWNING, R.A. (1974). The spontaneous firing patterns of forebrain neurones. 1. The effects of dopamine and non-dopamine depleting lesions on caudate unit firing patterns. Brain Res. 73, p. 241-262.

IBATA, Y., NOJYO, Y., MATSUURA, T. and SANO, Y. (1973). A correlative study with Fink-Heimer impregnation, fluorescence histochemistry and electron microscopy. Z. Zellforsch. 138, p. 333-344.

IVERSEN, L.L. (1967). The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press.

IVERSEN, L.L., IVERSEN, S.D., BLOOM, F.E., VARGO, T. and GUILLEMIN, R. (1978). Release of enkephalin from rat globus pallidus *in vitro*. Nature. 271, p. 679-681.

JACOBS, B.L., FOOTE, S.L. and BLOOM, F.E. (1978). Differential projections of neurones within the dorsal raphe nucleus of the rat: a horseradish prroxidase HRP study. Brain Res. 147, p. 149-153.

JACOBS, B.L., WISE, W.D. and TAYLOR, K.M. (1974). Differential behavioural and neurochemical effects following lesions of the dorsal or median raphe nucleii in rats. Brain Res. 79, p. 353-361.

JENNER, P. and MARSDEN, C.D. (1979). Mini review: The substituted benzamides - a novel class of dopamine antagonists. Life Sciences. 25, p. 475-486.

JENNER, P., MARSDEN, C.D. and TAYLOR, R.J. (1981). Behavioural changes induced by the unilateral intrastriatal administration of glutamate analogues in the rat. Brit. J. Pharmac. 72, p. 570-571P.

JESSEL, T.M. (1978). Substance P release from the rat substantia nigra. Brain Res. 151, p. 469-478.

JINNAI, K. and MATSUDA, Y. (1979). Neurones of the motor cortex projecting commonly on the caudate nucleus and the lower brain stem in the cat. Neuroscience Letters. 13, p. 121-126.

JOHNSON, T.N. (1961). Fiber connections between the dorsal thalamus and corpus striatum in the cat. Experimental Neurology. 3, p. 550-569.

JONES, B.E., HALARIS, A.E., McILHANY, M. and MOORE, R.Y. (1977). Ascending projections of the locus coeruleus in the rat. 1. Axonal transport in central noradrenaline neurones. Brain Res. 127, p. 1-21.

JONES, E.G., COULTER, J.D., BURTON, H. and PORTER, R. (1977b). Cells of origin and terminal distribution of corticostriatal fibers arising in the sensory-motor cortex of monkeys. J. Comp. Neurol. 173, p. 53-80.

JONES, E.G. and LEAVITT, R.Y. (1974). Retrograde axonal transport and the demonstration of non-specific projections to the cerebral cortex and striatum from thalamic intralaminar nucleii in the rat, cat and monkey. J. Comp. Neurol. 154, p. 349-378.

JONES, E.G. and WISE, S.P. (1979). Size laminar and collumnar distribution of efferent cells in the sensory-motor cortex of monkeys. J. Comp. Neurol. 175, p. 391-438.

JUNG, R. and HASSLER, R. (1960). American Handbook of Physiology. Section 1: Neurophysiology. p. 863.

KALIL, K. (1978). Patch-like termination of thalamic fibers in the putamen of the rhesus monkey: an autoradiographic study. Brain Res. 140, p. 333.

KANAZAWA, I., EMSON, P.C. and CUELLO, A.C. (1977). Evidence for the existence of substance P-containing fibers in striatonigral and pallidonigral pathways in rat brain. Brain Res. 119, p. 447-453.

KANAZAWA, I. and JESSEL, T. (1976). Post mortem changes and regional distribution of substance P in the rat and mouse nervous systems. Brain Res. 117, p. 362-367.

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

KANAZAWA, I. and YOSHIDA, M. (1980). Electrophysiological evidence for the existence of excitatory fibres in the caudatonigral pathway in the cat. Neuroscience Letters. 20, p. 301-306.

KATAOKA, K., BAK, I.J., HASSLER, R., KIM, J.S. and WAGNER, A. (1974). L-glutamate decarboxylase and cholineacetyltransferase activity in the substantia nigra and the striatum after surgical interruption of the strio-nigral fibres of the baboon.

KATAYAMA, Y. (1978). Physiological properties and function of caudate neurones: single unit responses to stimulation of nigrostriatal, cortico-striatal and thalamostriatal fibers. Nihon Univ. J. Med. 20, p. 187-223.

KEBABIAN, J.W. and CALNE, D.B. (1979). Multiple receptors for dopamine. Nature. 277. p. 93-96.

KEBABIAN, J.W., PETZHOLD, G.L. and GREENGARD, P. (1972). Dopamine-sensitive adenylate cyclase of rat brain and its similarity to the "dopamine receptor". PNAS. 69, p. 2145-2149.

KEMP, J.M. (1970). The termination of strio-pallidal and strio-nigral fibers. Brain Res. 17, p. 125-128.

KEMP, J.M. and POWELL, T.P.S. (1970). The cortico-striate projection in the monkey. Brain. 93, p. 525-546.

KEMP, J.M. and POWELL, T.P.S. (1971). The structure of the caudate nucleus in the cat. Light and electron microscopy. Phil. Trans. Roy. Soc. Lond. Ser. B. 262. p. 383-401.

KIM, J.S., BAK, I.J., HASSLER, R. and OKADA (1971). Role of γ-aminobutyric acid in the extrapyramidal motor system. 2: Some evidence for the existence of a type of GABA-rich strio-nigral neurones. Exp. Brain Res. 14, p. 95-104.

KIM, J.S., HASSLER, R., HAUG, P. and PAIK, K.S. (1977). Effect of frontal cortex ablation on striatal glutamic acid level in rat. Brain Res. 132. p. 370-374.

KIMMEL, D.L. (1942). Nigro-striatal fibres in the cat. Anat. Rec. 82, p. 425.

KITAI, S.T., KOCSIS, J.D., PRESTON, R.J. (1978). In Cholinergic Monoaminergic Interactions in the Brain. Physiologic and Morphologic characteristics of the caudate spiny neurone. p. 157-165. Academic Press.

KITAI, S.T., KOCSIS, J.D., PRESTON, R.J. and SUGIMORI, M. (1976a). Monosynaptic inputs to caudate neurones identified by intracellular injection of horseradish peroxidase. Brain Res. 169, p. 601-606.

KITAI, S.T., KOCSIS, J.D. and WOOD, J. (1976b). Origin and characteristics of the cortico-caudate afferents: an anatomical and electrophysiological study. Brain Res. 118, p. 137-141.

KITAI, S.T., PRESTON, R.J., BISHOP, G.A. and KOCSIS, J.D. (1979). Striatal projection neurones: morphological and electrophysiological studies. Advances in Neurology. 24, p. 45-51.

KITAI, S.T., SUGIMORI, M., KOCSIS, J.D. (1976c). Excitatory nature of dopamine in the nigro-caudate pathway. Exp. Brain Res. 24, p. 351-363.

KITAI, S.T., WAGNER, A., PRECHT, W. and OHNO, T. (1975). Nigro-caudate and caudato-nigral relationship: an electrophysiological study. Brain Res. 85, p. 44-48.

KLUVER, H. and BARRERA, E. (1953). A method for the combined staining of cells and fibres in the central nervous system. J. Neuropath. Exp. Neurol. 12, p. 400-403.

KOCSIS, J.D. and KITAI, S.T. (1977). Dual excitatory inputs to caudate spiny neurones from substantia nigra stimulation. Brain Res. 138, p. 271-283.

KOCSIS, J.D., SUGIMORI, M. and KITAI, S.T. (1977). Convergence of excitatory synaptic inputs to caudate spiny neurones. Brain Res. 124, p. 403-413.

KOCSIS, J.D. and VANDERMAELEN, C.P. (1977). The caudate projection of the retrorubral nucleus and its relationship to the substantia nigra in the cat. Anat. Rec. 187, p. 628-629.

KOCSIS, J.D. and VANDERMAELEN, C.P. (1979). A supernormal period in central axons following single cell stimulation. Exp. Brain Res. 36, p. 381-386.

KÖNIG, J.F.R. and KLIPPEL, R.A. (1963). The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins (Baltimore, U.S.A.).

KOPSCH, F. (1957). Nomina Anatomica. 5th Edit. Georg Thieme, Hamburg.

KRIEG, W.J.S. (1947). Connections of the cerebral cortex. 1. The albino rat, c. extrinsic connections. J. Comp. Neurol. 86, p. 267.

KUHAR, M.J., AGHAJANIAN, G.K. and ROTH, R.H. (1972). Tryptophan hydroxylase activity and synaptosomal uptake of serotonin in discrete brain regions after midbrain raphe lesions: correlations with serotonin levels and histochemical fluorescence. Brain Res. 44, p. 165-176.

KÜNZLE, H. (1975). Bilateral projections from precentral motor cortex to the putamen and other parts of the basal ganglia. Brain Res. 88, p. 195-210.

KÜNZLE, H. (1978). An autoradiographic analysis of the efferent connections from premotor and adjacent prefrontal regions (areas 6 and 9) in *Macaca fasicularis*. Brain Behaviour, Evolution. 15, p. 185-234.

KUYPERS, H.G.J.M., CATSMAN-BERREVOETS, C.E. and PADT, R.E. (1977). Retrograde axonal transport of fluorescent substances in the rat's forebrain. Neuroscience Letters. 6, p. 127-135.

KUYPERS, H.G.J.M., KIEVIT, J. and GROEN-KLEVANT, A.C. (1974). Retrograde axonal transport of horseradish peroxidase in rat's forebrain. Brain Res. 67, p. 211-218.

LAMMERS, H.J. and LOHMAN, A.H.M. (1957). Experimenteelanatomisch onderzoek naar de verbindingen van piriforme cortex en amygdalakern bij de kat. Ned. T. Geneesk. 101, p. 602-604.

LASHLEY, K.S. (1941). Thalamo-cortical connections of the rat's brain. J. Comp. Neurol. 75, p. 67-121.

LE GROS CLARK, W.E. and RUSSELL, W.R. (1939). Observations of the efferent connexions of the centre median nucleus. J. Anat. 73, p. 255-262.

LEVIN, P.M. (1936). The efferent fibres of the frontal lobe of the monkey *Macaca mulatta*. J. Comp. Neurol. 63, p. 369-419.

LEVINE, M.S., HULL, C.D., BUCHWALD, N.A. and VILLABLANCA, J. (1974). The spontaneous firing patterns of forebrain neurones. II. Effects of unilateral caudate nuclear ablation. Brain Res. 73, p. 411-424.

LEVINE, M.S., HULL, C.D., BUCHWALD, N.A., GARCIA-RILL, E., HELLER, A. and ERINOFF, L. (1977). The spontaneous firing patterns of forebrain neurones. III. Prevention of induced assymetries in caudate neuronal firing rates by unilateral thalamic lesions. Brain Res. 131, p. 215-225.

LIBET, B. (1967). Long latent periods and further analysis of slow synaptic responses in sympathetic ganglia. J. Neurophysiol. 30, p. 494-514.

LIBET, B. (1979). In The Neurobiology of Dopamine. Dopaminergic synaptic processes in the superior cervical ganglion: models for synaptic actions. Eds. Horn, Korf, Westerink. Academic Press.

LIGHTALL, J.W., PARK, M.R. and KITAI, S.T. (1981). Inhibition in slices of rat neostriatum. Brain Res. 212, p. 182-187.

LILES, S.L. (1973). Cortico-striatal evoked potentials in cats. Electroenceph. Clin. Neurophysiol. 35, p. 277-285.

LILES, S.L. (1974). Single unit responses of caudate neurones to stimulation of frontal cortex, substantia nigra and entopeduncular nucleus in cats. J. Neurophysiol. 37, p. 254-265.

LINDVALL, O. and BJÖRKLUND, A. (1974). The organization of the ascending catecholamine neurone systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Phys. Scand. Supp. 412, p. 1-48.

LINDVALL, O. and BJÖRKLUND, A. (1978). In Advances in Biochemical Psychopharmacology. The anatomy of the dopaminergic neurone systems in the rat brain. Eds P.J. Roberts, G.N. Woodruff and L.L. Iversen. 19, p. 1-23.

LIPSKI, J. (1981). Antidromic activation of neurones as an analytic tool in the study of the central nervous system. J. Neuroscience Methods. 4, p. 1-32.

LJUNDAHL, A., HÖKFELT, T., GOLDSTEIN, M. and PARK, D. (1975). Retrograde peroxidase tracing of neurones combined with transmitter histochemistry. Brain Res. 84, p. 313-319.

LOGAN, W.J. and SNYDER, S.H. (1971). Unique high affinity uptake systems for glycine, glutamic and aspartic acids in central nervous tissue of the rat. Nature. 234, p. 297-299.

LORENS, S.A. and GULDBERG, H.C. (1974). Regional 5-hydroxytryptamine following selective midbrain lesions in the rat. Brain Res. 78,p. 45-56.

LYNCH, G.S., FIBIGER, H.C., McGEER, P.L. and BROOKE, S. (1972). The demonstration of acetylcholinesterase containing neurones within the caudate nucleus of the rat. Brain Res. 45, p. 617-621.

LYNCH, G., SMITH, R.L. and ROBERTSON, R.T. (1973). Direct projections from brainstem to telencephalon. Exp. Brain Red. 17, p. 221.

McCARTHY, P.S., WALKER, R.J. and WOODRUFF (1977). On the depressant action of dopamine in rat caudate nucleus and nucleus accumbens. B.J.P. 59, p. 469P.

McCLENNAN, H. (1974). Actions of excitatory amino acids and their antagonism. Neuropharmacology. 13, p. 449-454.

McCLENNAN, H. (1981). In Glutamate as a Neurotransmitter. On the nature of the receptors for various excitatory amino acids in the mammalian central nervous system. Eds Di Chiara and Gessa. Raven Press, New York.

McCLENNAN, H. and LODGE, O. (1979). The antagonism of amino acid-induced excitation of spinal neurones in the cat. Brain Res. 169, p. 83-90.

McCLENNAN, H. and YORK, D.H. (1967). The action of dopamine on neurones in the caudate nucleus. J. Physiol. 189, p. 393-402.

McGEER, P.L., FIBIGER, H.C., MALER, L., HATTORI, T. and McGEER, E.G. (1974). Evidence for descending pallido-nigral GABA-containing neurones. Adv. Neurol. 5, p. 153-160.

McGEER, P.L. and McGEER, E.G. (1975). Evidence for glutamic acid decarboxylase-containing interneurones in the neostriatum. Brain Res. 91, p. 331-335.

McGEER, P.L., McGEER, E.G., FIBIGER, H.C. and WICKSON, V. (1971). Neostriatal cholinacetylase and cholinesterase following selective brain lesions. Brain Res. 35, p. 308-314.

McGEER, E.G., McGEER, P.L. and HATTORI, T. (1979). In Glutamic Acid: Advances in Biochemistry and Physiology. Glutamate in the striatum. Eds L.J. Filer *et al*. Raven Press, New York.

McGEER, P.L., McGEER, E.G., SCHERER, U. and SINGH, K. (1977). A glutamatergic corticostriatal path? Brain Res. 128, p. 369-373.

McGEER, P.L., McGEER, E.G., SINGH, V.K. and CHASE, W.H. (1974). Choline acetyltransferase localization in the central nervous system by immuno-histochemistry. Brain Res. 81, p. 373-379.

MADRID, J., ALVARADO, J., DUTTON, H. and RUDOMIN, P. (1979). A method for the dynamic continuous estimation of excitability changes of single fiber terminals in the central nervous system. Neuroscience Letters. 11, p. 253-258.

MALER, L., FIBIGER, H.C. and McGEER, P.L. (1973). Demonstration of the nigrostriatal projection by silver staining after nigral injections of 6-hydroxydopamine. Exp. Neurol. 40, p. 505-515.

MALLIANI, A. and PURPURA, D.P. (1967). Intracellular studies of the corpus striatum. II. Patterns of synaptic activities in lenticular and entopeduncular neurones. Brain Res. 6, p. 341-354.

MARCHI, V. (1886). Sulle degenerazioni consecutive all 'estirpazione totale e parziale del cervelletto. Rivista Sperimentale di Freniatria et Medicina Legale delle Alienazioni Mentali. 12, p. 50-56.

MARCO, L.A., COPACK, P. and EDELSON, A.M. (1973). Intrinsic connections of caudate neurones. Locally evoked intracellular responses. Exp. Neurol. 40, p. 683-698.

MARKSTEIN, R., HERRLING, P.L., BÜRKI, H.R., SOPER, H. and RUCH, W. (1978). The effect of bromocryptine on rat striatal adenylate cyclase and rat brain monoamine metabolism. J. Neurochem. 31, p. 1163-1172.

MARTIN, G.F. and HAMEL, Jr. E.G., (1967). The striatum of the oppossum, *didelphis virginiana*. Description and experimental studies. J. Comp. Neurol. 131, p. 491-516.

MATSUDA, Y. and JINNAI, K. (1980). Afferent inputs to caudate output neurones as monitored by antidromic spike invasion of the cell soma. Brain Res. 183, p. 560-565.

MENSAH, P. and DEADWYLER, S. (1974). The caudate nucleus of the rat: cell types and demonstration of a commisural system. J. Anat. 117, p. 281-293.

METTLER, F.A. (1942). Relation between pyramidal and extrapyramidal function. Assoc. Res. Nerv. Ment. Dis. 21, p. 150-227.

METTLER, F.A. (1943). Extensive unilateral cerebral removals in the primate. Physiologic effects and resultant degeneration. J. Comp. Neurol. 79, p. 185-243.

METTLER, F.A. (1945). Fibre connections of the corpus striatum of the monkey and baboon. J. Comp. Neurol. 82, p. 169.

METTLER, F.A. (1957). Le Congrès International des Sciences Neurologiques; vol publ à l'occasion de la l'ere Journée. Anatomy and Physiology of the Extrapyramidal Motor Passage. p. 11.

METTLER, F.A. (1970). Nigrofugal connections in the primate brain. J. Comp. Neurol. 138, p. 291-320.

MILLER, J.J., RICHARDSON, T.L., FIBIGER, H.C. and McLENNAN, H. (1975). Anatomical and electrophysiological identification of a projection from the mesencephalic raphe to the caudate putamen in the rat. Brain Res. 97, p. 133-138.

MISGELD, U. and BAK, I.J. (1979). Intrinsic excitation in the rat neostriatum mediated by acetylcholine. Neuroscience Letters. 12, p. 277-282.

MISGELD, U., OKADA, Y. and HASSLER, R. (1979). Locally evoked potentials in slices of rat neostriatum: a tool for the investigation of intrinsic excitatory processes. Exp. Brain Res. 34, p. 575-590.

MISGELD, U., WEILER, M.H. and BAK, I.J. (1980). Intrinsic cholinergic excitation in the rat neostriatum: nicotinic and muscarinic receptors. Exp. Brain Res. 39, p. 401-409.

MITCHELL, P.R. (1980a). Dopaminergic modulation of striatal [^{3}H]-glutamic acid release. Brit. J. Pharmacol. 70, p. 48-49P.

MITCHELL, P.R. (1980b). A novel GABA receptor modulates stimulus induced glutamate release from cortico-striatal terminals. Europ. J. Pharmacology. 67, 119-122.

MITCHELL, P.R. and DOGGETT, N.S. (1980). Modulation of striatal [^{3}H]-glutamic acid release by dopaminergic drugs. Life Sciences. 26, p. 2073-2081.

MOORE, R.Y. and BLOOM, F.E. (1978). In Annual Rev. of Neuroscience II. Central catecholamine neurone systems: anatomy and physiology of the dopamine systems. Eds. W.M. Cowan, Z.W. Hall and E.R. Kandel. Annual Reviews Inc.

MOORE, R.Y., WONG, S.C.R. and HELLER, R. (1965). Regional effects of hypothalamic lesions on brain serotonin. Arch. Neurol. 13, p. 346-362.

MOORE, R.Y., BHATNAGAR, R. and HELLER, A. (1971). Anatomical and chemical studies of a nigroneostriatal projection in the cat. Brain Res. 30, p. 119-135.

MORI, S. (1966). Some observations on the fire structure of the corpus striatum of the rat brain. Z. Zellforsch. 70, p. 461-488.

MORRIS, R., LEVINE, M.S., CHERUBINI, E., BUCHWALD, N.A. and HULL, C.D. (1979). Intracellular analysis of the development of responses of caudate neurones to stimulation of cortex, thalamus and substantia nigra in the kitten. Brain Res. 173, p. 471-487.

MROZ, E.A., BROWNSTEIN, M.J. and LEEMAN, S.E. (1977). Evidence for substance P in the striatonigral tract. Brain Res. 125, p. 305-311. 113, p. 597-599.

NAGY, J.I., CARTER, D.A. and FIBIGER (1978). Anterior striatal projections to the globus pallidus, entopeduncular nucleus and substantia nigra in the rat: the GABA connection. Brain Res. 158, p. 15-29.

NAUTA, W.J.H. (1961). Fibre degeneration following lesions of the amygdaloid complex in the monkey. J. Anat. 95, p. 515-531.

NAUTA, W.J.H. and GYGAX (1954). Silver impregnation of degenerating axon terminals in the central nervous system. Stain Tech. 26, p. 5.

NAUTA, W.J.H. and KUYPERS, H.G.J.M. (1958). In Reticular Formation of the Brain. Some ascending pathways in the brain stem reticular formation. Eds Jasper *et al*. Little Brown & Co. Boston.

NAUTA, W.J.H., PRITZ, M.B. and LASEK, R.J. (1974). Afferents to the rat caudoputamen studied with horseradish peroxidase. An evaluation of a retrograde neuroanatomical research tool. Brain Res. 67, p. 219-238.

NAUTA, W.J.H. and MEHLER, W.R. (1966). Projections of the lentiform nucleus in the monkey. Brain Res. 1, p. 3-42.

NIIMI, K., IKEDA, T., KAWAMURA, S. and INOSHITA, H. (1970). Efferent projections of the head of the caudate nucleus in the cat. Brain Res. 21, p. 327-343.

NISHI, S., SOEDA, H., KOKETSU, K. (1965). Studies in sympathetic B and C neurones and patterns of pregnaglionic innervation. J. Cell Comp. Physiol. 66, p. 19-32.

NORCROSS, K. (1978). Ph.D. Thesis. Northwestern University Medical School, Chicago, USA.

NORCROSS, K. and SPEHLMANN, R. (1978a). A quantitative analysis of the excitatory and depressant effects of dopamine on the firing of caudatal neurones: electrophysiological support for the existence of two distinct dopamine-sensitive receptors. Brain Res. 156, p. 168-174.

NORCROSS, K. and SPHELMANN, R. (1978b). Decreased sensitivity of caudatal neurones to microiontophoretic dopamine in dopamine depleted caudate nucleus. Brain Res. 156, p. 175-180.

OHYE, C., BOUCHARD, R., BOUCHER, R. and POIRIER, L.J. (1970). Spontaneous activity of the putamen after chronic interruption of the dopaminergic pathway : effect of L-dopa. J. Pharmacol. & Exp. Therapeutics. 175, p. 700-708.

OKA, H. (1980). Organization of the cortico-caudate projections. Exp. Brain Res. 40, p. 203-208.

OLPE, H-R. and KOELLA, W.P. (1977). The response of striatal cells on stimulation of the dorsal and median raphe nuclei. Brain Res. 122, p. 357-360.

OLPE, H-R. and KOELLA, W.P. (1978). The effect of some neuroleptics on the interaction of cortically and nigrally evoked potentials in the rat striatum. Nauyn. Schmiedeberg's Arch. Pharmacol. 303, p. 165-170.

PALKOVITS, M., BROWNSTEIN, M., SAAREDRA, J.M. and AXELROD, J. (1974). Norepinephrine and dopamine content of hypothalamic nuclei of the rat. Brain Res. 77, p. 137-149.

PAPEZ, J.W. (1938). Reciprocal connections of the striatum and pallium in the brain of Pithecus macacus rhesus. J. Comp. Neurol. 69, p. 329-349.

PAPEZ, J.W. (1941). A summary of fiber connections of the basal ganglia with each other and with other portions of the brain. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 21, p. 21-68.

PARK, M.R., LIGHTALL, J.W. and KITAI, S.T. (1980). Recurrent inhibition in the rat neostriatum. Brain Res. 194, p. 359-369.

PASIK, P., PASIK, T. and DI FIGLIA, M. (1976). Quantitative aspects of neuronal organization in the neostriatum of the macaque monkey. In Basal Ganglia. Ed. M.D. Yahr. p. 57-89. Raven Press.

PASIK, P., PASIK, T. and DI FIGLIA, M. (1979). The internal organization of the neostriatum in mammals. In The Neostriatum. Eds I. Divac and G. Oberg. Pergamon Press.

PASQUIER, D.A., FORBES, W.B., KEMPER, T.L. and MORGANE, P.J. (1977). Serotoninergic (dorsalis raphe nucleus) connections with the striatum. Anat. Rec. 187, p. 677-.

PENG, J.H., KIMURA, H., McGEER, P.L. and McGEER, E.G. (1981). Anti-choline acetyltransferase fragments antigen binding (Fab) for immuno-histochemistry. Neuroscience Letters. 21, p. 281-285.

PERKINS, M.N., STONE, T.W., COLLINS, J.F. and CURRY, K. (1981). Phosphonate analogues of carboxylic acids as amino acid antagonists on rat cortical neurones. Neuroscience Letters. 23, p. 333-336.

PETERS, D.A.V., McGEER, P.L. and McGEER, E.G. (1968). The distribution of tryptophan hydroxylase in cat brain. J. Neurochem. 15, p. 1431-1435.

PICKEL, V.M., SEGAL, M. and BLOOM, F.E. (1974). A radioautographic study of the efferent pathways of the nucleus locus coeruleus. J. Comp. Neurol. 155, p. 15-42.

PINNOCK, R.D., WOODRUFF, G.N. and TURNBULL, M.J. (1979). Sulpiride blocks the action of dopamine in the rat substantia nigra. Europ. J. Pharmacol. 56, p. 413-414.

POIRIER, L.J., McGEER, E.G., LAROCHELLE, L., McGEER, P.L. and BEDARD, P. (1969). The effect of brain stem lesions on tyrosine and tryptophan hydroxylase in various structures of the telencephalon of the cat. Brain Res. 14, p. 147-155.

POIRIER, L.J. and SOURKES, T.L. (1965). Influence of the substantia nigra on the catecholamine content of the striatum. Brain. 88, p. 181-192.

POLJAK, S. (1927). Association, callosal and projection fibres of the cerebral cortex of the cat. J. Comp. Neurol. 44, p. 192.

POWELL, T.P.S. and COWAN, W.M. (1954). The connexions of the midline and intralaminar nucleii of the thalamus of the rat. J. Anat. 88, p. 307.

POWELL, T.P.S. and COWAN, W.M. (1956). A study of thalamo-striate relations in monkeys. Brain. 79, p. 364-390.

PRECHT, W. and YOSHIDA, M. (1971). Blockade of caudate-evoked inhibition of neurones in the substantia nigra by picrotoxin. Brain Res. 32, p. 229-233.

PRESTON, R.J., BISHOP, G.A. and KITAI, S.T. (1980). Medium spiny neurone projection from the rat striatum: an intracellular horseradish peroxidase study. Brain Res. 183, p. 253-263.

PURPURA, D.P. and MALLIANI, A. (1967). Intracellular studies of the corpus striatum. 1. Synaptic potentials and discharge characteristics of caudate neurones activated by thalamic stimulation. Brain Res. 6, p. 325-340.

RANSON, S.W. and RANSON, S.W. Jr. (1942). Efferent fibres of the corpus striatum. Res. Publ. Assoc. Res. Nerv. Ment. Dis. 21, p. 69-76.

RANSON, S.W., RANSON, S.W. Jr. and RANSON, M. (1941). Fiber connections of the corpus striatum as seen in Marchi preparations. Arch. Neur. Psych. 46, p. 230-249.

RASMINSKY, M., MAURO, A.J. and ALBE-FESSARD, D. (1973). Projections of medial thalamic nuclei to putamen and cerebral frontal cortex in the cat. Brain Res. 61, p. 69-77.

REUBI, J.C. (1980). Comparative study of the release of glutamate and GABA, newly synthesized from glutamine, in various regions of the central nervous system. Neuroscience. 5, p. 2145-2150.

REUBI, J.C. and CUÉNOD, M. (1979). Glutamate release *in vitro* from corticostriatal terminals. Brain Res. 176, p. 185-188.

RICHARDSON, T.L., MILLER, J.J. and McCLENNAN, H. (1977). Mechanisms of excitation and inhibition in the nigrostriatal system. Brain Res. 127, p. 219-234.

ROBERTS, P.J. and ANDERSON, S.D. (1979). Stimulatory effect of L-glutamate and related amino acids on [3H] dopamine release from rat striatum: an *in vitro* model for glutamate actions. J. Neurochem. 32, p. 1539-1545.

ROBERTO, P.J. and ROWLANDS, G.J. (1980). Release of glutamate from cortico-striatal terminals is regulated by activation of dopamine receptors. Brit. J. Pharmacol. 70, p. 137-138P.

ROBERTS, P.J. and SHARIF, N.A. (1978). Effects of L-glutamate and related amino acids upon the release of [3H] dopamine from rat striatal slices. Brain Res. 157, p. 391-395.

ROBERTSON, R.T., LYNCH, G.S. and THOMPSON, R.F. (1973). Diencephalic distributions of ascending reticular systems. Brain Res. 55, p. 309-322.

ROBERTSON, R.T. and TRAVERS, J.B. (1975). Brain stem projections to the striatum: experimental morphological studies in the rat. Exp. Neurol. 48, p. 447-459.

ROCHA-MIRANDA, C.E. (1965). Single-unit analyses of cortex-caudate connections. Electroenceph. and Clin. Neurophysiol. 19, p. 237-247.

ROSEGAY, H. (1944). An experimental investigation of the connections between the corpus striatum and substantia nigra in the cat. J. Comp. Neurol. 80, p. 293-321.

ROWLANDS, G.J. and ROBERTS, P.J. (1980a). Activation of dopamine receptors inhibits calcium-dependent glutamate release from cortico-striatal terminals *in vitro*. Europ. J. Pharmacol. 62, p. 241-242.

ROWLANDS, G.J. and ROBERTS, P.J. (1980b). Specific calcium-dependent release of endogenous glutamate from rat striatum is reduced by destruction of the cortico-striatal tract. Exp. Brain Res. 39, p. 239-240.

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

ROYCE, G.J. (1978b). Cells of origin of subcortical afferents to the caudate nucleus: a horseradish peroxidase study in the cat. Brain Res. 153, p. 465-475.

RUDOMIN, P., ENGBERG, I., JANKOWSKA, E. and JIMÉNEZ, I. (1980). Evidence of two different mechanisms involved in the generation of presynaptic depolarization of afferent and rubrospinal fibers in the cat spinal cord. Brain Res. 189, p. 256-261.

RUSSELL, G.V., BARRAT, E.S., DEATON, S.M. and TAYLOR, R.R. (1968). Evoked responses in the putamen and globus pallidus following stimulation of the basolateral amygdala. Brain Res. 7, p. 459-462.

SALT, T.E. and HILL, R.G. (1981). Excitatory amino acids as transmitter candidates of vibrissae afferent fibres to the rat trigeminal nucleus caudates. Neuroscience Letters. 22(2), p. 183-188.

SATO, M., ITOH, K. and MIZUNO, N. (1979). Distribution of thalamocaudate neurones in the cat as demonstrated by horseradish peroxidase. Exp. Brain Res. 34, p. 143-154.

SCHOUSBOE, A. and HERTZ, L. (1981). In Glutamate as a Neurotransmitter. Role of astroglial cells in glutamate homeostasis. Eds DiChiara and Gessa. p. 103-114.

SCHULTZ, W. and UNGERSTEDT, U. (1978). A method to detect and record from striatal cells of low spontaneous activity by stimulating the cortico-striatal pathway. Brain Res. 142, p. 357-362.

SCHWARTZ, R., CREESE, I., COYLE, J.T. and SNYDER, S.H. (1978). Dopamine receptors localized on cerebral cortical afferents to rat corpus striatum. Nature. 271, p. 766-768.

SEDGEWICK, E.M. and WILLIAMS, T.D. (1967). The response of single units in the caudate nucleus to peripheral stimulation. J. Physiol. 189, p. 281-298.

SIGGINS, G.R., HOFFER, B.J., BLOOM, F.E. and UNGERSTEDT, U. (1976). In The Basal Ganglia. Cytochemical and electrophysiological studies of dopamine in the caudate nucleus. Ed. M.D. Yahr. ARNMD: 55.

SIGGINS, G.R., HOFFER, B.J. and UNGERSTEDT, U. (1974). Electrophysiological evidence for involvement of cyclic adenosine monophosphate in dopamine responses of caudate neurones. Life Sciences. 15, p. 779-792.

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

SIMON, J.R., CONTRERA, J.F. and KUHAR, M.J. (1976). Binding of [3H]-kainic acid, analogue of L-glutamate to brain membranes. J. Neurochem. 26, p. 141-147.

SKIRBOLL, L.R. and BUNNEY, B.S. (1979). The effects of acute and chronic haloperidol treatment on spontaneously firing neurones in the caudate nucleus of the rat. Life Sciences. 25, p. 1419-1434.

SKIRBOLL, L.R., GRACE, A.A. and BUNNEY, B.S. (1979). Dopamine auto- and postsynaptic receptors: electrophysiological evidence of differential sensitivity to dopamine agonists. Science. 206, p. 80-82.

SLOAN, N. and JASPER, H. (1950). The identity of spreading depression and "suppression". EEG Clin. Neurophysiol. 2, p. 59-78.

SOMOGYI, P., BOLAM, J.P. and SMITH, A.D. (1981). Monosynaptic cortical input and local axon collaterals of identified striatonigral neurones. A light and electron microscopic study using the Golgi-peroxidase transport-degeneration procedure. J. Comp. Neurol. 195, p. 567-584.

SOMOGYI, P., HODGSON, A.J. and SMITH, A.D. (1979). An approach to tracing neurone networks in the cerebral cortex and basal ganglia. Combination of Golgi staining, retrograde transport of horseradish peroxidase and anterograde degeneration of synaptic boutons in the same material. Neuroscience. 4, p. 1805-1853.

SPEHLMAN, R. (1975). The effects of acetylcholine and dopamine on the caudate nucleus depleted of biogenic amines. Brain. 98, p. 219-230.

SPEHLMAN, R. and STAHL, S.M. (1974). Neuronal hyposensitivity to dopamine in the caudate nucleus depleted of biogenic amines by tegmental lesions. Exp. Neurol. 42, p. 703-706.

SPENCER, H.J. (1976). Antagonism of cortical excitation of striatal neurones by glutamic acid diethyl ester: evidence for glutamic acid as an excitatory transmitter in the rat striatum. Brain Res.

SPENCER, H.J. and HAVLICEK, V. (1974). Alterations by anaesthetic agents of the responses of rat striatal neurones to iontophoretically applied amphetamine, acetylcholine, noradrenaline and dopamine. Can. J. Physiol. Pharmacol. 52, p. 808-813.

STAINES, W.A., ATAMADJA, S. and FIBIGER, H.C. (1981). Demonstration of a pallido-striatal pathway by the retrograde transport of an HRP-labelled lectin. Brain Res. 204, p. 253-268.

STAINES, W.A., KIMURA, H., FIBIGER, H.C. and McGEER, E.G. (1980). Peroxidase-labelled lectin as a neuroanatomical tracer: evaluation in a CNS pathway. Brain Res. 197, p. 485-490.

STAINES, W.A., NAGY, J.I., VINCENT, S.R. and FIBIGER, H.C. (1980). Neurotransmitters contained in the efferents of the striatum. Brain Res. 194, p. 391-402.

STARKE, K., REUMANN, W., ZUMSTEIN, A. and HERTLING, G. (1978). Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus *in vitro*. N.S. Arch. Pharmacol. 305, p. 27-36.

STARR, M.S. (1979). GABA-mediated potentiation of amine release from nigrostriatal dopamine neurones *in vitro*. Europ. J. Pharmacol. 53, p. 215-226.

STEG, G. (1969). In Third Symposium on Parkinson's Disease. Striatal cell activity during systemic administration of monoaminergic and cholinergic drugs. Eds F. Gillingham and I. Donaldson. p. 26-29. Livingstone, Edinburgh.

STEINDLER, D.A. and DENIAU, J.M. (1980). Anatomical evidence for collateral branching of substantia nigra neurones: a combined horseradish peroxidase and [3H]-wheat agglutinin axonal transport study in the rat. Brain Res. 196, p. 228-236.

STONE, T.W. (1975). Blockade by amino acid antagonists of neuronal excitation mediated by the pyramidal tract. J. Physiol. 257, p. 187-198.

STONE, T.W. (1976). Responses of neurones in the cerebral cortex and caudate nucleus to amantadine and dopamine. Br. J. Pharmacol. 56, p. 101-110.

STONE, T.W. (1979). Amino acids as neurotransmitters of corticofugal neurones in the rat: a comparison of glutamate and aspartate. Brit. J. Pharmac. 67, p. 545-551.

STONE, T.W. and BAILEY, E.V. (1975). Responses of central neurones to amantadine: comparison with dopamine and amphetamine. Brain Res. 85, p. 126-129.

STREIT, P. (1980). Selective retrograde labelling indicating the transmitter of neuronal pathways. J. Comp. Neurol. 191, p. 429-463.

STOOF, J.C., DEN BREEJEN, E.J.S., MULDER, A.H. (1979a). GABA modulates the release of dopamine and acetylcholine from rat caudate nucleus slices. Europ. J. Pharmacol. 57, p. 35-42.

STOOF, J.C., THIEME, R.E., VRYMOED-de-VRIES, M.C. and MULDER, A.H. (1979b). *In vitro* acetylcholine release from rat caudate nucleus as a new model for testing drugs with dopamine-receptor activity. N.S. Arch. Pharmacol. 309, 119-124.

SUGIMORI, M., PRESTON, R.J. and KITAI, S.T. (1978). Response properties and electrical constants of caudate nucleus neurones in the cat. J. Neurophysiol. 41, p. 1662-1675.

SWANK, R.L. and DAVENPORT, H.A. (1935). Chlorate-osmic-formalin method for staining degenerating myelin. Stain Tech. 10, p. 87.

SZABO, J. (1962). Topical distribution of the striatal efferents in the monkey. Exp. Neurol. 5, p. 21-36.

SZABO, J. (1967). The efferent projections of the putamen in the monkey. Exp. Neurol. 19, p. 463-476.

SZABO, J. (1970). Projections from the body of the caudate nucleus in the rhesus monkey. Exp. Neurol. 27, p. 1-15.

SZABO, J. (1971). A silver impregnation study of nigrostriate projections in the cat. Anat. Rec. 169, p. 441.

SZABO, J. (1972). The course and distribution of efferents from the tail of the caudate nucleus in the monkey. Exper. Neurol. 37, p. 562-572.

SZABO, J. (1977). Horseradish peroxidase study of the striatal and nigral efferents. Anat. Rec. 187, p. 726-.

TAKAGI, M. and YAMAMOTO, C. (1978). Suppressing action of cholinergic agents on synaptic transmissions in the corpus striatum of rats. Exp. Neurol. 62, p. 433-443.

TERNAUX, J.P., HÉRY, F., BOURGOIN, S., ADRIEN, J., GLOWINSKI, J. and HAMON, M. (1977). The topographical distribution of serotonergic terminals in the neostriatum of the rat and the caudate nucleus of the cat. Brain Res. 121, p. 311-326.

TSURUTA, K., FREY, E.A., CREWE, C.W., COTE, T.E., ESKAY, R.L. and KEBABIAN, J.W. (1981). Evidence that LY-141865 specifically stimulates the D-2 dopamine receptor. Nature. 292, p. 463-465.

TULLOCH, I.F., ARBUTHNOTT, G.W. and WRIGHT, A.K. (1978). Topographical organization of the striato-nigral pathway revealed by anterograde and retrograde neuroanatomical tracing techniques. J. Anat. 127, p. 425-441.

UNGERSTEDT, U. (1968). 6-Hydroxydopamine induced degeneration of central monoaminergic neurones. Europ. J. Pharmacol. 5, p. 107-110.

UNGERSTEDT, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Phys. Scand. (Suppl.). 367, p. 1-48.

UNGERSTEDT, U. and ARBUTHNOTT, G.W. (1970). Quantitative recording of rotational behaviour of rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. Brain Res. 24, p. 485-593.

URETSKY, N.J. and SCHOENFELD, R.I. (1971). Effect of L-dopa on the locomotor activity of rats pretreated with 6-hydroxy-dopamine. Nature, New Biol. 234, p. 157-159.

USUNOFF, K.G., HASSLER, R., RAMANSKY, K. and USUNOVA, R.P. (1976). The nigrostriatal projection in the cat. Part 1. Silver impregnation study. J. Neurol. Sci. 28, p. 265-288.

VAN DER HEYDEN, J.A.M., VENEMA, K. and KORF, J. (1980). *In vivo* release of endogenous GABA from rat striatum: inhibition by dopamine. J. Neurochem. 34, p. 1338-1341.

VANDER MAELEN, C.P., BONDUKI, A.C. and KITAI, S.T. (1979). Excitation of caudate-putamen neurones following stimulation of the dorsal raphe nucleus in the rat. Brain Res. 175, p. 356-361.

VAN DER KOOY, D. (1979). The organization of thalamic, nigral and raphe cells projecting to the medial versus lateral caudate-putamen in rat. A fluorescent retrograde double labelling study. Brain Res. 169, p. 381-387.

VAN DER KOOY, D. and CARTER, D.A. (1981). The organization of the efferent projections and striatal afferents of the entopeduncular nucleus and adjacent areas in the rat. Brain Res. 211, p. 15-36.

VAN DER KOOY, D., COSCINA, D.V. and HATTORI, T. (1981). Is there a non-dopaminergic nigrostriatal pathway? Neuroscience. 6, p. 345-357.

VAN DER KOOY, D. and HATTORI, T. (1980). Dopal raphe cells with collateral projections to the caudate putamen and substantia-nigra: a fluorescent retrograde double labelling study in the rat. Brain Res. 186, p. 1-7.

VAN DER KOOY, D. and KUYPERS, H.G.J.M. (1979). Retrograde fluorescent double labelling: axon collaterals in the ascending raphe and nigral projections. Science. 204, p. 873-875.

VAN DER KOOY, D. and WISE, R.A. (1980). Retrograde fluorescent tracing of substantia nigra neurones combined with catecholamine histofluorescence. Brain Res. 183, p. 447-452.

VERHAART, W.J.C. (1950). Fiber analysis of the basal ganglia gibbon. J. Comp. Neurol. 93, p. 425-440.

VERHAART, W.J.C. and KENNARD, M.A. (1940). Corticofugal degeneration followvng thermo-coagulation of areas 4, 6 and 4=0 in *Macaca mulatta*. J. Anat. 74, p. 239.

VONEIDA, T.J. (1960). An experimental study of the course and destination of fibres, arising in the head of the caudate nucleus in the cat and monkey. J. Comp. Neurol. 115, p. 75-87.

WALAAS, I. (1981). Biochemical evidence for overlapping neocortical and allocortical glutamate projections to the nucleus accumbens and rostral caudatoputamen in the rat brain. Neuroscience. 6, p. 399-406.

WALKER, R.J., KEMP, J.A., YAJUMA, H., KITAGAWA, K. and WOODRUFF, G.N. (1976). The action of substance P on mesencephalic reticular and substantia nigral neurones in the cat. Experientia. 32, p. 214-215.

WALL, P.D. (1958). Excitability changes in afferent fibre terminations and their relation to slow potentials. J. Physiol. 142, p. 1-21.

WAMSLEY, J.K., YOUNG, W.S. and KUHAR, M.J. (1980). Immuno-histochemical localization of enkephalin in rat forebrain. Brain Res. 190, p. 153-174.

WATKINS, J.C., DAVIES, J., EVANS, R.H., FRANCIS, A.A. and JONES, A.W. (1981). In Glutamate as a Neurotransmitter. Pharmacology of receptors for excitatory amino acids. Eds G. Di Chiara and G.L. Gessa. p. 263-273.

WEBSTER, K.E. (1961). Cortico-striate interrelations in the albino rat. J. Anat. p. 95, p. 532-544.

WEBSTER, K.E. (1965). The cortico-striatal projection in the cat. J. Anat. 99, p. 329-327.

WELKER, C. (1971). Microelectrode delineation of fine grain somatotopic organization of S ml cerebral neocortex in albino rat. Brain Res. 26, p. 259-275.

WELKER, C. and SINHA, M.M. (1972). Somatotopic organization of 5 ml cerebral neocortex in albino rat. Brain Res. 37, p. 132-136.

WESTFALL, T.C. (1974). Effect of muscarinic agonists on the release of [3H]-norepinephrine and [3H]-dopamine by potassium and electrical stimulation from rat brain slices. Life Sciences. 14, p. 1641-1652.

WESTFALL, T.C., BESSON, M.J., GIORGUIEFF, M.F. and GLOWINSKI, J. (1976). The role of presynaptic receptors in the release and synthesis of [3H]-dopamine by slices of rat striatum.

WHITLOCK, D.G. and NAUTA, W.J.H. (1956). Subcortical projections from the temporal neocortex in *Macaca mulatta*. J. Comp. Neurol. 106, p. 183-212.

WIITANEN, J.T. (1969). Selective silver impregnation of normal and degenerating axons and axon terminals in the central nervous system of the monkey. Brain Res. 14, p. 546-548.

WILLIAMS, J.M. (1953). The amygdaloid nucleus. A clinical study of its ablation and a theory as to its function. Confin. Neurol. 13, p. 202-221.

WILSON, S.A.K. (1914). An experimental research into the anatomy and physiology of the corpus striatum. Brain. 36, p. 427-492.

WILSON, C.J., CHANG, H.T. and KITAI, S.T. (1980). Involvement of a cortico-striatal axon reflex in the responses of striatal neurones to substantia nigra stimulation in the rat. Neuroscience Abstracts. 6, p. 187.

WISE, S.P. and JONES, E.G. (1977). Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex. J. Comp. Neurol. 175, p. 129-158.

YARBROUGH, G.G. (1975). Supersensitivity of caudate neurones after repeated administration of haloperidol. Europ. J. Pharmacol. 31, p. 367-369.

YORK, D.H. (1967). The inhibitory action of dopamine on neurones of the caudate nucleus. Brain Res. 5, p. 263-266.

YORK, D.H. (1970). Possible dopaminergic pathway from substantia nigra to putamen. Brain Res. 20, p. 233-249.

YORK, D.H. (1972). Dopamine receptor blockade - a central action of chlorpromazine on striatal neurones. Brain Res. 37, p. 91-99.

YORK, D.H. (1980). In Neurobiology of Dopamine. The neurophysiology of dopamine receptors. Eds A.S. Horn, J. Korf, B.H.C. Westerink. Academic Press (1979).

ZARZECKI, P., BLAKE, O.J. and SOMJEN, G.G. (1976). Interactions of nigrostriate synaptic transmission, iontophoretic o-methylated phenethylamines, dopamine, apomorphine and acetylcholine. Brain Res. 115, p. 257-272.

ZARZECKI, P., BLAKE, D.J. and SOMJEN, G.G. (1977). Neurological disturbances, nigrostriate synapses, and iontophoretic dopamine and apomorphine after haloperidol. Exp. Neurol. 57, p. 956-970.

CHERAMY, A., LEVIEL, V., GLOWINSKI, J. (1981). Dendritic release of dopamine in the substantia nigra. Nature. 289, p. 537-542.

LAURSEN, A.M. (1963). Corpus striatum. Acta. Phys. Scand. Supp. 211.

TOSSMAN, U. and UNGERSTEDT, U. (1981). Neuroleptic action on putative amino acid neurotransmitters in the brain studied with a new technique of brain dialysis. Neuroscience Letters. Supp. 6.