THE UNIVERSITY OF HULL

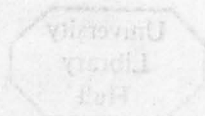
Functional Effects of Cholinergic Stimulation of the Substantia Nigra in the Rat

being a Thesis submitted for the
Degree of Doctor of Philosophy
in the University of Hull

by

Philip Winn B.A.

January 1981



To my Mother and Father, with much love.

Acknowledgements

I have many people to thank for their advice, assistance and encouragement in preparing and executing this thesis.

I am happy to acknowledge my debt to my supervisor, Dr. Ian Horrell, who was always able to offer support, encouragement and eminently sensible advice during my years in Hull.

Dr. Peter Redgrave, of the University of Sheffield, stimulated much of the research reported here by his initial direction of my studies and by his continuing enthusiastic support for my work. I am also indebted to him for his assistance in preparing some of the histological material.

Just as I must thank Peter Redgrave for his stimulation of much of the work, I must also thank Dr. Trevor Robbins of the University of Cambridge for helping me to complete this thesis. Dr. Robbins very kindly read the initial drafts of the entire thesis and was able to offer me a great deal of constructive criticism and advice. I am most grateful for the help of both Peter Redgrave and Trevor Robbins.

I also thank Professor J.M. Brener and Professor A.D.B.Clarke for their support of my research in their department. Dr. L.J. Herberg kindly allowed me to perform an experiment reported here while I was his Research Assistant in the Experimental Psychology Laboratory, Institute of Neurology at the National Hospital, London.

I also thank Dr. Herberg for his helpful advice and encouragement of my work. I would also like to thank Professor Oliver Zangwill, F.R.S. of the Department of Experimental Psychology, University of Cambridge for allowing me to complete a substantial proportion of this thesis in his department.

I had excellent technical assistance in running experiments at the University of Hull. The animals were attentively cared for by the animal technicians (principally Brian Hinch) under the expert guidance of Heather Rodmell. John Devine and his technical staff - especially Robin Dews and Ray Wallis - greatly helped in the development of equipment and in offering me technical facilities.

Perhaps the greatest technical achievement in the preparation of this thesis has been the typing, which has been executed swiftly and skillfully by Doreen Rawlinson. I thank her for completing this difficult task so well. All of the photographs contained in this thesis were prepared by Roy S. Hammans, to whom I am very grateful. Richard Phillips also deserves my thanks for his help with statistical analysis, histology and in many other areas. I should also like to thank Dr.Steve Maddison for his comments on early drafts of this thesis. Peter Kelsall assisted in running experiment one.

Financial support during the period of this research came from the Science Research Council of Great Britain, whom I thank. The Managers of the Pinsent-Darwin Studentship, University of Cambridge, also deserve thanks for providing much of the motivation necessary for the production of this work.

However, most of all I should like to thank my wife Jane. She has proof-read the entire manuscript and prepared many of the figures. She has given me constant encouragement both in successful periods and in adversity, and for this she deserves the highest praise of all.

Contents

Chapter 1: An Introduction 1 - 9

i. Behaviour, Anatomy and Chemistry: Coding and Relationships 1

ii. Techniques for Examining Neuronal Activity by Means of Behaviour: Chemical Stimulation v. Electrical Stimulation v. Lesion Techniques 2

iii. Chemical Stimulation of Brain Tissue 6

Chapter 2: The Substantia Nigra: Anatomy 10 - 32

i. General Structure 10

ii. Synapses Within the Substantia Nigra 10

iii. Neurones Within the Substantia Nigra 15

iv. Afferent and Efferent Projections of the Substantia Nigra 20

iva. Efferent Projections 20
ai. The Nigro-Striatal Projection 20
aii. The Nigro-Thalamic Pathway 22
aiii. The Nigro-Tectal Pathway 24
aiv. Other Substantia Nigra Efferent Systems 26

ivb. Afferent Projections 27
bi. Striato-Nigral Projections 27
bii. Other Substantia Nigra Afferent Systems 31

Chapter 3: The Nigro-Striatal Projection: Dopamine Within the Substantia Nigra 33 - 48

i. Dopamine Within the Ventral Midbrain 33

ii. Morphology of Nigro-Striatal Cells 33

iii. Origin, Organisation and Course of the Nigro-Striatal Projection 35

iv. Inputs to the Dopamine Neurones of Pars Compacta 39

iva. The GABA and SP Mediated Striato-Nigral Projections 40
ivb. DA Release Within the Substantia Nigra 43
ivc. Other Systems Interacting with DA in the Substantia Nigra 46

Chapter 4: Dopamine-Acetylcholine Interactions and the Nigro-Striatal Pathway 49 - 63

i. Acetylcholine-Dopamine Interactions in the Striatum 49

ii. Cholinergic Substances in the Substantia Nigra 52

iia. Acetylcholine in Substantia Nigra 52
iib. Choline Acetyltransferase in Substantia Nigra 54
iic. Acetylcholinesterase in Substantia Nigra 55

iii. Acetylcholine-Dopamine Interactions in the Substantia Nigra 57

iiia. Summary of Evidence Already Presented 57
iiib. Functional Studies of ACh-DA Interactions in Substantia Nigra: Electrophysiology 58
iiic. Functional Studies of ACh-DA Interactions in Substantia Nigra: Behaviour 59
iiid. Acetylcholine-Dopamine Interactions in the Substantia Nigra: Conclusions 62

Chapter 5: Behavioural Functions of the Nigro-Striatal Pathway 64 - 95

i. Catalepsy 64

ii. Turning Behaviour 66

iia. Dopamine, the Nigro-Striatal System and Turning Behaviour 66
iib. Cholinergic Stimulation and Turning Behaviour
iic. Turning Behaviour Elicited from Unlesioned Animals 71

iii. Stereotypy and Locomotion 73

iiia. Dopamine, the Striatum, Stereotypy and Locomotion 73
iiib. The Process of Stereotypy 77
iiic. Cholinergic Elicitation and Modification of Stereotypy 80

iv. The Nigro-Striatal DA System and Feeding Behaviour 83

iva. DA Systems in Relation to the Lateral Hypothalamic Syndrome 83
ivb. Dopaminergic Drugs and Feeding Behaviour 87
ivc. Stress-Induced Feeding and the Nigro-Striatal DA System 93
ivd. Summary of Section iv. 94

Chapter 6: Summary and Experimental Hypotheses 96 - 99

Chapter 7: Methods and Techniques 100 - 110

i. Animal Housing 100

ii. Surgical Procedure (Experiments 3-5) 101

iii. Cannula Construction 102

iv. Injection Procedure 103

v. Experiment 2: Methods and Techniques 104

va. Animal Housing 104
vb. Surgical Procedures 104
vc. Cannula Construction 105
vd. Injection Procedure 105

vi. Feeding Behaviour Tests 105

vii. Drugs 107

viii. Histological Procedures 107

ix. Statistical Treatment of Data 109

Chapter 8: Experimental Reports 111 - 154

Experiment One: The Effects of Systemically Applied d-Amphetamine on Behaviour 111

Purpose 111

Method 111

Results a. Consummatory Behaviour 113
b. Gnawing and Grooming 114

c. Locomotor Activity and Rearing 115
d. Stereotyped Behaviour 116

Discussion 116

Experiment Two: The Effects of Low Doses of d-Amphetamine Microinjected into the Striatum 119

Purpose 119

Method 119

Results 121

Discussion 123

Experiment Three: The Effect of Cholinergic Stimulation of the Substantia Nigra on Feeding Behaviour 128

Purpose 128

Method 129

Results 129

Discussion 133

Experiment Four: The Effect of Acetylcholinesterase Blockade Within the Substantia Nigra on Feeding Behaviour 136

Purpose 136

Method 136

Results 137

Discussion 140

Comparison of the Effects of ACh and Eserine Mixtures and Eserine Sulphate Alone on Feeding Behaviour 142

A Study of Correlations Between Feeding and Drinking Elicited by Cholinergic Stimulation of the Substantia Nigra 144

Experiment Five: Antagonism of Feeding Induced by 5.0μg ACh and Eserine using Nicotinic and Muscarinic Cholinergic Receptor Blocking Agents 147

Purpose 147

Method 147

Results 149

Conclusions 153

Chapter 9: General Discussion 155 - 185

i. Summary of the Main Experimental Findings 155

ii. Dopamine and Feeding: Involvement of the Striatum and Striatal Efferents 158

iii. Dopamine-Acetylcholine Interactions Within the Substantia Nigra 170

iv. What do Nigro-Striatal DA Neurones Do? 176

Retrospective. 185a.-c.

Appendix 186 - 189

i. A Note on the Terms Neurotransmitter and Neuromodulator 186

References. 190 - 233

Chapter 1: An Introduction

i. Behaviour, Anatomy and Chemistry: Coding and Relationships

The brain contains neurones in a vast and highly organised matrix. Cragg (1975) has estimated that one neurone in the human cerebral cortex receives information from 38,000 individual synapses. As the greatest number of synapses derived from one axon in this tissue, so far observed, is 60 (Marin-Padilla, 1968), it may be estimated that something like 600 separate neurones project to one other neurone. As the human brain is estimated to contain 10^{11} neurones (Hubel, 1979) we can readily understand why brain modelling and the unravelling of central nervous system (CNS) function and organisation are such daunting tasks.

We must, in order to make any advances, accept, first, that the brain is an organised and understandable structure, and second, the principle that alteration of the structure or chemistry of the brain will result in specific functional changes and be expressed by altered neuro-chemistry, physiology and behaviour. If such a change is demonstrated and proves replicable then we might assume that it has implications for the understanding of nervous system activity and organisation.

However, it must immediately be noted that while these physiology/ behaviour relationships are believed to hold good, the author of this thesis is reluctant to make automatic claims that because some physio-logical manipulation always results in a particular form of behavioural response, then that physiological system must be intimately associated with that behaviour. Indeed, it is often not immediately apparent which physiological system under stimulation is responsible for the observed behaviour. (Witness the continuing "fibres of passage vs.

intrinsic neurones" debate over the lateral hypothalamic syndrome, which is discussed in Chapter 5, section iv(a).) Moreover, it is possible to modify a behavioural response to physiolgical stimulation by altering, for instance, an animal's environment or previous experience (Rosenzweig et. al., 1958). The principle that physiology/behaviour relationships should hold good, be replicable and dose-dependent is not challenged. It simply has to be qualified by specifying that such things as previous experience and test conditions need to be maintained to assure replicability and before making broad claims, it must be made quite clear which neuroanatomical and neurochemical systems mediate the observed effects.

The author also considers that the important relationships between anatomy, chemistry and behaviour are only rarely stated, and that some description of them ought to be attempted. Rose (1980) has cogently argued that behaviour, chemistry and anatomy are simply vehicles for examining events. If behaviour occurs, it may be examined and explained <u>in terms of</u> behavioural science, anatomical events or by neurochemistry (or by many other means). Each is a valid approach, but one cannot be said to <u>cause</u> another. They are all simply expressions, in different languages as it were, of the same event. They are related, obviously, but not necessarily in a causal sense. Thus we may examine behaviour as a functional expression of anatomy and chemistry, bearing in mind that the three show an underlying unity.

ii. <u>Techniques for Examining Neuronal Activity by Means of Behaviour: Chemical Stimulation v Electrical Stimulation v Lesion Techniques</u>

As this section specifically deals with "Techniques for examining Neuronal Activity by means of Behaviour ......" we shall not review many

neurochemical, histological or electrophysical techniques which are available. Neurochemical and histological techniques may be very useful when investigating the effect of behaviour on neural substrates, but they are not generally thought of as tools for eliciting, facilitating, or modifying behaviour. Similarly, most electrophysiological work deals with the effects of intracranial manipulations in anaesthetised animals and as such is not directly concerned with behaviour.

Leaving aside these techniques, we may identify, in broad terms, two types of approach to the problems of brain/behaviour interaction: stimulation, by the application of chemicals or by the passage of electric current, and lesion, which involves the destruction by neurotoxic chemicals, electrocoagulation, microknife cuts or suction of specific areas of the brain. Either of these approaches may induce net excitatory or inhibitory effects on both physiology and behaviour. The activation of a fibre bundle whose net effect is excitatory may be achieved by direct and specific chemical stimulation, electrical stimulation or by the removal (lesion) of inhibitory influences on the cells of origin of the bundle.

Lesion studies have an important place within the neurobiologist's armoury. It must be borne in mind throughout this discussion that the purpose of any technique is to provide an answer to a question, and it is with this in view that we shall discuss the relative merits and demerits of each technique. If we wish to ask the question "what function does this structure control?" then the application of a lesion may be an appropriate step towards an answer. The pitfalls of lesion techniques are many though they may safely be used to answer specific questions of functional coding provided we bear in mind that:

(a) a gross lesion may destroy many anatomical elements within a particular structure. These anatomical elements may normally relate to behaviour in a complex and non-uniform manner.

(b) a gross lesion does not respect neurochemical differentiation and as such can provide little information as to the way in which neurotransmitters relate to one another during behavioural activation.

(c) any lesion may elicit gross behavioural changes which mask more subtle effects accessible only through less drastic intervention, and

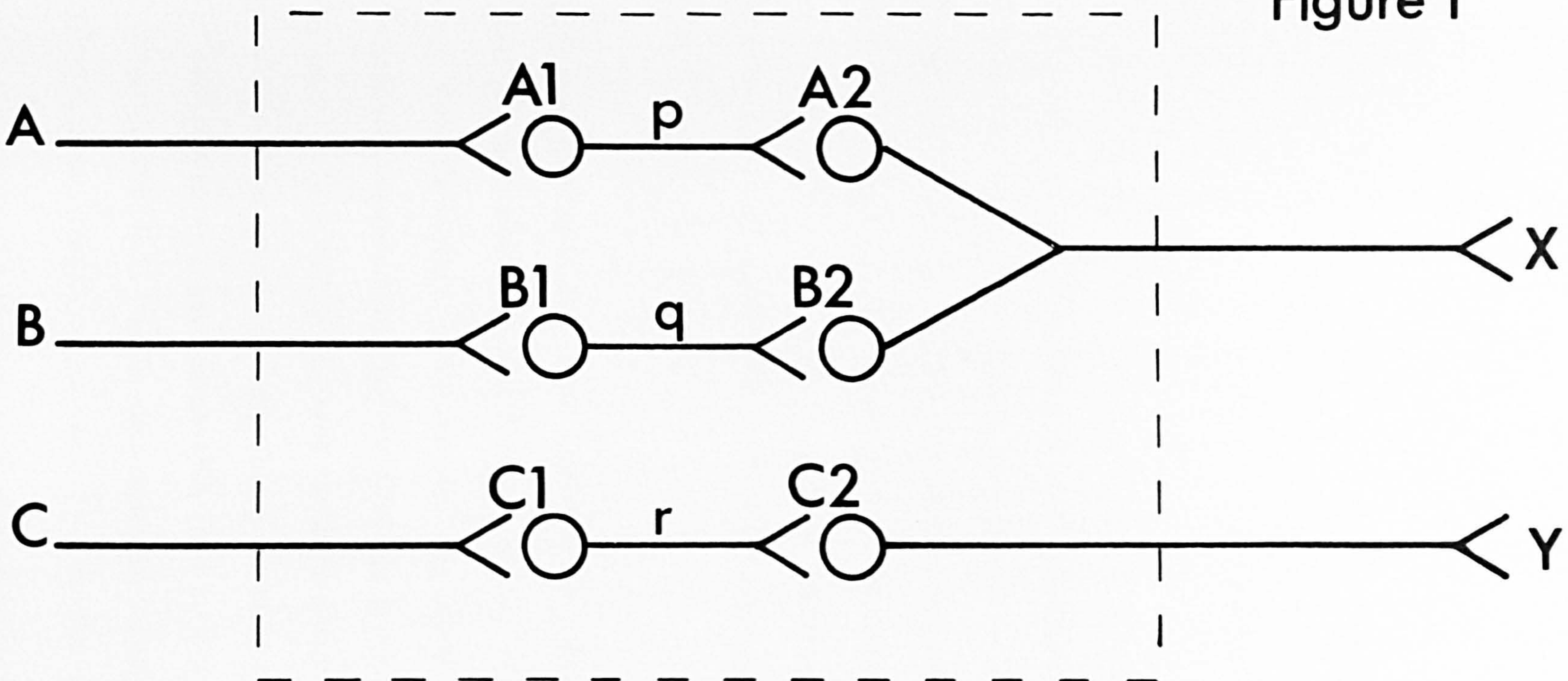
(d) it is possible that compensatory mechanisms may be activated following a lesion.

Essentially points (c) and (d) may apply to any gross CNS intervention. Finally, it is worth noting that not all functions may be located by lesion studies. Lashley's long series of selective ablations attempting to locate the neuroanatomical locus of memory led him to forlornly conclude: "I sometimes feel, in reviewing the evidence on the localisation of the memory trace, that the necessary conclusion is that learning just is not possible" (Lashley, 1950).

Stimulation techniques, as we noted, may be divided into two groups: electrical and chemical stimulation. <u>Electrical stimulation</u> is a valuable but indiscriminate tool. Reference to Figure 1 helps to make this apparent. If such an anatomical structure were stimulated by a microelectrode "the net result may be a most complicated and variable depolarisation of all the pathways in that region. Simultaneous changes in the ion flux of each nerve membrane would occur altogether in this segment of the three pathways." (Myers, 1974, p5).

<u>Figure 1.</u> An hypothetical brain structure. It receives afferent fibres (A, B, C) which make separate synaptic connections (A1, B1, C1) with interneurones (p, q, r) which then make separate synaptic connections (A2, B2, C2) with two different efferent projections (X and Y). (Adapted from R.D. Myers "Handbook of Drug and Chemical Stimulation of the Brain" (1974) p. 4.)

Figure 1



The result would without doubt be stimulating but would lack any specificity; functional differentiation of the pathways might prove to be impossible. Valenstein et. al. (1970) observed eating, drinking, grooming and gnawing as a result of stimulation of one electrode located in the diencephalon: no single behaviour was consistently observed. Such a phenomenon quite possibly represents the functional expression of simultaneous stimulation of overlapping neuronal systems. Similarly, Miller (1965) describes how, in trying to fix a discriminable effect in such a situation, he employed ever smaller electrodes until he observed no effect at all. The overriding problem of electrical stimulation is its lack of any discriminating properties. This does not, though, invalidate it as a technique: "specific techniques to answer specific questions" remains our motto. Indeed, the "non-specific" properties of electrical stimulation of the brain may be of interest for their own sake, and may elicit consistent behavioural patterns despite utilising a gross stimulation: self-stimulation of the MFB, for instance, shows this feature.

<u>Chemical stimulation</u> on the other hand has the potential to overcome the problem of neurochemical non-specificity. Again, reference to Figure 1 helps make this clear. If a cannula were aimed at this structure any of the different neurotransmitter* systems (A, B or C) or inter-neurones (p, q, r) active within the area could be specifically affected by the application of chemicals or drugs known to affect only one of the systems. Alternatively, any combination (A + B, B + C, A + C or A + B + C) could be affected. It is this specific ability to select for stimulation any one system from a varied collection that provides

---

*For definitions of "neurotransmitter" and other terms, see Appendix i.

the greatest advantage of chemical over electrical stimulation.

iii. Chemical Stimulation of Brain Tissue

R.D. Myers (1974) outlines four basic assumptions (which are not always borne out), made regarding the ability of chemical stimulation techniques to distinguish between interlaced networks of neurones. These are:

1. That the neuronal membrane at the dendrite will be sensitive to a particular chemical (or chemicals).
2. That cells in another functional category in surrounding tissue will remain inactive while excitation of another set of neurones occurs.
3. That there exists a specialized receptor for a specific substrate or class of compounds, and that recognition occurs between receptor and ligand.
4. That among a group of cells, collective excitation or inhibition will occur in synchrony.

Having accepted these primary assumptions the level of analysis at which examination is to occur has to be decided upon. Systemic administration of a chemical or drug is the most basic level, the substance being injected into the blood stream (intra-venous, iv) sub-cutaneously (sc) or intra-peritoneally (ip). The greatest problem of this approach is the effectiveness of transport into the brain. Peptide fractions may not pass the liver if injected iv, while the blood brain barrier(B.B.B)- itself not uniformly permeable and absent in some areas (for instance the median eminence) - may not permit the

passage into brain of compounds even of low molecular weight. Indeed, even if compounds do pass the B.B.B. they may act with varying degrees of efficacy at various anatomical sites. Peripheral and central effects may also differ and compete. Noradrenaline (NA), peripherally applied has an anorexic (Russek et. al., 1967) effect, though if applied centrally it has a well documented hyperphagic effect. (For instance, see Myers (1974) p361-362). Once again, one has to gauge this technique against the questions one is asking. If it is important to discover a central site of action, this technique alone is not enough. However if all that is required is, for instance, a model of human therapeutic drug administration, this technique may be perfectly adequate, especially if drugs are delivered by means of a slow release pellet or osmotic mini-pump.

For the analysis of central effects, a more appropriate level of analysis is to administer substances via the cerebral ventricles. This route avoids the problem of B.B.B. passage: the injected drug without doubt enters the brain, though the choice of ventricle affects exactly where the substance does go. Intra-lumen stoppers (Herz et. al., 1970) may be used to limit a drug to one particular ventricle. However, answers to questions of anatomical specificity are still not provided. The ventricular approach is "best considered as a highly useful but provisional approach, at least anatomically .... (it provides a) ...... provisional extrapolation from a general pot of stew to a specific vegetable". (Myers, 1974, p9).

If the question asked is "what function does this particular neuroanatomical structure relate to, and which neurotransmitter

facilitates this action" then one has to apply a technique which involves highly localised actions and which can deliver minute amounts of chemical. The microinjection technique aspires to these things. Guide cannulae may be accurately implanted giving access to, but not interfering with, particular areas of brain. Microinjection cannulae may then be passed through the guide to deliver precise quantities of chemical to the required area. While injection volumes and drug doses remain small the anatomical and chemical precision is maintained (Myers and Hoch, 1978). With larger amounts, diffusion away from the required site becomes a problem that may lead to a spurious specificity being assumed unless adequate control procedures are adopted. Crystals may alternatively be applied, though dose-response characterisation is difficult in this case, as is control over such factors as local pH and osmolarity. Temporal control over the release of chemical onto a particular structure may be achieved by using the push-pull perfusion technique, although this, reliant on larger cannulae, lacks the anatomical subtlety of the straightforward microinjection. The induction of regional hydrocephalus has also been a problem when using this technique, though this appears to have been skilfully minimised by some authors (Redgrave, 1977).

In passing, we must mention another chemical approach: that of micro-iontophoresis. At intervals two or more substances are ejected into extra-neuronal spaces from the tips of a fused array of multi-barrelled glass pipettes. Simultaneous recording shows whether or not the released chemicals have inhibitory or excitatory effects on the neural membrane. Control is the biggest drawback of the technique: local pH, trauma to tissue pCO_2 levels, and anaesthetic and paralysing effects of the solutions are all indeterminable. The technique is unsuited

to an analysis of function: its main value is in electrophysiological determination of _in vivo_ cellular activity. Chemical techniques of _in vitro_ analysis are also valuable: assay studies of metabolites, endogenous content studies, immunochemistry and histochemistry all provide valuable chemical and anatomical data, but are of limited value in directly assessing function.

We have presented this brief survey of available techniques to highlight a simple point, which is basic to any scientific enterprise. There are a variety of techniques available to us: lesion techniques, electrical stimulation and recording techniques, and chemical stimulation techniques, within which category there is further division, depending upon the level of analysis required. However, in selecting a technique one has to choose that which will most satisfactorily answer the question which has been raised. The principle question asked in this thesis is "Do cholinergic systems within the substantia nigra have a functional interaction with dopaminergic systems in that same structure?". In order to answer this question we need to use a technique or techniques which provide anatomical and chemical specificity of stimulation and allow for a functional expression of neural activity. For this reason the simple microinjection technique was chosen.

Chapter 2: The Substantia Nigra: Anatomy

i. General Structure

The substantia nigra was first described in the late eighteenth century by Vicq d'Azyr (1786) and Soemmering (1791): this description was undoubtedly aided by the fact that the substantia nigra is a recognizable nuclear mass, darkly stained by the pigment melanin. Functional effects of the nigra were noted by Blocq and Marinesco (1893) and Brissaud (1895) who observed a unilateral, left-sided Parkinsonism resulting from specific right-side substantia nigra destruction in the human patient. These observations were subsequently confirmed in the postencephalitic Parkinsonian (Tretiakoff, 1919) and by Hassler in the idiopathic (1938) and hereditary (1955) Parkinsonian state.

The substantia nigra (see Figure 2) lies above the crus cerebri throughout the entire extent of the midbrain. It is defined ventrally by a thin bundle of myelinated fibres (the cortico-bulbar and cortico-spinal tracts) while in more caudal aspects the nigra is delineated dorsally and dorsomedially by lemniscal fibres. The lateral portion of the nucleus is embedded in the most dorsolateral portion of the crus cerebri (Gulley and Wood, 1971). It is generally assumed to be divided into two regions, the cell-rich pars compacta and the relatively cell-poor zona reticulata which is the more lateral and ventral of the two, being situated directly above the fibres of the cerebral penduncles.

ii. Synapses Within the Substantia Nigra

Different authors have identified a varying number of synaptic bouton types within the substantia nigra. Bak et. al. (1967) identified

Figure 2. Showing the relative position of the substantia nigra (pars compacta and zona reticulata) in the ventral midbrain of the rat. (Adapted from the stereotaxic atlas of Konig and Klippell (1963).) The sections, enlarged by approximately 17.3X, are arranged from anterior (A) to posterior (K); the distance of each section from the inter-aural line is, in microns: A 3180, B 2970, C 2790, D 2580, E 2420, F 2180, G 1950, H 1760, I 1610, J 1270, K 1020. (Abbreviations: SNC, substantia nigra pars compacta; SNR, substantia nigra zona reticulata; SNL, substantia nigra pars lateralis; CC, Crus cerebri; CCS, Commissura colliculorum superiorum; d, nucleus Darkschewitsch; F, Columna fornicis; FMP, Fasciculus medialis prosencephali; FR, Fasciculus retroflexus; HI, Hippocampus; hp, nucleus posterior (hypothalami); H1, Forel's field H1; H2, Forel's field H2; i, nucleus interstitialis (Cajal); ip, nucleus interpeduncularis; LM, Lemniscus medialis; mmm, nucleus mammilaris medialis, pars medialis; SAM, Striatum album mediale colliculi superioris; SPCC, Splenium corpus callosi; sut, nucleus subthalamicus; ZI, Zona Incerta.)

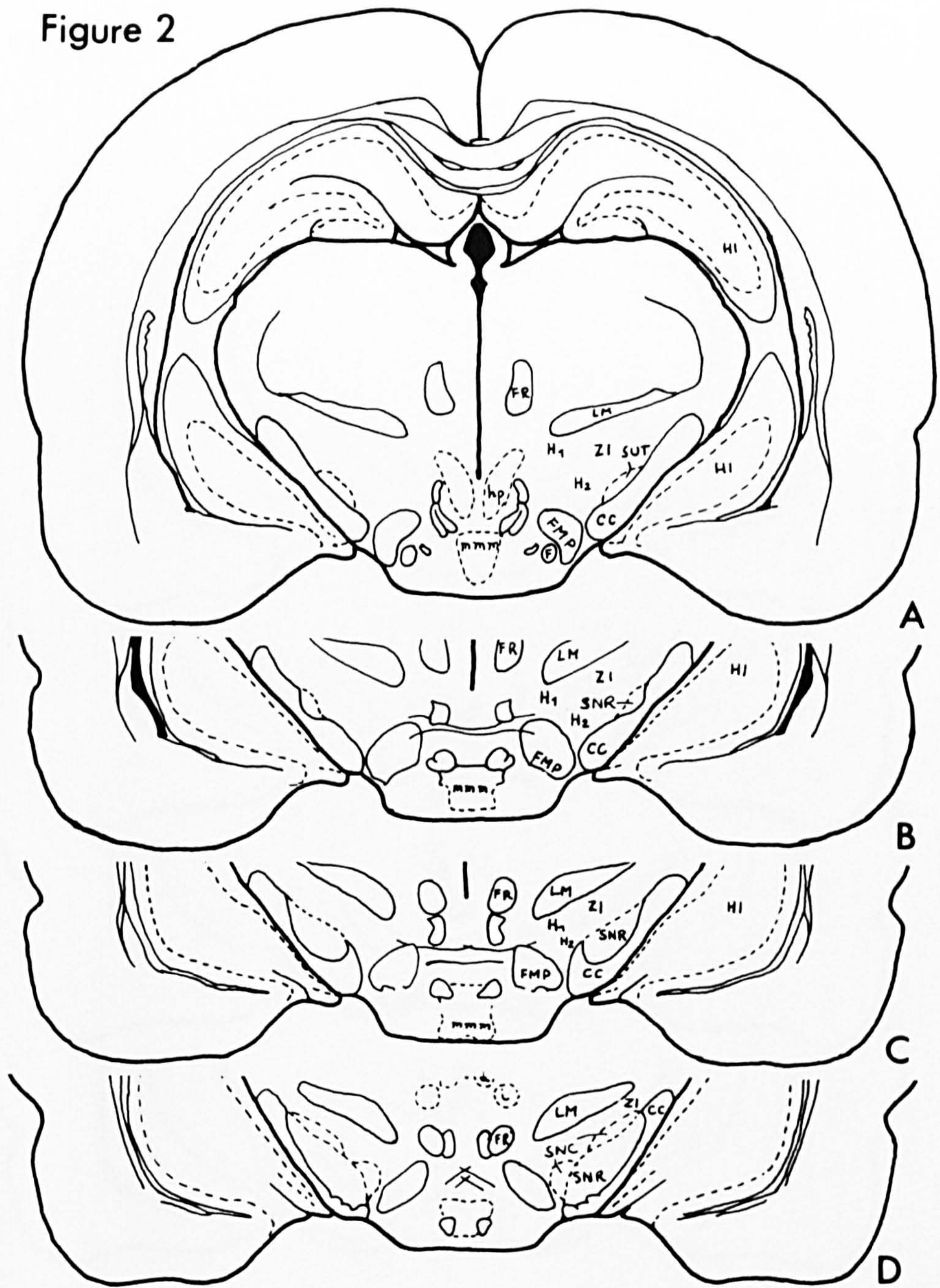


Figure 2

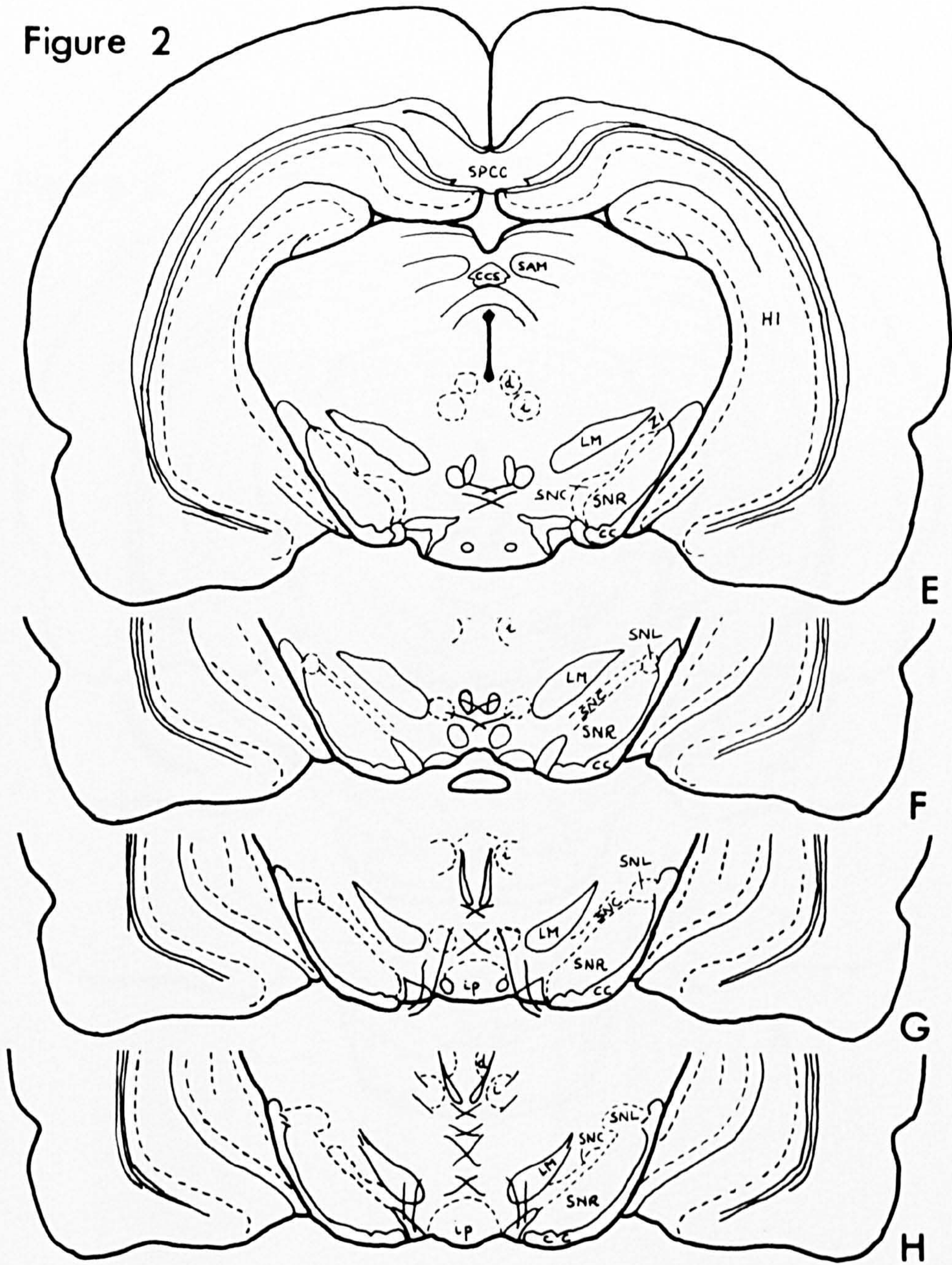
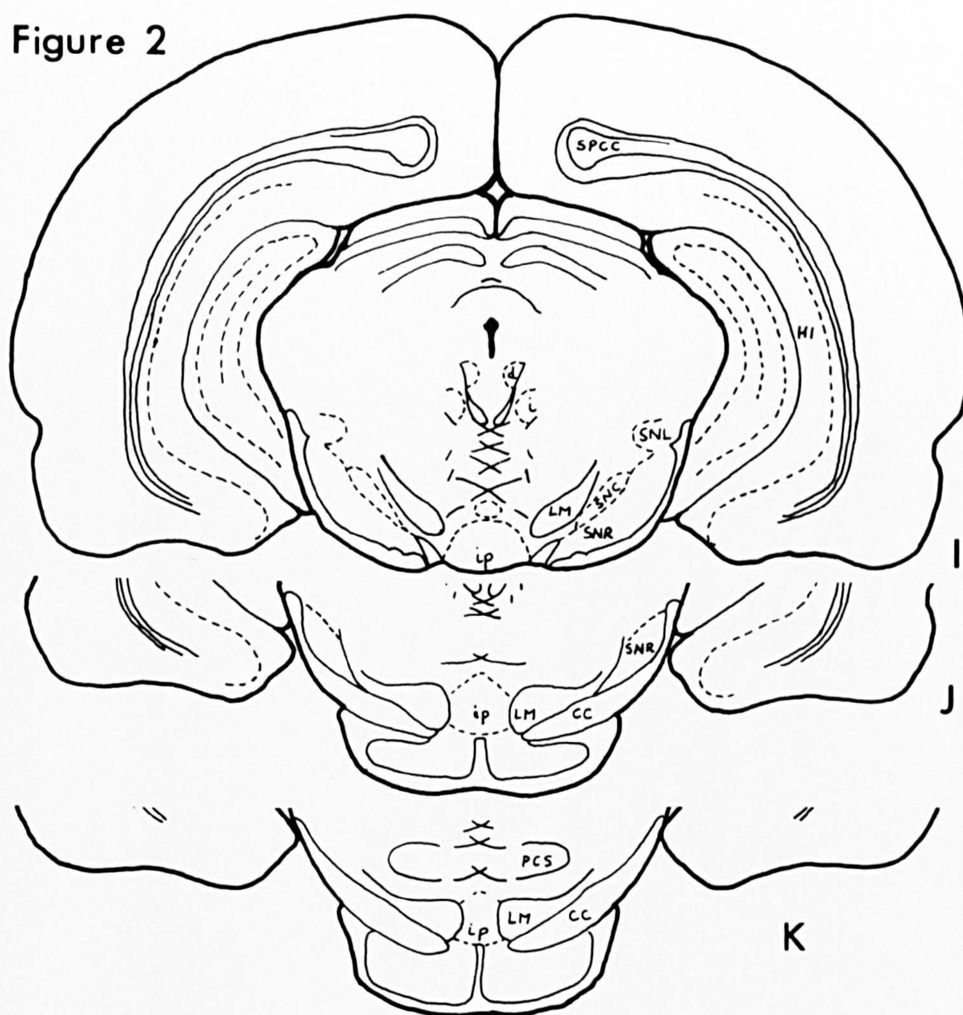


Figure 2

Figure 2



two synaptic types within the nigra - 'encircled' and 'sandwich' synapses. The encircled synapse observed involved large dendrites 2µ in diameter on which three synaptic boutons appeared to terminate by partially or completely encircling the process. Either small ($\sim$ 500Å) clear vesicles or a combination of large, dense-core and small clear vesicles were present, all of which might be contained within an envelope of astrocytic processes (Gulley and Smithberg, 1971). The sandwich synapse involved only small dendrites ($<$ 1µ diameter) surrounded by one or two synaptic boutons containing only small clear synaptic vesicles.

Bak's were the first observations made in an electron microscopic study of the nigra. The subsequent study by Gulley and Smithberg (1971) using electron microscopy and Golgi stained material, discovered more synaptic configurations. These authors observed three basic types of axo-dendritic synaptic endings and axo-axonic synapses. Their first category of axo-dendritic synapses is further divided into two sub-groups. Group A1 have a moderate number of 378Å clear vesicles, show an equal thickening of both pre- and post-synaptic membranes and are primarily located on cell bodies and primary segments of all neuronal types. Group A2 has many densely packed 378Å clear vesicles with equal thickening of both pre- and post-synaptic membranes and is found only on small dendrites of less than 0.8µ diameter. Group B are characterised by having large, dense core vesicles ($\sim$900Å) and 378Å clear vesicles. Post-synaptic and pre-synaptic membranes show a differing degree of thickening, the post-synaptic being the more prominent, and the synaptic cleft is widened. They are found on large and medium dendrites of all neuronal types. The synapses of Group C have large dense core vesicles and 581Å clear vesicles, and both pre- and post-synaptic membranes are equally thickened.

These are found in cell body and primary segments of all neurone types. The axo-axonic synapses observed by Gulley and Smithberg also occur in two varieties. The first is found on the axon hillock and has small boutons with one or two small mitochondria, clear vesicles (~600Å) which appear slightly elongated. The second type is found between two synaptic endings. These have only one bouton and small clear vesicles. They are not associated with a junctional complex, suggesting that they are post-synaptic.

Gulley and Smithberg note that dense core vesicles (Type B) are usually associated with aminergic nerve endings in the CNS (Bloom and Aghajanian, 1968; Bloom and Giarman, 1968). Furthermore, they report that type B was the only bouton type to selectively take up ^{3}HNE, again indicating that this bouton mediates catecholaminergic neurotransmission. These authors further speculate that bouton types A1 and A2 may be cholinergic and type C serotonergic.

Hajdu, Hassler and Bak (1973) in a further electron microscopic study of substantia nigra, identified six separate bouton types within substantia nigra as follows:

Type 1, Pleomorphic vesicle boutons, which have ovoid, elongated vesicles (~500Å diameter) mainly contacting neuronal perikarya and large dendrites, though also located on distal portions of dendrites. 40% of boutons are of this type.

Type II, Elongated vesicle boutons which have ellipsoidal vesicles (~400Å wide, 700/800Å long) contacting primarily large dendrites. 10% of all boutons are of this type.

Type III, Small, round vesicle boutons. These vesicles are approximately

400Å in diameter, usually contacting small dendrites, and accounting for 10% of all boutons.

Type IV, Large, round vesicle boutons which mainly contact distal portions of dendrites and whose vesicles have a diameter of approximately 600Å; 20% of all boutons.

Type V, Clear Terminal. 15% of all boutons are identified by clear terminal vesicles of varying size and loose arrangement, found on proximal portions of dendrites and occasionally neuronal perikarya. They are further characterised by the presence of microtubules and endoplasmic sacs.

Type VI, (Unnamed). Type VI boutons are typically identified by large dense-core vesicles (800-1200Å diameter) and clear round vesicles (600Å diameter). Only 5% of all boutons show these characteristics.

Hajdu, Hassler and Bak show that one bouton - the type 1, pleomorphic vesicle type - predominates (40%) in the nigra. Rinvik and Grofova (1970) also found that this type predominated, although they estimated it to account for 80-90% of all nigral boutons. (Though these authors worked only on cat substantia nigra, this may not be the source of their difference with Hajdu et. al.. Bak et. al. (1975) in a study of nigra in rat and cat concluded that the two species showed "great similarity" with regard to the substantia nigra.) Hajdu et. al. make no thorough attempt to identify the neurotransmitters at each bouton, though Bak et. al. observe only Type VI bouton atrophy following dorsal and median raphe nucleii destruction, suggesting that these boutons, probably equivalent to Gulley and Smithberg's Type C, utilise serotonin .

Dendro-axonic transmission within the substantia nigra has also been proposed recently by McGeer et. al. (1979). They observed that the

DA synthesising enzyme tyrosine hydroxylase (TH) is localised to microtubules and the smooth endoplasmic reticulum which approaches post-synaptic membranes. They also found that spiroperidol binding in the substantia nigra occurs on afferent striato-nigral neurones. Together these results suggest that DA is synthesised and released from dendrites within the substantia nigra and that the released DA acts on receptors located on neurones afferent to the nigra. McGeer and his colleagues point out, in a very apt simile that "communication at many C.N.S. synapses may involve a neurotransmitter dialog rather than a monolog" and proceed to sound the warning that "if true, this will affect interpretations of many pharmacological, behavioural and physiological experiments".

Similarly, dendro-dendritic synapses within the substantia nigra have been morphologically established (Hajdu, Hassler and Bak, 1973; Wilson et. al., 1977). It has been postulated that the pars compacta DA cells are able to release DA from their long dendrites. Bjorklund and Lindvall (1975) have established that potassium will cause the release of exogenous ^{3}HDA which had been previously taken up by the DA neurones, and that this release is calcium dependent. Nieoullon et. al. (1979) have extended this by demonstrating that tetrodotoxin (TTX) which blocks sodium channels and consequently axonic transmission, if added to the perfusion fluid in a push-pull cannulation of the substantia nigra, causes an increase in the release of ^{3}HDA from the nigra itself. This suggests that intra-nigral DA is not released by a process dependent on the activity of axonal sodium channels, but that instead, it is probably released dendritically. Such a release between the dendrites of DA neurones could cause the lateral inhibition of these neurones as suggested by Groves et. al. (1975a) and Llinas (1975).

The possibility of field effects being able to account for some neuronal communication within substantia nigra has also been suggested. Gulley and Wood (1971) observed that when pars compacta neurones were closely related, astrocytic processes intervened between the neurones. Such ensheathing has been suggested by Peters and Palay (1966) to ensure the electrical isolation of adjacent neurones and processes. However, in the lateral regions of substantia nigra neurones were seen by Gulley and Wood to appear in groups of two or three, the membranes of apposed neurones being separated by a space of merely 150-200Å for a distance of one or two microns. Neither glial intervention nor morphological specialisation of the apposed membranes could be detected, leading to speculation concerning the functional significance of this, and the possibility of field effects occurring.

iii. Neurones Within the Substantia Nigra

Gulley and Wood (1971) in a thorough study of the fine structure of neurones within the substantia nigra identified three types of neurone: large (25-40µ diameter); medium (15-20µ); and small (8-12µ).

The large neurone was found predominantly in the rostrolateral portion of the zona reticulata, though it occurred throughout the region never appearing in the pars compacta. It had an oval nucleus with a single nuclear infolding, evenly distributed chromatin and many nuclear pores. The rough endoplasmic reticulum (RER) consisted of many groups of short interconnected cisternae randomly distributed throughout the cytoplasm, except in the perinuclear regions. The Golgi apparatus had numerous cisternae and vesicles arranged concentrically around the nucleus and near the various groups of RER. The vesicles observed were dense

cored. The inclusions appeared as whorls of lamellae embedded in a fila - mentous mesh. Between three and five dendrites were noted, all apparently tapering, and having irregular contours and dendritic thorns.

The medium sized neurone was found to be the predominant neurone of the pars compacta. The nucleus of this neuronal type was oval and eccentric with only slight nuclear infoldings, which were filled with free ribosomes. Fine, evently distributed chromatin was observed with some clumping near the nuclear invaginations. A moderate number of nuclear pores were counted. The RER contained several groups of three or four cisternae, interconnected, and parallel to the nuclear envelope. A similar grouping of between four and seven cisternae parallel to the cell membrane in the cytoplasm and away from the nucleus was also observed. The Golgi apparatus appeared to consist of one group of cisternae and vesicles around the nucleus and a second group which projected into the primary dendrite segments. Dense cored vesicles were again observed. The inclusions of the medium sized neurone were seen as whorls of cisternae with ribosomes attracted to the inner and outermost membranes. Four to six dendrites were present. Neurotubules and glycogen were tightly packed in medium sized processes.

The small neurone was discovered in both pars compacta and zona reticulata and had an oval nucleus with much infolding and clumping of chromatin especially apparent around the nuclear invaginations. A fibrous inclusion was present but few nuclear pores were noted. The RER had scattered, single cisternae with frequent continuities between these and the nuclear envelope. A poorly developed Golgi apparatus was present; only a small group of cisternae and vesicles were present near the mouth of a nuclear invagination. The only inclusion observed was a fibrous, nuclear one. Dendrites took the form of three or four

stout processes with few organelles in the primary segment and loosely arranged neurotubules. Significantly, the axons of the small neurones observed were seen, in Golgi preparations, to be associated with the dendrites of other nigral neurones. This neurone is therefore presumed to be an interneurone: the lack of Nissl material and the relative paucity of cytoplasmic organelles is compatible with this interpretation, which is further supported by the observation that neurones with similar cytological features, but in other structures, have been categorised as interneurones. (For instance, in caudate nucleus (Adinolfi and Pappas, 1968), and in the dentate gyrus (Laatsch and Cowan, 1966). A short axoned neurone within the substantia nigra has been previously reported in Golgi preparations by Ramon y Cajal (1904), who described the axon of this neurone entering corpuscles formed by different axons and dendrites of nigral neurones. Taber (1961) also noted the presence of short-axoned nigral neurones with little or no Nissl substance.

Gulley and Wood conclude, rightly and obviously, that the varying size and characteristics of nigral neurones demonstrates the existence of a heterogeneous population of neurones. Their observation is clearly supported by many other studies. Several authors have described the nigra as containing neurones of varying sizes (Ramon y Cajal, 1904; Rioch, 1929; Gillilan, 1943; Taber, 1961 and Hanaway et. al., 1970) and as containing neurones with varying distributions of Nissl material (Malone, 1910, 1913; Morgan, 1927). Bak (1965) found the Golgi apparatus of various neurones within the substantia nigra to be associated with a non-uniform distribution of dense core vesicles. Finally, a functional difference has been clearly demonstrated by Anden et. al. (1964) and Fuxe (1965) who have reported the existence of dopamine fluorescence from

most, but not all, pars compacta neurones, but none at all from zona reticulata neurones.

Gulley and Wood believe that both location and number suggest that their medium sized neurone, most populous in pars compacta, corresponds to the fluorescent DA neurones known to be present there, and giving rise to the important nigro-striatal projection. The large amount and complex organisation of the Nissl material of this neurone is presumably related to the size (7.3µ diameter) and length of its axon. Alone of all the nigral neurones, these take up exogenously applied NA, a characteristic only of catecholamine neurones and a further suggestion that these utilise the most prodigious compacta catecholamine, dopamine (DA).

The large neurones found particularly within the zona reticulata - especially in the rostro-lateral portion which some authors have designated pars lateralis - are hypothesised to be the neurones which form the nigro-thalamic projection (Nauta and Mehler, 1969). The nigro-thalamic neurones are believed to possess large axons: this is certainly suggested by Gulley and Wood's findings of a large cell body with a well-developed granular reticulum. Carpenter and Strominger (1967) observed that lateral lesions in the substantia nigra resulted in heavier degeneration of the nigro-thalamic projection than did more medially placed lesions.

While other authors examining rat substantia nigra broadly agree with Gulley and Wood in their descriptions of neurones, there have been some disagreements. Hajdu, Hassler and Bak (1973) found most nigral neurones to be between 8 and 25µ in diameter, the majority being between

8 and 12μ. The neuropil they describe in the following manner: most of the observed neurones have long, straight dendrites with infrequent branches, oriented for the most part sagitally. The dendritic cytoplasm appeared rich in regularly arranged tubuli while dendritic spines were rare - a true spine apparatus (Gray, 1959) never being observed. Many fine myelinated and unmyelinated fibres were also observed, mostly between .15 and .25μ in diameter. Their description of six nigral bouton types we have already catalogued. Basically these authors are in good agreement with both Gulley and Wood (1971) and Gulley and Smithberg (1971).

However, Hajdu, Hassler and Bak could not discriminate three types of neurone - the 40μ large neurone was not observed, though two others were. The larger of these had a better granular endoplasmic reticulum and more free ribosomes and lysosomes. The smaller had a pale cytoplasm and a paucity of organelles. Bak et. al. (1975) also found that the largest neurone in substantia nigra was only 28μ in diameter, with most neurones in the reticulata being between 12 and 15μ. diameter.

The results of Hajdu, Hassler and Bak, and Gulley and Wood are not incompatible however, though they do only fit awkwardly with each other. As Hajdu's group are able to observe small (8-12μ) neurones which have a pale cytoplasm and a paucity of organelles, one would suggest that there is no question as to the existence of Gulley and Wood's small neurone which was similarly characterised. What difficulty remains lies in deciding whether Gulley and Wood's large (25-40μ) and medium (15-20μ) neurones - which presumably correspond to the "compacta output cells" (DA - medium sized) and the "reticulata output cells" (the nigro-thalamic cells, large neurones) - can in fact be discriminated morphologically. A third study suggests that they can be. Faull and Mehler (1978)

characterise the nigral efferents to three separate structures - the striatum, thalamus and tectum. The nigro-striatal cells they claim to have a diameter of 13.8μ (± 2.4μ); the nigro-thalamic 19.3 ± 3.4μ; and the nigro-tectal 15.8 ± 3.4μ. This clearly suggests that Gulley and Wood are correct in discriminating between the various efferent systems of substantia nigra by cell diameter, (the nigro-thalamic being bigger than the nigro-striatal) but it is also clear that Faull and Mehler, like Hajdu, Hassler and Bak, could not find the very large 40μ neurones.

iv. Afferent and Efferent Projections of the Substantia Nigra

In studying the projections to and from the substantia nigra, we shall focus our attention first on the efferent projections and second on the afferent projections. Figure 3 presents a summary diagram of the proposed connections.

iv.a. Efferent Projections

The substantia nigra has three major efferent pathways to the striatum, thalamus and tectum. Faull and Mehler (1978) in the most recent and comprehensive study of these, used relatively large injections of the retrogradely transported cell marker horseradish peroxidase (HRP) to define their cells of origin. Figure 4 shows the sources of the separate projections which Faull and Mehler (1978) define.

ai. The Nigro-Striatal Projection

This arises almost exclusively from the pars compacta, though a group of cells projecting to the striatum from the zona reticulata has

Figure 3. A schematic representation of the major afferent and efferent projections of the substantia nigra (pars compacta, snpc, and zona reticulata, snzr). (Circles represent cell bodies; open ends represent synaptic connections. The diagram is not to scale.)

Figure 3

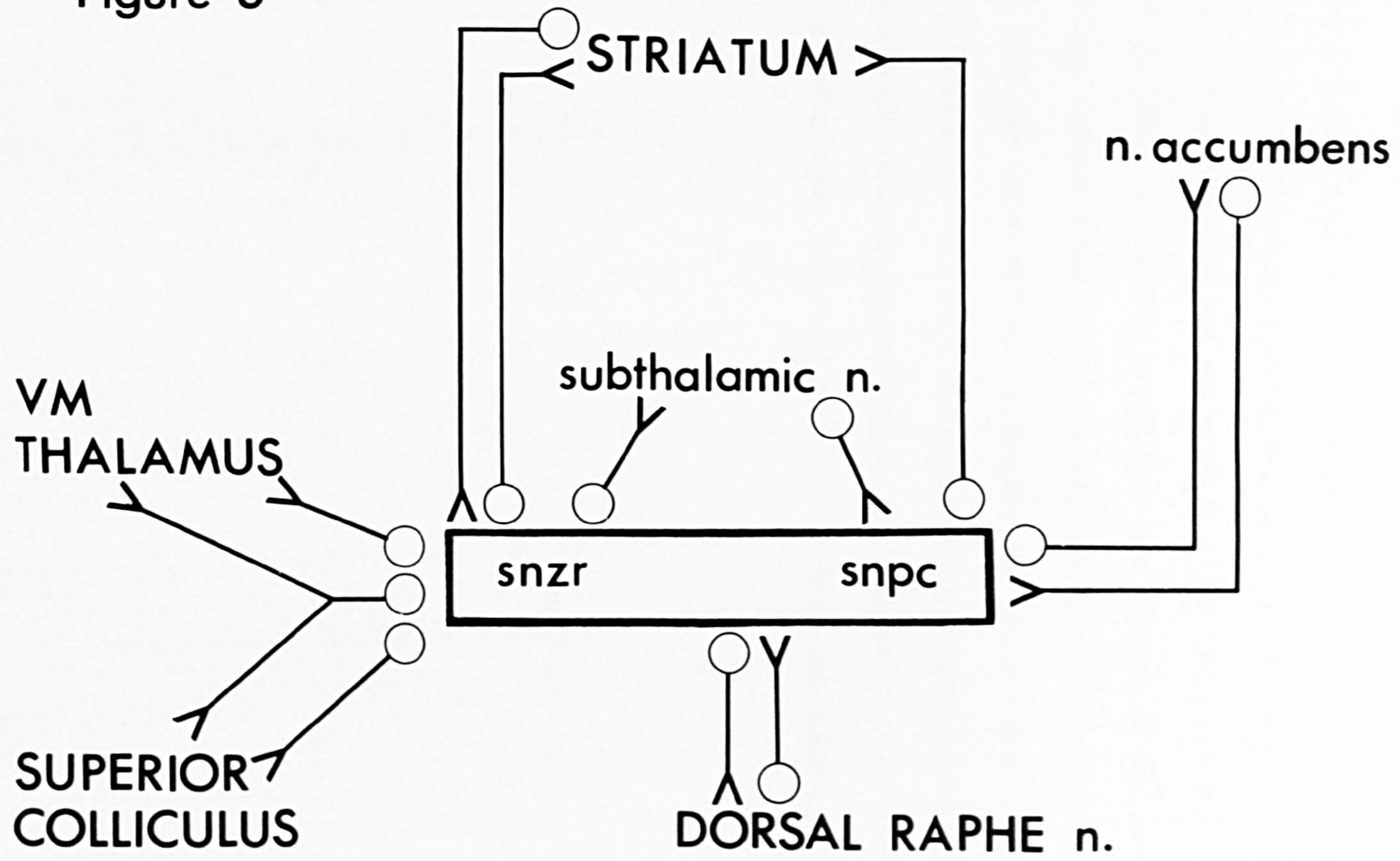
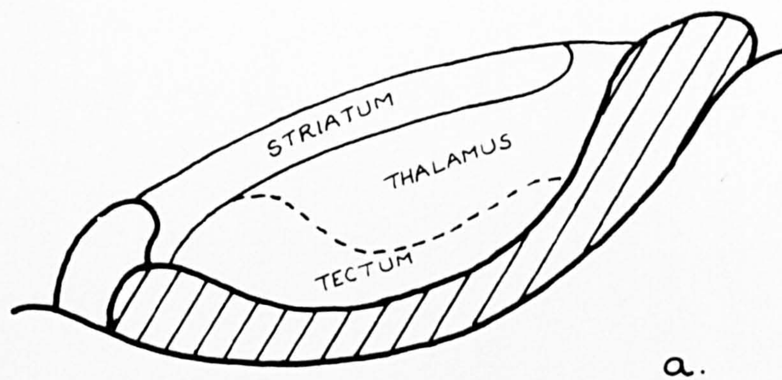
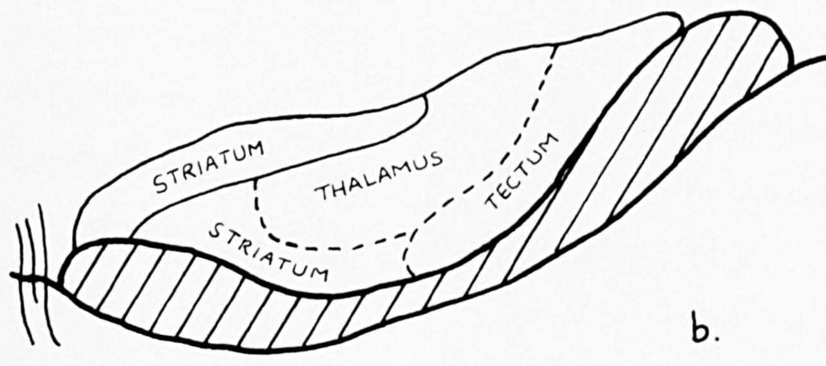


Figure 4. "Schematic summary diagram depicting the principal location of the nigral cells projecting to the tectum, thalamus and striatum within the rostral (a) and caudal (b) portions of the substantia nigra. Cells within the pars compacta division project to the striatum while the pars reticulata portion is divisible into three complementary longitudinal subnucleii projecting respectively to the tectum, thalamus and striatum." (From Faull and Mehler (1978) p. 999.) It must be noted that the clarity of the zona reticulata subdivisions has been criticised by other authors, who claim that the three reticulata projections overlap each other to a greater degree, and that a population of neurones exists which possess branched axons projecting to both the VM thalamus and the superior colliculus (Bentivoglio, Van Der Kooy and Kuypers, 1979).

Figure 4





been described by Faull and Mehler (1978), Bentivoglio et. al. (1979) and Butcher and Talbot (1978). However, these groups disagree as to the precise location of the zona reticulata nigro-striatal cells. Faull and Mehler place them within the caudal reticulata while Bentivoglio et. al. suggest that they are located primarily within the dorsal reticulata. Butcher and Talbot place them in the rostrolateral portion of the zona reticulata, which is referred to as the pars lateralis by some authors.

Faull and Mehler found that the pars compacta nigro-striatal cells had a medium sized soma (15.1 ± 2.7μ diameter), categorising them as the medium sized neurones of Gulley and Wood (1971). The striatal projection neurones of the reticulata Faull and Mehler observed appeared to be slightly smaller than those of the pars compacta (13.8 ± 2.4μ diameter) though both were found to have oval, multipolar cells and to be cytoarchitectonically similar. All the striatal projection cells were seen as being smaller than either the nigro-thalamic or nigro-tectal cells. Faull and Mehler did not observe any overlap between the zona reticulata-striatal neurones and any other cells.

The striatal projection cells of the pars compacta have been characterised as utilising the neurotransmitter dopamine (DA) (Anden et. al, 1964). The projection is monosynaptic, the pars compacta somata sending long axons to the striatum where they synapse predominantly on a system of cholinergic interneurones.

On the other hand, while some authors (Dahlström and Fuxe, 1965) suggest that the nigro-striatal cells of the zona reticulata are also dopaminergic, others (Guyenet and Aghajanian, 1978) suspect that they may not be.

We shall turn our attention to the dopaminergic nigro-striatal projection in due course, treating it in much greater detail. Meanwhile considerable attention has been directed recently towards the other major efferent pathways of the substantia nigra. Many authors (for instance, Di Chiara et. al., 1978; Olianas et. al., 1978a, 1978b) have argued that the substantia nigra acts as an output station for the striatum, impulses being relayed from the caudate-putamen and/or the globus pallidus back to the nigra by the striato-nigral projections. It is thus currently hypothesised that the GABA-ergic striato-nigral projection not only acts to provide feedback inhibition of the DA cells in pars compacta, but also activates other outputs from the zona reticulata.

aii. <u>The Nigro-Thalamic Pathway</u>

The nigro-thalamic cells lie within the central longitudinal region of the reticulata immediately ventral to the pars compacta. At rostral levels these cells occupy the dorsal two-thirds of the reticulata while at caudal levels they are found especially

in dorsal regions of its central two-thirds (Faull and Mehler, 1978). The cells which make up this projection were identified by Faull and Mehler as having an average cell diameter of 19.3 $\pm$ 3.4μ, the largest of the nigral neurones observed by them. This is in agreement with other authors, notably Gulley and Wood (1971) and Nauta and Mehler (1969).

The nigro-thalamic pathway has been shown to terminate principally in the ventro-medial nucleus of the thalamus (Faull and Carman, 1968; Clavier et. al., 1976; Faull and Mehler, 1978; Kultas-Ilinsky et. al., 1978; Di Chiara et. al., 1979a). Di Chiara et. al., (1979a) suggest that this pathway utilises GABA as a neurotransmitter, having observed terminal degeneration after kainic acid lesions of the cells of origin of the projection. In the kainic acid lesioned substantia nigra, tyrosine hydroxylase (TH) (a DA synthesising enzyme) levels fell by 58% compared to the unlesioned side while glutamic acid decarboxylase (GAD) (a GABA synthesising enzyme) levels fell by 18%. No effect on the cholinergic synthetic enzyme, choline acetyltransferase (CAT), was detected in the substantia nigra or in either the ventro-medial (VM) or ventro-basal (VB) thalamus, while, significantly, GAD levels fell to 35% of control in the VM thalamus, but not at all in the VB thalamus. This enzyme-specific loss of GAD rather than CAT in only the VM thalamus strongly suggests that GABA is indeed the transmitter utilised by the nigrothalamic projection.

aiii. The Nigro-Tectal Pathway

The nigro-tectal projection also arises in the reticulata, predominantly from the rostral portions of the medial two-thirds. The cells of origin of this projection Faull and Mehler (1978) identify as being on average smaller than those of the nigro-thalamic projection but larger than those of the nigro-striatal systems. The projection appears to terminate mainly within the intermediate grey layer of the superior colliculus (Faull and Mehler, 1978). The terminals of the projection have been labelled by axoplasmic transport (Vincent et. al., 1978) and are seen forming symmetrical synapses with major dendrites. They have moderately packed pleomorphic vesicles and 1-2µ diameter boutons. The axons which constitute the projection, labelled in the substantia nigra, appear to be myelinated. Electrophysiological data(Deniau et. al., 1978a) suggest that the pathway has a possible inhibitory function, an hypothesis which is sustained by the observation that GABA - which usually mediates synaptic inhibition - appears to be the pathway's primary neurotransmitter. Lesion of the substantia nigra by kainic acid (Di-Chiara et. al., 1979a) as well as reducing the VM thalamic GAD content also lowers levels of this enzyme in the superior colliculus to 40% of control. CAT levels, as in the thalamus, remain unchanged. These results confirm those of Vincent et. al. (1978) who found that substantia nigra lesions decreased GAD levels in superior colliculus to 60% of control while CAT levels fell only by 14%, and that the uptake of neither glycine nor aspartate was unaffected.

There is evidence that some nigral output neurones are branched. Deniau et. al. (1978b) found that neurones in the pars compacta were activated only from the striatum, the axons having a relatively slow

conduction velocity of between 0.33 and 1.0 m/sec. Some neurones in the reticulata however were activated from more than one structure. Antidromic stimulation of both the striatum and the superior colliculus was able to activate these neurones, which had a fast conduction velocity (1.9 - 10.00 m/sec). In a double labelling study, Bentivoglio et. al. (1979) also found that some neurones in the zona reticulata showed branching. Having first established that their individual tracers - red fluorescent Evans Blue and a fluorescent DAPI-primuline mixture - performed retrograde tracing individually as well as did the more common retrograde tracer, HRP, they systematically examined the fluorescent patterns resulting from simultaneous injections into any two of the major nigral output areas - striatum, thalamus or superior colliculus. Their results demonstrated that the only population of branched neurones was in the zona reticulata - as Deniau et. al. (1978b) found - but that they projected to the VM thalamus and superior colliculus. This is at variance both with Deniau et. al. (1978b) and Guyenet and Aghajanian (1978) who suggest, on the basis of electrophysiological data (antidromic stimulation), that nigro-striatal neurones send collaterals to the thalamus. Guyenet and Aghajanian provide an explanation for the discrepancy which Bentivoglio et. al. echo; that the electrophysiological work may involve unintentional stimulation and recording from fibres of passage.

It must also be noted that in their labelling study of the various nigral efferents, Bentivoglio et. al., while broadly agreeing with Faull and Mehler's (1978) comprehensive description (see Fig. 4) do show some disagreement. Bentivoglio et. al. especially emphasise that the nigrothalamic projection neurones arise not only from dorsal aspects of the nigra but also from medial and ventral parts adjoining the cerebral

University Library Hull

peduncle, thereby giving rise to a greater overlap with the tectal projection neurones and forming a morphological basis for the branching. They also claim that the dorsal nigro-thalamic neurones overlap with the reticulata striatal projection neurones. Thus they suggest that Faull and Mehler's clear subdivision of the zona reticulata into striatal, thalamic and tectal projection areas is a little too clear, and that in fact a greater degree of overlap occurs.

aiv. Other Substantia Nigra Efferent Systems

Aside from the three major nigral outputs, there are two smaller and less thoroughly studied systems which may prove to be of importance; a diffuse projection to the dosral raphe nucleus and a small projection to the subthalamic nucleus.

The nigro-raphe pathway arises from the dorsomedial portion of the nigra (Pasquier et. al., 1977) but fails to form a large projection, the neurones showing only a minimal degree of clustering. Pasquier et. al. were able to confirm the observations of Ramon y Cajal (1955) who also described the pathway as being diffuse and having an irregular distribution, as well as noting that some of the neurones had bifurcated axons.

The sub-thalamic projection has been extensively studied by Meibach and Katzman (1979) using the classic Falck-Hillarp histofluorescence technique. The spur for this investigation has been recent observations, using the radiolabelled 2-deoxy-D-glucose "metabolic mapping" technique, that systemic injections of the DA agonists apomorphine (Brown and Wolfson, 1978) and amphetamine (Wechsler et. al. 1979) increase glucose utilization within the subthalamic nucleus (S.T.N.), by 26% and 67% respectively. Meibach and Katzman found that, at a level through the

red nucleus, two rostrally directed groups of DA neurones appeared - a medial group of small oval cells and a lateral group of large and small cells, both in the zona reticulata. The DA cells of the pars compacta were seen to run dorsal to the S.T.N. and form the nigro-striatal pathway while the reticulata cells were observed to form synapses within the S.T.N. although whether the whole projection was confined to this nucleus or whether these were collaterals from a nigro-striatal projection was not clear. Further evidence for a dopaminergic system within the S.T.N. is found in studies which have demonstrated the existence of a DA-sensitive adenylate cyclase within the nucleus (Wolfson et. al., 1979). A projection from the S.T.N. to pars compacta has also been described, which will be examined in the next section.

Nauta et. al. (1978) in examining nucleus accumbens afferents from cell group A10 note that a few (and only a few) fibres project to the accumbens from the pars compacta. It has also been suggested that there is a DA projection from the ventral midbrain to the median eminence. A fall in DA levels within this area was found following lesions in the ventral midbrain (Kizer et. al., 1976), though NA levels remained unchanged. However, as the lesion made involved bilateral destruction of the A8, A9 and A10 areas, the precise location of the origin of this putative pathway could not be described.

ivb. Afferent Projections

bi. Striato-Nigral Projections

While there are three major nigral output systems, there appears to be only two major inputs, and both of these arise in the striatum. Studies using HRP injections into the substantia nigra have shown that it

receives projections from both the caudate putamen and the globus pallidus (Bunney and Aghajanian, 1976; Kanazawa et. al., 1976). Following HRP injection into the substantia nigra, Bunney and Aghajanian (1976) discovered that the caudato-nigral projection was topographically organised, and that all of the caudate-putamen complex, except for a central medial core, contained HRP reactive cells, which were predominantly of a medium size (12-20μ diameter). Moreover, they found that in HRP-positive areas, 30-50% of all cells were stained, an unusually large percentage. This would appear to confirm that the substantia nigra has a privileged position as a major midbrain output station of the striatum. Bunney and Aghajanian also observed labelled neurones in the globus pallidus, as did Kanazawa et. al. (1976) and Hattori et. al. (1975). Predominantly, large triangular neurones were labelled in the globus pallidus. Kanazawa et. al. and Hattori et. al. both found fewer reactive cells here than in the caudate-putamen.

There are as yet no definitive answers to two questions: do either of these projections specifically innervate the pars compacta or zona reticulata; and, which neurotransmitters are utilised by these systems? To the question of anatomic localisation, there is substantial difference of opinion. Hattori et. al.(1975) suggest that the globus pallidus preferentially innervates the pars compacta. However, Bunney and Aghajanian (1976) find that microinjections of HRP preferentially into the zona reticulata tend to label more globus pallidus neurones than caudate putamen. Microinjections into the pars compacta do not display this tendency. It must also be noted that these microinjections were made with extreme care by Bunney and Aghajanian: both vertical and oblique approaches with a narrow gauge (31 ga.) cannula were made to the nigra, where only 0.05μl of 25% HRP in distilled water was released.

Such caution inspires confidence in a study, but it must also be noted that Kanazawa et. al. (1976) confirmed the results of Hattori's group, albeit in a very small-sample study.

The question of neurotransmitters is further complicated. Bunney and Aghajanian (1976) suggest that GABA is the neurotransmitter of the pallido-nigral pathway. Transection between the basal ganglia and substantia nigra causes a fall of both GABA and GAD (but not ACh) within nigra, suggesting that GABA is indeed a striato-nigral transmitter (Fonnum et. al., 1974; McGeer et. al., 1974). That it is the transmitter of the pallido-nigral, but not the caudato-nigral projection is apparently demonstrated by the discovery that labelled GABA micro-injected into the globus pallidus subsequently appears in the substantia nigra, while equivalent injections into the caudate putamen fail to subsequently reveal radiolabelled GABA in substantia nigra (Fonnum et. al. , 1974). Bunney and Aghajanian speculate therefore, that the pallido-nigral projection is mediated by GABA while the caudato-nigral projection is not. The most likely other transmitter to be involved is the peptide, substance P. Several laboratories have produced evidence which indicates that the substance P content of the nigra - which is higher than any other brain structure - is mainly stored in terminal axons descending from the neostriatum. (Hong et. al., 1977; Kanazawa et. al., 1977; Mroz et. al., 1977). Indeed, depolarizing stimuli induce substantia nigra slices to release substance P (Reubi et. al., 1977). However, substance P is apparently not confined solely to the pallido-nigral projection. "....Substance P abounds in the axon terminals of specific pallido-nigral and caudato-nigral neurones in the rat ......" (Starr, 1978), and indeed, authentic substance P has been

identified in neurones which project from the caudate-putamen to the substantia nigra by Ben-Ari et. al. (1979). However, while the two neurotransmitters appear to be located in close proximity of each other they are claimed to exist within separate neurones (Gale et. al., 1978). Gale et. al. (1978) also observe that substance P and GABA played different roles in the mediation of striatal tyrosine hydroxylase activity elicited by haloperidol, and therefore suggested that both SP and GABA participated in the regulation of nigrostriatal DA system activity. (SP presumably being typically excitatory, GABA inhibitory - if we can presume even thus far, Starr (1979) having observed both excitation and inhibition mediated by intranigral GABA.) SP and GABA themselves interact within the nigra: the efflux of endogenous SP from nigral slices can be attenuated by GABA (Jessell, 1977) but is unaffected by DA, whereas this amine is able to liberate GABA from intranigral stores (Reubi et. al., 1978).

We can therefore state only the most basic facts concerning the striato-nigral projections with any degree of certainty. The substantia nigra receives afferents from both the caudate-putamen and the globus pallidus, but whether either or both of these selectively terminate in pars compacta or zona reticulata is unclear, evidence being available to favour a pallido-compacta or a pallido-reticulata scheme. Moreover, while it is clear that both substance P and GABA are striato-nigral transmitters, it is not clear whether the caudato-nigral projection utilises either or both SP and GABA, although separate GABA and SP pallido-nigral projections are strongly suggested. Starr (1978) sounds a warning that "... the peptidergic nature of these projections remains unresolved" and, indeed, much is still to be learnt about peptidergic transmission in general .

bii. Other Substantia Nigra Afferent Systems

As we noted in our discussion of the nigral afferents, the subthalamic nucleus and dorsal raphe both enjoy reciprocal connections with substantia nigra. While the substantia nigra zona reticulata gives rise to an efferent projection to the S.T.N. (Meibach and Katzman, 1979), this nucleus appears to innervate the pars compacta (Kanazawa et. al. 1976), thereby creating a short loop, the function of which is unclear. The nigral afferent originating in the dorsal raphe nucleus has been demonstrated using both retrograde-tracing with HRP (Bunney and Aghajanian, 1976; Fibiger and Miller, 1977), and anterograde tracing (Taber Pierce et. al., 1976). This, as with the nigro-raphe efferent, is believed to utilise serotonin to mediate neurotransmission (Conrad et. al., 1974; Dahlström and Fuxe, 1964; Kuhar et. al., 1972; Parizek et. al., 1971) and appears to regulate the activity of pars compacta DA neurones. Microintophoretically applied 5HT has little effect on reticulata neurones but blocks glutamate-induced excitation of pars compacta cells (Aghajanian and Bunney, 1974). Fibiger and Miller (1977) suggest that the pathway exerts a phasic inhibitory control over pars compacta neurones. Serotonin and DA appear to enjoy a similar antagonistic relationship within the corpus striatum (Waldmeier and Delini-Stula, 1979). While neither of these two reciprocal systems are thoroughly understood, their existence is beyond doubt, and important neurochemical discoveries relating to them are beginning to make their influences over the larger nigral systems more understandable.

In passing, it must also be noted that other structures have been reported to project to the substantia nigra. Nauta et. al. (1978) note

that the nucleus accumbens, projects not only to cell group A10 but also to the medial pars compacta. "It thus appears that the accumbens projects to a region of the nigral complex considerably larger than that from which it receives .... fibers, and hence ..... (that accumbens - ventral tegmental connections are).... not organised in a mode of simple point-for-point reciprocity" (Nauta et. al., 1978).

In addition, Bunney and Aghajanian (1976) observed a few labelled cells in the hypothalamus and the lateral habenula, as well as in areas of the pre-frontal cortex. Indeed, a relationship between the cortex and the substantia nigra has been demonstrated by Lindvall and Bjorklund (1978) who observed that many areas of the cortex receive projections from the nigra and the adjacent ventral tegmental area. The extent and importance of these connections remains to be discovered.

It also remains to be seen whether or not there exists a specific glycinergic projection. The nigra has one of the highest concentrations of glycine of any human brain structure (Perry et. al., 1971) and appears to be active in both pars compacta and zona reticulata of the rat (Dray and Straughn, 1976). However, whether or not it has neurotransmitter properties in the substantia nigra, and whether or not it has a specific location, remain to be seen.

Chapter 3: The Nigro-Striatal Projection: Dopamine within the Substantia Nigra

i. Dopamine within the Ventral Midbrain

The histochemical technique of Falck and Hillarp offered the first proof of cellular localisation of monoamines within the central nervous system (Carlsson et. al., 1962a and 1962b; Falck and Hillarp, 1959). The existence of a nigro-striatal DA system has been demonstrated in a number of studies emanating mainly from Sweden, which offer a combination of biochemical and histochemical evidence (Anden et. al., 1964; Anden et. al., 1965; Anden et. al., 1966; Hökfelt and Ungerstedt, 1969; Ungerstedt, 1968). Similarly, a "mesolimbic" DA system with terminals in the nucleus accumbens and the olfactory tubercle, has been described following histochemical study. (In the classification of Dahlström and Fuxe (1965), these systems arise in A9 (substantia nigra) and A10 (the Ventral Tegmental Area, V.T.A.); see Figure 5). These two DA systems converge at a level dorsal to the nucleus interpeduncularis and ascend together into the forebrain (Ungerstedt, 1971a) the mesolimbic system ascending just medial to the nigro-striatal. The nigro-striatal projection predominantly innervates the caudate-putamen complex, while the mesolimbic system innervates, in one branch, the nucleus accumbens, as well as dorsal aspects of the nucleus interstitialis striae terminalis, and, in a separate latero-ventral branch, the olfactory tubercle.

ii. Morphology of Nigro-Striatal Cells

Faull and Mehler (1978) identify the nigro-striatal projection cells

Figure 5. "Sagittal projection of the Dopamine pathways. The stripes indicate nerve terminal areas." (From Ungerstedt, 1971a, p. 27.) Four groups of DA cell bodies are identified: the A9 DA cell group in the pars compacta; the A8 group, just caudal to the substantia nigra, dorsal to the medial lemniscus ,the axons of which probably join those of the A9 group as they show retrograde degeneration after lesions of the corpus striatum (Ungerstedt, 1971a); the A10 group, whose axons ascend with those of the A9 group but innervate the n. accumbens and olfactory tubercle. The cell bodies of this group lie just dorsal to the n. interpeduncularis. DA Cell bodies are also found in the n. arcuatus of the hypothalamus; these form the A12 cell group.

DOPAMINE

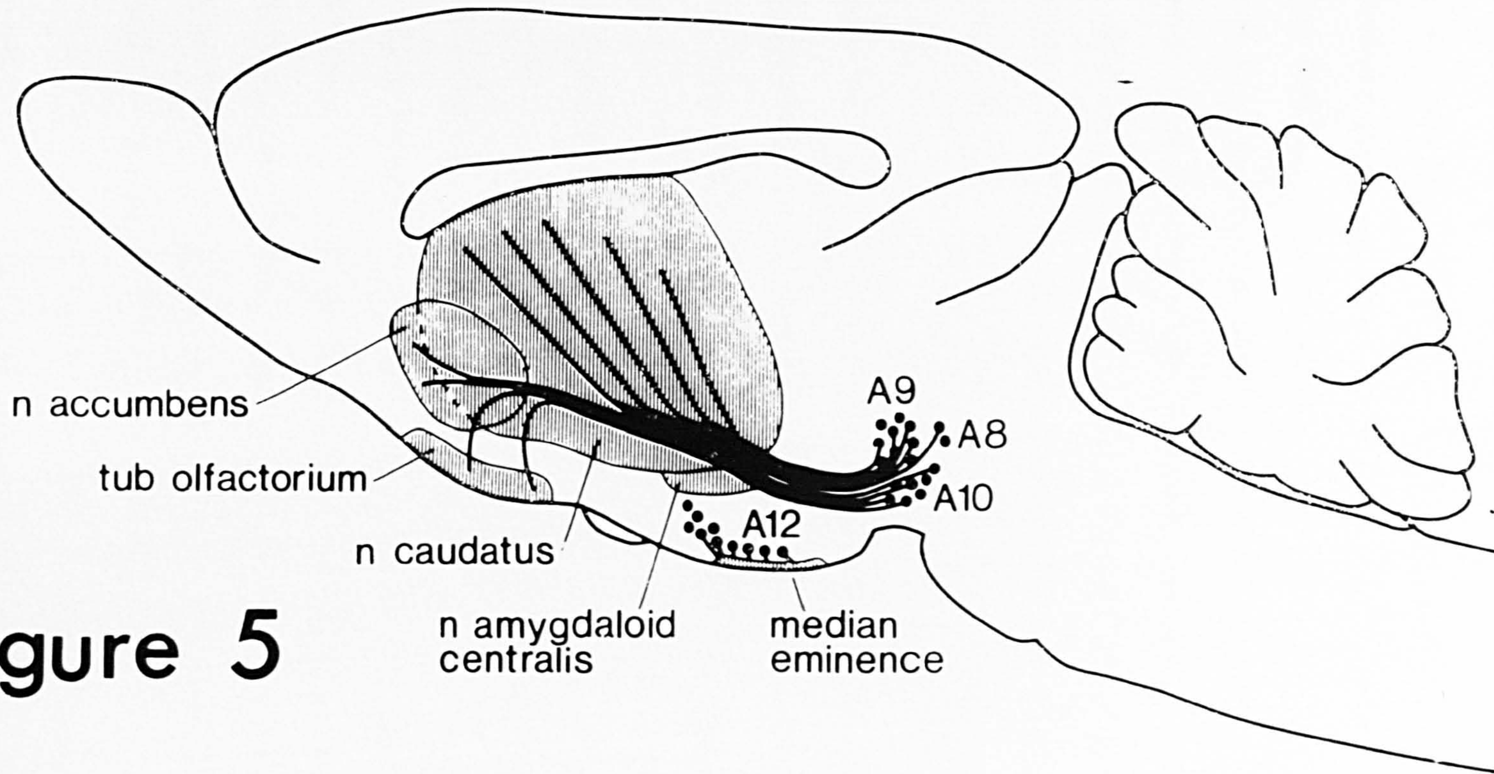


Figure 5

as being generally of medium size (15.1 $\pm$ 2.7μ average diameter). These are probably equivalent to the medium sized neurones whose morphology has been described by Gulley and Wood (1971) and which we have already noted. The pars compacta nigro-striatal neurones are described by Faull and Mehler as being oval and multipolar, though Fallon et. al. (1978), following treatment for the glyoxylic acid histofluorescence technique, described them as being pyramidal. The pars compacta DA neurones are also known to possess dendrites - Gulley and Wood (1971) estimate between four and six per neurone - which possess fluorescent varicosities (Fallon et. al., 1978) and project ventrally and laterally into the zona reticulata.

Fallon et. al., (1978) also noted the presence of histofluorescent neurones within the pars compacta which were fusiform, not pyramidal, and which possessed non-varicose dendrites extending medially and laterally but contained solely within the pars compacta. These neurones formed a dorsal sheet above the others. Two other important aspects of the striatal projections originating in substantia nigra need to be emphasised. Both Faull and Mehler (1978) and Bentivoglio et. al. (1979) observed a nigro-striatal projection originating in the zona reticulata, the neurones of which were slightly smaller than those of the pars compacta though cytoarchitecturally similar. These reticulata-striatal neurones are thought by some to be dopaminergic (Dahlström and Fuxe, 1965) but by others to be non-dopaminergic (Guyenet and Aghajanian, 1978). (See also Chapter 2, section iv ai). It must also be noted that the pars compacta does not contain exclusively DA cells. Both Fallon et. al. (1978) and Sotelo (1971) observed the presence of small, non-dopaminergic neurones within pars compacta, which appear as a non-specific fluorescent population. Moreover, Sotelo (1971) identified

these neurones as noradrenergic.

The firm anatomic distinction between mesolimbic and nigro-striatal projections is being questioned by some authors. The compacta neurones observed by Fallon et. al. (1978) to form a dorsal sheet above the "classic" neurones of this area were also found to be labelled by HRP retrogradely transported from the lateral olfactory tubercle or the amygdala- accepted mesolimbic DA projection areas - while the more numerous "classic" pyramidal cells were labelled by striatal HRP, as expected. Fallon et. al. conclude that the 'V.T.A./A10/mesolimbic' and 'substantia nigra/A9/nigro-striatal' distinction is inadequate. "Both ventral tegmental and nigral DA neurones form a continuous group which can be subdivided on the basis of neuronal morphology and differential projections into distinct functional groups" (Fallon et. al., 1978). This attitude is echoed by some anatomists in considering terminal regions, where, for example, the nucleus accumbens has been suggested to be a ventral extension of the striatum (Nauta et. al., 1978).

While such anatomical debates do not prevent us from studying the substantia nigra or, more specifically, the pars compacta, it must be recognised that there is more to it than one projection, and that other areas, even within the substantia nigra and adjacent areas, are still at liberty to project to the striatum.

iii. Origin, Organisation and Course of the Nigro-Striatal Projection

That the nigro-striatal pathway is primarily derived from the pars compacta has been demonstrated by several techniques. (Anterograde

degeneration (Carpenter and Peter, 1972); retrograde degeneration (Butcher and Talbot, 1978); HRP retrograde tracing (Kuypers et. al., 1974; Nauta et. al., 1974)). The projection is believed to display a medio-lateral topographical organisation (Butcher and Talbot, 1978). Consequently, medial aspects of the pars compacta display the greatest cell loss following lesion of medial portions of striatal terminal areas while lateral striatal lesions cause cell loss within lateral aspects of the nigra. Such medio-lateral organisation, as Butcher and Talbot (1978) point out, explains why the posterior portion of the caudate-putamen is innervated by the lateral pars compacta. This is because posterior aspects of the striatum are, in mammals, extensions of the lateral striatum. Several observations bear this out; lesions of substantia nigra which spare lateral portions result in much greater terminal degeneration within anterior rather than posterior portions of the neostriatum (Moore et. al., 1971) while 6OHDA lesions of the caudo-lateral substantia nigra reduce caudate-putamen DA levels preferentially in posterior regions (Ungerstedt, 1971a).

The nigro-striatal fibres initially course rostro-medially and dorsally from the pars compacta (Petrovicky, 1972). Fibres derived from more caudal regions maintain this course until they merge with the fibres arising from rostral regions, after which the whole pathway turns sharply towards the midline (Lindvall and Bjorklund, 1974). However, the fibres do not extend as far as the midline, the most medially placed axons remaining at least 0.4mm away from it (Butcher and Talbot, 1978). While coursing through the midbrain, the medio-lateral organisation is maintained: fibres from the medial pars compacta lie medio-ventrally while those from lateral pars compacta appear latero-dorsally (Butcher and Talbot, 1978).

Having formed a compact bundle in the anterior mesencephalon, the nigro-striatal projection turns rostrally and courses through field H_2 of Forel (Dahlström and Fuxe, 1965; Lindvall and Bjorklund, 1974). At this point most of the tract lies dorso-lateral to the medial forebrain bundle (M.F.B.) and not within it. Only a minor ventro-medial segment appears within the M.F.B. (Thus small dorso-lateral hypothalamic lesions result in a severe reduction in the number of pars compacta cell bodies while extensive M.F.B. lesions spare these somata almost entirely (Butcher and Talbot, 1978).)

While in field H_2 of Forel the nigro-striatal pathway lies immediately dorso-medial to the subthalamic nucleus (S.T.N.) (Lindvall and Bjorklund, 1974; Butcher and Talbot, 1978), a nucleus which, as previously seen, receives fibres from, and projects to, the substantia nigra. Whether or not the nigro-striatal pathway sends collaterals which synapse within the S.T.N. is not clear. The nigro-striatal path continues rostrally through the ventral thalamus, initially ventral to the rostral portion of the zona incerta and subsequently inferior to the caudal two-thirds of the thalamic reticular nucleus. (Jacobowitz and Palkovits, 1974), eventually taking a position adjacent to and partially within the ventro-medial portion of the internal capsule (Hedreen, 1971; Lindvall and Bjorklund, 1974; Ungerstedt, 1971a). As the projection travels rostrally through the ventral thalamus it gradually diminishes in size as constituent fibres arch laterally, dorsally and rostrally through the internal capsule toward the ipsilateral corpus striatum (Lindvall and Bjorklund, 1974; Ungerstedt, 1971a). This dissociation begins caudally in the S.T.N. (Lindvall and Bjorklund, 1974) and the entopeduncular region (Hedreen, 1971) and ends rostrally at central levels of the globus pallidus (Jacobowitz and Palkovits, 1974; Lindvall and Bjorklund, 1974). The most dorsal

nigro-striatal fibres leave caudally while the ventral fibres only depart at rostral levels (Lindvall and Bjorklund, 1974). While within the ventral thalamus the fibres are arranged not just dorso-ventrally, as in the ventral mesencephalic tegmentum (V.M.T.), but also, to a lesser extent, medio-laterally. This dorso-lateral/ventro-medial topography has been described by Lindvall and Bjorklund (1974); dorso-laterally situated axons are derived from the lateral portions of the pars compacta while the ventral axons may be divided into two groups, superior and inferior. The superior group arises in the medial pars compacta and projects to the centro-medial and rostro-medial portions of the corpus striatum. The inferior group, on the other hand, emerges from the lateral pars compacta and projects to centro-lateral and rostro-lateral aspects of the striatum.

Whilst within the internal capsule, the nigro-striatal neurones arrange themselves in the pattern which will determine where within the striatum the fibres will arrive and terminate. Butcher and Talbot (1978) describe this particularly lucidly. "Especially when viewed in horizontal sections .... the pattern of nigro-neostriatal axons crossing the internal capsule resembles an open fan, the hub of which is the ventromedial edge of the internal capsule . Not surprisingly, the 'spokes' of the fan are arranged such that those radiating across the internal capsule caudally, centrally and rostrally are directed towards the caudal, central and rostral neostriatum respectively" (p.69). The nigro-striatal projection may be divided into three components, best classified by reference to their striatal terminal areas as a caudal group, centro-medial and rostro-medial group, and centro-lateral and rostro-lateral group. The projection arborizes extensively on entering the striatum. This accounts for several observations; the

larger a striatal lesion is, the greater the degree of nigral cell loss (Anden et. al., 1965a); lesions of the substantia nigra - even those which are only partial - cause dense homogeneous terminal degeneration within the striatum (Shimizu and Onishi, 1973); despite the prodigious size of the striatum, nigral efferents (at least in rodents) are mainly arranged medio-laterally rather than rostro-caudally. Indeed, it would appear that the nigral input to the striatum is more extensive than the cortical one, which in primates at least is often restricted to irregular clusters or bands of terminals (Goldman and Nauta, 1977; Jones et. al., 1977). This anatomical imbalance suggests that the substantia nigra has a more powerful control over the basal ganglia than does the cortex. Cortico-striatal fibres are presumed to relay analyses of sensory-motor information to the striatum topographically (Jones et. al., 1977) while the nigral afferents have a more long-term role in modifying the activity of striatal neurones. Indeed, the profound movement disorders of Parkinson's disease are associated with a marked cell loss in the pars compacta (Hassler, 1938) which clearly points to the tremendous importance of these fibres in normal motor functioning.

iv. <u>Inputs to the Dopamine Neurones of Pars Compacta</u>

When we studied the anatomy of the substantia nigra (Chapter 2, section ivb) we noted that it received two major inputs, and three smaller ones; the GABA and substance P afferents which descend from the globus pallidus and the caudate-putamen complex, the diffuse serotonergic projection from the dorsal raphe nucleus, the small, apparently dopaminergic projection from the subthalamic nucleus, and a projection from the nucleus accumbens. Now, in an attempt to

understand from where the nigro-striatal projection receives afferent input, these projections will be examined in terms of their relationships with the dopaminergic neurones of pars compacta, as well as discussing the role of dendritically released DA itself.

iva. The GABA and SP Mediated Striato-Nigral Projections

Bunney and Aghajanian (1976), as we have already seen, have demonstrated the existence of a striato-nigral pathway which arises in the caudate-putamen complex, while a pathway from the globus pallidus to the nigra has been demonstrated by Kanazawa et. al. (1976). It appears possible that the pallido-nigral projection utilises GABA as a neurotransmitter while the caudato-nigral projection is mediated by substance P. (However, this distinction is not absolutely clear; see Chapter 2, section ivb).

The striatal input to the substantia nigra has recently been investigated by Arbuthnott (1978). Having traced the course of the pathways following radioleucine labelling he describes the results. "The close similarity between the topographical arrangement of the DA system to the striatum and the striatal input to the same area was striking, and suggested that parts of the striatum are linked to particular areas of nigra by connections in both directions. This arrangement would perhaps be an advantage if the striato-nigral fibres carried information about the effectiveness of a DA input back to its source in substantia nigra" (p.240). That an inhibitory striato-nigral GABA projection fulfilled such a feedback role had previously been hypothesised by Bunney et. al.(1973) who observed that blockade of DA receptors by neuroleptic drugs increased both DA turnover in the

striatum and the firing of pars compacta neurones. d-Amphetamine had exactly the reverse effects. These authors conjectured that these effects were caused by neuroleptics effectively cutting off a feedback pathway, releasing the pars compacta neurones from an inhibitory influence. This concept of an inhibitory neuronal feedback, GABA-mediated, gained rapid, widespread acceptance. However, it has recently been observed that lesions which decrease both the GABA and GAD content of the substantia nigra without directly affecting DA terminals in the ipsilateral striatum, do not alter DA turnover in that structure, and have no impact on the effects of haloperidol or apomorphine in either contra- or ipsilateral striata (Arbuthnott, 1978). Groppetti et. al. (1978) confirm this, showing that crus cerebri lesions, which should effectively cut the supposed feedback pathway fail to affect the haloperidol induced increases in striatal DA metabolite concentration, again suggesting very strongly that the role of the striato-nigral GABA pathway is not a simple one of feedback on to the DA cells. The likelihood that a SP pathway instead fulfills this role is not strong. Arbuthnott (1978), in discussing his findings, raises the possibility that the lesions which he reported were too small to be effective. However, he was forced to dismiss this, and the alternative idea of SP action, by taking into account results which demonstrated that far more extensive lesions interrupting striato-nigral transmission have the same effect. Indeed, extensive lesions of pallido-nigral and striato-nigral paths similarly fail to affect the supposed feedback pathway. Thus we may conclude that the striato-nigral pathways do not fulfill a simple feedback role, though there is evidence that they do indeed interact with the DA neurones of pars compacta. (See this Chapter, section ivb.)

The GABA pathway is hypothesised as being a major striatal output. It appears that DA activity in the striatum stimulates this descending pathway which then activates nigral neurones in the zona reticulata, which project to the thalamus, tectum and the dorsal raphe nucleus. Anatomical evidence for such a system has recently been presented by Somogyi et. al. (1979) who show connections between descending striato-nigral neurones and zona reticulata neurones projecting to the thalamus. There is also a growing list of behaviours previously ascribed to nigro-striatal DA neuronal control, which may be linked to other nigral projection areas. (For instance, catalepsy elicited from the thalamus (Di Chiara, et. al., 1979b); rotation from the superior colliculus (Crossman and Sambrook, 1978; Winterkorn and Meikle, 1980); stereotyped gnawing from the superior colliculus (Dean et. al., 1980) and substantia nigra zona reticulata (Arnt and Scheel-Krüger, 1980); aphagia following thalamic lesions (Shumway and Lindholm, 1978).)

It is becoming apparent that the DA neurones of pars compacta interact to a large extent with the striato-nigral GABA system. Within the striatum, as we noted, the topographical arrangement of each path bears close similarity (Arbuthnott, 1978). As such it is now thought that the GABA system relays much dopaminergically mediated information back through the substantia nigra to the thalamus and tectum. As such, the nigra is seen as an output station for striatal efferents.

The precise role of substance P within the substantia nigra is harder to define; presumably it acts as an excitatory neurotransmitter (or possibly neuromodulator*). In support of this are observations of an

*For definition of "neuromodulator", see Appendix i).

increased striatal DA metabolism following SP infusion (Waldmeier et. al., 1978) or perfusion (Cheramy et. al., 1977) into the substantia nigra. Moreover, Michelot et. al. (1979) observed SP to stimulate DA release in the striatum and simultaneously reduce the dendritic release of DA within the ipsilateral substantia nigra. Neither contra-lateral striatum, nor substantia nigra were similarly affected by the treatment. However, the behavioural responses to intra-nigral SP have been unusual. If the peptide had a straight-forward stimulating action on pars compacta DA neurones, then characteristic dopaminergic behaviours might be expected. Kelley and Iversen (1978) were able to elicit whole body locomotion with either the first or second intra-nigral micro-injections, but the third or fourth elicited only grooming behaviour. Neither of these would be anticipated if SP simply excited DA systems.

While much effort is currently being expended in reassessing the role of the striato-nigral pathways - from feedback functions to striatal output - it must not be forgotten that these neurones do interact with nigral DA neurones. Using slices of substantia nigra, Reubi et. al. (1978) found that DA released within the nigra binds only to GABA containing neurones, and not to those containing substance P. These authors discuss their results and speculate that "the selective enhance-ment of the spontaneous release of GABA .... may provide a .... subtle mechanism for controlling the firing rate of dopaminergic neurones; thus during intense striato-nigral inhibiting activity the excitatory effects of DA ... (released in nigra on to the afferent GABA neurones) ... may be abolished."

ivb. <u>DA Release within the Substantia Nigra</u>

The demonstration by Reubi et. al. (1978) alerts us now to the fact

that DA is active within the substantia nigra, where DA has been shown to be liberated from dendrites (Geffen et. al., 1976). These dendrites " ... project and ramify abundantly into the pars reticulata ... (from the pars compacta) ... to form a DA-containing varicose dendritic network" (Bjorklund and Lindvall, 1975). Reubi and Sandri (1979) have investigated potential morphological correlates of this dendritic release. They have shown that dendro-dendritic synapses are not formed: the pars compacta cell dendrites are always separated from post-synaptic structures by glial elements which in turn are connected by multiple gap junctions. They also observed, rather curiously, no dendro-axonic synapses but noted the presence of large, specialised symmetrical junctions which could form the morphological basis of dendritic DA release. Hefti and Lichtensteiger (1978a) have shown, by examination of particle formation in response to amphetamine and potassium, that dendritic and terminal release of DA from nigro-striatal neurones are different. They also observed (Hefti and Lichtensteiger, 1978b) that dendrites were able to form particles which behaved like synaptosomes on density gradient centrifugation, and which they termed dendrosomes. They estimate from the proportion of DA found in the particulate fractions that between 40 and 60% of the nigral DA is found in dendrites.

Evidence for the existence of DA receptors in the substantia nigra is also strong. A DA-sensitive adenylate cyclase has been discovered there (Phillipson and Horn, 1976; Spano et. al., 1977) which appears to lie outside the DA neurones themselves (Kebabian and Saavedra, 1976; Gale et. al., 1977). Moreover, spiroperidol has been found to bind selectively to neurones which descend from the striatum (McGeer et. al., 1979), suggesting that the DA receptors are located on these afferents.

In support of this, Gale et. al. (1977) found that intranigral injections of 6OHDA failed to abolish the response of adenylate cyclase to DA, although lesions of the striato-nigral pathways did. As previously noted, Reubi et. al. (1978) have isolated which of the two striato-nigral pathways is contacted by dendritic DA: only GABA, and not substance P, is liberated by intra-nigral DA. This interaction between the DA neurones and the descending GABA neurones is intriguing, control over the firing of the DA neurones probably being exerted both by descending GABA fibres and the DA neurones themselves. This appears to represent a good example of McGeer's (1979) postulate that "communication at many C.N.S. synapses may involve a .... dialog rather than a monolog".

Glowinski and his collaborators have noticed that the terminal release of DA in the nigra is inversely correlated with the terminal release of DA in the ipsilateral caudate nucleus, but that it is positively correlated with the release of DA in the contralateral caudate. Thus dopaminergic agonists released within the substantia nigra (of the cat) by push-pull perfusion enhance dendritic release of DA, decrease DA activity in the ipsilateral caudate but increase it in the contralateral caudate; the reverse was found to be the case if dopaminergic antagonists were perfused into the nigra (Leviel et. al., 1979a). This pattern of activity has been observed by Glowinski's group following unilateral stimulation of the cerebellar dentate nucleii, unilateral sensory stimulation, or unilateral stimulation of the motor cortex, and appears to confirm the important role these DA systems, and indeed the substantia nigra as a whole, have in modulating sensory-motor activity. Bilateral changes in DA activity have also been observed following unilateral nigral application of GABA-ergic drugs or glycine (Leviel et.al., 1979b), though, as previously

noted, unilateral perfusion of SP in substantia nigra does not alter contralateral DA activity, only unilateral (Michelot et. al., 1979).

However, although the French group have built an impressive array of evidence concerning dendritic DA release and concomitant striatal activity, results from another laboratory (Wuerthle et. al., 1979) indicate different results. Using rats, these authors failed to alter the concentration of DOPAC in the striatum following unilateral intra-nigral microinjections of the DA agonist apomorphine, but reduced both DA and DOPAC within the nigra. They concluded that "if DOPAC concentrations reflect nigro-striatal dopaminergic neuronal activity, the present results suggest that dopaminergic agonists and antagonists acting in substantia nigra do not exert a major influence on the activity of these neurones". Whether the discrepancy between these authors' findings and those of the French group are due to incorrect assumptions concerning DOPAC, technical differences, or species differences, remains to be seen.

ivc. <u>Other Systems Interacting with DA in the Substantia Nigra</u>

Serotonin-Dopamine interactions within the nigro-striatal system have been studied by Waldmeier and Delini-Stula (1979). They found that uptake inhibitors of serotonin were able to potentiate haloperidol induced catalepsy and to potentiate the antagonistic effects of haloperidol on apomorphine induced stereotypy. However, it is not possible to ascertain whereabouts within the nigro-striatal DA system these effects were mediated. Lesion studies have shown that the striatum is innervated by a serotonergic projection from the dorsal raphe nucleii (Geyer et.al., 1976; Ternaux et. al., 1977) and the

substantia nigra also receives a projection from this area. (See Chapter 2, section ivb.)

We have already seen that glycine has effects upon both ipsilateral and contralateral DA systems following unilateral nigral infusion. It is hypothesised to act as a tonic inhibitory neurotransmitter (Cheramy et. al., 1978). Glycine reduced the amount of DA released in both ipsi- and contra-lateral striata: this effect was abolished by strychnine and was mimicked by diazepam. Potassium or GABA induced the opposite effect (Leviel et. al., 1979b).

Opiates also appear to be active within the substantia nigra. While levels are not high, amounts of met-enkephalin have been found within the nigra (Yang et. al., 1978) and opiate receptors have been observed (Pollard et. al., 1978). Gale et. al., (1979) found that the binding of ^{3}Henkephalinamide within substantia nigra was not affected by 6OHDA lesions of the nigro-striatal path, but were decreased by approximately 40% after brain hemitransection, suggesting localisation on neurones descending from the striatum. They also observed that morphine injected into the substantia nigra failed to affect DA metabolism in the striatum but blocked haloperidol induced increases in striatal tyrosine hydroxylase. Gale and her colleagues conjectured that the opiate affected DA activity by adopting a neuro-modulatory role with regard to the striato-nigral neurones. Interestingly, characteristic DA behaviours have been observed by Iwamato and Way (1977) following manipulation of nigral opiate systems.

It is clear that the nigro-striatal DA system receives information from a variety of sources: the striatum, via GABA and SP mediated

systems, the dorsal-raphe nucleus, through an inhibitory serotonergic projection and possibly through the intervention of other putative neurotransmitters and neuromodulators such as enkephalin and glycine. Indeed, DA also appears able to modify the release of some of these neurochemicals and selectively to enhance or depress their effects by a mechanism of dendritic release. Even reciprocal control between the two DAergic nigro-striatal systems has been postulated by Glowinski and his colleagues.

However, there remains one interaction which we have not yet discussed in detail. This interaction, which has been sadly neglected, is between acetylcholine, one of the first neurotransmitters to be identified, and dopamine, and it will be the subject of the next chapter.

Chapter 4: Dopamine-Acetylcholine Interactions and the Nigro-Striatal Pathway

The dopaminergic nigro-striatal pathway interacts with cholinergic systems in both the striatum and the substantia nigra. Each of these will be examined, but most emphasis will be placed on the interaction within the substantia nigra, the real point of interest of this thesis.

i. Acetylcholine-Dopamine Interactions in the Striatum

The dopaminergic nigro-striatal projection appears to synapse in the striatum upon a dense population of cholinergic inter-neurones (Butcher and Butcher, 1974a; Hattori et. al., 1976).

A wealth of pharmacological evidence indicates that DA released from nigro-striatal neurones has an inhibitory influence on cholinergic interneurones. Dopaminergic agonists increase striatal ACh concentration and decrease turnover, while antagonists have the reverse effect. Table 1 presents a summary of some of these findings, which have used pre- and post-synaptically acting agonists, neuroleptics, atypical neuroleptics and specific D_2 (Kebabian and Calne, 1979) receptor agonists and antagonists. All of this evidence points to DA having an inhibitory influence on cholinergic interneurones in the striatum.

On the other hand, there are electrophysiological studies which demonstrate that DA might have an excitatory function in the striatum (Kitai et. al., 1976 and 1978; Norcross and Spehlmann, 1977 and 1978). Kitai et. al. (1978) further claim that "the initial

Table 1

Drug	Effect on Striatal ACh concentration	Effect on striatal ACh turnover	Reference
DA agonists			
Apomorphine	Increase		Consolo et. al. 1974
	Increase		Guyenet et. al. 1975
	Increase		Sethy & Van Woert 1974
		Decrease	Racagni et. al. 1976
		Decrease	Trabucchi et. al. 1975
Amphetamine	Increase		Consolo et. al. 1974
		Decrease	Trabucchi et. al. 1975
Piribedil	Increase		Consolo et. al. 1974
L-DOPA	Increase		Sethy & Van Woert 1974
		Decrease	Trabucchi et. al. 1975
Trivastal	Increase		Sethy & Van Woert 1974
Bromocriptine*	Increase		Sethy 1979
Lergotrile*	Increase		Sethy 1979
Desipramine	Increase		Consolo et. al. 1974
Amantadine	Increase		Consolo et. al. 1974
DA antagonists			
Chlorpromazine	Decrease		Guyenet et. al. 1975
	Decrease		Sethy & Van Woert 1974
	No effect on steady-state concentration	Increase	Trabucchi et. al. 1974
Haloperidol	Decrease		Sethy & Van Woert 1974
	Decrease		Consolo et. al. 1975
	No effect on steady-state concentration	Increase	Trabucchi et. al. 1974
	Decrease		Guyenet et. al. 1975
	Decrease		Sherman et. al. 1978
	No effect on steady-state concentration	Increase, and reverses apomorphine induced decrease	Racagni et. al. 1976
Clozapine	Dose dependent decrease	No effect, but reverses apomorphine induced decrease	Racagni et. al. 1976
	Failed to reverse apomorphine induced increase		Consolo et. al. 1975
Spiroperidol	Decrease		Sherman et. al. 1978

Table 1 continued

Drug	Effect on Striatal ACh concentration	Effect on striatal ACh turnover	Reference
Metoclopramide	Decrease		Sethy 1979
Molindone*	Decrease		Sethy 1979
Reserpine	Decrease		Consolo et. al. 1975
Pimozide	Decrease Decrease		Sethy & Van Woert 1974 Consolo et. al. 1975
Fluphenazine	Decrease		Sherman et. al. 1978
L-Fenfluramine	Failed to reverse apomorphine induced increase		Consolo et. al. 1975
Trihexphenidyl (anti-muscarinic)	Decrease		Consolo et. al. 1974

Table 1: Summary of the Effects of Various Dopaminergic Agonists and Antagonists on Acetylcholine Concentration and Turnover in the Striatum

*These drugs are specific D_2 agonists or antagonists (Kebabian and Calne, 1979) Sethy (1979) concludes that the receptors on the ACh neurones are D_2 (non-adenylate cyclase linked); D_1 agents were not tested.

response of caudate neurones to either substantia nigra or nigro-caudate pathway stimulation is almost always excitatory" (Kitai et. al., 1978, p.170). There are several possible explanations for these results, the first being simply that nigro-striatal DA does have genuine excitatory and inhibitory properties. The DA receptors within the striatum do not appear to be of one type (Kebabian and Calne, 1979) some being mediated by DA-sensitive adenylate cyclase, while others are not, the two types having different affinities for various dopaminergic drugs and differing distributions throughout the striatum (Cools and Van Rossum, 1976). A possible source of the controversy lies in the technique. Difficulties of microiontophoresis have already been noted (Chapter 1, section iii), but it is of more interest to note that all of the authors cited above as observing excitatory DA effects examined the modification of the effects of substantia nigra or nigro-striatal pathway stimulation using microiontophoretic application of dopaminergic agents in the striatum. In a study examining the effects of microiontophoretically applied DA on spontaneous neural activity in the striatum, Bloom et. al. (1965) predominantly observed a depression of firing. A complex relationship exists between the nigro-striatal terminals and the cholinergic interneurones (see below). It might thus be hypothesised that the application of DA to terminals where strong activity has already been induced will show different effects compared to the effects of DA applied to non-activated neurones, inducing an apparent excitation instead of inhibition.

Cholinergic receptors are known to exist in the striatum. The presence of nicotinic receptors, labelled by snake toxins, has been demonstrated (Salvaterra et. al., 1975; Tindall et. al., 1978) although other authors report either low binding or an absence of

either α-bungarotoxin or naja naja siamensis α toxin binding (Polz-Tejera et. al., 1975; Morley et. al.,1977; Speth et. al., 1977). Muscarinic binding has been demonstrated using radio-labelled quinuclidinyl benzilate (QNB) (Kobayashi et. al., 1978; Kuhar and Yamamura, 1975 and 1976). Little controversy surrounds the existence in the striatum of muscarinic cholinergic receptors. However, it is worth noting that some authors are cautious when identifying CNS receptors for ACh. McLennan and Hicks (1978) observe that "... the nature of the ... (cholinergic) ... receptors in the rat is not easily described as being either nicotinic, muscarinic or mixed; they rather appear to lack selectivity toward the pharmacological agents with which they interact."

Evidence has also been presented to show that pre-synaptic receptors exist on the dopaminergic nerve terminals in the striatum. De Belleroche and Bradford (1978) demonstrated that ACh stimulated the release of DA from synaptosomes prepared from rat or sheep striata. In the presence of the muscarinic antagonist atropine, ACh stimulated DA release, whereas in the presence of nicotinic receptor antagonists ACh inhibited DA release, presumably indicating the existence of excitatory nicotinic and inhibitory muscarinic pre-synaptic receptors on the terminals of the nigro-striatal pathway. McGeer et. al.,(1979) identified nicotinic presynaptic receptors in the striatum, as well as muscarinic receptors on neuronal perikarya and dendrites. Both muscarinic and nicotinic presynaptic receptors have been identified by Giorguieff et. al. (1977) in a clever series of pharmacological studies. Moreover, Giorguieff-Chesselet et. al. (1979) have shown that ACh stimulates the release of DA from inactive nigro-striatal terminals, but has no effect on them once these neurones have been

activated by SP infusion into the substantia nigra. A complex rate-dependent effect thus appears to exist between the presynaptic receptors and DA neurones.

ii. Cholinergic Substances in the Substantia Nigra

iia. Acetylcholine in Substantia Nigra

Jacobowitz and Goldberg (1977) localised ACh to both the pars compacta and zona reticulata, slightly higher concentrations being observed in the reticulata (0.28 ± 0.06 pmoles/μg protein in zona reticulata; 0.20 ± 0.01 pmoles/μg in pars compacta.) These amounts are not large, being approximately 50% of the very high levels found in the striatum (Caudate-putamen, 0.41 ± 0.04 pmoles/μg protein). The question must thus arise, is there a physiologically significant amount of ACh within the substantia nigra? Butcher and Talbot (1978) conclude that there is, basing their argument on the relative amounts and relationships between ACh and choline acetyltransferase (CAT) (the synthetic enzyme of ACh) in the striatum and substantia nigra. They reason as follows:

"From data reported by Kuczenski et. al. (1975), the level of CAT in the rat nigra is 17.7% that of the relatively very high enzyme activity in the caudate-putamen nucleus of the same species. Although lower nigral/neostriatal CAT percentages in the rat are derived from other studies (2.5% and 8.9% for whole substantia nigra: Cheney et. al. 1975 and McGeer et. al., 1973 respectively; 8.8 and 3.1% for the pars compacta and pars reticulata of the substantia nigra respectively: Kobayashi et. al., 1975), the level of ACh in

the rat nigra is itself 18.3% that of the relatively very high caudate-putamen level of ... (ACh) ... in the same species (derived from Cheney et. al., 1975), corresponding closely to the nigral/ neostriatal CAT percentage calculated from Kuczenski et. al. (1975). Considering that the quantity of ACh found in the rat neostriatum is between 81 and 98 nM/g tissue ... (a very high level) ... (Butcher and Butcher, 1974b; Butcher et. al., 1976), there is reason to believe that the substantia nigra as a whole contains a physiologically significant amount of ACh" (Butcher and Talbot, 1978, pp. 72-73).

Such an argument is actually able to prove very little. Although the nigral/striatal ACh and CAT ratios can, by selecting the appropriate figures, show a similarity, this does not necessarily imply that the same functional and physiological relationships are maintained. It is thought, for instance, that the regulation of ACh in the striatum is different from that in any other brain region (Sherman, et. al., 1979). However, the observation that ACh and CAT show similar ratios across the substantia nigra and striatum (where a physiological role for cholinergic systems is not doubted) is of interest. As will shortly be seen, other cholinergic substances within the substantia nigra (Acetylcholinesterase; this Chapter, Section iic) do show marked responses to physiolgical stimulation, and modification of nigral cholinergic systems results in behavioural changes (this Chapter, section iiic). All of these together suggest that a cholinergic system is active within the substantia nigra, both physiologically and functionally. The presence of cholinergic receptors is further evidence: De Montis et. al. (1979) demonstrated muscarinic receptors to be present in the whole substantia nigra, and the receptor density to fall by 17% following kainic acid lesions. (Separate analyses of the pars compacta and zona reticulata were not

made; nor was any examination of nicotinic receptors made.) Kobayashi et. al. (1978) also observed muscarinic receptor binding in substantia nigra following (^{3}H) QNB labelling. Although the level they found was low (116 $\pm$ 7 pmol/g protein (^{3}H) QNB) when compared to the caudate-putamen (625 $\pm$ 43 pmol/g protein (^{3}H) QNB), where a high density of muscarinic receptors exist, it is interesting to note that Butcher and Talbot's (1978) argument for a physiologically significant amount of ACh in substantia nigra can be extended to receptors, where the nigra contains 18.56% the amount of (^{3}H) QNB binding of the striatum, a comparative figure for CAT being 17.7% and for ACh, 18.3%.

iib <u>Choline Acetyltransferase in Substantia Nigra</u>

As became apparent in the preceding section, choline acetyltransferase (CAT) has been found within substantia nigra (Kuczenski et. al., 1975; Cheney et. al., 1975; McGeer et. al., 1973; Kobyayashi et. al., 1975). While CAT is known to be present its exact location is something of a mystery. Transection between the striatum and the substantia nigra causes nigral GABA and GAD levels to fall, while levels of ACh and CAT remain unchanged (Fonnum et. al., 1974; McGeer et. al., 1974). McGeer et. al. (1973) have shown that hemitransections posterior, as well as anterior, to the nigra, also have no effect on cholinergic parameters there. These observations might be conveniently explained by hypothesising that the CAT is contained within the interneurones observed by Gulley and Wood (1971). (See Chapter 2, section iii.) This would be consistent with Kuczenski et. al.'s (1975) observation that nigral CAT is most commonly associated with cell bodies. Unfortunately, one piece of evidence stands in direct opposition to this hypothesis. Nagy et. al. (1977) found the

CAT content of substantia nigra to be unchanged following kainic acid lesions (which destroy cell bodies by binding to glutamate receptors). They concluded that CAT could not be contained within either interneurones or efferent projections. It is possible that for some reason, the kainic acid lesions spared a hypothetical cholinergic interneurone. As Nagy et. al. (1977) used a dose (5 nmoles kainic acid) and injection rate (1µl/5 min) that other authors have concluded are effective (McGeer and McGeer, 1978) for inducing lesions the most likely reason for failure might be an absence of glutamate receptors on the interneurones. This, however, is speculative: the exact location of CAT within substantia nigra remains in doubt.

iic <u>Acetylcholinesterase in Substantia Nigra</u>

Acetylcholinesterase (AChE) is the most prevalent cholinergic substance within substantia nigra, and indeed, within nigro-striatal neurones. It appears to be transported somato-fugally along the nigro-striatal pathway, colchicine injections (which block axonal transport) giving rise to a proximal accumulation of AChE in fibres of this system (Lehmann et. al., 1979). Moreover, AChE has been localised to striato-nigral fibres (Olivier et. al., 1970; Kaiya et. al., 1979) although other authors have been unable to show an accumulation of AChE in these following colchicine injections (Lehmann et. al., 1979).

The existence of AChE within nigro-striatal neurones has been known for many years (Shute and Lewis, 1963, 1967; Jacobowitz and Palkovits, 1974; Butcher, Talbot and Bilezikjian, 1975; Butcher and Bilezikjian, 1975; Lehmann and Fibiger, 1978; Butcher and Talbot, 1978; Meibach and Weaver, 1979). While these demonstrations have

clearly shown AChE to be contained within nigro-striatal neurones, none has provided the critical demonstration that AChE is contained within dopaminergic nigro-striatal neurones. Recently, however, this has been done. By using fluorescence histochemistry and a pharmaco-histochemical treatment for AChE, on the same brain section, Butcher and Marchand (1978) were able to show that DA and AChE co-exist within the same nigro-striatal neurones.

Despite this demonstration of co-existence, we cannot be sure of the point of cholinoception. It is generally agreed that AChE does not reliably indicate this - there is for example, no correspondence between the distribution of AChE and (^{3}H) QNB (muscarinic receptor) binding in the feline neostriatum (Brand, 1980). It is also recognised that AChE is synthesised within somata and transported somato-fugally along axons (Jeffrey and Austin, 1973) and as we know that cholinergic receptors exist on the terminals of the nigro-striatal pathway we can assume that some of the AChE is used there. Indeed, Lehmann and Fibiger (1978) have argued that this is its sole function.

However, the precise localisation of nigro-striatal AChE must also be considered. Following histochemical treatment to display AChE-containing neurones, Butcher, Talbot and Bilezikjian (1975) observed that "some processes, probably dendrites, of the more ventrally situated neurones of the rostral zona compacta projected toward and into zona reticulata; this feature was observed more frequently in the medial and central portions of the rostral zona compacta than in the lateral aspect". The stained neurones possessed dendrites which also contained AChE, and these neurones, as Butcher and Marchand (1978) were later to show, are the DA neurones which form the nigro-striatal projection. Presumably this AChE is either synthesised within somata

and transported along the dendrites or synthesised <u>in situ</u> in the dendrites. In either case, it can hardly be destined for use at the striatal terminals. As such, we must presume that it has a function <u>within</u> the substantia nigra. Confirmation not simply of the existence of AChE in pars compacta dendrites but also of its release under stimulation has recently been provided by Greenfield et. al. (1980), greatly strengthening the argument in favour of a cholinergic function. Using cats implanted bilaterally with nigral and caudate nucleus push-pull cannulae, they observed a spontaneous release of AChE within both structures. Application of 30 mM potassium to one substantia nigra increased the local release of AChE and was accompanied by remote changes in enzyme release within the other three structures, which were associated with, but were different to, changes in DA release. AChE release was potentiated in the contralateral caudate and inhibited in the ipsilateral caudate and contralateral substantia nigra, while DA release was enhanced within all four structures.

We might therefore conclude that AChE is contained within the dendrites of pars compacta DA neurones and that it is released both spontaneously and under physiological stimulation. It must also be concluded that its most likely function is to hydrolyse ACh; that is, to inactivate a cholinergic input to the DA neurones.

iii. <u>Acetylcholine-Dopamine Interactions in the Substantia Nigra</u>

iiia. <u>Summary of Evidence Already Presented</u>

This evidence rests entirely with acetylcholinesterase which, as has just been observed (this Chapter, section iic) is contained within the DA neurones of the pars compacta (Butcher and Marchand, 1978).

While some of this AChE is probably synthesised in these somata but destined for transport to striatal terminals, some undoubtedly has a function within the substantia nigra. Demonstrations of dendritic storage (Butcher and Marchand, 1978) and release both spontaneously and under physiological stimulation (Greenfield et. al., 1980) suggest very strongly that there exists a cholinergic input to these neurones. The existence within substantia nigra of acetylcholine itself (Jacobowitz and Goldberg, 1977) choline acetyl-transferase (Kuczenski et. al., 1975) and muscarinic cholinergic receptors (Kobayashi et. al., 1978) make such an interaction possible.

iiib. Functional Studies of ACh-DA Interactions in Substantia Nigra: Electrophysiology

Several electrophysiological studies demonstrate that nigral ACh can affect nigro-striatal DA cells. Javoy et. al. (1974) found that carbachol increased the striatal content of DA; Aghajanian and Bunney (1974) observed ACh, microiontophoretically applied, to have no effect on pars compacta cells but to excite zona reticulata cells; Lichtensteiger et. al. (1976) found that peripherally applied physostigmine decreased striatal HVA concentration and inhibited the firing of nigro-striatal DA neurones, while nicotine induced a transient decrease in unit activity for 1 minute followed by a sustained increase accompanied by a marked rise in cellular fluorescence intensity; Dray and Straughn (1976) found that microionto-phoretically applied ACh excited cells in both the pars compacta and the zona reticulata.

Together, these results indicate that ACh might inhibit, excite or have no effect upon pars compacta DA neurones. However, all of

these studies adopted a similar approach in that they used anaesthetised or paralysed animals. (Javoy et. al. (1974) used a halothane/nitrous oxide/oxygen mixture; Aghajanian and Bunney (1974) immobilised with gallamine; Lichtensteiger et. al. (1976) urethane.) It can be argued that this approach might lead to misleading data by examining, as an analogy, the effects of d-amphetamine on striatal activity in anaesthetised and unanaesthetised animals. In anaesthetised animals, d-amphetamine produces a long-lasting depression of neuronal activity, occasionally preceded by a transient excitation (Groves et. al., 1974, 1975b; Rebec and Groves, 1975; Bunney et. al., 1973). However, in both awake cats (Trulson and Jacobs, 1979) and rats (Hansen and McKenzie, 1979) systemically administered d-amphetamine (1-10 mg/kg) induces a high rate of discharge which lasts for the duration of the drug's effects. Moreover, Huff et. al. (1979) have shown this to be accompanied by a dose-dependent increase in caudate DA release. Hansen and McKenzie (1979) almost point out the obvious in saying that "the discrepancy between data derived from freely moving and immobilised animals suggests that striatal activation ... may depend on sensory feedback from behaviour". As such it might be argued that the studies on anaesthetised animals might produce data which does not necessarily have any relevance to either the normal functioning of the nigro-striatal pathway, or the cholinergic/dopaminergic interaction within the substantia nigra.

iiic. Functional Studies of ACh-DA Interactions in Substantia Nigra: Behaviour

Very few studies have examined the behavioural effects of cholinergic substances applied to the substantia nigra. Two studies have examined

the effects of carbachol (an analogue of ACh with anti-cholinesterasic properties) and cholinergic antagonists injected into the zona reticulata, on turning behaviour. De Montis et. al. (1979) observed ipsilateral turning following unilateral intra-nigral carbachol injections and contralateral turning following scopolamine, a muscarinic receptor antagonist. Bilateral injections of scopolamine induced stereotyped behaviour while bilateral carbachol induced catalepsy, which was not antagonised by apomorphine, indicating its independence from DA systems. All of these injections were made into posterior aspects of substantia nigra, presumably therefore, missing the pars compacta. These authors concluded that cholinergic systems controlled posture by activating zona reticulata output neurones which GABA-ergic systems normally inhibit.

James and Massey (1978) present very similar results. Hemicholinium 3 (HC3), which causes a reduction in neuronal ACh content by blocking choline uptake, elicited contralateral rotation, while carbachol elicited ipsilateral when injected into the zona reticulata. However, James and Massey (1978) attempt to interpret their data as demonstrating an inhibitory cholinergic influence on the DA neurones of pars compacta. They claim that haloperidol attenuated the effects of intra-nigral HC3 while striatal DA and HVA levels in the striatum changed following nigral cholinergic modifiation. However, on closer inspection their data might be explained by cholinergic modification of zona reticulata output, as De Montis et. al. (1979) argue, because: (i) the dose of haloperidol used to attenuate the contralateral turning elicited by HC3 (2.5 mg/kg) is quite possibly sedative, and thus could not be said to have specifically attenuated the cholinergic induced rotation; (ii) HC3 depleted striatal DA and

increased HVA levels, while carbachol produced a non-significant rise (7%) in striatal DA and lowered HVA concentration. James and Massey interpret this as showing a net cholinergic inhibition of nigro-striatal transmission, by an action within substantia nigra on pars compacta cells. However, striatal DA and HVA changes over the time allowed (15 minutes) could have been mediated by nigro-thalamic/thalamo-striatal (Van Der Kooy, 1979) systems or have been induced by behavioural action alone; (iii) James and Massey observe that intra-nigral injection of α-flupenthixol (a DA receptor antagonist) blocks carbachol induced rotation. This they interpret as demonstrating a dopaminergic link in the action of ACh on pars compacta DA neurones. However, as nigral DA receptors have been localised to GABA-ergic afferent fibres (Reubi et. al. (1978) -Chapter 3, section ivb) it might be hypothesised that blockade of such receptors would prevent dopaminergic control of these neurones and consequently permit a GABA-ergic inhibition of pars compacta DA cells, or possibly zona reticulata cells. As it is recognised that these neurones do have a role in rotational behaviour (Chapter 5, section iia) a possible response competition masking the cholinergic response might have been established.

While the possibility of cholinergic inhibition as formulated by James and Massey is undoubtedly real, it might be more parsimonious to accept that their behavioural data reflect exactly the phenomena observed by DeMontis et. al. (1979), especially as all the injections were made into zona reticulata. The modification of the cholinergic response by haloperidol is quite conceivably due to a sedative action of the drug: doses of haloperidol as low as 0.4 mg/kg have been used to achieve non-sedative DA antagonism (Redgrave and Taha, 1980, in press) much lower than the 2.5 mg/kg used by James and Massey. The

neurochemical estimations might also be explained by various means. Until a direct action can be shown the more parsimonious explanation must be that carbachol elicits ipsilateral rotation from the zona reticulata by an action on non-dopaminergic output neurones.

Cholinergic substances injected into substantia nigra have also been shown to induce other characteristic dopaminergic behaviours. Smelik and Ernst (1966) observed that intranigral injections of crystalline physostigmine (an AChE inhibitor) in a high dose elicited compulsive gnawing, a behaviour commonly associated with DA stimulation (Ljungberg and Ungerstedt, 1978). Smelik and Ernst concluded that AChE inhibition had caused a local accumulation of ACh and in consequence, stimulation of the nigro-striatal pathway. This conclusion was reinforced by their demonstration that L-DOPA or apomorphine injections into the striatum also elicited compulsive gnawing (Ernst and Smelik, 1966). Similar results have been shown by Decsi et. al. (1978) who found that high doses of carbachol (10-40 μg) injected bilaterally into the substantia nigra elicit a dose-dependent stereotyped behaviour indistinguishable from that produced by systemic apomorphine. Decsi et. al. (1978) also observed that intra-caudate injections of apomorphine induced stereotyped behaviour.

These results appear to demonstrate that cholinergic stimulation in the substantia nigra can produce behaviour identical to that elicited by dopaminergic stimulation of the striatum.

iiid. <u>Acetylcholine-Dopamine Interactions in the Substantia Nigra: Conclusions</u>

i. AChE is present within pars compacta neurones and is released

from dendrites both spontaneously and under physiological stimulation. It is presumably present to inactivate a cholinergic input.

ii. Electrophysiological studies of nigral ACh-DA interactions show conflicting data: excitation, inhibition or no effect of ACh on pars compacta neurones has been shown. However, as these studies were performed on anaesthetised animals, and as radical differences in neuronal responding to pharmacological stimulation in conscious or unanaesthetised animals have been demonstrated, their relevance to the conscious, functional, state is questioned.

iii. Results of behavioural studies suggest the presence of two cholinergic systems in substantia nigra; a zona reticulata system activating output independently of DA systems (De Montis et. al., 1979), and a pars compacta system, stimulation of which elicits behaviour characteristic of high-level DA stimulation of the striatum (Smelik and Ernst, 1966; Decsi et. al., 1978). It is hypothesised, therefore, that acetylcholine has two roles within the substantia nigra: excitation of zona reticulata output neurones and excitation of the dopaminergic nigro-striatal pathway.

Chapter 5: Behavioural Functions of the Nigro-Striatal Pathway

In order to understand the effects of cholinergic agents in relation to the nigro-striatal DA system, it would be prudent to survey the behavioural functions which this system has previously been reported to subserve.

i. Catalepsy

Catalepsy is a term given to a state of immobility perhaps best characterised in animals by the ability to maintain an imposed posture. It is not to be equated with schizophrenic catatonia, which has the symptoms of catalepsy as well as other features (Baruk, 1950; Bleuler, 1924). However, in recent studies, the presence or absence of rigidity has been used in an attempt to discriminate between catalepsy and catatonia. Rigidity may be estimated by the ability of a rat to remain suspended between two book holders (Bloom, et al., 1976), remain seated with an erect trunk or from a clinical estimation of muscle tone (Segal et. al., 1977). The presence of rigidity is thought to indicate catatonia, while its absence is believed to indicate catalepsy, and on this basis opioid treated rats have been classified as catatonic (Segal et. al., 1977) while neuroleptic treated rats have been classified as cataleptic. If this distinction could be made, a second problem would have to be solved - how is catalepsy different from ataxia? However, other authors have shown that rigidity is a component of neuroleptic-induced catalepsy as well as opioid-induced "catatonia" (Steg and Ungerstedt, 1978; Jurna, 1976). This suggests that rigidity is not an adequate factor for differentiating between catalepsy and catatonia. As opioid and neuroleptic movement disorders are, however,

visibly different, it might be best to simply categorise them as "opioid catalepsy" and "neuroleptic catalepsy", rather than attempt to force them into the misleadingly "different" terms catalepsy and catatonia (Di Chiara et. al., 1979b).

Neuroleptic catalepsy is primarily associated with DA receptor blockade in mesolimbic and striatal areas, and would appear to be the converse of the increase in activity seen following administration of drugs which release DA and act as psychomotor stimulants (see below, this chapter, section iiia). Whether the response is dependent upon a specific anatomical locus or type of DA receptor is not clear. Carter and Pycock (1979) are prepared to speculate that "... a distinct site of action for the production of this response has not been, and probably cannot be, forwarded". However, lesions of the corpus striatum have been observed to attenuate neuroleptic catalepsy, while, interestingly, potentiating opioid catalepsy (Koffer et. al., 1978).

It has proved possible to elicit catalepsy not only by neuroleptic administration, but also by manipulation of striatal output and input systems - a feature of DA induced behaviour which we shall see over and again. Di Chiara et. al. (1979b) established catalepsy by bilateral injection of nanogram quantities of muscimol, a GABA agonist, in the ventromedial and ventro-anterior regions of the thalamus. Serotonin has also been implicated in the elicitation of catalepsy. 5HT uptake inhibition was found to potentiate the catalepsy induced by the neuroleptic butyrophenone haloperidol (and, moreover, to augment the action of haloperidol in blocking apomorphine induced stereotypy) (Waldmeier and Delini-Stula, 1979). Similarly, the

action of high doses of amphetamine or apomorphine in potentiating haloperidol induced catalepsy has been found to be due to the action of these drugs in releasing serotonin (Carter and Pycock, 1979).

We may thus conclude that (a) catalepsy may be produced by several drugs, including neuroleptics which block DA receptors; (b) that at present, a particular DA projection or receptor population has not been identified which alone mediates catalepsy, and (c) that, as with other DA induced behaviours, it is possible to modify or induce the cataleptic response by manipulation of other striatal afferent and efferent systems.

ii. Turning Behaviour

iia Dopamine, the Nigro-Striatal System and Turning Behaviour

Turning behaviour - its neuroanatomy, neurochemistry and pharmacology - is a surprisingly large and complex field of study, which is largely beyond the scope of this thesis. (For a thorough review, see Pycock, 1980.) Unilateral stimulation of the brain has been known to provoke postural assymmetries and rotational behaviour since the last century (Ferrier, 1873) but in recent years it has been widely used as an index of unilateral dopaminergic activity. This is primarily the consequence of several demonstrations in Sweden that unilateral lesion of the nigro-striatal pathway by 6OHDA results in animals turning towards the side of the lesion (Ungerstedt, 1968; Ungerstedt and Arbuthnott, 1970; Ungerstedt 1971b), the animals apparently turning away from the side of greatest striatal dopaminergic activity. Indeed, there is a positive correlation between the

rate of circling following DA stimulation and the degree of DA deficiency in the striatum (Thornburg and Moore, 1975). Mesolimbic DA projections do not appear to mediate a rotational response. Neither direct electrical stimulation of the A10 cell bodies (Arbuthnott and Crow, 1971) nor 6OHDA lesions of either nucleus (n) accumbens or olfactory tubercle (Fleisher and Glick, 1975; Kelly, 1975; Costall et. al., 1976) elicits rotation. However, while mesolimbic projection manipulation alone will not induce circling behaviour, it has been suggested that it does form an important component of the response. Bilateral injections of DA into the nucleus accumbens are known to induce locomotion (Pijnenburg and Van Rossum,1973; see below, this chapter, section iiia), and intact DA terminals within this nucleus are known to be required for the elicitation of rotation (Kelly et. al., 1975). Kelly and Moore (1976) have proposed that rotation might be elicited in the rat as a result of (i) a striatal DA imbalance which causes a postural assymetry and (ii) stimulation of mesolimbic DA systems - especially within the nucleus accumbens - which provides the locomotor component necessary to convert postural assymetry into circling behaviour.

The literature dealing with rotation following peripheral or central administration of dopaminergic drugs is extensive, and a complete review is beyond the scope of this thesis. Suffice to say that in the unilaterally lesioned rat, DA drugs can and do elicit rotation whether applied centrally or peripherally. However, two interesting discoveries concerning turning behaviour deserve special mention. The first is that, in the unilaterally nigro-striatal lesioned animal, amphetamine, which acts at pre-synaptic loci to potentiate DA release, induces ipsiversive rotation, while apomorphine,

which acts directly at DA receptors, elicits contraversive rotation (for instance, Ungerstedt, 1971b and 1971c respectively). This phenomenon has been ascribed to the development of a denervation supersensitivity among the receptor population of the lesioned side, enabling apomorphine to have maximal effect on that side, while amphetamine, reliant on intact presynaptic terminals, acts maximally on the unlesioned side. The second interesting phenomenon is the observation by Cools and colleagues that in cats, different striatal sites support either contraversive or ipsiversive turning. Low doses of DA injected into the medial two-thirds of the striatum elicit contralateral turning, which is antagonised by haloperidol (Cools and Van Rossum, 1970) while at other intracaudate sites these doses elicit ipsilateral turning which is insensitive to haloperidol blockade (Cools et. al., 1975). Large doses of DA at either of these sites cause ipsilateral turning (Cools and Van Rossum, 1970; Cools, 1971). These data are thought to demonstrate the existence of two (or more) types of DA receptor, one excitatory, the other inhibitory (see Chapter 4, section i.).

It has proved possible to influence rotational behaviour by manipulation of many different neurotransmitter systems: ACh, noradrenaline, serotonin, GABA, SP and enkephalin have all been reported to affect rotation (see Pycock, 1980). As we observed with the modification of catalepsy by non-DA drugs, it may be possible to link some of these effects to other striatal afferent or efferent systems. Unilateral destruction of nigral cells by kainic acid elicits a rotational response which is unaffected either by DA receptor blockade or 6OHDA lesion of nigral DA cells (Di Chiara et. al., 1977; Olianas et. al., 1978a); unilateral thalamic stimulation by muscimol induces ipsilateral rotation

which is converted to contralateral rotation by i.p. administration of apomorphine (Di Chiara et. al.,1979c); amphetamine induced circling is susceptible to nigral GABA level manipulation (Thiebot and Soubrie, 1979); superior colliculus lesions support circling (Crossman and Sambrook, 1978) which is independent of DA systems (Reavill et. al., 1979). All of these results are presumed to indicate that nigral efferents (principally those to the thalamus and tectum) may mediate striatal effects relayed via the GABA-ergic striato-nigral system. Whether or not these systems support rotation with complete independence of the nigro-striatal DA system, or if this pathway has a primary role, is not yet absolutely clear.

iib <u>Cholinergic Stimulation and Turning Behaviour</u>

Cholinergic drugs have been reported to influence turning behaviour, showing effects in rats with unilateral nigro-striatal lesions as well as modifying the effects of dopaminergic drugs. Such modification of dopaminergic activity is not surprising when one considers that the nigro-striatal projection is known to make extensive terminal connections in the striatum with the large population of cholinergic interneurones present there(See Chapter 4, section i). However, "studies reporting turning behaviour and postural asymmetries elicited following unilateral injection of cholinergic/anti-cholinergic drugs into various regions of the basal ganglia are confusing and contradictory" (Pycock, 1980). In the rat, unilateral injections of cholinomimetics into the caudate n. are reported to cause ipsiversive head turning (Costall et. al., 1972a; McKenzie et. al., 1972) or contraversive head turning and asymmetry (Dill et. al., 1968; Matthews and Chiou, 1979). In contrast, anticholinergics are reported to induce

contralateral posturing (Costall et. al., 1972a; Costall and Naylor, 1974) or to have no effect (McKenzie et. al., 1972). Similar striatal injections in other species have been reported to elicit different results.

These data may be explained by the presence within the striatum of a non-homogeneous population of cholinergic receptors. It is known that different portions of this structure support different behaviours (see below, this Chapter, section iiia), that the DA projection is not uniform (Chapter 4, section i), and that efferent pathways are not homogeneously organised. (The striato-nigral GABA-ergic pathway for instance shows a topographical arrangement similar to that of the nigro-striatal DA pathway .) Under these circumstances, it would be surprising if striatal cholinergic systems were to display a functional homogeneity.

Within the substantia nigra, microinjections of cholinergic drugs elicit turning behaviour in the rat, and as with cholinergic injections into the striatum, considerable confusion exists among the reports; species differences may account for part of this confusion. However, within the studies on the rat, variation in results might be explained by the location of the microinjection within substantia nigra. Following unilateral stimulation by carbachol of posterior portions of substantia nigra (presumably into caudal zona reticulata), De Montis et. al.,(1979) observed dose-dependent ipsilateral turning which could be antagonised by scopolamine given either systemically or intranigrally. These authors also found that scopolamine injected alone at these sites elicited contraversive turning. Furthermore, bilateral injections of carbachol elicited catalepsy which was resistant to apomorphine treatment, but reversed by scopolamine. Similar injections

of picrotoxin also elicited catalepsy, but in this case, both apomorphine and scopolamine reversed the effect. De Montis et. al. concluded that a cholinergic system in the reticulata, mediated by muscarinic receptors played "a role in the control of posture opposite to that of nigral GABA receptors". James and Massey (1978) also observed contraversive turning following injection of hemicholinium and ipsilateral turning following carbachol injection in zona reticulata (Chapter 4, section iiic). Costall et. al. (1972a) follow this trend in finding that cholinergic stimulation of the nigra elicits ipsilateral turning (though they found atropine injections to be ineffective) but provide no intra-nigral localisation of the injections. As yet, no study has examined the effects of cholinergic stimulation of the pars compacta on turning behaviour.

iic <u>Turning Behaviour Elicited from Unlesioned Animals</u>

Rotational behaviour can be elicited by dopaminergic drugs in unlesioned animals, although the rate of rotation is usually much slower than that seen following similar pharmacological treatment of unilaterally lesioned animals. Amphetamine (Jerussi and Glick, 1974; Thiebot and Soubrie, 1979) and apomorphine (Jerussi and Glick, 1975; Keren and Korczyn, 1978) both induce rotation, usually in opposite directions in the same animal (Jerussi and Glick, 1976). Amphetamine induced rotation is blocked by DA synthesis inhibition, while both this and apomorphine induced rotation are blocked by haloperidol. Moreover, at certain doses, both L-DOPA and haloperidol itself have been known to induce circling (Jerussi and Glick, 1976). Jerussi and Glick (1974) initially hypothesised that this circling reflected a natural imbalance between the nigro-striatal pathways and have attributed amphetamine induced rotation to an asymmetry in striatal DA concentration

(Glick et. al., 1974; Jerussi and Glick, 1976); apomorphine induced rotation apparently reflects an asymmetry amongst the post-synaptic receptor populations in the striata of normal rats (Jerussi et. al., 1977).

Other drugs have also been discovered to elicit rotation in unlesioned animals: tetrahydrocannabinol, morphine, LSD and serotonergic drugs all share this facility (see Pycock, 1980). Scopolamine and atropine are both reported to potentiate the effects of amphetamine, and to induce similar rotation when administered alone (Jerussi and Glick, 1976; Korczyn and Eshel, 1979). Pilocarpine - a cholinergic stimulant- has also been observed to antagonise amphetamine induced rotation (Jerussi and Glick, 1976).

In addition to pharmacologically stimulated rotation in non-lesioned animals, Korczyn and Eshel (1979) have reported a spontaneous circling in rats. While the circling was slow and not "nose-to-tail" - in contrast to the unilateral lesion studies - the animals exhibited a statistically significant preference to rotate (as measured over a 17 hour period). These authors have therefore claimed that the rotation induced pharmacologically is simply a reflection of a hyperactive component of the drugs - and moreover that as non-DA drugs appear to induce rotation, then the "striatal asymmetry" model might not be valid. We might thus usefully conclude this review of turning behaviour with the following observations.

(i) Animals may show a strong preference to circle in a particular direction when neither lesioned nor pharmacologically treated.

(ii) Dopaminergic drugs may however release a circling behaviour in

unlesioned animals which is phenomenologically different from that spontaneously demonstrated.

(iii) That in unlesioned animals, asymmetries appear to exist in both striatal DA concentration and amongst post-synaptic striatal DA receptors. Whether or not asymmetries are responsible for the spontaneously occurring rotation is not clear; similarly, whether or not the psychomotor stimulants amphetamine and apomorphine elicit circling rather than locomotion by playing upon (and possibly exaggerating) these asymmetries is not clear. Both of these are valid hypotheses.

(iv) That non-dopaminergic drugs, including cholinergics, might affect rotation by an alteration of striatal afferent or efferent systems which normally interact with the DA system.

iii. <u>Stereotypy and Locomotion</u>

While turning behaviour in unlesioned animals has been reported, following systemic administration of dopaminergic agonists such as apomorphine and d-amphetamine, stereotyped behaviour and locomotion are the more commonly reported effects of these drugs.

iiia <u>Dopamine, the Striatum, Stereotypy and Locomotion</u>

At lower levels of dopaminergic stimulation a potentiation of general locomotor activity is observed, followed, at rather higher levels, by stereotyped behaviour. First classified by Randrup and Munkvad (1968), stereotypy usually takes the form of repetitive movements of the head and/or forelimbs, repetitious walking on a fixed path, sniffing, licking, oral behaviour or gnawing, dependent on the drugs used, dosage and the animals. At very high levels of stimulation,

retropulsion has been observed, though this may be attributable to the activation of serotonergic systems (Lees et. al., 1979) rather than catecholaminergic ones.

It has proved possible to show an anatomical dissociation between locomotor activity and stereotyped head and oral movements. Locomotion has been elicited by microinjection of dopaminergic agonists into the nucleus accumbens, the principal terminal region of the mesolimbic DA system (Pijnenburg and Van Rossum, 1973; Pijnenburg et. al., 1973; Pijnenburg et. al., 1976) while d-amphetamine induced locomotion has been blocked by injections of haloperidol into this area (Pijnenburg et. al., 1975). On the other hand stereotypy has been elicited by microinjections of d-amphetamine into the caudate n. (Costall et. al., 1972b). Moreover, lesions of the caudate n. have been found to abolish or attenuate oral stereotypy elicited by dopaminergic stimulants (Fog et. al., 1970). That locomotor activity and oral stereotypy are dissociable effects has been demonstrated by Kelly et. al., (1975) who observed that 6OHDA lesions which depleted the caudate n. of DA by 50%, but which spared n. accumbens and olfactory tubercle, substantially reduced the stereotyped behaviour elicited by 5.0 mg/kg of d-amphetamine applied systemically. On the other hand, locomotor activity elicited by 1.5 mg/kg of this drug was unaffected by caudate n. lesions, but was attenuated by 6OHDA lesions of the n. accumbens (79% DA reduction) which apparently spared the caudate n..These lesions in turn spared the stereotypic activity of the higher dose. Creese and Iversen (1975) showed a rather more complex pattern following selective lesions. As expected, 1.5 mg/kg d-amphetamine elicited locomotion but not stereotypy in control animals. However, behaviour elicited by the drug following 6OHDA lesions within the

caudate n. appeared to be dependent on the severity of the lesion. In caudate-DA depleted animals, the drug elicited bizarre locomotor activities and stereotypy but in less severely DA depleted animals it elicited twice the locomotor activity of controls and no stereotypy. Creese and Iversen (1975) concluded that "... both locomotor and stereotyped responses induced by amphetamine are dependent on the functional integrity of the nigro-striatal DA pathways".

The simple involvement of mesolimbic DA with locomotion and nigro-striatal DA with stereotypy may prove inadequate for several reasons. As we have previously observed, the anatomical distinctions are not as clear as once thought. The substantia nigra (Chapter 3, section ii and Chapter 2, section iv.a iv) has been observed to project not only to the striatum but also to the n. accumbens, while the distinction between A9 (substantia nigra) and A10 (the ventral tegmental area) has been questioned by others; moreover, some anatomists now consider the n. accumbens might best be considered as a ventral extension of the striatum, while this might best be considered not as a homogeneous whole but as a heterogeneous structure, which, within specific portions, contains various afferent and efferent neurones. In respect of this last point, Creese and Iversen (1975) observed an abolition of stereotyped behaviour with lesions restricted to ventral aspects of the caudate n., whereas electrolytic lesions of the dorsal striatum have no effect on amphetamine-induced stereotypy (Neill et. al., 1974a). Different areas of the striatum also appear capable of mediating the effects of DRL responding (Neill and Herndon, 1978), avoidance behaviour (Winocur, 1974), spontaneous wheel running (Neill et. al., 1974b) and feeding behaviour (Neill and Linn, 1975; Neill 1976; Neill and Grossman, 1973). For some functions,

the n. accumbens or certain aspects of striatum appear to exert equal influence. Neill et. al., (1978) observed that crystal or solution injections of DA or d-amphetamine into the ventral anterior striatum, but not dorsal or posterior aspects increased rates of lateral hypothalamic self-stimulation: these authors noted that injections of these drugs into the n. accumbens were as effective as those in the ventral anterior striatum.

Moreover, the effects of drugs whose primary action may be to stimulate DA activity within the striatum need not be confined within that structure. Peripherally administered amphetamine (Wechsler et. al., 1979) or apomorphine (Brown and Wolfson, 1978) increase neuronal 2 deoxy-D-glucose utilization (accepted as an index of metabolic activity (Sokoloff et. al., 1977)) in structures at a distance from the striatum -the subthalamic nucleus, the zona reticulata and the superior colliculus (see Brown and Wolfson (1978), Figure 1, p. 189) . These structures all receive, directly or indirectly, information from the striatum: presumably amphetamine acts to release DA here, this stimulation activating striato-nigral GABA (and possibly SP) neurones which in turn synapse within the substantia nigra to stimulate thalamic and tectal efferent neurones as well as establishing a possible feedback relationship with the pars compacta DA neurones themselves.

As with both catalepsy and rotation, stereotyped behaviours may be elicited or manipulated through these striatal output systems. Koob et. al., (1978) observed potentiation of stereotyped behaviour - including that elicited by d-amphetamine - following potentiation of GABA transmission in substantia nigra; unilateral or bilateral destruction

of zona reticulata cell bodies by kainic acid results in, respectively, turning and stereotyped behaviour, insensitive to both DA receptor blockade and DA cell body destruction (Di Chiara et. al., 1977; Olianas et. al., 1978a); stimulation of thalamic neurones (by morphine) in the terminal regions of the nigro-thalamic pathway, has been reported to elicit compulsive gnawing (Bergmann et. al., 1974), as have microinjections of picrotoxin (a GABA antagonist) into intermediate and deep layers of the superior colliculus (Dean et. al., 1980). Clearly, stereotyped behaviours may be manipulated by excitation or inhibition of striatal output systems, and so to tie stereotypy unqualified, to the striatum, or even aspects of the striatum, leaves a great deal unsaid. What appears to be the case is that certain types of behaviour - sniffing and rearing for instance - are mediated by or through the striatum, and not the n. accumbens. Alternatively, locomotor activity (or, it might be suggested, stereotyped walking (Schiorring, 1979)) appears to be mediated by the n. accumbens or the n. accumbens and the striatum.

iiib The Process of Stereotypy

Two questions remain unanswered. First, to take the n. accumbens and striatum together, how far can we localise particular behaviours, and indeed components of those behaviours, to specific areas and neurones? And second, how is the process of stereotyping (for it must always be borne in mind that this is a description of behaviour, not a "behaviour" in itself) actually brought about? How do behaviours such as sniffing, walking and rearing come to be stereotyped? How does the process of stereotypy - repetition of an invariant sequence of behaviour - come to dominate the animal's behavioural repertoire?

To the first of these questions, this thesis has no answer, other than to restate that (i) different areas of the striatum have been observed to mediate different behaviours, and (ii) we have repeatedly observed how dopaminergically induced behaviours may be elicited by the appropriate stimulation of others parts of the brain known to receive information from the originally manipulated DA systems. (For instance, picrotoxin injected into deep layers of the superior colliculus elicits a gnawing response (Dean et. al., 1980) as does apomorphine stimulation of the striatum (Ernst and Smelik, 1966): presumably the striato-nigral and nigro-tectal pathways link the two.) We might tentatively hypothesise therefore, that certain areas and nucleii are specifically tied to certain behavioural functions, but that the connections of these areas may modify or even induce that behaviour.

The process of stereotypy has, on the other hand been examined and must be considered. Lyon and Robbins (1975) have examined in depth the behavioural effects of amphetamine, and advance a hypothesis concerning the actions of the drug - and similarly acting psychomotor stimulants - which appears to explain its behavioural actions within a single framework. In searching for a general principle of amphetamine action, Lyon and Robbins observe that one of the very few consistent generalisations to emerge from the literature is that of Dews (1958) who observed that "... when the control performance includes long inter-response times then low effective doses of amphetamine will tend to reduce their number and length. Conversely, at higher doses, the number of very short inter-response times would be reduced" (Lyon and Robbins p.81). They extend this conception of rate changes to demonstrate that "the increasing stereotypy yields higher and higher rates of activity but in more and more limited categories

of response" (p. 82), the behavioural repertoire being limited by perseveration within certain response categories - those most amenable to performance at a high rate - to the exclusion of others. This process continues until the animal enters a state of virtual catalepsy, being unable to initiate and maintain even the simplest movement. Thus, paradoxically, as Lyon and Robbins put it, "perhaps the oddest feature is that the animal finally ceases to respond not because of inhibition, but because of maximal excitation" (p. 89).

Lyon and Robbins are thus able to set out a formal "General Hypothesis of the Actions of Amphetamine" (which is believed to apply equally well to other psychomotor stimulant drugs) and which might be put forward as an explanation of the process of stereotypy. This General Hypothesis states that:

"The action of amphetamine is such that as the dose response within the central nervous system increases, the repetition of all motor activities will increase with the result that the organism will tend to exhibit increasing response rates within a decreasing number of response categories" (p. 85).

These authors go further and describe four discriminable stages of action of the drug, all following the principle that the general increase in CNS activity will be made apparent in the relative proportion of time spent in the possible unconditioned behaviours available to the animal in a given situation. In the first instance, complex behaviours and behavioural chains are eliminated - especially those requiring long pauses. As the level of stimulation increases, those behaviours capable of repetition without long pauses and amenable

to a high rate of performance will dominate the animal's behaviour (for instance, locomotion and rearing) and gradually shorter and shorter response sequences (for instance, biting, forepaw movements) become more dominant, until only tremor is possible." In the final stages, all overt activity will cease because the nervous system is in such a rapidly changing repetition pattern that no activity of consequence can be completed" (p. 89). Conditioned behaviours, as well as unconditioned, are susceptible to this process, though the degree of training, recency, and the type of test and response required affect the quality of the stereotyped behaviour.

To summarise, Lyon and Robbins perceive stereotyped behaviour to be the product of an overstimulation of nervous activity which increasingly allows only behaviours capable of performance at ever increasing rates to be displayed. As the dose response increases the observed behaviour will be selected from a diminishing range: fewer categories of overt behaviour are observed, but these are executed at a higher rate of performance than is normal.

iiic <u>Cholinergic Elicitation and Modification of Stereotypy</u>

We have already noted that stereotypy may be modified by striatal afferent and efferent systems other than the dopaminergic one (this Chapter, section iiia). However, as we have also noted that the nigro-striatal DA system interacts with cholinergic systems in both the striatum (Chapter 4, section i) and the substantia nigra (Chapter 4 section iii), a cholinergic modification of stereotypy might be expected.

On this point the literature is quite clear: a cholinergic

mechanism in the brain inhibits amphetamine stereotypy (Arnfred and Randrup, 1968). Systemic atropine, a muscarinic receptor antagonist, potentiates intra-striatally (Costall et. al., 1972b) or systemically (Phillips, 1974) elicited amphetamine stereotypy. Physostigmine (an AChE inhibitor) on the other hand, attenuated amphetamine stereotypy (Phillips, 1974). The effect of atropine alone, systemically applied, is dose-dependent. Phillips (1974) found 10 mg/kg atropine ineffective, whereas Decsi et. al., (1979) observed stereotypy at doses between 12.5 and 50 mg/kg. Moreover, this effect was abolished by intra-caudate injections of triperidol, a DA antagonist.

An interaction between DA and ACh in the striatum has been observed by Sahakian et. al. (1978), who found that the stereotypy elicited by systemic apomorphine could be blocked by direct stimulation of striatal muscarinic receptors without necessarily affecting striatal ACh levels. Indirectly acting cholinergic agonists (physostigmine and choline chloride) increased striatal ACh levels but lacked an immediate effect on apomorphine stereotypy. This observation is consistent with the belief that nigro-striatal DA neurones synapse on a population of cholinergic neurones within the striatum (Chapter 4, section i).

Characteristic dopaminergic stereotypies have been observed following direct microinjection of high doses of either cholinergic or dopaminergic drugs into the striatum or the substantia nigra. Atropine microinjected into the striatum elicited stereotypy which was abolished by systemic triperidol (Zambo et. al., 1979). Costall et. al., (1972b) found that high doses of amphetamine (25-400µg) microinjected into the striatum also elicited stereotypy: sniffing was observed although

the characteristic features of high level peripheral amphetamine administration - gnawing, biting and licking - were all absent despite the large doses used. Systemic haloperidol or arecoline potentiated the effects while atropine abolished them. Ernst and Smelik (1966) however observed stereotypic gnawing following implantation of crystalline apomorphine (approximately 100µg) into the striatum.

Cholinergic injections into the substantia nigra elicit characteristic dopaminergic stereotypy. Winn and Redgrave (1979) observed stereotyped biting and gnawing following microinjection of 5µg carbachol into substantia nigra. Decsi et. al. (1978) also observed carbachol induced stereotypy at doses between 10 and 40µg. The stereotypy observed by Winn and Redgrave (1979) could be blocked by administration of low, non-sedative doses of systemic haloperidol (Redgrave and Taha 1980). Physostigmine (approximately 30µg) has also been observed to elicit biting when implanted into the substantia nigra (Smelik and Ernst, 1966).

These observations might be used to conclude that:

i. As potentiation of dopaminergic neurotransmission within the striatum by large doses of microinjected apomorphine or amphetamine elicits stereotyped behaviour, and as a similarly microinjected cholinergic antagonist also elicits stereotyped behaviour, it might be concluded that DA acts within the striatum to inhibit cholinergic neurones. The observation that muscarinic receptor blocking agents, in doses which do not affect striatal ACh levels, can antagonise apomorphine-induced stereotypy tends to support this.

ii. As potentiation of cholinergic neurotransmission in the substantia nigra by large doses of microinjected carbachol or physostigmine also elicit stereotyped behaviour, which may be antagonised by low, non-sedative doses of haloperidol, it might be concluded that a cholinergic system within the substantia nigra stimulates activity in the nigro-striatal pathway.

iv. <u>The Nigro-Striatal DA System and Feeding Behaviour</u>

There are three lines of evidence which suggest that the nigro-striatal DA system might have an important role in the regulation of feeding behaviour.

iva <u>DA Systems in Relation to the Lateral Hypothalamic Syndrome</u>

Lesions of the lateral hypothalamus (LH) have long been known to induce a syndrome of severe aphagia and adipsia (Anand and Brobeck, 1951; Teitelbaum and Stellar, 1954). Feeding and drinking are eventually recovered by LH lesioned animals if they are maintained through the immediate post-operative period by intragastric feeding (Teitelbaum and Stellar, 1954). Recovery appears to involve several discrete stages, which have been catalogued and analysed in detail (Teitelbaum and Epstein, 1962). However, even following recovery, these animals appear to have several persistent deficits, which include:

a. failure to drink in the heat (Epstein and Teitelbaum, 1964).

b. impaired responding to cellular dehydration (Epstein and Teitelbaum, 1964) - though it has also been reported that drinking does occur in response to intracellular (or intravascular) fluid compartment

dehydration if testing is allowed to continue over several hours (Stricker, 1976).

c. not drinking in the absence of food (Epstein and Teitelbaum, 1964) and (alternatively), drinking only in association with eating (Kissileff, 1969).

d. failure to eat in response to insulin (Epstein and Teitelbaum, 1967).

e. overeating in a cold environment (Epstein and Teitelbaum, 1967).

f. body weight changes (Powley and Keesey, 1970) and body weight set point changes (Keesey et. al., 1978).

g. Extreme finickiness in food choices (Teitelbaum and Epstein, 1962).

Additionally, there appear to be sensory-motor deficits which have an important role in creating the initial aphagia and adipsia (Marshall et. al., 1974; Marshall and Teitelbaum, 1974). Unilateral LH lesion produces sensory-motor deficits on the side contralateral to the lesion, while bilateral lesions, naturally, induce bilateral deficits. These deficits include a defective response to sensory stimuli - unilateral lesions cause a neglect of contralateral visual, tactile, and olfactory stimuli - orientation failures, failure to use limbs properly in situations requiring precise control, a marked changed in limb muscle tone and a tendency to move in ipsilateral rather than contralateral directions following unilateral lesion, all in addition to the well known consummatory deficits (Marshall et. al., 1974; Marshall and Teitelbaum, 1974).

A great deal of controversy has surrounded what has become known as the lateral hypothalamic syndrome, particularly in respect of two

points. First, are neurones intrinsic to the LH responsible for the syndrome of aphagia and adipsia and/or the sensory-motor neglect, and second, how far does the one syndrome contribute to the other? (Specifically, can the aphagia be accounted for by the sensory-motor neglect?) In terms of the work we shall examine,these discussions are of little relevance. For the moment, suffice to say that until recently, neurones not synapsing within the LH (fibres of passage) were thought by some authors to be of paramount importance, though some recent evidence appears to demonstrate that the LH itself plays a crucial role. Injections of kainic acid - a neurotoxin with a specific target in cell bodies - into the LH have been reported to elicit a classic aphagic and adipsic syndrome, strongly suggesting that intrinsic hypothalamic neurones may mediate this effect (Grossman et. al., 1978; Stricker, et. al., 1978). Moreover, bilateral LH destruction in infant rats has been reported to induce, even in adult life, consummatory deficits without sensory neglect or attentuated arousal (Almli et. al., 1979) indicating that the LH neurones have specific feeding behaviour/body weight regulation functions.

Despite this recent work specifying the role of the lateral hypothalamus in inducing the LH syndrome, earlier work indicated that other structures could mediate similar effects. In particular, destruction of hypothalamic fibres of passage at locations outside this structure appeared to induce identical LH syndromes. In particular, bilateral 6OHDA injections into the nigro-striatal pathway at the level of the substantia nigra or globus pallidus "produce a syndrome of feeding impairments strikingly similar to that seen after LH electrocoagulation" (Marshall et. al., 1974). Indeed, by 1974 it was already known that extensive striatal lesions abolished feeding behaviour,

the degree and co-ordination of the surviving feeding abilities being correlated with the extent of striatal survival (Sorensen and Ellison, 1970) and that intraventricular 6OHDA induced a "LH syndrome" which could be similarly overcome by intragastric feeding and intensive care (Zigmond and Stricker, 1973). Kainic acid lesions of the striatum also induce aphagia and body weight loss (Sanberg and Fibiger, 1979).

Work continued to demonstrate that fibres of passage, coursing through the LH, could induce LH effects. Parasagittal knife cuts along the lateral border of the diencephalon or coronal cuts across either lateral or medial components of the medial forebrain bundle (M.F.B.) reproduced most of the glucoprivic and hydrational challenge deficits of rats which had sustained electrolytic lesions of the LH or 6OHDA lesions (McDermott et. al., 1977). Transection of fibres crossing the LH borders were reported to induce fragmented spontaneous ingestive patterns resembling those of LH lesioned animals (Rowland, 1979).

Clearly of most importance to the present discussion are the repeated demonstrations that lesions of the striatum (mechanically or by neurotoxin), or the nigro-striatal pathway (by intra-nigral 6OHDA, transsection in the diencephalon or M.F.B., or by globus pallidus lesions) can induce a syndrome of which aphagia is clearly a component. Indeed, total or sub-total 6OHDA lesions of substantia nigra (and the V.T.A.) induce striatal DA depletions of varying degrees of severity, greater caudate n. DA depletion leading to more severe aphagia (Oltmans and Harvey, 1976). Whether this aphagic state is in addition to, or a consequence of, sensory

neglect is an open question. A recent report suggests that lesions of the nigra - or its forebrain connections - produce sensory neglect, while LH lesions which predominantly spare the fibres of passage (within the internal capsule) do not (Feeney and Wier, 1979). Marshall (1979) presents data in support of this, in that following V.T.A. 6OHDA lesions, the severity of somatosensory inattention is highly correlated with neostriatal DA depletion. (Though, he notes, mesolimbic DA terminals may also contribute to the defects.) An hypothesis suggesting that nigro-striatal lesions induce sensory neglect while LH lesions induce consummatory/body weight regulation deficits is consistent with recent reports. However, the relationship between nigro-striatal sensory-motor deficits and aphagia is not clear. Three hypotheses might be presented:

(i) nigro-striatal lesions induce two separate syndromes - sensory-motor neglect and aphagia/adipsia.

(ii) nigro-striatal lesions induce a sensory-motor neglect which in consequence disrupts feeding behaviour

(iii) nigro-striatal lesions induce a general sensory-motor neglect, all or part of which involves a sensory-motor system specific for feeding behaviour.

This thesis cannot discriminate between these hypotheses, and, indeed, does not seek to: the important consideration for our present purposes is that nigro-striatal lesions do induce a syndrome of aphagia.

ivb <u>Dopaminergic Drugs and Feeding Behaviour</u>

A variety of evidence suggests that DA drugs - both agonists and antagonists - can influence feeding behaviour. With reference to the

aphagia induced by substantia nigra 6OHDA lesions, it has been found that peripheral administration of the DA agonist apomorphine, in low doses, can reinstate co-ordinated eating 24-48 hours after the lesion (Ljungberg and Ungerstedt, 1976). Predictably, higher doses of apomorphine failed to reinstate eating, but instead, elicited stereotyped behaviours, including non-food directed gnawing. More interesting is the observation by these authors that LH electrocoagulation-induced aphagia could not be reversed by apomorphine, a possible interpretation of this being that neurones intrinsic to the LH support feeding independently of the fibres of passage. Similarly, Schwartz (1978) found that, as predicted, caudate-putamen glucose consumption decreased following electrolytic LH lesion or nigral 6OHDA lesion. However, Schwartz also noted that this decreased glucose consumption was reversed by systemic apomorphine. (Presumably restoration of caudate n. activity following LH lesion is not sufficient to reinstate eating as an intra-hypothalamic system has also been destroyed.)

One might assume from this work that drugs eliciting a release of DA in the striatum would elicit eating, and conversely, that neuroleptic or other DA blocking drugs would abolish feeding, being the pharmacological equivalent of the nigro-striatal 6OHDA lesion. Work to support both of these hypotheses is available within the literature. Costa et. al. (1972) describe how 0.3 mg/kg i.v.(+) amphetamine caused an increase in striatal DA turnover but not tel-diencephalic NA turnover. Indeed, such low doses have been reported to elicit feeding behaviour, (although not by Costa et. al., - quite the reverse in fact!) Blundell and Latham (1978) report that 0.125 mg/kg d-amphetamine in rats increased food intake measured over a 24 hr. period. Holtzman (1974) reported that 0.3 mg/kg d-amphetamine increased food intake in

the rat over a 2 hour test period. Dobrzanski and Doggett (1976) demonstrated 0.5 - 2.00 mg/kg + amphetamine to have "markedly increased the negligible food intake of free-feeding mice between 12.00 and 14.00h, an effect which rapidly disappeared at higher doses". Eichler and Antelman (1979) report that low doses of apomorphine - a direct DA receptor stimulant - can also induce feeding when administered in very low doses.

Work in support of the converse-DA antagonist blockade of feeding - is readily available. Zis and Fibiger (1975) report that neuroleptics induced food and water intake deficits similar to the LH syndrome. Rolls et. al. (1974) found doses of spiroperidol greater than 0.316 mg/kg attenuated or abolished feeding; Heffner et. al. (1977) found that 0.5 mg/kg spiroperidol was effective in reducing food intake. In a study using three different butyrophenones, Rowland and Engle (1977) found the following doses to abolish or attenuate feeding to a statistically significant degree: 0.2 mg/kg droperidol and 0.2 mg/kg haloperidol while 0.1 mg/kg spiroperidol reduced intake, but not to a statistically significant degree (in agreement with Heffner et. al., 1977). All of these drugs also caused a dose-dependent decrease in water intake.

From these studies a clear picture emerges: nigro-striatal DA lesions abolish feeding. Neuroleptic administration abolishes feeding. Low doses of d-amphetamine, increasing striatal DA turnover, are reported to increase food intake. Unfortunately, this picture is clouded by a great many other results which indicate quite a different scheme; d-amphetamine is acknowledged by many as being a classic <u>anorexic</u> agent, while reports also exist showing that doses of spiro-

peridol even lower than those noted for their anorexic properties, actually stimulate feeding (0.016 mg/kg (Rolls et. al., 1974); 0.02 mg/kg (Rowland and Engle, 1977)).

The explanation of these biphasic dose-response effects is far from clear. For the actions of amphetamine however, there are hypotheses which might account for its effects. That d-amphetamine is indeed a potent and specific anorexic agent is beyond doubt. Blundell et. al. (1976) give a clear demonstration of this, and furthermore, show that its main effect is to increase the inter-meal interval. These authors interpret this as being an effect on hunger (whereas fenfluramine another potent anorexic agent reduces meal size and is consequently believed to affect satiety mechanisms). However, these authors, not unusually in amphetamine anorexia studies, used a dose of amphetamine (1.75 mg/kg) sufficient to induce both an increase in locomotor activity (for instance, see Mumford, et. al., 1979) and stereotyped behaviour (for instance, see Pechnick et. al., 1979). How far do these behaviours interact and conflict? Moreover, at this dose, does amphetamine have widespread or localised effects on brain DA (and NA) systems?

To solve this puzzle it is best to initially examine the localised effects of amphetamine. Applied to the striatum this drug is known to induce stereotyped behaviour (Costall et. al., 1972b), and although not commented on by these authors this would presumably preclude feeding and represent an anorexic effect. Additionally, injections of amphetamine into the lateral hypothalamus induce an anorexic state in rats. Booth (1968) found that injections of amphetamine into the LH suppressed feeding, while injections of noradrenaline at the same site potentiated

it, therefore concluding that amphetamine elicited anorexia by an action on an adrenergic system - not dopaminergic - within the LH. Leibowitz (1974) also found that lateral hypothalamic injections of amphetamine induced anorexia, and found this to be blocked by the noradrenergic beta-blocker propranolol but not the alpha-receptor blocker phentolamine. Haloperidol (in a dose probably sufficient to block both NA and DA systems)also abolished the amphetamine anorexia.

These data lead back to the work previously discussed on the relationship between "lateral hypothalamic anorexia" and "fibres of passage anorexia". It seems entirely plausible that amphetamine, while acting on a noradrenergic and/or dopaminergic system within the hypothalamus induces anorexia by an action on a feeding system but may also, independently, act on striatal DA systems to induce a similar anorexic state by behavioural competition. Thus inhibition of only NA synthesis does not abolish the anorexic effects of amphetamine, (Franklin and Herberg, 1976) presumably because it is still able to act at DA synapses in the striatum. (Unfortunately, the converse of this does not appear to hold good: nigro-striatal destruction apparently attenuates the anorexic effects of amphetamine (Carey and Goodall, 1975).)

While some (not all) of the evidence points to anatomically dissociable effects of amphetamine, the behavioural question remains unresolved. It is possible that a "genuine" feeding system exists within the LH upon which amphetamine may act to induce aphagia; but is the striatal system concerned purely with feeding, or is it a sensory-motor system - or indeed, both? As yet, no conclusive evidence has been

presented to resolve this conundrum. However, it is well worth noting that the effects of amphetamine upon avoidance behaviour (Lyon and Randrup, 1972) and in quieting hyperactive children (Robbins and Sahakian, 1979) have both been ascribed to stereotypic effects of this drug. It is not too wild a suggestion that the actions of amphetamine - certainly within the striatum if not the hypothalamus - in inducing anorexia, might be ascribed to sensory-motor, stereotypic dysfunctions.

The low dose effects of neuroleptics in inducing eating is a far less well researched area: indeed it has been almost totally neglected. However, besides the postulates that different neurochemical and/or neuroanatomical mechanisms might account for the observed biphasic effects, there is an explanation for the data, which has a long history. In 1963, Carlsson and Lindqvist reported that chlorpromazine and haloperidol could stimulate the metabolism and synthesis of brain catecholamines, and these authors speculated that blockade of DA receptors might influence a negative feedback system. In a more recent version of this scheme Carlsson (1975) has proposed that the feedback function might be achieved by a more localised mechanism: presynaptic autoreceptors. The speculation runs that at low doses, these neuroleptics act at autoreceptors and produce the reverse of their higher dose post-synaptic effects. Carlsson also accounts for the biphasic actions of apomorphine in this manner.

Thus, overall, the initial picture - DA agonists inducing feeding, antagonists and lesions aphagia - might remain sound, although not without complications. Anatomical, chemical and behavioural alternatives make possible a completely reversed scheme, but do not

necessarily invalidate the one presented.

ivc Stress-Induced Feeding and the Nigro-Striatal DA System

Stress in the form of a mild tail-pinch has remarkable effects in inducing feeding behaviour. Eating is the predominant response in this syndrome, although gnawing and licking are also observed (Antelman et. al., 1975). DA appears to be critically involved in the syndrome: the relatively specific DA antagonists spiroperidol and pimozide both abolish the response while similar administration of NA blocking drugs (phentolamine and sotalol) have no effect. (Although it has been reported that tail-pinch increases brain NA turnover (Antelman et. al., 1975).) 6OHDA lesions of the nigro-striatal DA system are able to attenuate the response, although this treatment blocks the initiation of tail-pinch eating in only 44% of trials despite extensive striatal DA depletion (Antelman et. al., 1975). Tail-pinch also appears able, like systemic administration of apomorphine (Ljungberg and Ungerstedt, 1976) to induce sufficient ingestion of food in the newly LH lesioned rat to enable it to survive until spontaneous eating occurs (Antelman et. al., 1976). Failure to treat animals during this period otherwise almost certainly results in death. Stressing animals which display LH lesion or nigro-striatal lesion-induced sensory-motor dysfunction by placing them in a sink of water will also reverse the defects, though unfortunately they are corrected for no more than 4 hours (Marshall et. al., 1976). Interestingly, Marshall and Berrios (1979) note that aged rats showing,spontaneously, signs of DA neurone loss-induced sensory-motor dysfunction, undergo a marked improvement in swimming performance when treated with either apomorphine or L-DOPA!

The picture that emerges from these results, is that the sensory-motor inattention induced by DA depleting lesions can be overcome by stress. Moreoever, stressing healthy animals elicits a clear syndrome, of which eating is the major component. Thus low levels of stress appear to possess dopaminergic properties: its effects are blocked by specific DA antagonists and indeed its effects mimic those of low level apomorphine stimulation (in reversing the effects of nigro-striatal lesions) and low level amphetamine stimulation (in eliciting, spontaneously, feeding behaviour).

ivd Summary of Section iv

The nigro-striatal DA system appears capable of mediating eating behaviour. This is suggested by the observations that:

i. 6OHDA lesions of the nigro-striatal DA system provoke a syndrome of aphagia, adipsia and sensory-motor neglect, strikingly similar to that elicited by LH lesions.

ii. Low doses of the DA stimulant amphetamine (or apomorphine) induce feeding; higher doses are thought to induce anorexia by either acting on a lateral hypothalamic system which appears to be unrelated to fibres of passage or striatal DA dysfunction, or by acting within the striatum and/or mesolimbic areas to induce locomotion and/or stereotyped behaviours incompatible with feeding.

iii. Neuroleptic drugs induce consummatory deficits similar to LH lesion. Low dose stimulant effects of these DA antagonists might be explained by effects on different neuroanatomic structures or on pre-synaptic receptors.

iv. Stress induces a syndrome of which eating is a major component. DA antagonists block this. Moreover stress can reverse the aphagic

and sensory-motor deficits induced by nigro-striatal lesions or LH lesions.

v. In total this evidence points to the nigro-striatal DA system being able to mediate eating, if stimulated appropriately, though it is not clear to what extent the aphagia observed following lesion is due to a specific effect on a putative striatal feeding system or a result of sensory-motor defects.

Chapter 6: Summary and Experimental Hypotheses

In Chapter 2 the anatomy of the substantia nigra was examined, and we observed this structure to be composed of a heterogenous population of neurones, varying in respect of size, morphology and projection areas. The possibility of the nigra containing inter-neurones was also discussed. Projections to and from the striatum (caudate-putamen and globus pallidus) were noted as were efferent connections with the superior colliculus and the ventro-medial thalamus. Reciprocal connections with the sub-thalamic nucleus and the raphe nucleii were also examined. The striatal connections of the substantia nigra emerged as its most thoroughly studied and, to date, important projections. The nigro-striatal projection, mediated by DA, is clearly the most thoroughly investigated efferent projection of the substantia nigra, passing information to the striatum where it synapses upon a dense population of cholinergic interneurones, which are primarily inhibited by DA. The GABA mediated striato-nigral pathway is topographically organised in a manner strikingly similar to that of the DA projection from pars compacta, although presumably it serves not only to relay DA-mediated information back to substantia nigra but also to receive independent intra-striatal input of its own. This pathway appears to synapse within the zona reticulata upon large neurones which project to the thalamus and tectum.

It has been hypothesised that these GABA-ergic striato-nigral neurones relay information back to the DA cells of pars compacta. The observation that DA receptors within the nigra are found on GABA-ergic striato-nigral neurones supports this; the observation that decreases in both GABA and GAD content of substantia nigra fail to

alter striatal DA turnover does not. The evidence from receptor localisation studies suggests that a DA/GABA interaction exists, while the failure of GABA depleting lesions to alter striatal DA parameters suggests that the action of GABA is not to directly inhibit DA cells. Perhaps the activity of GABA neurones is modified by DA activity within the nigra? Such an hypothesis might reconcile these otherwise contradictory findings.

Little is known about input to the DA cells of pars compacta. SP released from a striato-nigral pathway appears to excite them, and stimulate DA release within the caudate n.. However, the neurotransmitter properties of SP (and other newly discovered neuro-peptides) remain to be fully confirmed, while behavioural responses to intranigral infusion of SP do not elicit strikingly "dopaminergic" responses. Serotonin, released from raphe-nigral neurones is thought to inhibit DA cells in the pars compacta. While the DA neurones themselves have been extensively studied and DA effects within the striatum examined, little work has been done to investigate potential afferents to these cells.

Perhaps the most probable neurotransmitter candidate for stimulating DA neurones is ACh. We may advance a variety of evidence to support this:

i. ACh and CAT are contained within substantia nigra, possibily within interneurones.
ii. Muscarinic cholinergic receptors exist within substantia nigra.
iii. AChE is stored within and released from the dendrites of pars compacta DA neurones, presumably to inactivate a cholinergic input.

iv. Electrophysiological evidence generally shows ACh to affect DA cells; some studies show excitation, others inhibition.

v. Following high doses of carbachol or physostigmine infused into substantia nigra characteristic dopaminergic stereotypies have been observed.

Chapter 5 examines the behavoural functions of the nigro-striatal pathway. The clearest generalisation concerning these neurones might be that they are involved in motor control; the concept of rate-dependency appears to be useful in further understanding their actions. At the very highest levels of stimulation catalepsy has been observed, but this is also known to follow neuroleptic treatment. This paradox is explained by hypothesising that under extreme stimulation, these neurones are in a constantly repeating state of activation at such a high level that no behavioural act can be either initiated or completed. Lowering the level of stimulation reveals short behavioural patterns repetitiously performed (stereotypy); continued lessening of the stimulation shows response chains of longer duration and greater co-ordination.

A few reports exist documenting the presence of feeding behaviour at very low levels of dopaminergic stimulation. An involvement of the dopaminergic nigro-striatal projection with feeding behaviour might be predicted because:

i. Dopaminergic stereotypies frequently show an oral and/or olfactory characteristic - gnawing, sniffing - which could represent components of eating behaviour fragmented by over-stimulation.

ii. Lesion of the nigro-striatal DA pathway by lateral hypothalamic or substantia nigra lesions can elicit a syndrome of aphagia and adipsia similar to the LH syndrome.

Three hypotheses could account for the aphagic syndrome seen following nigro-striatal pathway destruction:

(a) nigro-striatal lesions induce two separate syndromes - sensory-motor neglect and aphagia/adipsia
(b) nigro-striatal lesions induce a sensory-motor neglect which in consequence disrupts feeding behaviour
(c) nigro-striatal lesions induce a general sensory-motor neglect all or part of which involves a sensory-motor system specific for feeding behaviour.

The following experimental hypotheses might thus be presented:

1. DIRECT OR INDIRECT LOW LEVEL STIMULATION OF DOPAMINE RELEASE FROM TERMINALS OF THE NIGRO-STRIATAL PROJECTION SHOULD ELICIT FEEDING BEHAVIOUR, WHILE HIGHER LEVELS OF STIMULATION ELICIT STEREOTYPED BEHAVIOUR.
2. LOW-LEVEL STIMULATION OF THE NIGRO-STRIATAL PATHWAY AT THE LEVEL OF THE SUBSTANTIA NIGRA SHOULD ALSO ELICIT BEHAVIOURAL PHENOMENA SIMILAR TO THOSE OBSERVED BY DIRECT STIMULATION OF DOPAMINE RELEASE. CHOLINERGIC SUBSTANCES SHOULD PROVIDE SUCH STIMULATION. MOREOVER, ACETYLCHOLINESTERASE INHIBITION SHOULD INDUCE FEEDING BEHAVIOUR. RECEPTOR BLOCKING AGENTS SHOULD ANTAGONISE ANY SUCH CHOLINERGIC RESPONSE.

Chapter 7: Methods and Techniques

Specific details relating to individual experiments are provided separately: the following (i-iv) are general details relating to experiments 1, 3, 4 and 5.

i. Animal Housing

All the animals used were male black hooded rats (PVG/C strain) weighing between 210 and 330 grammes immediately before surgery, (Experiment 3), 310 and 385 gms (Experiment 4) and 325 to 535 gms (Experiment 5). All were housed individually under constant temperature ($\sim 24^{o}C$) and lighting conditions. (8 hours darkness: 2200 hours to 0600 hours or 2300 hours to 0700 hours, dependent on British Summer Time.) Food (Oxoid No. 41B) and water were freely available at all times; in addition, animals were allowed four sticks of dry spaghetti (approximately 25cm. each) every day. Sawdust bedding material was always available; cages were routinely cleaned once every week.

During the experimental sessions animals were housed in individual boxes (30 cm long, 25cm wide and 17.5cm deep). No bedding material was present in these boxes, which were thoroughly cleaned after each experimental session. Animals were either tested between 0930 hours and 1200 hours or between 1330 hours and 1600 hours. The relevant indwelling stylets were removed from the implanted animals at the start of the test session and replaced at the end, prior to replacing the animals in their home cages. The experimental material available to the animals is detailed in the individual experimental methods.

ii. Surgical Procedure (Experiments 3-5)

Prior to being positioned in a stereotaxic frame (David Kopf Instruments), in the orientation of De Groot for implantation using the atlas of Pellegrino and Cushman (1967), animals were anaesthetised by administration (ip) of 'Sagatal' (May and Baker Limited; pentobarbitone sodium), following light anaesthetisation by ether. The top of the head was shaved immediately following anaesthetisation. When in the stereotaxic frame, animals' temperature was maintained by means of a homeothermic blanket, temperature being monitored by a rectal probe. (Homeothermic Blanket Control 8142, C.F. Palmer Limited.)

Having exposed the skull following a midline incision (and controlled any bone bleeding by the application of 'Gelfoam' (the Upjohn Corporation Kalamazoo, Michigan)) holes to accommodate skull screws (12BA x 3/16") and guide cannulae (23ga, 11mm, 5.5mm remaining above dura) were drilled using Ash Round number 1 burrs held in a dental drill mounted on the stereotaxic frame. The cannulae and skull screws were then surrounded by cranioplast. (Cranioplastic liquid and powder, Plastic Products Co. Limited.) Care was taken to avoid excess application of cranioplast, and to prevent rough edges from forming on or around the implant. If necessary, the wound was then sealed with a suture anterior and/or posterior to the implant as necessary. (Mersilk non-capillary sterile braided silk suture.) Guide cannulae carriers having been removed, indwelling stylets were positioned in the guides and wound dressing powder applied. Immediately following removal from the stereotaxic and homeothermic blanket control, animals were returned to their home cages, where the sawdust bedding material had

been replaced by shredded paper, to prevent infection of the wound, and to maintain temperature. Until the animals had fully recovered consciousness, a strong light was placed directly above the cage: this had the effect of both maintaining temperature and providing a stimulus for movement. Aureomycin (an antibiotic) was administered to the animals via the water supply for a week following surgery . Animals were allowed a minimum of two weeks to recover from surgery before experiments began.

iii. Cannula Construction

All guide cannulae were constructed from 23ga stainless steel hypodermic tubing. (Internal diameter .013", external diameter .025".) Cannulae were constructed with bevelled ends, to allow easy passage through tissue; the protruding end had a drop of solder applied to provide an adequate surface for cranioplast to adhere to once the implant was secured (See Fig. 6).

Injection cannulae were constructed from 29 or 30ga stainless steel hypodermic tubing. (29ga: ID .007", OD .013"; 30ga: ID .005", OD .012".) All were bevelled. A 23ga collar was fitted to the needle: adjustment of the cannula to the correct length for injection was made by the addition of a measured piece of PP20 polythylene tubing below the collar. (See Figure 7). Indwelling stylets were constructed from 29ga tubing, with a 23ga top soldered in position. Stylets were constructed to fit snugly into guide cannulae and to be flush with the guide at the tip, to prevent clogging of the guide cannula by necrotic tissue (See Figure 6).

Figure 6. A diagram showing the implantation of guide cannulae into the CNS. The guide cannulae (E)are constructed of 23ga. stainless steel hypodermic tubing, the tip of which is bevelled. These are held in place by cranioplastic cement (A) which is held to the skull surface by four stainless steel skull screws. Solder applied at the top of the guide cannulae (B) aids the cranioplast in holding the guide cannulae. Stylets, constructed from 30ga. tubing (D) with a 23ga. cap soldered in place (C) prevent occlusion of the cannulae. Vertical co-ordinates are taken not from the skull surface (G) but from dura (F).

Figure 6

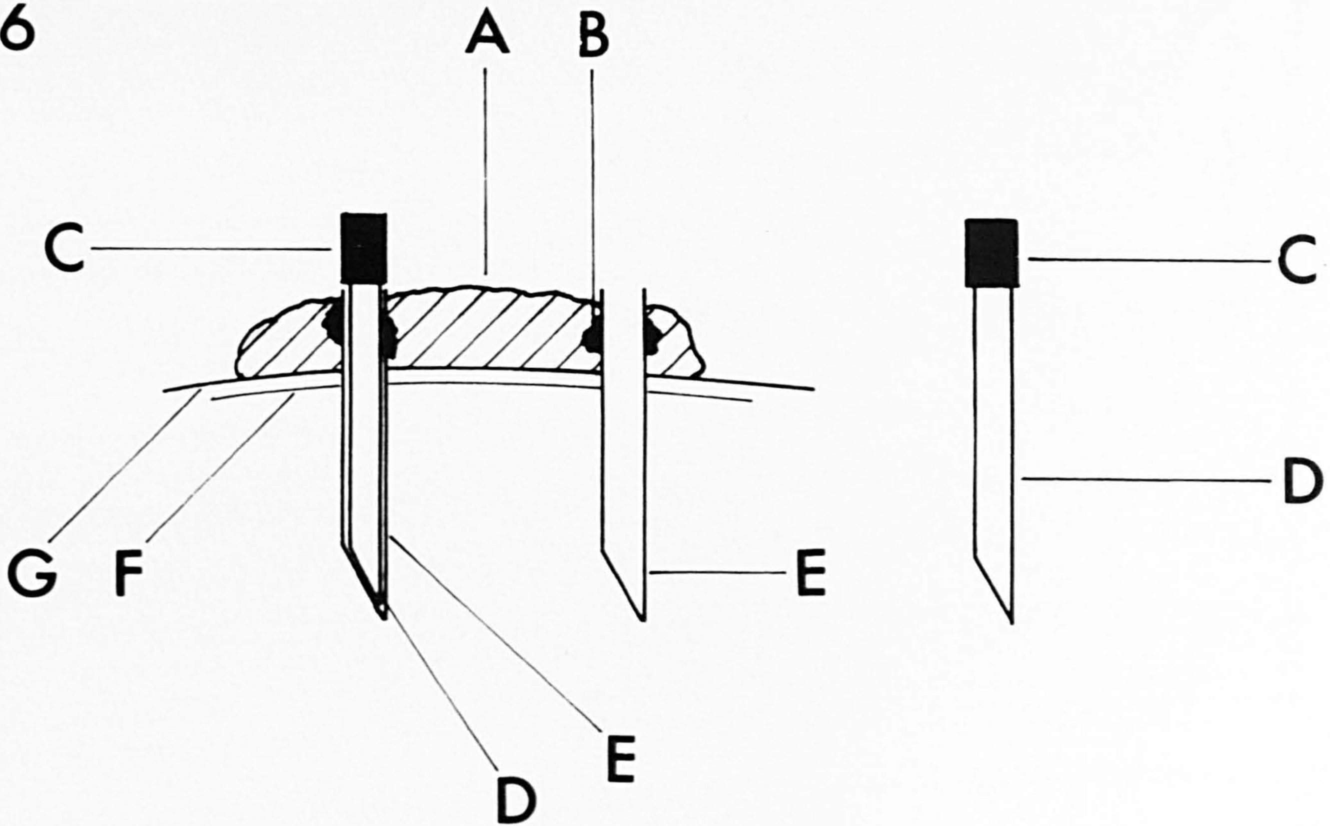
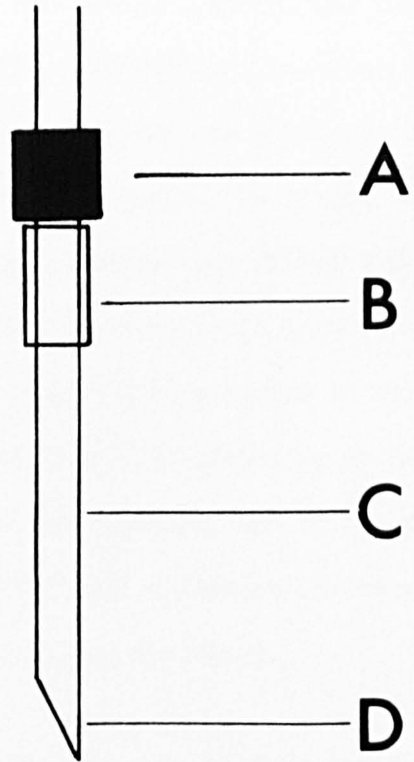


Figure 7. Microinjection Cannula. This is constructed from 30ga stainless steel tubing (C) and is bevelled at the tip (D). A 23ga. collar is soldered in position (A) and an adjustable collar, cut from PP20 polyethylene tubing, is fitted (B) allowing for adjustment of cannula length.

Figure 7



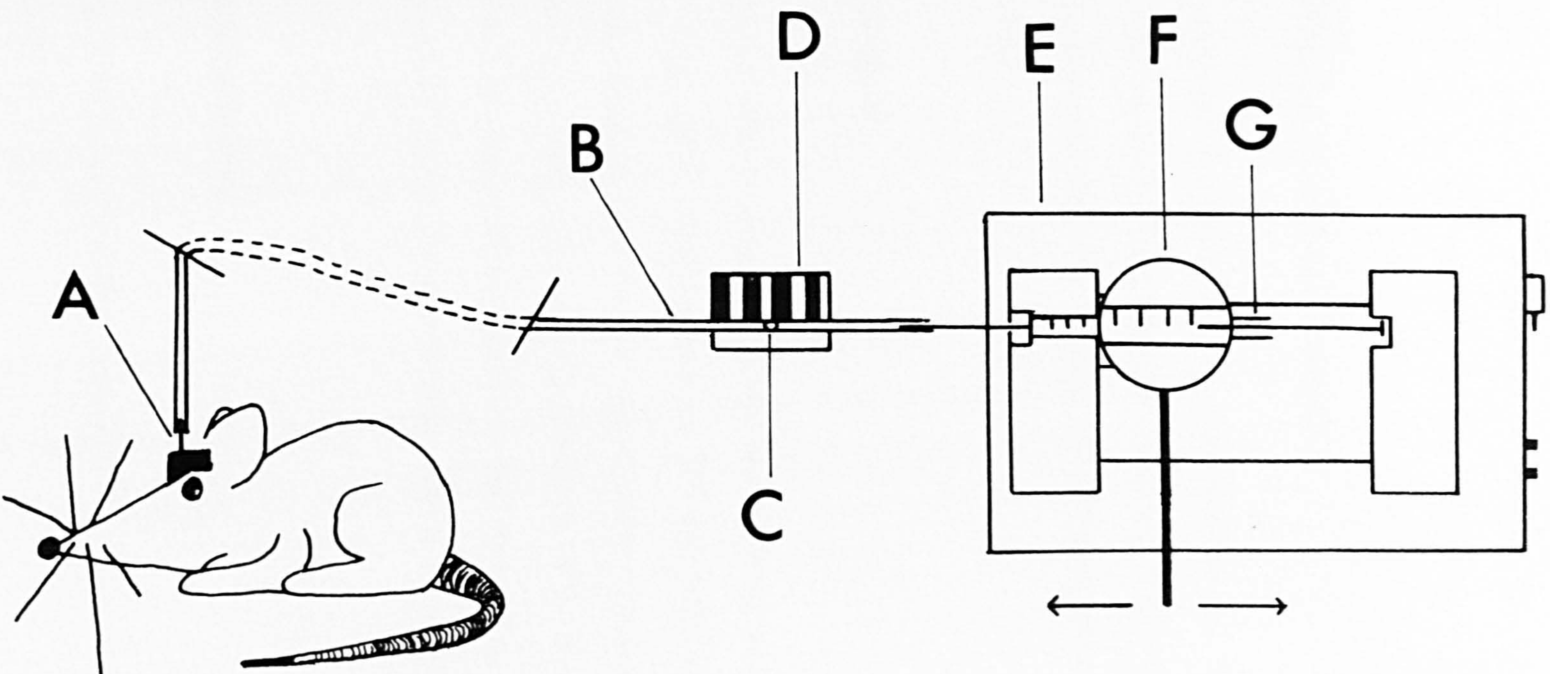
iv. Injection Procedure (See Figure 8.)

All microinjections were made unilaterally. The injection cannula, held by the collar in artery clamps, was connected by PP10 polyethylene tubing to a 1ml syringe fitted with a 30ga needle, containing a solution of the drug to be injected. This solution would then be flushed through the tubing and cannula. After this procedure the syringe and needle were carefully disconnected, avoiding the introduction of air into the tubing. However, one small air bubble was deliberately introduced into the open end of the tubing before connecting it to a ten microlitre Hamilton syringe filled with absolute alcohol. The microlitre syringe was then mounted in a Harvard Apparatus infusion/withdrawal pump (Model 901) modified to accept microlitre syringes. The pump would then be switched on to expel any air accidentally introduced into the cannula, and to bring the one deliberately introduced air bubble into a section of tubing loosely held in a clamp graduated in 0.5µl divisions.

When ready to inject, the cannula was removed from the artery clamps and held between first finger and thumb of the right hand while the animal to receive the injection was picked up in the left hand. (Stylets were always removed from the guides immediately after taking them from their home cages.) The injection cannula was then carefully inserted into the guide cannula, causing the animal as little distress as possible, before gently returning him to the experimental box. The animal having settled, the pump was then switched on for 26 seconds to pass 0.5µl of fluid through the cannula. The flow of solution was monitored by the movement of the air bubble and by direct observation (though a lens) of the microlitre syringe . After 26

<u>Figure 8.</u> The Microinjection. A 30ga. microinjection cannula is inserted into the rat's brain via permanently implanted guide cannulae (A; see also figures 6 and 7). This is connected by PP10 polyethylene tubing (B), filled with the fluid to be injected, to a 10μl Hamilton syringe (G) mounted in an infusion pump (E). The flow of fluid can be monitored at two points: (1) by examination of the microlitre syringe through a lens (F) and (2) by following the progress along the polyethylene tubing of a small air bubble (C) allowed to form in the open end of the tubing before connecting it to the alcohol filled microlitre syringe. During the injection the rate of passage of the bubble is observed by lightly clamping the tubing against a graduated (0.5μl divisions) scale (D).

Figure 8



seconds the pump was switched off; the cannula was left in place for a further 30 seconds to allow for dispersion of the solution.

If, for any reason, injections were found to be faulty, experiments were terminated, and any data collected discarded.

v. Experiment 2: Methods and Techniques

This experiment was performed in the Experimental Psychology Laboratory, Institute of Neurology, The National Hospital, London. The author is grateful for the facilities made available by Dr. L.J. Herberg.

va Animal Housing

The animals used in this experiment were Wistar rats (Bantin and Kingman Ltd., Hull) weighing between 325 and 360g immediately before surgery. Animals were singly housed under natural lighting and at an ambient temperature of approximately 24°C. Food (Oxoid No. 41B and 4 25cm sticks of spaghetti a day) and water were freely available. Sawdust bedding was provided; cages were cleaned once every week.

vb Surgical Procedures

Similar surgical procedures were adopted as for experiments 3-5, except that:

a. a Stoelting stereotoxic device was used, not a David Kopf.

b. a homeothermic blanket was not used.

c. dental cement was used in place of 'cranioplast'.

d. aureomycin was not administered.

In all major respects, surgical procedures were identical in all experiments involving intra-cerebral administration of drugs.

vc Cannula Construction

Guide cannula of the appropriate length were made from Plastic Products Guide Cannulae. These are identical to those used previously, except for having a plastic screw-thread covering that portion of the cannula above the skull. Indwelling stylets were constructed as previously described,as were injection cannulae.

vd Injection Procedure

The same procedure was adopted as for experiments 3-5, except that for these injections an 'Agla' syringe mounted in a home-made pump was used. 0.5µl of fluid was delivered in exactly 30 seconds, not 26 seconds as in the previous experiments.

vi. Feeding Behaviour Tests

Feeding behaviour was measured in all experiments by determining the amount (length, in cms) of dry spaghetti consumed in a given period of time. The spaghetti was available from a glass tube (Figures 9 and 10) which had an opening from which animals could take it. As free feeding tests (i.e. tests which did not involve an operant response intervening between animals and food) were used throughout, this was found to be the most efficient method for measuring consumption

Figure 9. Experimental apparatus. Animals were tested in a clean cage, at one end of which food and water were continuously available. Water was available from a calibrated tube fitted with a standard drip feed. Dry spaghetti was available from a glass tube. An opening was present at the bottom of this tube through which the rat was able to obtain the spaghetti. The position of food and water in the apparatus was kept constant. (See also figure 10.)

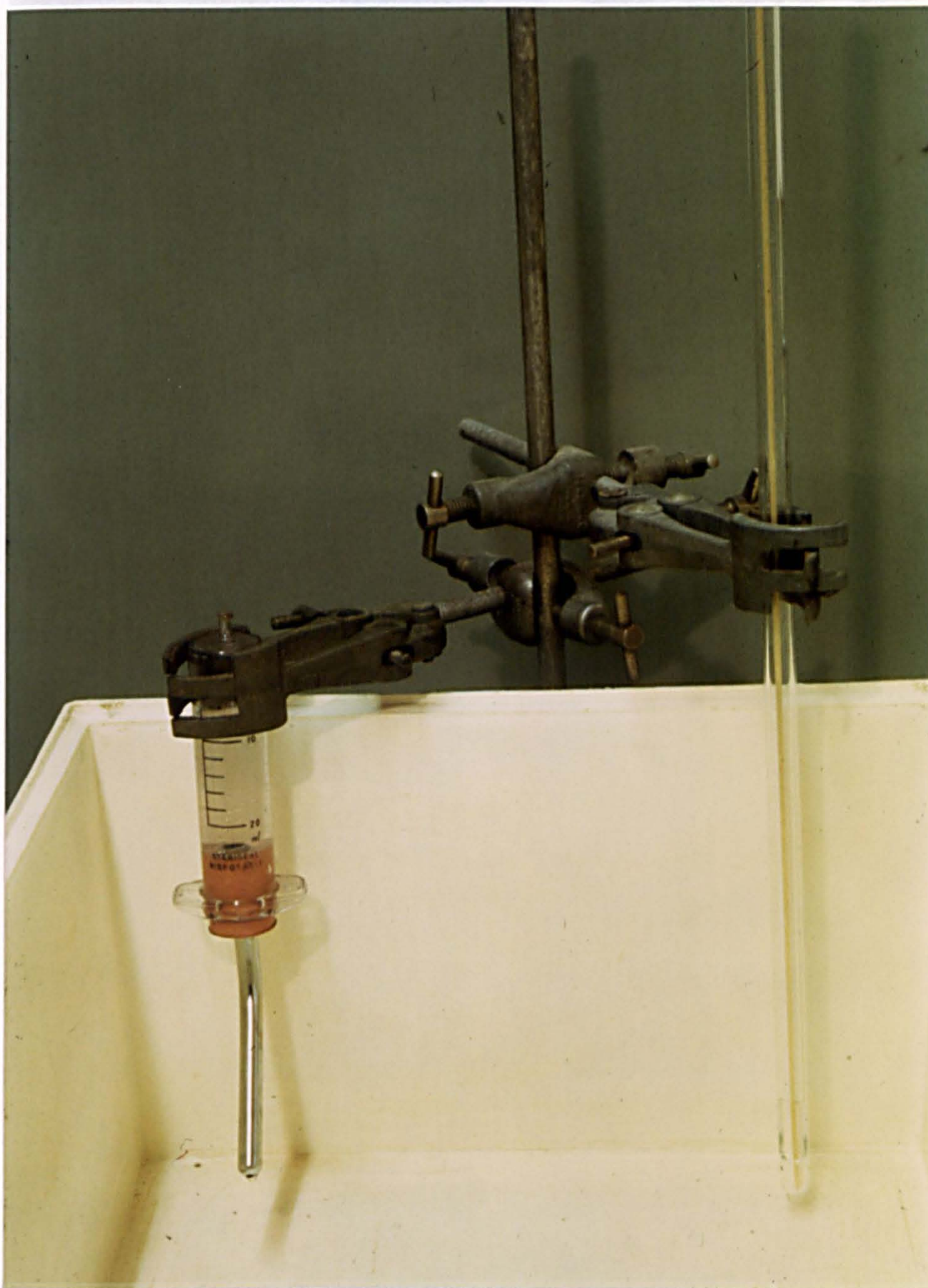
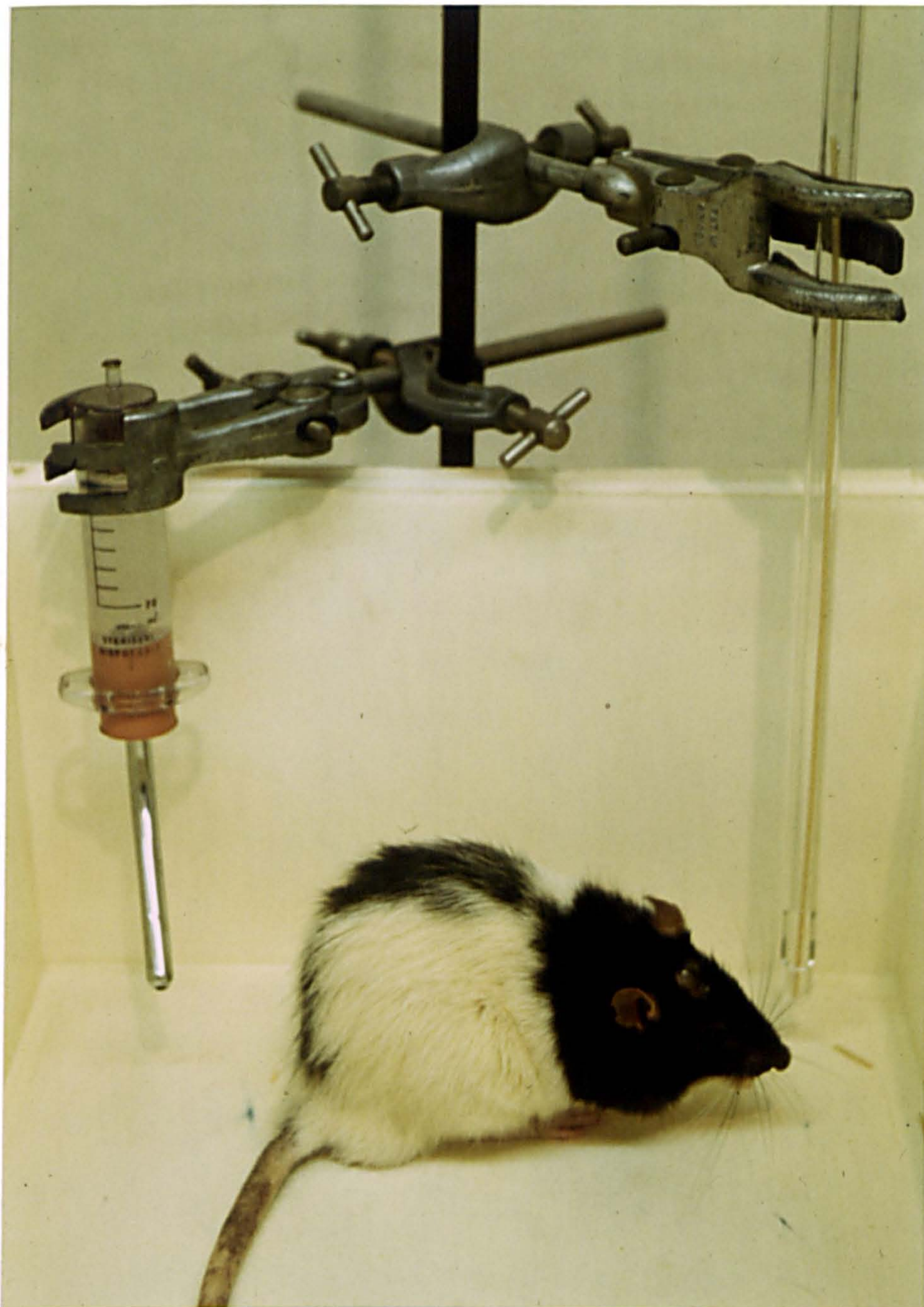


Figure 10. Showing a rat in the test apparatus obtaining dry spaghetti from the glass tube. (See also figure 9.)



because:

a) The amount of spaghetti consumed could be easily determined by substracting the amount remaining from the amount given.

b) Spillage could be minimised. Fragments of uneaten spaghetti could be collected and measured, avoiding the problems of counting half eaten food pellets and estimating wastage as dust, which is negligible with spaghetti.

c) The problem of delivery in a non-operant test was solved. Presentation of a collection of pellets invariably results in scattering,spoiling and wastage, while presentation of spaghetti kept food continuously available without running such risks.

Animals were trained to eat spaghetti from glass tubes by:

a) Presenting spaghetti alone in the home cage.

b) Familiarising the animals with the apparatus in the experimental cages.

c) If the animals had failed to take food from the tubes after this they were left overnight with a tube and a generous supply of spaghetti, but no lab diet, in their home cage. Once this procedure was successful, operation (b) was again tried. Of all the animals tested, fewer than 5% failed to take spaghetti from the tubes.

The latency and duration of feeding behaviour was also recorded. The beginning of the first bout of feeding lasting for more than 30 seconds was taken as a measure of latency. Duration of feeding was assessed by taking the sum total of time spent eating during the test

period.

vii. Drugs

All drugs administered centrally, with the exception of d-amphetamine, were dissolved in artificial cerebro-spinal fluid, made to the formula of Myers (1971) and pH adjusted to 7.0. (7.46g NaCl; 0.19g KCl; 0.14g $CaCl_2$ (Anhydrous); 0.19g $MgCl_2 6H_2O$; 1.76g $NaHCO_3$; 0.18g Na_2HPO_4; 0.61g glucose, all dissolved in 1 litre of distilled water. PO_4 and HCO_3 salts dissolved separately and added to 9/10ths of the final solution.) d-Amphetamine was dissolved in 0.9% sterile saline ("Steriflex", Allen and Hanburys). When administered peripherally a volume of 0.25ml per 100g body weight was used. It was chosen as a DA stimulant for its reported ability to release DA from pre-synaptic sites rather than block re-uptake mechanisms (it can do this but only at high doses), or directly stimulate receptors as apomorphine does (Scheel-Kruger, 1972). The d-isomer has 10X the potency of the l-isomer in releasing DA (Chiueh and Moore, 1974).

The following drugs were used: Acetylcholine chloride (Sigma), Eserine sulphate (Physostigmine sulphate) (Sigma), Atropine sulphate (Sigma), Mecamylamine hydrochloride (Sigma) and Dexamphetamine sulphate (Dexedrine) (which was a gift to the author from Smith, Kline and French).

viii. Histological Procedures

Histological verification of all microinjection sites was carried out. On completion of behavioural testing, all animals were killed by an overdose of barbiturate, administered following light anaesthetisation by ether. Animals were then perfused intra-

cardially with a 0.9% saline solution. Brains were then promptly removed and stored in 10% formalin until histological sectioning was undertaken.

Prior to sectioning, brains were blocked in the plane of Konig and Klippel (1963) or (Experiment 3 only), Pellegrino and Cushman (1967) and the formalin removed by thorough flushing with cold tap water. Brains were then mounted in a freezing microtome (MSE freezing stage microtome) or in a cryostat. 25μ sections were cut. Cresyl violet, or the more basic aniline stain thionin, were used to stain nucleii and the glial borders of cannulae tracks.

The following steps were involved in the staining procedure, once the sections had been mounted on glass slides smeared with either glycerin albumin or albumin

1. Water rinse (for a few minutes).
2. Progressive alcohol rinses (70%, 90%, 95%, 100% , 2-5 minutes in each). These steps gradually dehydrate the sections.
3. Xylene (20 minutes). This step removes cellular lipid substances which, if not removed, impair staining.
4. Alcohol rinses (100%, 95%, 90%. 70%, 2-5 minutes in each) followed by a water rinse (2-5 minutes): these stages gradually rehydrate the sections.
5. Staining; cresyl violet or thionin (2-10 minutes).
6. Water rinse (briefly).
7. Decolourization. This is achieved by briefly agitating the slides in an acid-alcohol solution (1 part 10% acetic acid to 9 parts absolute alcohol). Alternatively, on some occasions, the slides

were taken back through the four alcohol rinses and xylene in order to provoke decolourisation.

9. Slides were cover slipped using Depex mounting medium.

Sites were then verified by light microscopy, and the positions recorded in the representative frontal sections shown in the figures following each experimental report.

ix. Statistical Treatment of Data

The experiments reported here adopt a paradigm in which each animal receives each dose of drug and the vehicle alone in an individually-determined random order. Each animal thus serves as its own control. The author holds the view that while all the reported microinjections in a particular experiment may be contained solely within one anatomical structure (though with substantia nigra this is unlikely) there is no guarantee that they are aimed at exactly the same point within that structure. Indeed, it is virtually certain that they are not, and as such, it might be argued that each injection represents a discrete experiment involving a particular dose of drug, at a particular neuroanatomical site, in a particular animal.

As non-parametric statistical tests require fewer assumptions about population variance than equivalent parametric tests, and as they rarely are much less powerful than parametric tests, non-parametric statistical tests were used throughout (see Table 2).

Wherever appropriate, a non-parametric analysis of variance (ANOVA) has been used (Friedman two-way ANOVA: $\chi_r 2$) to initially test

Table 2

Non-Parametric Test	Power
Friedman Two Way ANOVA	Not known, but Siegel (1956) shows it to have essentially the same power as a parametric ANOVA (p.172)
Wilcoxon Matched-Pairs Signed-Ranks Test	Small samples = 95%, Large samples = 95.5%, the power efficiency of the equivalent parametric t test.
Sign Test	N = 6: 95% power efficiency of the t test. This declines to 63% as the sample size increases.
Mann Whitney U Test	95.5% of equivalent t test

Table 2. Comparisons of Power-Efficiency of Non-Parametric Tests and Equivalent Parametric Tests, when all the Assumptions of the Parametric Test Have Been Met. (From Siegel, 1956.)

the data. When significance (set at $p < .05$) has been achieved, further analysis of the data has employed the Wilcoxon Matched-Pairs Signed-Rank test (or, when n is low (< 6), the Sign Test). When appropriate, the Mann-Whitney U Test has been used to examine independent samples. Possible associations between feeding, drinking and the site of injection have been tested using Spearmans Rank Correlation Co-efficient. All of these tests are outlined and discussed in Siegel (1956).

Chapter 8: Experimental Reports

Experiment One: The Effects of Systemically Applied d-Amphetamine on Behaviour

Purpose

Two reports exist detailing the effects of low doses of d-amphetamine, a drug which acts to enhance the release of catecholamines (and at high doses, other monoamines), in potentiating feeding behaviour in rats (Blundell and Latham, 1978; Holtzman, 1974). At very low doses, systemic administration of this drug is believed to accelerate striatal DA turnover (Costa et. al., 1972). As no study exists showing a complete dose-response from very low doses to doses large enough to induce behavioural stereotypy, this experiment was performed to initially establish the principle that increasing levels of dopaminergic stimulation lead to changes in behaviour ranging from co-ordinated eating up to behavioural stereotypy.

Method

Five male black-hooded rats (PVG/C strain), approximately 120 days old and weighing 350-400g at the start of the experiment, were used. The animals were individually housed under controlled temperature and lighting conditions, and were allowed ad lib access to food and water. Sticks of dry spaghetti were included in the animals' diet.

Prior to testing all of the animals were trained to eat sticks of dry spaghetti from glass tubes. Animals were allowed three habituation sessions in the test apparatus before experiments began,

being placed into individual boxes (marked off into 4 equal quadrants) where hard and soft wood chips, dry spaghetti in glass tubes and water (in a calibrated bottle) were freely available. On subsequent alternate weekdays the animals were injected intra-peritoneally with a single dose of d-amphetamine sulphate ("Dexedrine",Smith, Kline and French) dissolved in 0.9% sterile saline, or a control injection of the vehicle alone immediately before being tested. The time of day at which testing took place was held constant for each animal. Each animal received all five doses of d-amphetamine (0.125, 0.25, 0.50, 1.00 and 4.00 mg/kg) and the control injection in an individually randomised order. The volume injected was also standardised - 0.25ml per 100g body weight.

Seven different measures of activity were observed and recorded, the rat's current behaviour and position being recorded at 5 minute intervals, giving a total of 36 observations.

1. Eating was measured in centimetres of spaghetti; latency of onset was also recorded.

2. Drinking was measured in mls. of water consumed.

3. Stereotyped behaviour was assessed at five minute intervals on the following 5-point scale:

0 Absence of stereotyped sniffing, etc.

2 Slight sniffing and licking, with ambulation.

4 Strong sniffing and licking, with ambulation.

6 Strong sniffing and licking; immobility.

8 Incessant sniffing and licking; immobility; retropulsion.

All points from 0 - 8 were used (i.e., odd number scores were

used); the latency of stereotyped behaviour was also recorded. (This was taken as the first score of 1 or more.)

4. Locomotor activity was assessed by recording, once every five minutes, the quadrant in which the animal was present. Quadrant changes (1 = change, 0 = no change) between subsequent 5 minute periods were summed for each animal over the test period to provide an index of activity.

5. The total time spent gnawing wood chips was recorded in seconds.

6. The number of times each animal reared (both forepaws off the floor of the cage) was recorded.

7. The total time spent grooming was recorded.

Observation was maintained for three hours following injections, after which animals were returned to their home cages.

Results

a. Consummatory Behaviour

Reference to Table 3 shows that d-amphetamine had a clear dose-dependent bitonic effect on feeding behaviour. Analysis of Variance showed this effect to be statistically significant ($\chi_r^2 = 20.89$, df = 5, $p < .001$). Further analysis using the Sign Test showed that significant differences existed between the 1.00mg/kg condition and all others, and the 4.00mg/kg condition and all others; the difference between the 0.250 mg/kg condition and control was also statistically significant. (All n = 5, X = 0, $p < .031$.) It might thus be concluded that d-amphetamine stimulates feeding at low doses and suppresses it at high doses. The latency of feeding also showed overall significant differences between the various conditions ($\chi_r^2 = 18.29$, df = 5, $p < .01$). Sign tests showed these differences to be between the 1.00mg/kg

	mg/kg d-ampehtamine					
	Saline	0.125	0.250	0.500	1.000	4.000
Feeding (cms)						
$\bar{x}$	164.54	185.74	201.14*	120.60	72.46*	0*
±SE	47.40	42.23	43.76	29.48	24.34	0
median	107.80	135.30	149.20	102.60	65.30	0
Drinking (mls)						
$\bar{x}$	1.20	0.80	0.60	0.80	0.20	0
±SE	0.49	0.37	0.29	0.37	0.12	0
median	1.00	1.00	0.50	1.00	0	0
Latency of feeding (mins)						
$\bar{x}$	18.20	31.00	18.00	42.00	85.00*	180.00*
±SE	8.65	8.86	7.00	10.07	13.04	0
median	15.00	20.00	15.00	45.00	75.00	180.00
n =	5	5	5	5	5	5

Table 3: Mean (± SE) and median amounts of Food and Water consumed in 3 hours following systemic administration of various doses of d-amphetamine; latency of feeding following d-amphetamine.

(n = no. of animals tested.)

(Feeding: $\chi_r^2 = 20.89$, df = 5, $p < .001$. Latency of Feeding: $\chi_r^2 = 18.29$, df = 5, $p < .01$. Sign tests were used to examine differences between the doses. * = sig. diff. to saline control, n = 5, x = 0, $p < .031$. Drinking: not tested; 14 of 30 observations = 0.)

dose and all other conditions. (All $n = 5$, $X = 0$, $p < .031$.) The 4.00mg/kg dose, which did not induce any feeding at all, quite obviously also showed such differences. It might thus be suggested that while lengthening the latency for feeding might account for the decrease seen in feeding at high levels of stimulation, (though other explanations for this might be advanced), a shortening of the latency cannot account for the increased feeding seen after low doses of d-amphetamine.

No significant amount of drinking was ever observed; following the highest dose of d-amphetamine drinking was completely absent. No increase in drinking was found to parallel the increase in feeding following low dose stimulation.

b. Gnawing and Grooming

The mean duration of wood-chip gnawing observed following stimulation is shown in Table 4. While these mean values display an apparent dose response curve, maximum gnawing being seen at low to middle range doses (0.250 - 0.500 mg/kg), the median values perhaps provide a more realistic picture of the effects. Gnawing was observed on only 10 of the 30 (5 animals x 6 conditions) possible occasions. The greatest amount of gnawing seen was for 310 seconds (5min. 10sec.) out of a total time of 180 minutes; on only five occasions did the total time spent gnawing by any animal exceed 1 minute; two of the five animals never showed any gnawing, while a third did so on only two occasions; each of the remaining animals showed gnawing on four of the six trials. This, and the fact that no statistically significant differences are present, strongly suggests that wood-chip gnawing was not in any way induced or affected by d-amphetamine.

	mg/kg d-amphetamine					
	Saline	0.125	0.250	0.500	1.000	4.000
Gnawing (secs)						
$\bar{x}$	13.80	53.00	81.40	69.60	52.00	0
$\pm$SE	10.46	33.16	58.95	60.55	52.00	0
median	0	39.00	0	0	0	0
Grooming (secs)						
$\bar{x}$	492.80	461.00	430.40	544.00	528.60	75.60*
$\pm$SE	53.81	29.28	90.07	108.90	66.24	15.79
median	480.00	483.00	344.00	488.00	524.00	70.00
n =	5	5	5	5	5	5

Table 4: Mean ($\pm$ SE) and median time spent Gnawing wood chips and Grooming in the 3 hours following systemic administration of various doses of d-amphetamine.

(n = no. of animals tested.)

(Grooming: $\chi_r^2 = 12.43$, df = 5, $p < .005$. Sign tests were used to examine differences between the doses. * = sig. diff. to saline control, n = 5, x = o $p < .031$. Gnawing: not tested; not observed on 20 of 30 trials.)

The mean amount of time which animals spent grooming is also shown in Table 4. Unlike the gnawing data, this does show an overall statistical significance ($\chi_r^2 = 12.43$, df = 5, $p < .005$). Sign Tests show this difference to be the product of the large fall in time spent grooming following administration of 4.00mg/kg d-amphetamine. (Significantly different to all other conditions; n = 5, X = 0, $p < .031$.)

c. Locomotor Activity and Rearing

The mean locomotor score for each condition is shown in Table 5. d-Amphetamine induced a significant, dose-responsive increase in locomotor activity ($\chi_r^2 = 22.14$, df = 5, $p < .001$). Sign Tests were used to further analyse the pattern of statistical significance: the results of these tests are presented in Table 6. Locomotor activity clearly increases as does the dose of d-amphetamine, until a maximal activation is achieved at 1.00mg/kg. The pattern of statistical significance shown in Table 6 reveals that:

i) no locomotor activation, or inactivation is shown following administration of the lowest dose of amphetamine (0.125mg/kg), while a slight increase is shown by 0.25mg/kg. (In as much as it induces a statistically significantly greater degree of activation than the lowest dose, but not saline.)

ii) All of the middle-to-high doses (0.50 - 4.00mg/kg) show a significantly greater potentiation of activity than either saline or the low doses (0.125, 0.25mg/kg).

iii) The middle-to-high doses show no significant differences amongst themselves.

The mean number of times animals reared on to their hind legs is

	mg/kg d-amphetamine					
	Saline	0.125	0.250	0.500	1.000	4.000
Locomotion						
$\bar{x}$	8.40	8.20	12.40	19.80*	22.80*	19.60*
±SE	1.96	2.06	2.64	1.28	2.27	2.60
median	8.00	10.00	16.00	20.00	23.00	19.00
Rearing						
$\bar{x}$	20.00	28.20	39.60*	47.60*	90.20*	49.80
±SE	4.64	2.75	9.44	11.89	30.48	29.11
median	19.00	31.00	31.00	35.00	71.00	25.00
n =	5	5	5	5	5	5

Table 5: Mean (±SE) and median values for Locomotion and Rearing observed in the 3 hours following systemic administration of various doses of d-amphetamine.

(n = no. of animals tested.)

(Locomotor activity: $\chi_r^2 = 22.14$, df = 5, $p < .001$. Rearing: $\chi_r^2 = 16.43$, df = 5, $p < .01$. Sign tests were used to examine differences between the doses. * = sig. diff. to saline control, n = 5, x = 0, $p < .031$.)

	mg/kg d-amphetamine				
	0.125	0.250	0.500	1.000	4.000
Saline	0	0	1	1	1
0.125	–	1	1	1	1
0.250		–	1	1	1
0.500			–	0	0
1.000				–	0
4.000					–

Table 6: Locomotor Activity: sign tests comparing between the various conditions.

(1:n = 5, x = 0, $p < .031$; 0 = not significantly different.)

shown in Table 5. This data shows an overall statistical significance (χ_r^2 = 16.43, df = 5, $p < .01$) which was further broken down by sign tests; the results of these are shown in Table 7. Rearing activity increased with dosage, reaching a maximum at 1.00 mg/kg before falling away, in fact quite dramatically, at 4.00mg/kg. (The median value, also shown in Table 5 gives a clearer indication of this than the mean value, which is raised substantially by one animal showing a very marked increase at the higher dose while all four others showed a less marked but clear decline.)

d. Stereotyped Behaviour

Classifiable stereotyped behaviours only emerged at the two highest doses employed, and as such there is a quite obvious dose-response effect, which can be seen in Table 8. This is further confirmed by Sign Tests comparing the two stereotypy-eliciting doses: a greater total score, a shorter latency and a longer duration are all shown by the 4.00mg/kg dose when compared to the 1.00mg/kg dose. (All n = 5, X = 0, $p < .031$.) At the highest dose stereotyped behaviour appeared as sniffing and rearing interspaced with periods of ambulation. The 1.00mg/kg dose elicited a similar response but with rearing rather more prevalent.

Discussion

The effects of systemically administered d-amphetamine are quite clearly dose-dependent, in that at very low doses feeding behaviour is potentiated while higher doses diminish feeding, potentiate locomotor activity and, at the highest levels examined, induce behavioural stereotypies consisting primarily of sniffing and rearing. This effect is shown in Table 9 which gives the ranked mean

	mg/kg d-amphetamine				
	0.125	0.250	0.500	1.000	4.000
Saline	0	1	1	1	0
0.125	-	0	1	1	0
0.250		-	0	1	0
0.500			-	1	0
1.000				-	0
4.000					-

Table 7: Rearing Activity: sign tests comparing between the various conditions.

(1:n = 5, x = 0, $p < .031$; 0: not significantly different)

	mg/kg d-amphetamine					
	Saline	0.125	0.250	0.500	1.000	4.000
Stereotypy						
$\bar{x}$	0	0	0	0	0.36*	3.30**
$\pm$SE	0	0	0	0	0.04	0.15
median	0	0	0	0	0.39	3.39
Latency (mins)						
$\bar{x}$	180	180	180	180	15.00*	8.00**
$\pm$SE	0	0	0	0	2.00	1.22
median	180	180	180	180	15.00	10.00
Duration (mins)						
$\bar{x}$					31.00	128.00**
$\pm$SE					2.45	4.64
median					30.00	130.00
n =	5	5	5	5	5	5

Table 8: Mean ($\pm$SE) and median values of the mean Stereotypy rating, Latency and Duration of Stereotypy shown in three hours following systemic administration of d-amphetamine.

(n = no. of animals tested)

(Stereotypy: analysis of variance not performed. Sign tests were used to make comparisons between the doses: * = sig. diff to control, n = 5, x = 0, $p < .031$. ** = sig. diff. to control and 1.00 mg/kg d-amphetamine, n = 5, x = 0, $p < .031$)

		mg/kg d-amphetamine					
		Saline	0.125	0.250	0.500	1.000	4.0000
Mean Drinking	(mls)	1	=2		=2		
Mean Feeding	(cms)		2	1*			
Mean Wood-Chip Gnawing	(secs)			1	2		
Mean Grooming	(secs)				1	2	
Mean Cumulative Locomotor Score	(Counts)				2*	1*	
Mean Cumulative Rearing Activity	(Counts)					1*	2*
Mean Stereotypy Rating						2*	1*

Table 9: Dose-responsive effects of d-amphetamine. The figures in this table are arrived at by taking the mean (n=5) values for each behaviour, under each condition, and ranking them across the six conditions; only the highest ranks are shown (1 = highest, 2 = second highest).

(* sig. diff. to control (saline) using sign test: $n = 5$, $x = 0$, $p < .031$.)

values observed for each behaviour under each drug condition. (Only the highest two ranks are shown for the sake of clarity.) The order in which the behaviours are listed is clearly contrived to show those susceptible to low-dose stimulation at the top (i.e. feeding) and those only susceptible to high-dose stimulation (i.e. stereotypy) at the bottom, effectively ranking them with respect to the level of drug stimulation at which they are maximally potentiated. While such speculation is not amenable to testing, it does appear that the most co-ordinated behaviours appear first on the dose-response curve (i.e. feeding, if we exclude drinking which is a non-significant effect, maximal under saline "stimulation"). The level of behavioural complexity then appears to decline, feeding being followed by gnawing and grooming (neither of them potentiated significantly), locomotor activity, and rearing until, finally, the stereotypic state is reached.

We might thus conclude that d-amphetamine has stimulating effects on behaviour regardless of dose, but that the dose administered determines the type and complexity of the behaviour subsequently observed. This behavioural pattern might be accounted for by the hypothesis of Lyon and Robbins (1975), (Chapter 5, section iiib). "The action of amphetamine is such that as the dose response within the CNS increases, the repetition rate of all motor activities will increase with the result that the organism will tend to exhibit increasing response rates within a decreasing number of response categories" (Lyon and Robbins, 1975, p.85). It is possible to conceive of d-amphetamine initially stimulating eating - a complex co-ordinated response requiring fairly long pauses - which, as the level of stimulation increases is actually broken down: less eating

is apparent as repetitious behaviours requiring only short pauses and amenable to a high rate of performance are increasingly stimulated. Gradually these change in character from a total locomotor activity to the quicker, faster rearing activities before even these are displaced by repetitive oral/facial behaviour.

d-Amphetamine is known to stimulate the release of both catecholamines and indoleamines - DA, NA and serotonin primarily - but at low doses (0.3 mg/kg) it has been reported to affect the turnover of striatal DA (Costa et. al., 1972) and to have no effect on tel-diencephalic NA turnover. It might be suggested that the potentiation of feeding behaviour seen following systemic administration of 0.250 mg/kg d-amphetamine is related to the acceleration of DA turnover in the striatum. This possibility is investigated in Experiment 2.

Experiment Two: The Effects of Low Doses of d-Amphetamine Microinjected into the Striatum.

Purpose

It was found in the previous experiment that d-amphetamine potentiated food consumption in non-deprived animals at doses which have been reported to stimulate the turnover of DA in the striatum (Costa et. al., 1972). The purpose of this experiment was to examine the hypothesis that low level striatal DA stimulation can potentiate feeding behaviour, by microinjection of low doses of d-amphetamine directly into areas of the striatum believed to be innervated by DA neurones.

Method

In each of four male Wistar rats (approximately 350g at the start of the experiment), stainless steel guide cannulae were implanted giving access to areas of the striatum thought to contain terminals of the nigro-striatal DA pathway. (In the atlas of Pellegrino and Cushman (1967) AP 3.0; Lat ± 2.5; Vert - 2.5.) Three of the animals were prepared with bilateral cannulae, the fourth unilateral. After recovering from surgery the animals were trained to eat sticks of dry spaghetti from glass tubes.

Animals were tested in circular plastic bowls designed to automatically record locomotor activity. The bowl was 32cm. in diameter and rested on a central pivot; six microswitches were equally spaced around the circumference. A second bowl served as a lid and formed an enclosure 26cm. high. Counts were automatically recorded when one or more microswitches was depressed. Locomotor activity by

the rat caused such activation while grooming, small postural changes and drug-induced stereotypy did not. Locomotor counts were cumulated automatically at 500 second intervals, giving a total of 12 counts in the 100 minute period. (This apparatus has been successfully used to measure locomotor activity in other studies; see for instance Bentall and Herberg, 1980.) The floor of the bowl was covered with sawdust; the lid had three holes to allow a glass tube containing dry spaghetti and a calibrated water bottle to be present within the bowl. The third, central, hole was present to allow examination of the animals' behaviour. Food and water were freely available throughout the experiment. Animals were also assessed for stereotyped behaviour at five minute intervals, as was described in experiment one. The latency of onset of feeding (as defined in Chapter 7, section vi) was recorded as the first 500 second period in which feeding occurred. (1 = first 500 sec. period; 12 = last 500sec. period; 13 = no feeding observed.) Each animal was allowed two 150 minute habituation sessions.

At the beginning of a test session animals were placed in the apparatus and allowed to settle for 50 minutes with food and water freely available. Animals were then microinjected (as described in Chapter 7, section v d) with 0.5µl of a 0.9% sterile saline solution and their behaviour recorded for 100 minutes. In subsequent tests, three different doses of d-amphetamine sulphate (0.7, 2.0 and 6.0µg) in 0.5µl 0.9% sterile saline solution were administered in an order which was individually randomised for each animal. Successive injections were separated by a minimum of 48 hours. Following d-amphetamine administration a second microinjection of the vehicle alone was made at four of the six striatal sites

examined, (the remaining two being inaccessible due to blockage of guide cannulae). As only four such injections were made, summary data (mean $\pm$ SE and median values) are shown, but the data are not included in the statistical analysis.

On completion of behavioural testing the animals were killed by an overdose of barbiturate and perfused intra-cardially with a 0.9% saline solution, followed by a solution of isotonic saline containing 10% formalin, prior to treatment by normal histological procedures.

<u>Results</u>

Data summarising feeding, drinking and locomotor activity during the 50 minute period preceding microinjection are shown in Table 10. Feeding and locomotion both show non-significant effects over the period (ANOVA: Feeding $\chi_r^2 = 1.98$, df = 3, $p < .7$; Locomotion $\chi_r^2 = 0.96$, df = 3, $p < .9$). Drinking was not tested by ANOVA because of the high number of zero observations (16 from a possible 24 (4 drug conditions x 6 striatal sites)).

The effects of intra-striatal microinjections of d-amphetamine on consummatory behaviour are shown in Table 11. The effects on feeding behaviour are also shown in Figure 11. Drinking was rarely observed: including the four second saline injection trials, drinking was observed on only 5 occasions of a possible 28. After stimulation of three of the six striatal sites examined drinking was never observed; the drinking which did occur was more evenly distributed across the five conditions, only one of which shows a zero score.

	Drug or vehicle condition subsequently administered				
		µg d-amphetamine			
	Saline 1	0.7	2.0	6.0	Saline 2
Feeding (cms)					
$\bar{x}$	100.95	106.60	95.32	103.43	123.88
±SE	40.11	51.49	41.31	47.45	66.63
median	77.35	78.20	69.70	84.80	123.30
Drinking (mls)					
$\bar{x}$	0.33	0.20	0.33	0.75	1.00
±SE	0.21	0.20	0.33	0.40	0.71
median	0	0	0	0.25	0.50
Locomotion					
$\bar{x}$	58.00	50.60	60.33	66.00	78.50
±SE	21.08	19.41	14.41	22.78	32.99
median	43.50	28.00	59.50	67.00	79.00
n =	6(4)	5(3)	6(4)	6(4)	4(2)

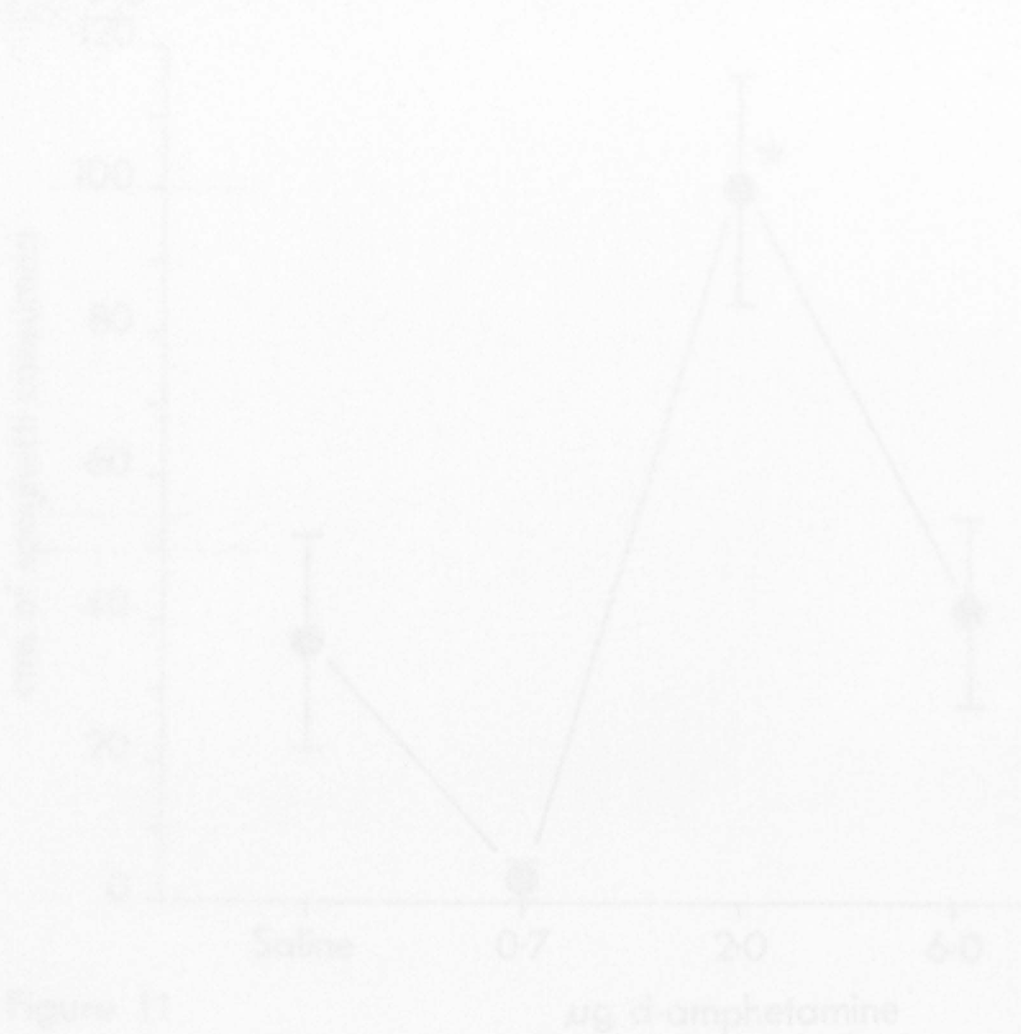
Table 10: Mean (±SE) and median values of Feeding, Drinking and Locomotion (cumulative score of each animal) in the 50 minutes preceding intra-striatal microinjection of d-amphetamine or saline solution. (n = no. of striatal sites examined. Figures in parentheses indicate the no. of rats tested.)
(Feeding: $\chi_r^2 = 1.98$, df = 3, $p < .7$; Locomotion: $\chi_r^2 = 0.96$, df = 3, $p < .9$. Drinking was not tested - 16 of the 24 observations = 0.)

	Saline 1	0.70	2.00	6.00	Saline 2
		— μg d-amphetamine —			
Feeding (cms)					
$\bar{x}$	37.55	3.20	99.93**	41.05	109.00
$\pm$SE	14.25	3.20	16.21	23.32	33.55
median	36.75	0	105.55	10.25	94.20
Drinking (mls)					
$\bar{x}$	0.17	0	0.25	0.58	0.13
$\pm$SE	0.17	0	0.17	0.58	0.13
median	0	0	0	0	0
Latency of Feeding (mins)					
$\bar{x}$	87.50	106.66	52.78*	81.94	54.17
$\pm$SE	6.72	1.67	9.04	11.27	7.22
median	79.17	108.33	54.17	87.50	58.33
n =	6(4)	5(3)	6(4)	6(4)	4(2)

Table 11: Mean ($\pm$SE) and median amounts of Food and Water consumed in 100 minutes following intra-striatal microinjection of various doses of d-amphetamine or the vehicle alone and the Latency of the feeding response. (This was recorded as the period in which feeding occurred; each period = 500 sec.; 1 = 0-500 sec.... 12 = 5500-6000 sec.; 13 = no feeding. Raw scores converted to minutes before mean ($\pm$SE) and median calculated.) (n = no. of striatal sites Figures in parentheses indicate the no. of rats tested). (Feeding: $\chi_r^2 = 11.16$, df = 3, $p < .02$. Latency of Feeding: $\chi_r^2 = 3.66$, df = 3, $p < .3$. Drinking: not tested, having been observed on only 5 occasions.

P.T.O.

Figure 11. Mean (±SE) amount of dry spaghetti consumed in 100min. following intra-striatal administration of various doses of d-amphetamine and 0.9% sterile saline solution (★ sig. diff. to saline control. Wilcoxon test: N = 6, T = 0, $p < .025$ (1 tailed test).)



Wilcoxon tests were used to examine differences between the doses, in respect of feeding behaviour. * = sig. diff. to saline control, $N = 6$, $T = 0$, $p < .025$ (1 tailed test). Sign tests were used to examine the latency of feeding. ** = sig. diff. to saline control, $n = 5$, $x = 0$, $p < .031$.)

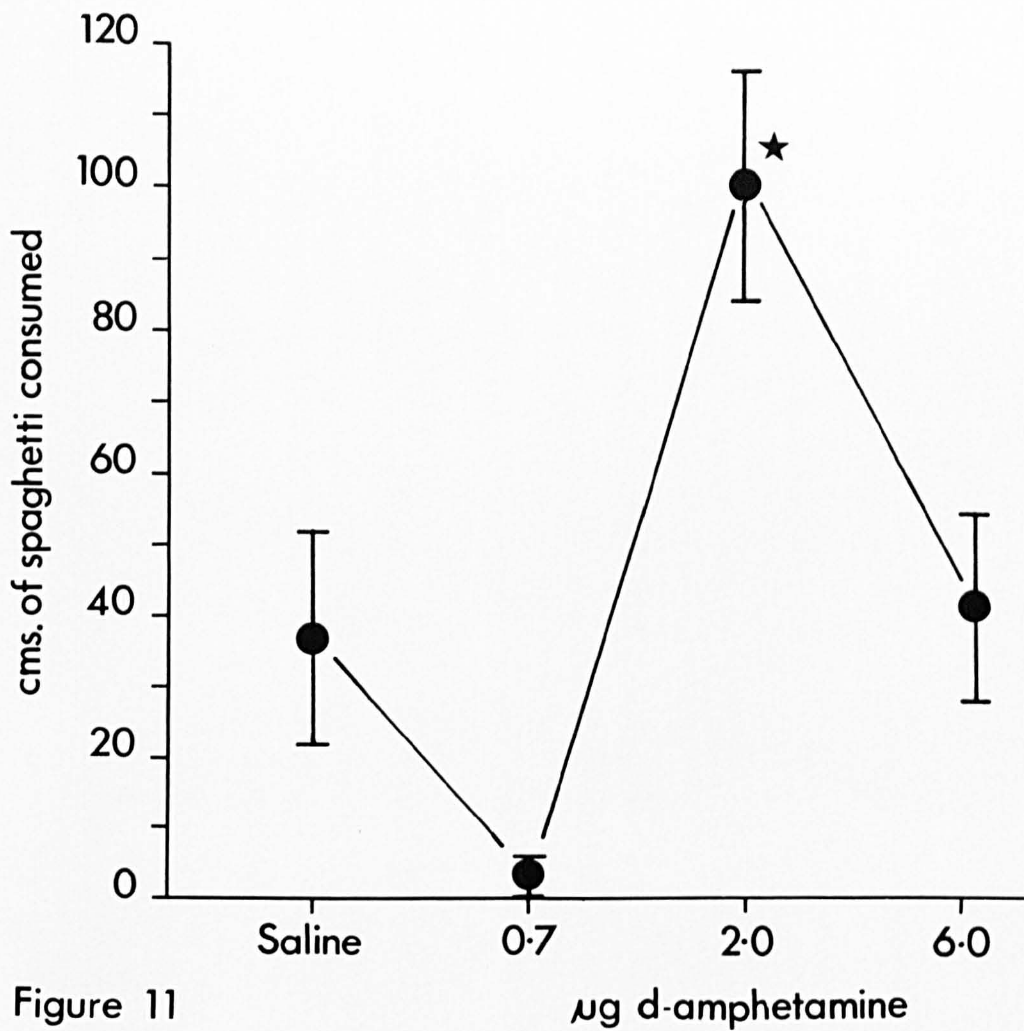


Figure 11

Feeding, however, was significantly potentiated by the drug. This is revealed by overall ANOVA ($\chi_r^2 = 11.16$, df = 3, $p < .02$), and by analysing the 2.0µg and first-saline conditions with the Wilcoxon Test (N = 6, T = 0, $p < .025$ (1-tailed test)). No difference between any other two conditions is statistically significant. It might therefore be concluded that d-amphetamine potentiates feeding behaviour in a dose dependent manner, by activation of striatal DA. Feeding, under stimulation by 2.0µg d-amphetamine also appears to show a significantly shorter latency than that elicited by control injections (Saline 1). This is revealed by Sign Test (n = 5, X = 0, $p < .031$) but not by ANOVA across all four conditions, ($\chi_r^2 = 3.66$, df = 3, $p < .3$). However, the latency even of the 2.0µg dose is very long - an average of 52.78 minutes, (range: 33 minutes - 75 minutes). The measure of latency used here is however crude: the onset of feeding was timed to no nearer than the first 500 sec. period in which it occurred.

Locomotor activity was not potentiated by any dose of d-amphetamine administered (ANOVA: $\chi_r^2 = 2.58$, df = 3, $p < .5$) (See Table 12.). While response competition might account for this lack of locomotor stimulation when feeding was potentiated (2.0µg d-amphetamine), it cannot at the other doses. It might be suggested therefore that feeding behaviour does not immediately decline as the level of stimulation increases because of a response competition from generalised locomotor activity. Classifiable stereotyped behaviours were never observed at any level of stimulation.

Histological verification of the injection sites was made. Unfortunately, as there was a long delay between the completion of

	Saline 1	µg d-amphetamine 0.7	2.0	6.0	Saline 2
$\bar{x}$	62.50	57.20	74.67	67.17	106.75
$\pm$SE	24.42	32.64	19.00	35.42	54.12
median	42.50	14.00	76.00	36.50	83.50
n =	6(4)	5(3)	6(4)	6(4)	4(2)

Table 12: Mean ($\pm$SE) and median values of the cumulative Locomotor Activity score for each animal following intra-striatal micro-injection of various doses of d-amphetamine or vehicle alone. (n = no. of striatal sites examined. Figures in parentheses indicate the no. of rats tested). Locomotor activity: $\chi_r^2 = 2.58$, df = 3, $p < .5$.)

behavioural testing and sacrifice the cannulae tracks became blurred by gliosis. In addition a technical problem in dealing with formalin fixed tissue was encountered. (The formation of crystals leads to the presence of a lattice of holes in the tissue .) However, as far as can be determined, it appears that cannulae were located within frontal portions of the caudate n. (Figure 12).

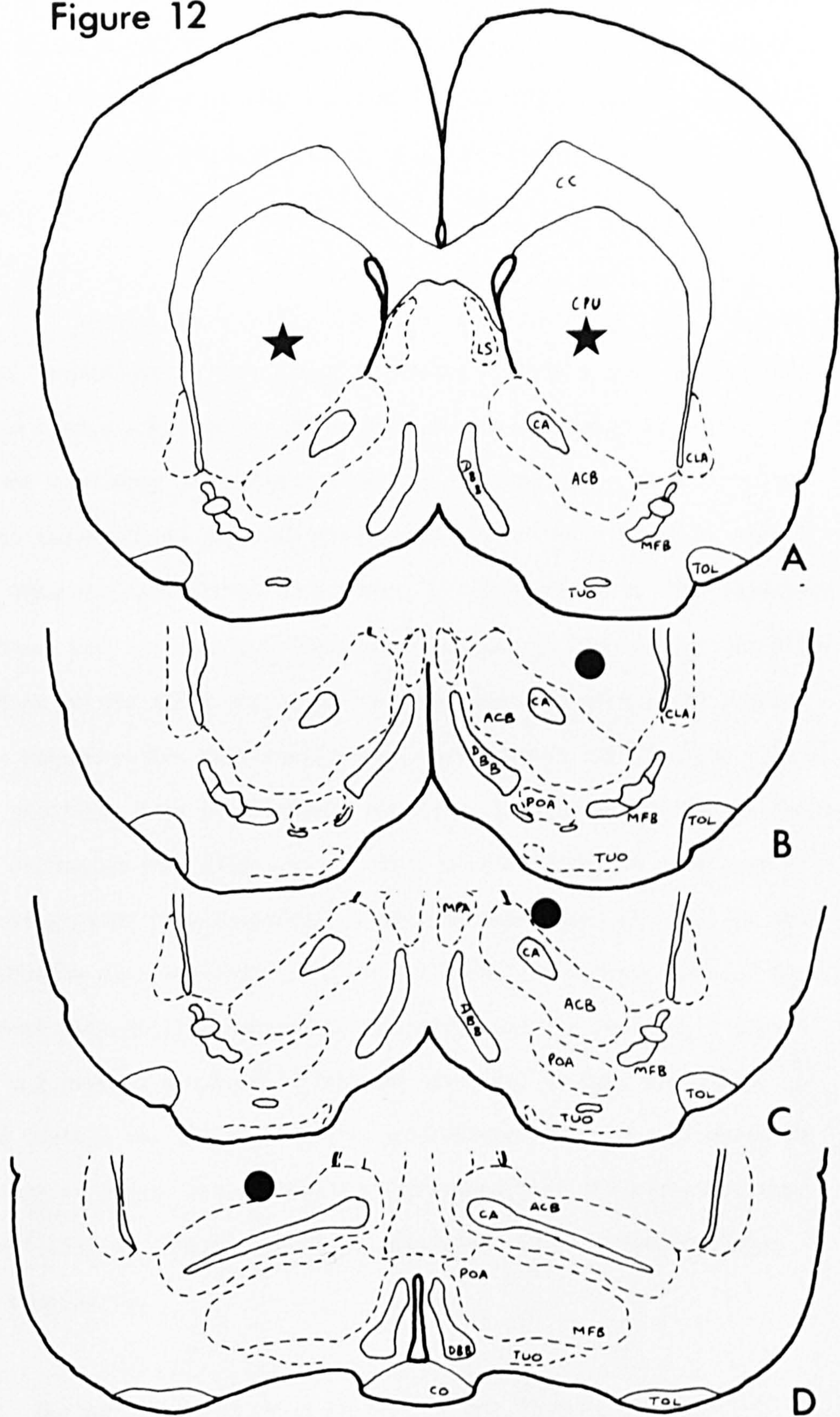
Discussion

The results of experiment two confirm that low level stimulation of striatal DA release can potentiate feeding behaviour. Drinking is not similarly affected. Locomotor activity is not potentiated by doses of d-amphetamine which do not themselves potentiate feeding. These data appear to confirm the prediction that striatal DA systems mediate the potentiation of feeding observed following low doses of systemically administered d-amphetamine.

However, it is of some concern that the latency of feeding was so long - an average 52.78 minutes, compared with only 18 minutes for 0.25mg/kg ip d-amphetamine in experiment one. It is difficult to understand why this should be. Wistar rats rather than Black-hoods, tested in an enclosed rather than an open cage, were used, but it is rather improbable that this should cause such an effect. Diffusion to an extra-striatal active site would similarly appear to be an unlikely cause of the effect as only 0.5µl was injected, though diffusion to an active site within the striatum might be possible. On the other hand, the use of such a small volume might have had an adverse effect in raising the concentration of the drug, which might have established a depolarisation blockade of local neurones. Only when this had worn off would normal neuronal stimulation be apparent.

Figure 12. Representative frontal sections of the rat brain, modified from the stereotaxic atlas of Pellegrino and Cushman (1967), indicating the placement of three of the injection sites (●) in Experiment 2 and the points at which cannulae were aimed (★). (All sections are anterior of bregma, the zero reference point: A 3.0mm, B 3.2mm, C 3.0mm, D 2.2mm .) Only 3 of the 6 injection sites could be identified with any degree of accuracy. Having examined all histological material available, an independent observer was of the opinion that " a fair statement would be that anterior caudate tissue will have almost certainly been affected by .... (the) .....injections." (Abbreviations: ACB, n. accumbens septi; CA, Commissura anterior; CC, Corpus callosum; CLA, Claustrum; CO, Chiasma opticum; CPU, Caudate n, putamen; DBB, diagonal band of Broca; LS, n. lateralis septi; MFB, medial forebrain bundle; MPA, area parolfactoria medialis; POA, area praeoptica lateralis; TOL, tractus olfactorius lateralis; TUO, Tuberculum olfactorium.)

Figure 12



If this is correct the 6.0μg dose might also have stimulated eating, but at an even later (and probably post-test) time. In this light it is interesting to note that the eating which occurred following 6.0μg stimulation (four out of six sites supported eating) was at a longer latency than following 2.0μg stimulation.

It is equally possible that the concentration of the drug was too low, the effect of this being expressed through a long latency rather than a diminished amount of eating. Costall, Naylor and Olley (1972b) found that very much higher doses of d-amphetamine microinjected into the striatum elicited stereotyped behaviour. Doses in excess of 50μg all facilitated stereotypy, but 25μg did not. The injection volume used was 5.0μl, giving a concentration at 25μg of 5μg per 1.0μl. (close to the 4.0μg per 1.0μl of the 2.0μg dose used here), while the concentration for stereotypy-inducing doses was 10μg per 1.0μl, or greater. When peripherally applied, d-amphetamine induces feeding at 0.25mg/kg (experiment one), while stereotypies are only seen at doses greater than 1.0mg/kg; a dose between 4 and 8X greater than 0.25mg/kg will reliably elicit a low level stereotypy. Thus if we accept that 2μg intra-striatally applied elicits feeding, a dose 4 to 8 X greater would still only be at most, 16.0μg. Why then did Costall et. al. only observe stereotyped behaviour at doses in excess of 50μg? The possibility therefore that the effective concentration of d-amphetamine in this study was too low must also be considered.

Another possible cause of such a long latency might be rebound neurochemical effects. Striatal DA, on this hypothesis, is stimulated in a manner appropriate for feeding but it is a con-

sequence of this stimulation - neurochemical activation at a distance - which actually elicits eating. While this is plausible, the long latency suggests a particularly subtle effect at considerable distance. As there is a suggestion from the previous experiment, and the work of Costa et. al. (1972), that systemic d-amphetamine stimulates feeding at a dose which causes an increase in striatal DA turnover, the previous hypothesis - that drug concentration was too low (or possibly too great) - might be considered a more plausible explanation of the long latency.

The involvement of striatal DA systems - namely, the nigro-striatal projection - with feeding behaviour has been suspected for some time. Lesions of the lateral hypothalamus which transect this projection, or 6OHDA lesions of the substantia nigra, elicit a profound aphagic and adipsic state (Chapter 5, section iva). However, the converse of this - that activation of these neurones should stimulate eating - has not previously been examined. This study is therefore able to provide evidence that low level stimulation of DA release within the striatum potentiates feeding behaviour while other possible behaviours (drinking, locomotor activity, stereotypy or circling), remain unaffected.

While stereotyped behaviour was observed in experiment one, it was not in experiment two, which used a much narrower range of doses. However, stereotyped behaviour is known to be associated with the striatum. Kelly et. al. (1975) and Creese and Iversen (1975) both showed that the stereotypic effects of 5.0mg/kg d-amphetamine were abolished by lesions of the striatum but not the n.accumbens. Moreover, Costall et. al. (1972b) found that high doses of d-amphetamine (25-400µg) microinjected directly into the striatum elicited stereo-

typic behaviour predominantly characterised by sniffing. (Other characteristic high-dose stereotypic effects such as gnawing, biting and licking were not observed.) While the work presented here demonstrates that the low dose effects of systemic d-amphetamine may be associated with the striatum, this previous work suggests that stereotypy may also be. (This discussion will be returned to in the General Discussion in Chapter 9.)

While it may be claimed that the stimulation of feeding reported here represents the converse of nigro-striatal lesion-induced aphagia, the question of adipsia induced by these lesions must not be forgotten. Stimulation of drinking was not observed following either central or peripheral administration of d-amphetamine. The following reasons might be advanced for this:

i. The adipsia observed following lesions is a consequence of the aphagia and as such, would not necessarily be potentiated under stimulation as feeding would be.

ii. If the nigro-striatal pathway carries information relating to sensory-motor components of feeding behaviour then stimulation of these neurones when the animal is in the presence of food might induce feeding but not drinking (except, possibly, as a consequence of feeding).

iii. If in fact the nigro-striatal pathway mediates only general sensory-motor information, (lesion-induced aphagia therefore being a consequence solely of sensory-motor debilitation), then under stimulation, any sensory-motor behaviour with a high probability of occurrence might be potentiated. As feeding, but not drinking had already occurred (Table 10), this might be considered a high probability behaviour. The apparent palatability of spaghetti might add to this.

These questions relate to a more fundamental one of "what information do nigro-striatal neurones mediate?", and take us back to the possible relationships between sensory-motor neglect and aphagia discussed in Chapter 5, section iva. The present experiments cannot in fact positively discriminate between these, or offer more than these suggestions as to why drinking was not potentiated. As the main thrust of this thesis is directed toward the nigro-striatal projection and its role in the control of feeding behaviour, we must be satisfied with saying that the experiments reported here appear to demonstrate that low level stimulation of striatal DA potentiates feeding behaviour. This appears to show that the converse of nigro-striatal lesion induced aphagia holds good.

Experiment Three: The Effect of Cholinergic Stimulation of the Substantia Nigra on Feeding Behaviour

Purpose

It has been demonstrated in experiments one and two that d-amphetamine elicits feeding behaviour in a dose-dependent manner. This action has been ascribed to a low level potentiation of DA activity within the striatum. If this is genuinely the case, then activation of nigro-striatal neurones at their source within the substantia nigra pars compacta should also elicit a dose-dependent feeding response.

Acetylcholine is believed to mediate an excitatory input to these neurones. Biochemical evidence appears to demonstrate the existence of ACh, CAT and muscarinic receptors within substantia nigra; electrophysiological evidence apparently demonstrates the existence of an ACh/DA interaction within substantia nigra, with some evidence to show that ACh excites DA neurones and some to show the opposite; behavioural evidence suggests that cholinergic activation within substantia nigra can, at high levels, elicit characteristic dopaminergic stereotypies; and that DA neurones of pars compacta possess AChE, stored within and released from dendrites, presumably to inactivate a cholinergic input.

It might thus be hypothesised that low level cholinergic stimulation of the substantia nigra, in the region of the pars compacta, should elicit a feeding response similar to that seen following potentiation of DA release within the striatum.

Method

Into each of nine male black-hooded rats (PVG/C strain), 230-270g, bilateral stainless steel guide cannulae(23ga) were implanted giving access to the substantia nigra. (Pellegrino and Cushman, 1967 ,(De Groot orientation): AP -2.4; Lat 0.5-2.0; Vert. -5.5 from dura.) A further six animals were prepared with unilateral cannulae. After recovering from surgery, animals were trained to eat sticks of dry spaghetti from glass tubes, feeding behaviour being measured in centimetres of spaghetti consumed. At the beginning of a test session animals were placed in individual boxes and allowed to settle for one hour with food and water freely available. Animals were then injected with 0.5µl of an artificial cerebro-spinal fluid (CSF), pH adjusted to 7.0, containing equal quantities of ACh and eserine (physostigmine sulphate, an anti-cholinesterase). Three different doses of ACh and eserine (2.5, 5.0 and 10.0µg of each) and a control injection of the vehicle alone were administered in an order which was individually randomised for each animal; successive injections were separated by a minimum of 48 hours.

On completion of behavioural testing, the animals were killed by an overdose of barbiturate and perfused intra-cardially with a 0.9% saline solution according to standard histological procedures.

Results

The mean (± SE) and median amounts of food and water consumed during the hour preceding microinjection are shown in Table 13. No significant differences exist between the various "conditions" . (ANOVA: Feeding $\chi_r^2 = 2.92$, df = 3, p $<.5$; Drinking: not tested.)

	µg and ACh and Eserine				
	CSF	2.5	5.0	10.0	Overall
Feeding (cms)					
$\bar{x}$	115.39	157.27	134.63	161.02	141.83
±SE	18.35	22.19	16.93	21.25	9.91
median	119.90	150.30	147.30	180.10	147.30
Drinking (mls)					
$\bar{x}$	0.29	0.39	0.50	0.28	0.37
±SE	0.17	0.15	0.22	0.12	0.08
median	0	0	0	0	0
n =	19(15)	19(15)	19(15)	18(15)	75(15)

Table 13: Mean (±SE) and median amounts of Food and Water consumed in 1 hour preceding microinjection of various doses of ACh and Eserine or a CSF control into the substantia nigra. (n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested).

(Feeding: $\chi_r^2 = 2.92$, df = 3, $p < .5$. Drinking: not tested - 51 of 75 observations = 0)

Table 14 shows the mean ($\pm$ SE) and median amounts of food and water consumed in the 30 minutes immediately following microinjection. The dose-dependent nature of the feeding response is more clearly displayed in Figure 13. Analysis of Variance reveals that this feeding response shows statistical significance ($\chi_r^2 = 15.15$, df = 3, $p < .01$) and further analysis using the Wilcoxon Test shows that both the 2.5µg and 5.0µg doses induce feeding significantly greater than control (2.5µg v CSF: T = 16.5, N = 15, $p < .01$; 5.0µg v CSF: T = 0 N = 19, $p < .005$; both 1-tailed tests) while the 5.0µg and 10.0µg doses also show statistically significant differences (T = 33, N = 18, $p < .01$, 1-tailed test).

The amounts of water drunk under the various conditions of stimulation were not analysed statistically. Drinking occurred on only 14 occasions out of a possible 76 (19 nigral sites examined X 4 conditions) and the median values accurately reflect the fact that no water at all was drunk in the majority of cases. One animal drank far more than any other, a total, over the four conditions of 5mls. The grand total amount of water drunk by all animals under all conditions was only 15.5mls . This animal was found to have the most anterior injection site of all.

The mean ($\pm$ SE) and median latency and duration of feeding are shown in Table 15. (Data for these measures is available for 9 of the 19 sites examined.) Analysis of variance shows that the various doses of ACh and eserine did not significantly alter animals' latency to feed ($\chi_r^2 = 4.6$, df = 3, $p < .3$). A similar analysis examining the duration of the response is not possible, owing to the differing number of animals eating under each condition. Sign Tests were applied

	CSF	μg ACh and Eserine 2.5	5.0	10.0
Feeding (cms)				
$\bar{x}$	11.17	32.55*	45.16**	22.41***
$\pm$SE	3.92	8.41	6.34	6.31
median	0	23.30	37.90	12.20
Drinking (mls)				
$\bar{x}$	0.21	0.24	0.16	0.22
$\pm$SE	0.09	0.13	0.08	0.15
median	0	0	0	0
n =	19(15)	19(15)	19(15)	18(15)

Table 14: Mean ($\pm$SE) and median amounts of Food and Water consumed in the first 30 minutes following microinjection of various doses of ACh and Eserine mixtures, or an artificial CSF control, into the substantia nigra.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.) (Feeding: $\chi_r^2 = 15.15$, df = 3, $p < .01$. Drinking: not tested - only 14 of 75 sites supported drinking. Differences between the doses were examined using the Wilcoxon test. * = sig. diff. to CSF control, T = 16.5, N = 15, $p < .01$; ** = sig. diff. to CSF control, T = 0, N = 19, $p < .005$; *** = sig. diff. to 5.0 μg ACh and eserine, T = 33, N = 18, $p < .01$. (All 1 tailed tests)).

Figure 13. Mean ($\pm$SE) amount of dry spaghetti consumed following administration of various doses of equal quantities of ACh and eserine, and artificial CSF, into the rat substantia nigra. (● : 0-30 min. following microinjection; ○ : 30-90 min. following microinjection; ▲ : 0-90 min. following microinjection.)

(▲ : sig. diff. to CSF control. (2.5 μg vs. CSF: $T = 16.5$, $N = 15$, $p < .01$; 5.0 μg vs CSF: $T = 0$, $N = 19$, $p < .005$.)

▲▲: sig. diff. to 5.0 μg ACh and eserine. ($T = 33$, $N = 18$, $p < .01$)

All Wilcoxon test, 1 tailed.)

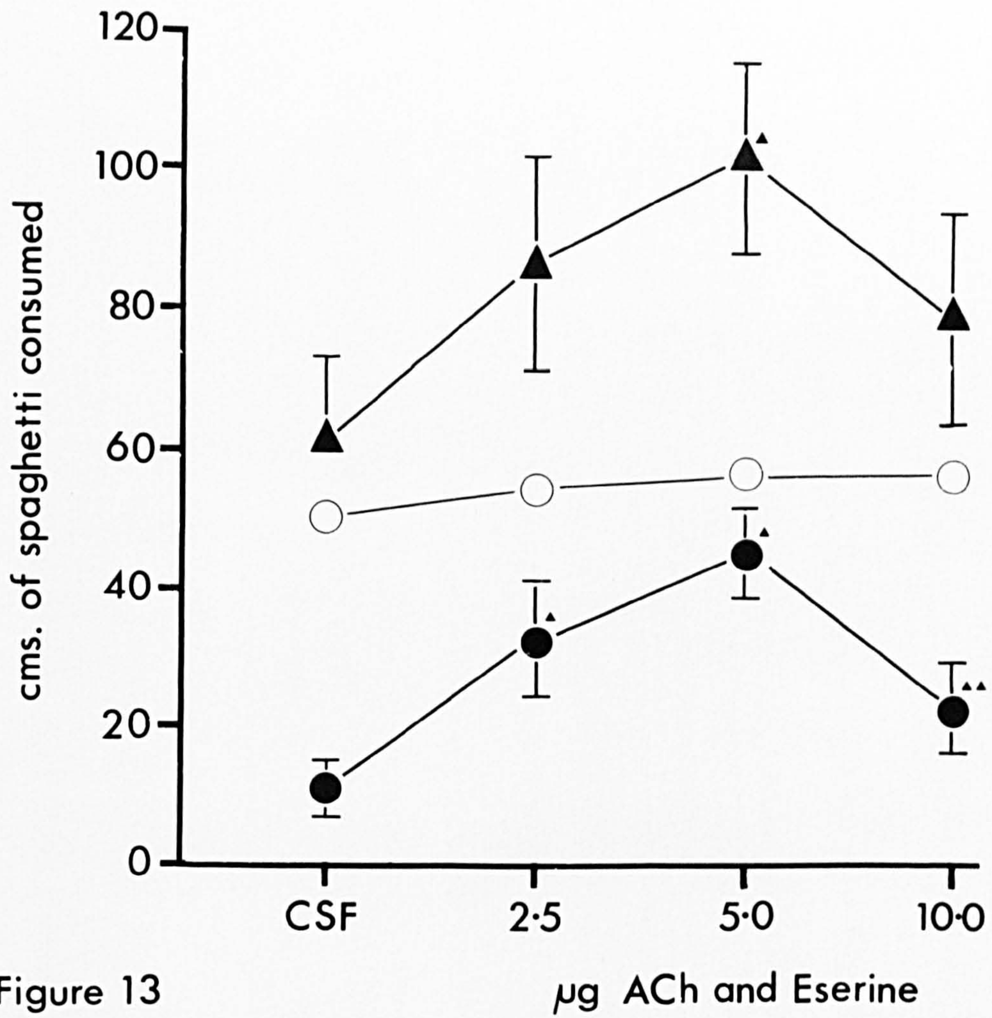


Figure 13

	CSF	2.5	5.0	10.0
		μg ACh and Eserine		
Latency of Feeding (min)				
$\bar{x}$	25.78	17.89	15.00	24.56
±SE	2.80	3.91	1.00	2.19
median	30.00	12.00	15.00	28.00
n(Latency) =	9(8)	9(8)	9(8)	9(8)
Duration of Feeding (min)				
$\bar{x}$	4.00	12.20	13.89	6.20
±SE	1.00	3.07	0.96	2.03
median	4.00	15.00	14.00	7.00
n. of sites supporting	2/9	5/9	9/9	5/9
n(Duration) =	2(2)	5(5)	9(8)	5(5)

Table 15: Mean (±SE) and median values of the Latency and Duration (in minutes) of Feeding behaviour in the thirty minutes following microinjection of various doses of ACh and Eserine mixtures into the substantia nigra.

(n(Latency): no. of nigral sites examined. Figures in parentheses indicate no. of rats tested. n(Duration): no. of nigral sites giving rise toduration of feeding scores > 0. Figures in parentheses indicate no. of rats tested. For latency, zero feeding scores were counted as 30 min, the maximum score.For duration, zero scores were discarded. This accounts for the values of n. given for each measure (Latency: $\chi_r^2 = 4.6$, df = 3, $p < .3$; Duration: not analysed by ANOVA. Sign tests: 5.0μg vs. 10.0μg, N = 5, x = 0, $p < .031$. 2.5μg vs. 5.0μg N = 5, x = 2, $p < .50$.)

where possible, and these show that the duration of the response differs significantly between the 5.0 and 10.0µg conditions (N = 5, X = 0, $p < .031$) but not the 2.5 and 5.0µg conditions (N = 5, X =2, $p < .5$). Table 15 also shows the number of animals eating in response to cholinergic stimulation. These data suggest that 5.0µg ACh and eserine induce a greater feeding response than either CSF control injections or other doses by:

i. Potentiating feeding behaviour in all animals studied rather than only a proportion (See Table 18).

ii. By a longer duration of feeding.

iii. As the latency of the response to 5.0µg does not significantly differ from other doses or control, a faster rate of eating might also be hypothesised.

The strength of the feeding induced by 5.0µg ACh and eserine is further indicated by Tables 16 and 17 and Figure 13. Table 16 shows that between 30 and 90 minutes following microinjections, the animals ate, on average, a little over 50cm. of spaghetti, regardless of the stimulation received. (Any differences here are clearly non-significant. $\chi_r^2 = 1.13$, df = 3, $p < .8$.) While there was still very little drinking observed, significant differences do emerge in this period.

There are too many zero scores to permit an effective ANOVA, but the Wilcoxon test shows the drinking elicited by 2.5µg ACh and eserine to be significantly greater than either the CSF control (T = 12.5, N = 12, $p < .05$) and 5.0µg (T = 0, N = 9, $p < .01$; both 2-tailed tests). Table 17 shows the feeding and drinking responses in the 90 minutes following microinjection, and over this period,

	μg ACh and Eserine			
	CSF	2.5	5.0	10.0
Feeding (cms)				
$\bar{x}$	50.42	53.95	56.12	56.01
$\pm$SE	10.48	10.87	11.61	10.61
median	46.90	57.60	50.60	52.55
Drinking (mls)				
$\bar{x}$	0.08	0.45	0.11	0.28
$\pm$SE	0.06	0.13	0.08	0.12
median	0	0.50	0	0
n =	19(15)	19(15)	19(15)	18(15)

Table 16: Mean ($\pm$SE) and median amounts of Food and Water consumed between 30 and 90 minutes following microinjection of various doses of ACh and Eserine mixtures into the substantia nigra. (n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested). (Feeding: $\chi_r^2 = 1.13$, df = 3, $p < .80$. Drinking: ANOVA not used - 56 of the 75 observations made = 0. *: Wilcoxon tests: 2.5μg ACh and eserine vs. CSF: T = 12.5, N = 12, $p < .05$. 2.5μg vs. 5.0μg ACh and eserine. T = 0, N = 9, $p < .01$. (Both 2 tailed tests)).

	CSF	µg ACh and Eserine 2.5	5.0	10.0
Feeding (cms)				
$\bar{x}$	61.59	86.50	101.27*	78.42
±SE	12.01	15.47	13.49	15.08
median	56.50	84.50	92.30	81.45
Drinking (mls)				
$\bar{x}$	0.29	0.68**	0.26	0.50
±SE	0.10	0.18	0.10	0.17
median	0	0.50	0	0
n =	19(15)	19(15)	19(15)	18(15)

Table 17: Mean (±SE) and median amounts of Food and Water consumed between 0 and 90 minutes following microinjection of various doses of ACh and Eserine mixtures into the substantia nigra. (n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested).
(Feeding: $\chi_r^2 = 8.88$, df = 3, $p < .05$. * = Wilcoxon test, 5.0µg ACh and eserine vs. CSF, T = 17, N = 19, $p < .005$ (1 tailed test). Drinking: ANOVA not used - 45 scores from 75 = 0. ** 2.5 vs. 5.0µg ACh and eserine: T = 16, N = 13, $p < .05$ (2 tailed test).)

	CSF	2.5	5.0	10.0
		μg ACh and Eserine		
0-30 minutes				
Feeding	8	12	19	13
Drinking	5	3	4	2
30-90 minutes				
Feeding	13	13	17	16
Drinking	2	10	2	5
0-90 minutes				
Feeding	14	15	19	16
Drinking	6	11	6	7
TOTAL NUMBER OF SITES:	19	19	19	18*
NUMBER OF RATS TESTED:	15	15	15	15

Table 18: Number of sites from which Feeding and Drinking could be elicited in various periods following microinjections of cholinergic substances or artificial CSF into the substantia nigra. (* One guide cannula blocked before the full dose response could be examined.)

significant differences remain. (Feeding: ANOVA $\chi_r^2 = 8.88$, df = 3, $p < .05$. The Wilcoxon Test indicates that the difference lies between the CSF control and 5.0µg T = 17, N = 19, $p < .005$, 1-tailed test. Drinking: Wilcoxon test reveals significant differences between 2.5µg and 5.0µg T = 16, N = 13, $p < .05$, 2-tailed test.) Figure 13 shows the mean amount of feeding elicited by the various conditions in the three time periods.

Overall it might therefore be concluded that cholinergic stimulation of the substantia nigra elicits dose-dependent feeding behaviour. 5.0µg ACh and eserine significantly potentiates feeding behaviour in the 90 minutes following microinjection into the substantia nigra. Moreover, the potentiation appears to occur within a very short period; as the average latency of the response is 15.00 minutes, and as no significant potentiation occurred in the 30-90 minute period, it might be suggested that it is in the period 15-30 minutes following injection that the potentiation typically occurs. Prolonging the duration of eating in this period and increasing the rate of eating might be suggested as the cause.

Histological examination of all injection sites was made; representative frontal sections showing the positions of all of these are shown in Figure 14. For each animal the value of two factors was determined. (a) A measure of the effectiveness of cholinergic stimulation in eliciting feeding was made by determining the differences between the amount of drug-induced feeding and CSF-induced feeding at each substantia nigra site examined. The following formula was used to calculate this value:

Figure 14. Representative frontal sections of the rat brain, modified from the stereotaxic atlas of Pellegrino and Cushman (1967) indicating the placement of injection sites in Experiment 3. The sections are arranged from anterior (A) to posterior (J); the distance of each from bregma (zero point) is (in mm); A - 1.6, B - 2.2, C - 2.4, D - 2.6, E - 2.8, F - 3.0, G - 3.2, H - 4.2, I - 4.4, J - 4.8. ● : effective site ○ : ineffective site ★ : represents the site of injection in the animal with the greatest overall food intake elicited by cholinergic stimulation. (Abbreviations: arh, n. arcuatus hypothalami; cl, n. subthalamicus; dtd, decussatio tegmenti dorsalis; hp, fasciculus retroflexus; ip, n. interpeduncularis; lm, lemniscus medialis; mp, n. mamillaris posterior; ot, tractus opticus; pc, pedunculus cerebri; ph, n. posterior hypothalami; pvg, substantia grisea centralis; re, n. reuniens thalami; sn, substantia nigra; vtn, n. ventralis tegmenti; zi, zona incerta.)

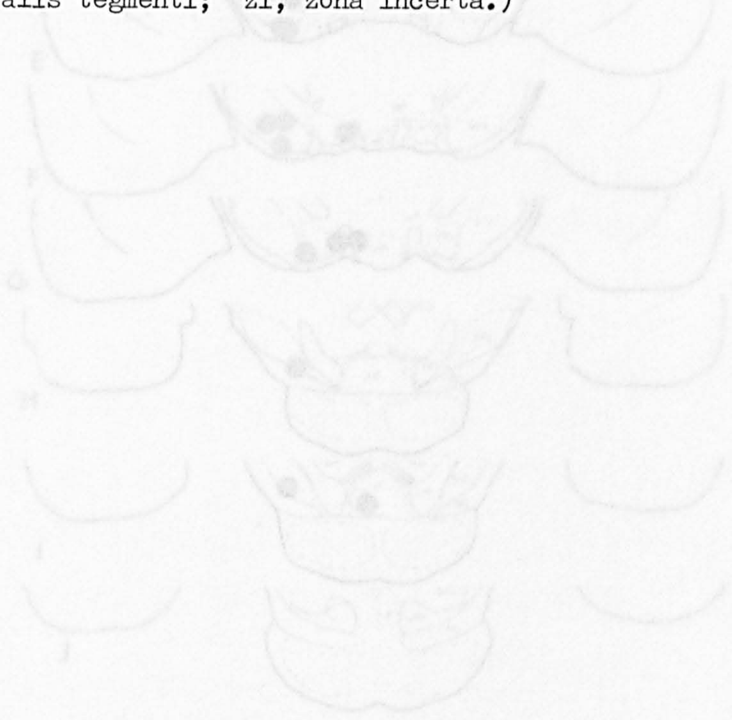
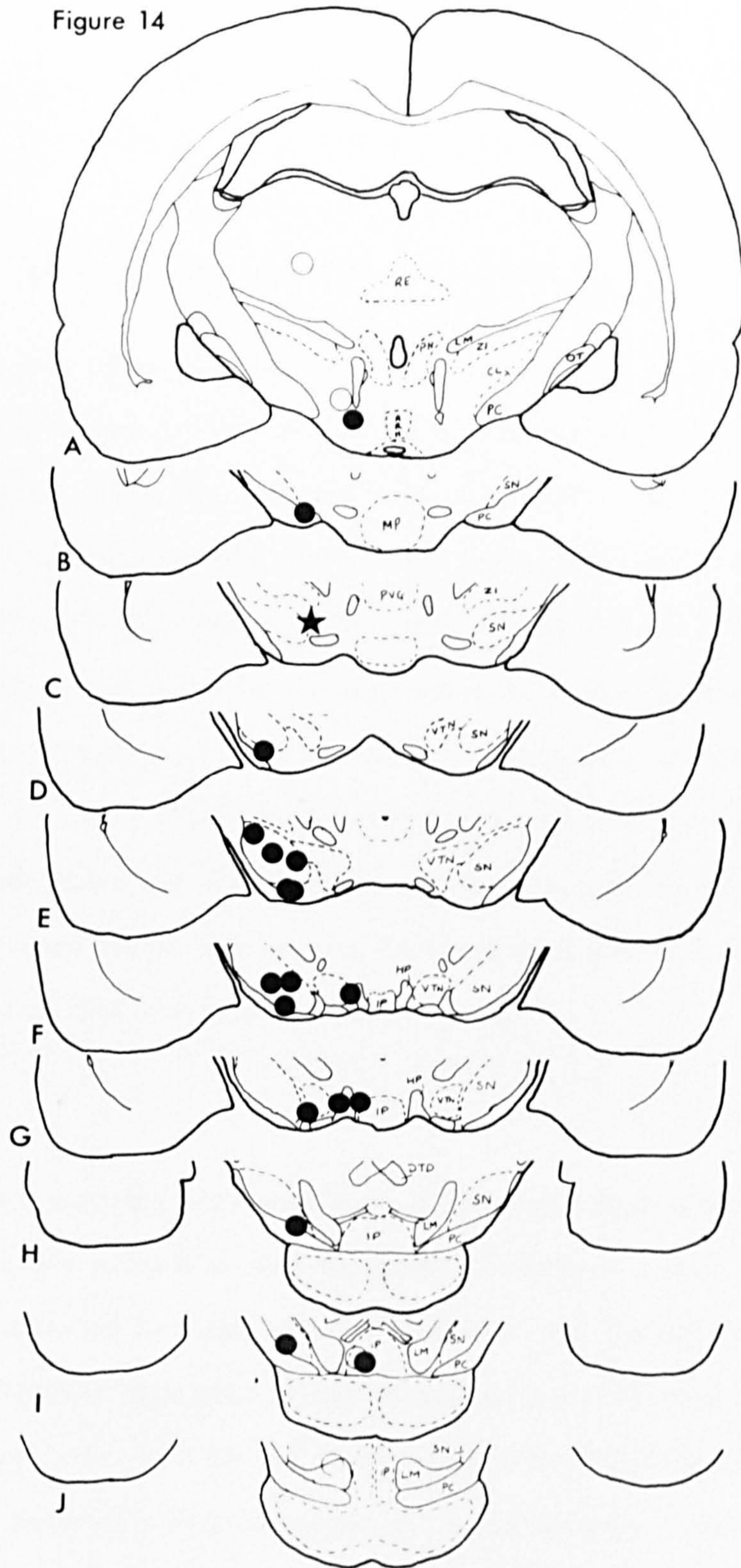


Figure 14



$$\text{Overall Effectiveness} = \frac{(D1-CSF) + (D2-CSF) + \ldots\ldots + (Dn-CSF)}{n}$$

where D1 = feeding elicited by first dose.

D2 = feeding elicited by second dose.

Dn = feeding elicited by nth dose.

CSF = feeding elicited by CSF control.

The values found for each site were then ranked (1 = highest feeding, 19 = lowest). (b) A measure of the physical distance between the site of injection and the site from which most feeding was obtained (i.e. the site with the highest overall effectiveness). These values were then ranked, (19 = most active site, 1 = least). This site was found to be in the pars compacta region of substantia nigra and is identified in Figure 14. A statistically significant ($t = 2.44$, $df = 2$, $p < .05$, two-tailed test) negative correlation of -0.51 between these two measures was obtained indicating that the feeding response became weaker with increasing distance of the injection site from the pars compacta region.

Discussion

The data reported here show that cholinergic stimulation of the substantia nigra elicits a dose-dependent feeding response. Feeding is most potentiated by 5.0μg ACh and eserine; the latency of the response following this dose is not significantly different to that following any other dose in the first 30 minutes following micro-injection. Moreover, this dose does not significantly potentiate feeding in the 30-90 minutes following microinjection. However, as 5.0μg ACh and eserine elicits significantly more eating in the thirty minutes following injection, and over ninety minutes, it might be concluded that a powerful stimulation of eating occurs

between 15 and 30 minutes following administration. The duration of responding as far as it can be examined, appears to account for some of this stimulation; the fact that all animals tested ate following only the 5.0µg dose and no other must also account for a proportion of the effect; and as latency is not a factor and duration only, apparently, marginally so, it might be suggested that the rate of eating was also potentiated.

Drinking showed no increases during the period when feeding was potentiated. Moreover, significant amounts of drinking were only rarely observed, animals more often than not showing no water consumption.

The results of this experiment strongly suggest that acetylcholine has a functional role within the substantia nigra. Moreoever the data reported here, taken in conjunction with that presented in experiment two, suggest that cholinergic stimulation within substantia nigra induces eating by activation of the nigro-striatal DA pathway. The evidence supporting this runs along two lines: (1) the feeding response observed following cholinergic stimulation of substantia nigra is greatest when injections are made into the pars compacta region - scurce of the dopaminergic nigro-striatal projection - and become weaker with increasing distance from this region. (2) At low levels, both dopaminergic stimulation within the striatum and cholinergic stimulation of pars compacta elicit a strong eating response, which is not accompanied by drinking. In other words, it might be suggested that activation of the nigro-striatal pathway, either by stimulation of DA release from terminals or by activation at source, can elicit similar behaviours.

As the DA neurones of the pars compacta are known to both contain and release AChE, a more effective method of examining the hypothesis that it is these neurones which mediate cholinergically induced feeding might be a blockade of AChE activity within the region, which would allow any endogenous cholinergic substances to have maximal effect.

Experiment Four: The Effect of Acetylcholinesterase Blockade Within the Substantia Nigra on Feeding Behaviour

Purpose

The previous experiments have shown that stimulation of DA release within the striatum and cholinergic stimulation of the substantia nigra both induce feeding behaviour. It has been hypothesised that these both represent functional expressions of activity in the dopaminergic nigro-striatal pathway.

The clearest evidence for cholinergic stimulation of pars compacta DA neurones lies in the observations that these cells both contain AChE and release it under physiological stimulation; the function of this is probably the inactivation of a cholinergic input to these cells. It should therefore be possible to potentiate feeding behaviour not only by cholinergic stimulation of the pars compacta but also by blockade of AChE, which would have the effect of enhancing the activity of any endogenous ACh present by inactivation of its degradative enzyme.

Method

Into each of six male black-hooded rats (PVG/C strain), 310-385g, bilateral stainless steel guide cannulae (23 gauge) were implanted giving access to the substantia nigra. (In the atlas of Pellegrino and Cushman (1967) (De Groot orientation) AP -2.4; Lat ± 2.0; Vert - 5.5). After recovery from surgery animals were trained to eat sticks of dry spaghetti from glass tubes, feeding behaviour being measured in centimetres of spaghetti consumed. At the beginning of a test session animals were placed in individual

boxes and allowed to settle for one hour with food and water freely available. Animals were then microinjected unilaterally with 0.5µl of artificial CSF, pH adjusted to 7.0, containing one of three different doses of eserine sulphate (2.5, 5.0 or 10.0µg) and a control injection of the vehicle alone. Drugs and control were administered in an order which was individually randomised for each animal; successive injections at any site were separated by a minimum of 48 hours.

On completion of behavioural testing the animals were killed by an overdose of barbiturate and perfused intracardially with a 0.9% saline solution according to standard histological procedures.

Results

The mean (±SE) and median amounts of food and water consumed during the 60 minutes preceding microinjection are shown in Table 19. No significant differences exist between the various conditions (ANOVA: Feeding: $\chi_r^2 = 3.56$, df = 3, $p < .5$). Drinking was not tested; the median values given in Table 19 indicate that drinking was very rarely observed. Drinking actually occurred on 6 occasions out of a possible 37. (37 = 10 sites examined x 4 conditions - 3 injections not made due to guide cannula blockage.)

Table 20 presents the mean (±SE) and median amounts of food and water consumed in thirty minutes following microinjection of various doses of eserine sulphate into the substantia nigra. Eserine appears to have a dose-dependent effect on feeding behaviour. Moreover, this dose-response curve is strikingly similar to that seen following ACh and eserine mixtures. (Compare Figures 13 and 15, and see also Figure 17.) The eserine-induced feeding is statistically

	µg Eserine sulphate				
	CSF	2.5	5.0	10.0	Overall
Feeding (cms)					
$\bar{x}$	107.76	75.24	78.97	104.05	91.16
±SE	16.76	15.23	25.81	25.78	10.23
median	114.35	88.90	100.70	98.70	97.60
Drinking (mls)					
$\bar{x}$	0.25	0.30	0.28	0	0.22
±SE	0.20	0.21	0.22	0	0.09
median	0	0	0	0	0
n =	10(6)	10(6)	9(6)	8(6)	37(6)

Table 19: Mean (±SE) and median amounts of Food and Water consumed in 1 hour preceding microinjection of various doses of eserine sulphate or a CSF control into the substantia nigra. (n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.)

(Feeding: $\chi_r^2 = 3.56$, df = 3, $p < .50$. Drinking: not tested - 31 scores from 37 = 0.)

	CSF	2.5	5.0	10.0
		μg Eserine sulphate		
Feeding (cms)				
$\bar{x}$	1.11	30.10*	43.22**†	31.64***
±SE	0.85	10.49	9.82	6.55
median	0	20.25	32.00	34.55
Drinking (mls)				
$\bar{x}$	0	0	0	0
±SE				
median	0	0	0	0
n =	10(6)	10(6)	9(6)	8(6)

Table 20: Mean (±SE) and median amounts of Food and Water consumed in the first 30 minutes following microinjection of various doses of eserine sulphate into the substantia nigra.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested).

(Feeding: $\chi_r^2 = 11.91$, df= 3, $p < .01$. Wilcoxon tests were used to test between the doses.

* CSF vs. 2.5μg: $T = 0$, $N = 9$, $p < .005$.

** CSF vs. 5.0μg: $T = 0$, $N = 9$, $p < .005$.

*** CSF vs. 10.0μg: $T = 0$, $N = 7$, $p < .01$.

\+ 5.0 vs. 2.5μg: $T = 6$, $N = 9$, $p < .025$.)

Figure 15. Mean (±SE) amount of dry spaghetti consumed following administration of various doses of eserine sulphate, and artificial CSF, into the rat substantia nigra. (● : 0-30 min. following microinjection; ○ : 30-90 min. following microinjection, ▲ : 0-90 min. following microinjection.)

(Wilcoxon tests. ▲ sig. diff. to CSF control (CSF vs. 2.5µg: T = 0, N = 9, $p < .005$; CSF vs. 5.0µg T = 0, N = 9, $p < .005$; CSF vs. 10.0µg T = 0, N = 7, $p < .01$) ▲▲ sig. diff. to 5.0µg. (T = 6, N = 9, $p < .025$).)

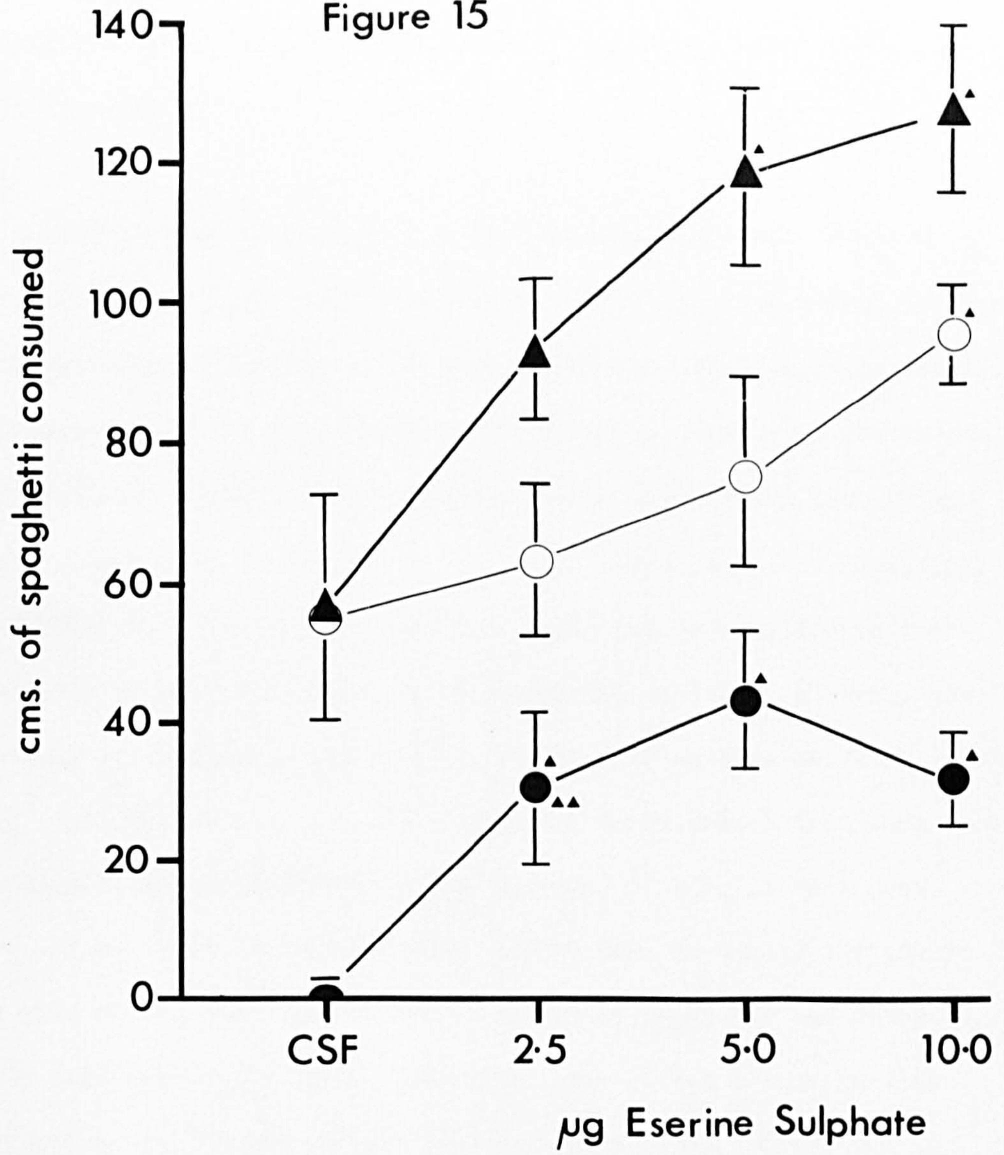


Figure 15
140
120
100
80
60
40
20
0
cms. of spaghetti consumed
CSF
2·5
5·0
10·0
µg Eserine Sulphate

significant, (ANOVA: $\chi_r^2 = 11.91$, df = 3, $p < .01$); Wilcoxon tests show the feeding induced by each of the doses to be significantly different to control feeding. (CSF v 2.5µg T = 0, N = 9, $p < .005$; CSF v 5.0µg T = 0, N = 9, $p < .005$; CSF v 10.0µg T = 0, N = 7, $p < .01$; all 1-tailed tests.) In addition 5.0µg eserine-induced a significantly greater feeding response than 2.5µg (T = 6, N = 9, $p < .025$). No drinking was observed in this period.

The latency of feeding was also affected, whereas duration apparently was not. Table 21 shows the mean (±SE) and median latency and duration of feeding under each condition. Statistically significant differences do not exist between the doses in respect of the duration of feeding. (ANOVA is not possible due to the unequal numbers in each group, Sign Tests between the three doses reveal no significant differences. The control condition could not be included in this analysis as only two sites tested supported eating.) However, the latency to feed was significantly affected by eserine sulphate (ANOVA: $\chi_r^2 = 13.80$, df = 3, $p < .01$). Wilcoxon tests reveal that each dose is significantly different to CSF control, (T = 0, in each case, $p < .01$ at least (1-tailed test)) though this is hardly surprising in view of the fact that of the 10 sites at which CSF was injected, only two supported eating; thus eight out of ten scores in this condition are 30, the cut-off point in this study. There was also a significant effect of drug dosage, 2.5µg inducing feeding with a significantly shorter latency than 10.0µg (T = 0, N = 7, $p < .01$, 1-tailed).

The effect of dosage on the latency of feeding can be further seen in both Table 22 and Figure 15. Between 30 and 90 minutes

		μg Eserine sulphate		
	CSF	2.5	5.0	10.0
Latency of Feeding (min)				
$\bar{x}$	29.30	14.60*[**]	14.11*	17.88*
±SE	0.60	2.48	1.65	2.01
median	30.00	13.00	14.00	17.00
n(Latency)	10(6)	10(6)	9(6)	8(6)
Duration of Feeding (min)				
$\bar{x}$	1.50	9.00	11.44	11.57
±SE	0.50	2.47	2.26	1.39
median	1.50	7.00	9.00	12.00
n(Duration)	2(2)	9(6)	9(9)	7(6)
no. of sites supporting eating	2/10	9/10	9/9	7/8

Table 21: Mean (±SE) and median values of the Latency and Duration (in minutes) of Feeding Behaviour in the 30 minutes following microinjection of various doses of eserine sulphate into the substantia nigra.

(n(Latency): no. of nigral sites examined. Figures in parentheses indicate no. of rats tested. n(Duration): no. of nigral sites giving rise to duration of feeding scores > 0. Figures in parentheses indicate no. of rats tested. For latency, zero feeding scores were counted as 30 min, the maximum score. For duration, zero scores were discarded. This accounts for the values of n. given for each measure.

(Duration: ANOVA not used - unequal n. in each group. Sign tests reveal no sig. diffs. between doses. Latency: $\chi_r^2 = 13.80$ df = 3, $p < .01$. * Wilcoxon tests show each dose sig. diff. to control, T = 0, N = n(latency), $p < .01$. ** 2.5μg vs 10.0μg: T = 0, N = 7, $p < .01$. (All 1 tailed).)

	CSF	2.5	5.0	10.0
		μg Eserine sulphate		
Feeding (cms)				
$\bar{x}$	55.66	63.01	74.89	95.13*
±SE	15.46	10.98	14.02	5.66
median	60.25	65.90	65.50	99.25
Drinking (mls)				
$\bar{x}$	0.95	0.25	0.17	0.88
±SE	0.37	0.13	0.12	0.47
median	0.25	0	0	0.25
n =	10(6)	10(6)	9(6)	8(6)

Table 22: Mean (±SE) and median amounts of Food and Water consumed between 30 and 90 minutes following microinjection of various doses of eserine sulphate into the substantia nigra.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested).

(Feeding: $\chi_r^2 = 3.99$, df = 3, $p < .3$. * Wilcoxon test, CSF vs. 10.0μg eserine sulphate, N = 8, T = 1, $p < .01$ (1 tailed test.)

Drinking: not tested - 23 of 37 scores = 0.)

following microinjection, 10.0µg eserine significantly potentiated feeding behaviour, (N = 8, T = 1, $p < .01$, 1-tailed test); ANOVA however does not indicate significant differences overall; $\chi_r^2 = 3.99$, df = 3, $p < .3$). This clearly suggests that 10.0µg eserine potentiates feeding, but with increasing strength over time. (The mean latency for this dose being 17.88 minutes, but the greatest effect being between 30 and 90 minutes post injection.)

This powerful stimulation of feeding over 90 minutes can be seen in Table 23. The feeding induced by eserine over this period is statistically significant. (ANOVA: $\chi_r^2 = 9.17$, df = 3, $p < .05$; Wilcoxon tests show that 5.0µg (N = 9, T = 1) and 10.0µg (N = 8, T = 0) differ significantly from CSF, $p < .005$, 1-tailed test.) This long term potentiation of feeding is in marked contrast to the ACh and eserine induced feeding (see Figures 13 and 17) which appears to have immediate effects (0-30 minutes) but less effect in the subsequent hour.

As in all the experiments so far reported, drinking behaviour was never significantly potentiated. Table 24 reveals that while eserine induced feeding from nearly all sites examined with all doses used, drinking was much more rarely observed. Indeed, drinking was never observed in the first 30 minutes following injection, while in the subsequent hour it appeared on only 14 of a possible 37 occasions.

Histological examination was made of all the sites and these are shown in Figure 16. A similar analysis to that undertaken in experiment three was made to discover whether the feeding response became weaker with increasing distance from the site which supported

		µg Eserine sulphate		
	CSF	2.5	5.0	10.0
Feeding (cms)				
$\bar{x}$	56.77	93.11	118.11*	126.76*
±SE	15.66	10.09	12.54	10.98
median	60.25	100.60	134.50	133.40
Drinking (mls)				
$\bar{x}$	0.95	0.25	0.17	0.88
±SE	0.37	0.13	0.12	0.47
median	0.25	0	0	0.25
n =	10(6)	10(6)	9(6)	8(6)

Table 23: Mean (±SE) and median amounts of Food and Water consumed between 0 and 90 minutes following microinjection of various doses of eserine sulphate into the substantia nigra.

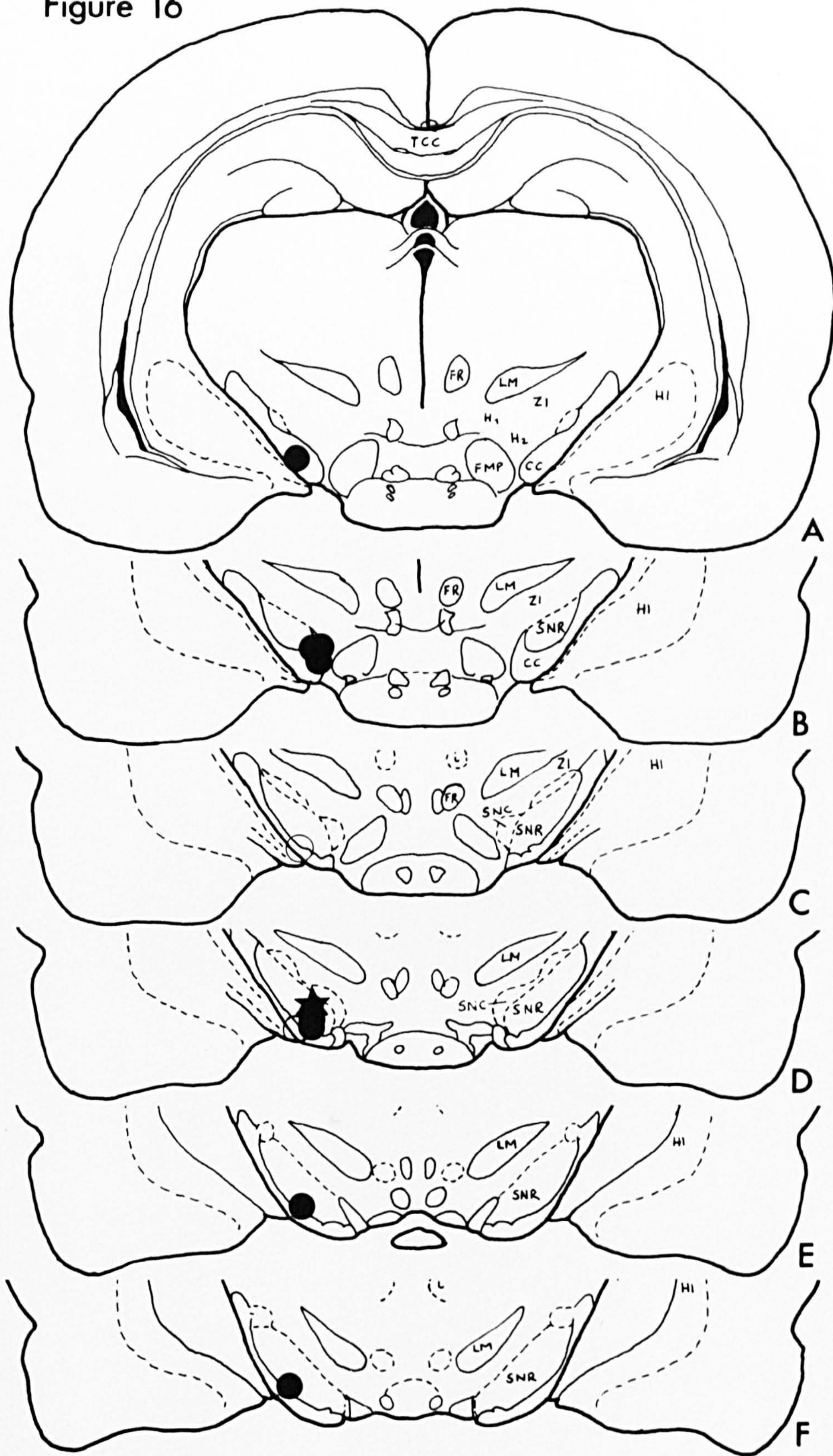
(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested. (Feeding: $\chi_r^2 = 9.17$, df = 3, $p < .05$. * sig. diff. to CSF control. Wilcoxon tests: CSF vs. 5.0µg, T = 1, N = 9, $p < .005$. CSF vs. 10.0µg, T = 0, N = 8, $p < .005$. (Both 1 tailed). Drinking: not tested - 23 of 37 scores = 0.)

	CSF	2.5	5.0	10.0
		µg Eserine sulphate		
0-30 minutes				
Feeding	2	9	9	7
Drinking	0	0	0	0
30-90 minutes				
Feeding	7	9	8	8
Drinking	5	3	2	4
0-90 minutes				
Feeding	7	10	9	8
Drinking	5	3	2	4
TOTAL NUMBER OF SITES:	10	10	9	8
NUMBER OF RATS	6	6	6	6

Table 24: Number of sites from which Feeding and Drinking could be elicited in various periods following microinjections of cholinergic substances of artificial CSF into the substantia nigra. (The unequal total numbers were caused by 3 guide cannula blockages preventing completion of dose-responses.)

Figure 16. Representative frontal sections of the rat brain modified from the stereotaxic atlas of Konig and Klippell (1963) indicating the placement of injection sites in Experiment 4. The sections are arranged from anterior (A) to posterior (F): the distance of each from the inter-aural line (zero reference point) is, in microns: A 2970, D 2790, C 2580, D 2420, E 2180, F 1950. ● : effective site, ○ : ineffective site ★ : represents the site of injection in the animal with the greatest overall food intake elicited by cholinergic stimulation. (Abbreviations: CC, Crus cerebri; d, n. Darkschewitsch; FMP, fasciculus medialis prosencephali; FR, Fasciculus retroflexus; HI, Hippocampus; H1, Forel's field H1; H2, Forel's field H2; i, n. interstitialis; LM, Lemniscus medialis; SNC, substantia nigra pars compacta; SNR, substantia nigro zona reticulata; TCC, Truncus corpus callosi; zi, zona incerta.)

Figure 16



most eserine induced feeding. Unlike the previous experiment, no such correlation could be shown between histology and a general measure of drug-induced eating in either the 0-30 minute condition ($r_s = 0.16$) or the 0-90 minute condition ($r_s = -0.38$).

Discussion

The data presented here clearly show that inhibition of AChE within the substantia nigra elicits a feeding response similar to that seen following either nigral stimulation by cholinergic substances or stimulation of DA release within the striatum. Eserine sulphate has clear dose-dependent effects on the amount of food eaten, the latency of responding and, apparently, the duration of the response. No significant stimulation of drinking was observed.

The results of this experiment suggest two things. First, that as inhibition of AChE activity induces a response similar to that seen after application of exogenous cholinergic substances, it might be presumed that endogenous ACh has a similar functional role in normal activity. Second, that as cholinergic stimulation in the region of the pars compacta induces a response similar to that elicited by stimulation of DA release within the striatum, and as AChE inhibition also induces a similar response, it might be suggested that it is the activity of the AChE known to be present within the dendrites of these pars compacta DA neurones which is inhibited by eserine. It might therefore be suggested that these neurones are stimulated by endogenous ACh.

While it might be argued that no correlation exists between the site of injection and the strength of the eserine response, it must

also be noted that the dendrites of the pars compacta neurones, which contain AChE, ramify extensively into the zona reticulata (see Chapter 3, section ii). The absence of such a correlation might not, therefore, be a crucial point against the hypotheses presented above.

We might conclude, therefore, from this experiment that ACh endogenous to the substantia nigra can mediate feeding behaviour. It might further be suggested, by similarity of the responding, that ACh activates DA neurones within the substantia nigra.

Before proceeding to the final experiment - an investigation of the nature of the receptors mediating this cholinergic response - a comparison between the effects of ACh and eserine, and eserine alone, on feeding and drinking, might provide further evidence of a role within substantia nigra for ACh, given the pharmacological similarities existing between the two forms of stimulation.

Comparison of the Effects of ACh and Eserine Mixtures and Eserine Sulphate Alone on Feeding Behaviour

Experiments 3 and 4 clearly show that both solutions containing equal quantities of ACh and eserine, and eserine sulphate alone can elicit feeding behaviour in a dose-dependent manner. Statistical analysis of both sets of data together reveals that in the first 30 minutes following microinjection equivalent doses of the two stimuli do not elicit significantly different responses in terms of either the amount of food eaten (Table 25) or the latency of feeding (Table 26). However, the feeding elicited by 10.0μg of eserine sulphate has a significantly longer duration than the feeding elicited by 10.0μg ACh and eserine, (mean values 12.00 minutes (eserine sulphate) and 6.20 minutes (ACh and eserine); $p < .037$). This potentiation in the first 30 minutes following microinjection is apparently maintained, for in the subsequent hour (30-90 minutes) eserine elicits a greater degree of feeding at this dose than ACh/eserine mixture. (Eserine: 95.13cms, ACh and eserine 56.01cms (mean values); $p < .01$ (1-tailed test); Table 25.) Moreover, the increased effect of 10.0μg eserine over 10.0μg ACh and eserine is seen in the ninety minutes following microinjection. (Mean values: eserine 126.76cms; ACh and eserine 78.42cms; $p < .01$ (1-tailed test); Table 25.) Apart from this dose, no significant differences in food consumption, latency or duration can be seen.

Why such a difference should exist following the highest level of stimulation is not absolutely clear; presumably the presence of ACh is critical in the variation but whether this is due to its independent action at this dose or to an interaction with the anti-

Condition:	ACh/Eserine	Eserine	n_1	n_2	U	p
0-30 minutes						
	CSF	CSF	10	19	68	ns
	2.5µg	2.5µg	10	19	86.5	ns
	5.0µg	5.0µg	9	19	83	ns
	10.0µg	10.0µg	8	18	46.5	ns
30-90 minutes						
	CSF	CSF	10	19	87	ns
	2.5µg	2.5µg	10	19	80	ns
	5.0µg	5.0µg	9	19	59	ns
	10.0µg	10.0µg	8	18	22	$<.002$
0-90 minutes						
	CSF	CSF	10	19	93.5	ns
	2.5µg	2.5µg	10	19	81	ns
	5.0µg	5.0µg	9	19	56.5	ns
	10.0µg	10.0µg	8	18	24	$p<.02$

Table 25: Comparison of the amount of dry spaghetti eaten under stimulation by ACh and eserine mixtures or by eserine sulphate alone using the Mann-Whitney U test. (Values given for p are two-tailed.)

Condition:	ACh/Eserine	Eserine	n_1	n_2	U	p
Latency of Feeding						
	CSF	CSF	9	10	15 scores=30	ns
	2.5μg	2.5μg	9	10	41.5	ns
	5.0μg	5.0μg	9	9	36.5	ns
	10.0μg	10.0μg	8	9	18.5	ns
Duration of Feeding						
	CSF	CSF	2	2	n too small for test	
	2.5μg	2.5μg	5	9	15.5	ns
	5.0μg	5.0μg	9	9	29.5	ns
	10.0μg	10.0μg	5	7	5.5	$p < .037$

Table 26: Comparison of the Latency and Duration (in minutes) of the Feeding elicited by ACh and eserine, or by eserine sulphate, in 30 minutes following microinjection into the substantia nigra, using the Mann-Whitney U Test.

Figure 17. Mean (±SE) amounts of dry spaghetti consumed in 0-30, 30-90 and 0-90 minutes following microinjection of various doses of either ACh and eserine (mixed in equal quantities) (●—●) or eserine sulphate alone (○—○) or 0.5µl **artificial** CSF solution into the rat substantia nigra. (▲ sig. diff. to CSF control at least, $p < .01$; △ sig. diff. to 5.0µg dose at least $p < .025$.)

The two groups (ACh/eserine vs. eserine alone) show significant differences ($p < .02$) only at the 10.0µg dose, in both the 30-90 min. and 0-90 min conditions.

Wilcoxon tests:

<u>ACh and Eserine</u>:	0-30 min.	CSF vs 2.5µg:	$T = 16.5$,	$N = 15$,	$p < .01$;
		CSF vs 5.0µg:	$T = 0$,	$N = 19$,	$p < .005$;
		5.0µg vs 10µg:	$T = 33$,	$N = 18$,	$p < .01$;
	30-90 min.	no sig. diffs.;			
	0-90 min.	CSF vs 5.0µg:	$T = 17$,	$N = 19$,	$p < .005$;
<u>Eserine sulphate</u>	0-30 min.	CSF vs 2.5µg:	$T = 0$,	$N = 9$,	$p < .005$;
		CSF vs 5.0µg:	$T = 0$,	$N = 9$,	$p < .005$;
		CSF vs 10.0µg:	$T = 0$,	$N = 7$,	$p < .01$;
		5.0µg vs 2.5µg:	$T = 6$,	$N = 9$,	$p < .025$;
	30-90 min.	CSF vs 10.0µg:	$T = 1$,	$N = 8$,	$p < .01$;
	0-90 min.	CSF vs 5.0µg:	$T = 1$,	$N = 9$,	$p < .005$;
		CSF vs 10.0µg;	$T = 0$,	$N = 8$,	$p < .005$;

(All 1 tailed tests).

Mann Whitney U tests:

10.0µg ACh and eserine vs 10.0µg eserine; 30-90 min ($n_1 = 8$, $n_2 = 18$, $U = 22$, $p < .002$);

10.0µg ACh and eserine vs 10.0µg eserine; 0-90 min ($n_1 = 8$, $n_2 = 18$ $U = 24$, $p < .02$).

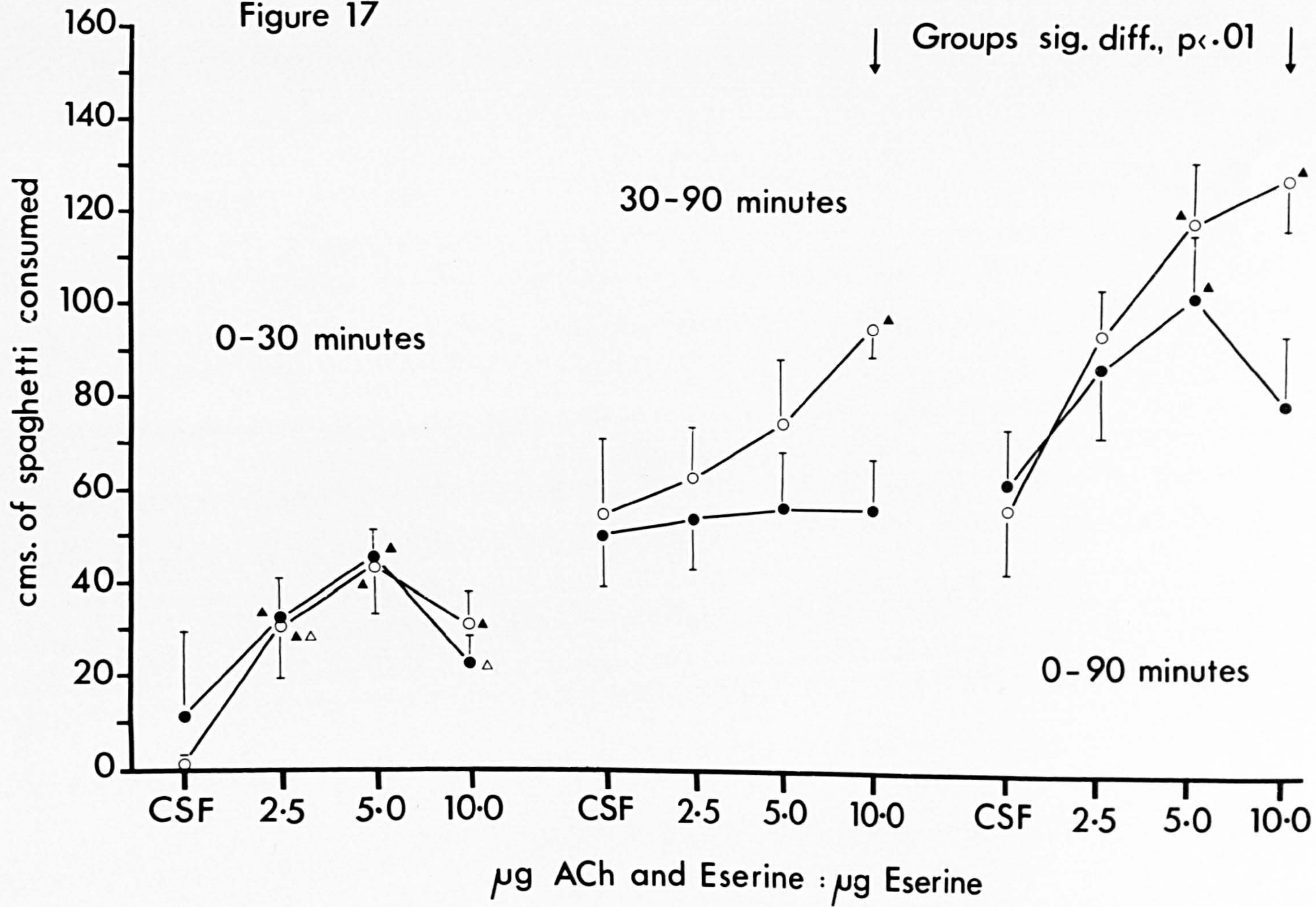


Figure 17
Groups sig. diff., p‹·01
30-90 minutes
0-30 minutes
0-90 minutes
cms. of spaghetti consumed
160
140
120
100
80
60
40
20
0
CSF
2·5
5·0
10·0
CSF
2·5
5·0
10·0
CSF
2·5
5·0
10·0
µg ACh and Eserine : µg Eserine

cholinesterase is not clear. It might be suggested that as 10.0μg eserine appears to successfully inhibit the activity of endogenous AChE, the addition of 10.0μg exogenous ACh to the endogenous ACh freed from cholinesterasic limitation most probably induced a gross overstimulation. Whether or not the decline in feeding seen following administration of 10.0μg ACh and eserine is thus equivalent to the decline in feeding seen following increased d-amphetamine stimulation is not clear. An equally plausible explanation of the difference between ACh and eserine and eserine alone at this dose might be that the excess ACh causes a depolarisation blockade of neurones in the region of the injection.

Overall, we might conclude that at doses below 10.0μg, ACh/eserine mixtures and eserine sulphate alone do not induce different feeding responses, as neither the amount consumed, the latency of feeding, nor the duration of the response appear to be significantly different at equivalent levels of each form of stimulation. However, the 10.0μg dose does show significant differences between the conditions: duration of the response and the volume eaten over 90 minutes show differences, eserine alone potentiating eating to a greater degree than 10.0μg ACh and eserine. Additive effects of endogenous and exogenous ACh and/or depolarisation blockade might account for this phenomenon.

A Study of Correlations Between Feeding and Drinking Elicited by Cholinergic Stimulation of the Substantia Nigra

Experiments 3 and 4 clearly show that cholinergic stimulation of the substantia nigra induces a dose-dependent feeding behaviour, while drinking is rarely observed. As both lateral hypothalamic and nigro-striatal pathway lesions induce a syndrome of aphagia and adipsia, this might be thought curious: if stimulation potentiates feeding, why does it not also potentiate drinking? The data reported here have shown only the quantity of water drunk in response to stimulation, and this has not shown any significant pattern. However, it is possible that drinking is related to feeding, but that a temporal factor is involved. For instance, does potentiation of feeding in the first 30 minutes following microinjection enhance drinking in the 30-90 minute period? In order to examine such interactions, the data from those sites examined in experiments 3 and 4 were ranked with respect to feeding and drinking in each time period (0-30, 30-90 and 0-90 minutes) at each dose (2.5, 5.0 and 10.0µg, and CSF control) and subjected to analysis by Spearman's Rank Correlation Co-efficient. The results are shown in Table 27.

Of the 44 correlation co-efficients shown in this table, only 3 show statistical significance. The highest of these (0.549) accounts for no more than 30.14% of the variance between the two conditions (2.5µg ACh and Eserine; Feed 30-90 mins vs. Drink 30-90 mins). Moreover, as the value set for statistical significance is $p < .05$, then we may estimate that approximately one correlation in every 20 will show statistical significance simply by chance. As 44 correlations are shown here and only three show significance, and as these are all

Experimental Condition	ACh and Eserine r_s	ACh and Eserine p	Eserine Alone r_s	Eserine Alone p
Following CSF injections				
Feed 0-30min. vs. Drink 0-30min.	0.052	ns		
vs. Feed 30-90min.	0.252	ns	0.424	ns
vs. Drink 30-90min.	-0.271	ns	0.441	ns
Drink 0-30min. vs. Feed 30-90min.	-0.150	ns		
vs. Drink 30-90min.	-0.216	ns		
Feed 30-90min. vs. Drink 30-90min.	0.410	$<.05$	0.560	ns
Following 2.5µg injections				
Feed 0-30min. vs. Drink 0-30min.	0.332	ns		
vs. Feed 30-90min.	0.304	ns	-0.714	ns
vs. Drink 30-90min.	0.190	ns	0.612	ns
Drink 0-30min. vs. Feed 30-90min.	0.183	ns		
vs. Drink 30-90min.	0.116	ns		
Feed 30-90min. vs. Drink 30-90min.	0.549	$<.05$	0.612	ns
Following 5.0µg injections				
Feed 0-30min. vs. Drink 0-30min.	-0.085	ns		
vs. Feed 30-90min.	0	ns	-0.357	ns
vs. Drink 30-90min.	0.304	ns	-0.267	ns
Drink 0-30min. vs. Feed 30-90min.	-0.068	ns		
vs. Drink 30-90min.	-0.129	ns		
Feed 30-90min. vs. Drink 30-90min.	-0.070	ns	0.490	ns
Following 10.0µg injections				
Feed 0-30min. vs. Drink 0-30min.	0.121	ns		
vs. Feed 30-90min.	0.232	ns	0.393	ns
vs. Drink 30-90min.	0.137	ns	-0.139	ns
Drink 0-30min. vs. Feed 30-90min.	0.273	ns		
vs. Drink 30-90min.	-0.216	ns		
Feed 30-90min. vs. Drink 30-90min.	0.277	ns	0.120	ns
Overall 0-90 minutes				
CSF Feed vs. CSF Drink	-0.008	ns	0.560	ns
2.5µg Feed vs. 2.5µg Drink	0.543	$<.05$	-0.204	ns
5.0µg Feed vs. 5.0µg Drink	-0.108	ns	0.223	ns
10.0µg Feed vs. 10.0µg Drink	0.395	ns	-0.139	ns

Table 27: Spearman's Rank Correlation Co-Efficients Between Feeding and Drinking elicited by various doses of two types of cholinergic stimulation in various periods following administration (ns = not significant; or $p < .05$). Correlations including "Eserine alone: drinking 0-30 mins. are not shown as no drinking was observed in this period. All animals with a full dose-response were used for these correlations. (ACh and Eserine, n = 18; eserine alone, n = 7 matched sets of data, where n= no. of sites examined. ACh and eserine = 15 individual rats, eserine alone = 5 individual rats.)

very low correlations, it would not be unreasonable to claim that there is at best a weak correlation between the amount of food eaten and water consumed within or between the time periods examined, or at the doses used.

It is argued in this thesis that because lesions of the lateral hypothalamus which destroy ascending fibres of passage, or destruction of those fibres alone, induce a syndrome of aphagia and adipsia, then stimulation of those pathways might be expected to induce consummatory behaviour. While various manipulations believed to enhance or mimic the activity of the pathway involved have been shown to induce or potentiate eating, drinking has never been observed. Moreover, it is not observed following tail-pinch stimulation which appears able to induce many consummatory behaviours (Marques et. al., 1979; Antelman et. al., 1976b) and which appears to involve activation of the nigro-striatal pathway. (See Chapter 5, section ivc.) While the failure to induce drinking is not a crucial point against the hypothesis that nigro-striatal activation should elicit feeding behaviour, it is nevertheless a puzzling anomaly.

Perhaps the most startling claim available to help account for this anomaly is that in fact, if tested appropriately, LH lesioned rats appear to show sufficient regulatory drinking to ensure their survival (Rowland et. al., 1979). Stressful ip sodium chloride injections and inadequate test periods, it has been argued, both serve to hide the true regulatory capacities of LH lesioned animals. Non-painful intravenous injections, longer test periods (up to 24 hr), and chronic osmotic challenge are thought to enable demonstrations of the real capacities of LH lesioned animals (Rowland et. al., 1979).

While this may account for the adipsia following LH lesions, the adipsia seen following 6OHDA lesions of substantia nigra may be accounted for by additional lesion of the zona incerta. This area lies above the substantia nigra and might easily be affected by lesion techniques or even by cannula tracks aimed at postero-lateral areas of the nigra; zona incerta-lesioned rats are known to show several drinking behaviour impairments (Rowland et. al., 1979).

It might thus be possible that neither nigro-striatal pathway nor LH lesions induce a true adipsic state: improper test procedures and/or additional zona incerta disruption might account for the observed adipsic effects. Consequently, it might not necessarily be expected that stimulation of the substantia nigra should elicit drinking, though feeding would be expected.

Experiment Five: Antagonism of Feeding Induced by 5.0μg ACh and Eserine using Nicotinic and Muscarinic Cholinergic Receptor Blocking Agents

Purpose

Experiment 3 demonstrated that cholinergic substances microinjected into the substantia nigra could potentiate feeding behaviour in a dose-dependent manner. Experiment 4 carried this work further by demonstrating that blockade of AChE activity could also facilitiate eating. The suggestion was made that this resulted from an increased activity of endogenous ACh. To further examine the claim that an endogenous cholinergic system within substantia nigra can facilitate eating, an attempt to block the response by using cholinergic receptor antagonists was made. Characterisation of these receptors was made by using both a muscarinic receptor antagonist (atropine) and a nicotinic receptor antagonist (mecamylamine), microinjected into the substantia nigra 15 minutes before similar microinjection of ACh and eserine, in a dose known to potentiate feeding, (5.0μg of each).

Method

Into each of twelve male black-hooded rats (PVG/C strain), 325-535g, bilateral stainless steel guide cannulae (23 ga.) were implanted, giving access to the substantia nigra. (In the atlas of Pellegrino and Cushman (1967), AP -2.4; Lat ± 2.0; Vert. -5.5 below dura.) After recovering from surgery, the animals were trained to eat sticks of dry spaghetti from glass tubes, feeding behaviour being measured in centimetres of spaghetti consumed. The animals were then tested for their responsiveness to 5.0μg ACh and eserine, and to an artificial CSF control, using the methods described in experiment three. Sites

which supported cholinergic-induced feeding were further examined.

For the next sessions, animals were placed in individual boxes and allowed to settle for 45 minutes with food and water freely available. Animals were then microinjected with either 0.5μl artificial CSF, or 0.5μl artificial CSF containing one of three doses of atropine sulphate (0.5, 1.0 or 5.0μg) or one of three doses of mecamylamine hydrochloride (0.5, 1.0 or 5.0μg) depending on which group they had been assigned to, (atropine group or mecamylamine group). Fifteen minutes after this animals were again microinjected with 5.0μg ACh and eserine, as previously described.

The order of administration (CSF control or drug preinjection) was randomised. Within each group 3 injections of each dose (0.5, 1.0 or 5.0μg) were made. At six of the nine sites examined in each group both CSF control and drug were administered, at one site in each group two different drug doses, and no control, were tested; at one site in each group, one dose of drug and no control was administered. Successive tests were separated by a minimum of 48 hours.

(This experimental design was not chosen deliberately. When only a very few animals had been tested it became apparent that repeated injections into the same substantia nigra site were affecting the responses. The data used here requires 6 repeated injections at any one site: CSF alone, 5.0μg ACh and eserine and 2 pre-injections both followed by 5.0μg ACh and eserine. If more than 6 injections are made, the feeding response appears to decline regardless of the drug injected. Only the data from the first 6 microinjections was taken from sites which had received more when this phenomenon became

apparent. This accounts for the lack of control data from certain sites.)

On completion of behavioural testing the animals were killed by an overdose of barbiturate and perfused intracardially with a 0.9% saline solution and treated according to standard histological procedures. Tissue was not treated with 10% formalin solution.

Results

All of the 10 sites that were fully examined supported eating following administration of 5.0μg ACh and eserine, in the first 30 minutes following the microinjection, while drinking was seen on only one occasion. The amount of food eaten was statistically significantly greater than the amount consumed following CSF administration, but not significantly greater than the amount consumed in the CSF pre-injection plus 5.0μg ACh and eserine condition, (i.e. in 30 minutes following administration of the cholinergics). (ANOVA across all three conditions: $\chi_r^2 = 18.00$, df = 2, $p < .001$. Wilcoxon Tests: 5.0μg ACh and eserine vs. CSF control: N = 16, T = 0, $p < .005$; 5.0μg ACh and eserine vs. CSF pre-injection plus 5.0μg ACh and eserine: N = 12, T = 34, not significant, $p > .05$; CSF pre-injection plus 5.0μg ACh and eserine vs. CSF control N = 12, T = 0, $p < .005$; all 1-tailed tests.) The mean (±SE) and median amounts of food and water consumed in all three conditions are shown in Table 28.

It might be concluded that 5.0μg ACh and eserine microinjected into the substantia nigra induced feeding behaviour (as was observed in Experiment 3) and that this response was not attenutated by pre-injection at the same site, 15 minutes beforehand, of 0.5μl artificial

	5.0μg ACh + Eserine	CSF	CSF pre-injection + 5.0μg ACh + Eserine
Feeding (cms)			
$\bar{x}$	48.22*	4.66	40.28**
$\pm$SE	6.89	2.98	7.27
median	45.30	0	30.20
Drinking (mls)			
$\bar{x}$	0.19	0	0
$\pm$SE	0.19	0	0
median	0	0	0
n =	16(12)	16(12)	12(11)

Table 28: Mean ($\pm$SE) and median amounts of Food and Water consumed in thirty minutes following microinjection into the substantia nigra of 5.0μg ACh and eserine or an artificial CSF control, or a combination of the two. (n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested). (Feeding: $\chi_r^2 = 18.00$, df = 2, $p < .001$. Wilcoxon tests were used to examine differences between the three conditions. * 5.0μg ACh and eserine vs. CSF control: N = 16, T = 0, $p < .005$. ** CSF pre-injection + 5.0μg ACh and eserine vs.CSF control: N = 12, T = 0, $p < .005$. (1 tailed tests). Drinking: not tested - 43 of 44 observations = 0).

CSF. As such it should be possible to examine the antagonistic effects of cholinergic receptor blocking agents on this feeding response by using the pre-injection technique.

Table 29 shows the mean (±SE) and median amounts of food and water consumed in the 30 minutes following microinjection of 5.0µg ACh and eserine into the substantia nigra, at sites which, 15 minutes previously, had been treated with various doses of either atropine or mecamylamine. Table 30 shows the same data, after each observation had been expressed as a percentage of the matched score for that site of 5.0µg ACh and eserine alone. Mean (±SE) and median percentage values are shown. Tables 31 and 32 show mean (±SE) and median values of food and water consumption following cholinergic stimulation of substantia nigra sites pretreated with receptor blocking agents for, respectively, 30 to 90 minutes following microinjection of 5.0µg ACh and eserine, and 0-90 minutes following microinjection. No statistically significant differences exist between the three "baseline" conditions (5.0µg ACh and eserine; CSF alone, and CSF preinjection followed by 5.0µg ACh and eserine) in either period. (ANOVA; 30-90 minutes, $\chi_r^2 = 1.17$, df = 2, $p < .7$, 0-90 minutes, $\chi_r^2 = 2.17$, df = 2, $p < .5$.)

Table 33 shows the feeding elicited by pre-injected drugs (CSF, atropine and mecamylamine) in the 15 minutes between their administration and the subsequent microinjection of 5.0µg ACh and eserine; mean (±SE), where n permits, and ± SD of the CSF control mean and median values are shown. As drinking occurred only twice (1.0 and 0.5ml. drunk) it is not tabulated. Table 34 shows mean (± SE where n permits) and median values of both the latency and duration (in minutes) of feeding following cholinergic stimulation alone, or

	Atropine Sulphate			Mecamylamine Hydrochloride		
	0.5µg	1.0µg	5.0µg	0.5µg	1.0µg	5.0µg
Feeding (cms)	Followed by 5.0µg ACh and Eserine					
$\bar{x}$	17.97	21.13	43.63	41.87	14.57	56.60
±SE	9.11	17.66	23.93	39.54	8.32	32.60
median	24.30	7.20	48.40	4.70	14.90	30.40
Drinking (mls)						
$\bar{x}$	0	0.83	0	0	0	0
±SE	0	0.60	0	0	0	0
median	0	0.50	0	0	0	0
n =	3(3)	3(3)	3(3)	3(2)	3(2)	3(3)

Table 29: Mean (±SE) and median amounts of Food and Water consumed in 30 minutes following intra-nigral administration of 5.0µg ACh and eserine at sites which had been pre-treated with cholinergic receptor antagonists.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.)

% Feeding (cms)	CSF pre-injection	Atropine Sulphate 0.5µg	1.0µg	5.0µg	Mecamylamine Hydrochloride 0.5µg	1.0µg	5.0µg
		Followed by 5.0µg ACh + Eserine					
$\bar{x}$	94.55	46.44	41.51	79.91	33.60	45.87	99.39
±SE	9.31	23.47	30.70	43.33	30.38	28.26	37.75
median	99.46	63.78	23.08	90.81	6.56	40.22	94.62
n =	12(11)	3(3)	3(3)	3(3)	3(2)	3(2)	3(3)

Table 30: Feeding induced by 5.0µg ACh and Eserine (0-30 minutes post-injection) at sites pre-treated with cholinergic receptor antagonists, expressed as a percentage of the feeding elicited from the same site by 5.0µg ACh and eserine, without pre-treatment.

(n = no. of nigral sites examined. Figures in parentheses indicate no of rats tested).

	5.0µg ACh and eserine	0.5µlCSF	0.5µlCSF	Atropine Sulphate 0.5µg	1.0µg	5.0µg	Mecamylamine Hydrochloride 0.5µg	1.0µg	5.0µg
			Followed by 5.0µg ACh and eserine						
Feeding (cms)									
$\bar{x}$	74.53	67.66	74.07	49.20	69.13	20.27	69.40	133.70	64.17
±SE	11.59	11.72	8.83	25.70	7.43	14.72	34.20	38.11	32.98
median	65.45	77.30	74.25	60.90	75.80	11.90	35.60	118.40	83.00
Drinking (mls)									
$\bar{x}$	1.19	0.78	1.25	1.17	2.0	1.0	0.50	1.33	1.17
±SE	0.30	0.29	0.45	0.93	0	0.58	0.29	1.33	0.60
median	1.50	0	1.0	0.50	2.0	1.0	0.50	0	1.50
n =	16(12)	16(12)	12(11)	3(3)	3(3)	3(3)	3(2)	3(2)	3(3)

Table 31: Mean (±SE) and median values of Food and Water consumed between 30-90 minutes following cholinergic stimulation of substantia nigra at sites pre-treated with cholinergic receptor antagonists.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.)

	5.0µg ACh and eserine	0.5µl CSF	0.5µl CSF	Atropine Sulphate 0.5µg	Atropine Sulphate 1.0µg	Atropine Sulphate 5.0µg	Mecamylamine Hydrochloride 0.5µg	Mecamylamine Hydrochloride 1.0µg	Mecamylamine Hydrochloride 5.0µg
			Followed by 5.0µg ACh and Eserine						
Feeding (cms)									
$\bar{x}$	122.75	72.26	114.34	67.17	90.27	63.90	111.27	148.33	120.77
±SE	13.13	12.35	9.15	26.74	10.25	15.25	38.02	36.52	11.23
median	116.25	85.95	120.80	60.90	83.00	48.90	142.50	118.40	121.40
Drinking (mls)									
$\bar{x}$	1.38	0.78	1.25	1.17	2.83	1.00	0.50	1.33	1.17
±SE	0.38	0.29	0.45	0.93	0.60	0.58	0.29	1.33	0.60
median	1.50	0	1.00	0.50	2.50	1.00	0.50	0	1.50
n =	16(12)	16(12)	12(11)	3(3)	3(3)	3(3)	3(2)	3(2)	3(3)

Table 32: Mean (±SE) and median values of Food and Water consumed between 0-90 minutes following cholinergic stimulation of substantia nigra at sites pre-treated with cholinergic receptor antagonists.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.)

	0.5µlCSF	Atropine Sulphate			Mecamylamine Hydrochloride		
		0.5µg	1.0µg	5.0µg	0.5µg	1.0µg	5.0µg
Feeding (cms)							
$\bar{x}$	22.25	4.0	14.03	14.80	25.83	0	18.97
±SE (±SD)	8.35 (28.92)	4.0	7.79	8.05	13.35	0	17.25
median	11.40	0	15.20	16.70	32.90	0	3.50
n =	12(11)	3(3)	3(3)	3(3)	3(2)	3(2)	3(3)

Table 33: Mean (±SE and (CSF control only) ±SD) and median amounts of Food consumed in 15 minutes following treatment with cholinergic receptor antagonists, prior to administration of 5.0µg ACh and eserine.

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.)

	5.0µg ACh and eserine	0.5µlCSF	0.5µlCSF	Atropine Sulphate 0.5µg	Atropine Sulphate 1.0µg	Atropine Sulphate 5.0µg	Mecamylamine Hydrochloride 0.5µg	Mecamylamine Hydrochloride 1.0µg	Mecamylamine Hydrochloride 5.0µg
Latency (min)			← Followed by 5.0µg ACh and Eserine →						
$\bar{x}$	18.44	25.56	15.42	20.00	22.67	17.00	24.00	23.33	15.33
± SE (±SD)	1.27(5.07)	2.11	1.85	5.77	5.90	6.56	4.16	3.38	5.33
median	19.00	30.00	16.00	20.00	27.00	12.00	26.00	21.00	10.00
Duration (min)									
$\bar{x}$	10.44	4.75	10.75	8.50	10.50	17.00	12.00	5.50	13.67
±SE	1.24	2.59	1.68						6.12
median	10.50	5.00	11.50						12.00
n =	16(12)	16(2)	12(11)	3(3)	3(3)	3(3)	3(2)	3(2)	3(3)

Table 34: Mean (±SE) and median values of Latency and Duration (both in minutes) of Feeding in the 30 minutes following cholinergic stimulation of substantia nigra: Effect of receptor antagonists.

(n= no. of nigral sites examined. Figures in parentheses indicate no. of rats tested).

	5.0µg ACh and eserine	0.5µl CSF	0.5µl CSF	Atropine Sulphate			Mecamylamine Hydrochloride		
				0.5µg	1.0µg	5.0µg	0.5µg	1.0µg	5.0µg
			Followed by 5.0µg ACh and Eserine						
<u>15-0 mins.</u>									
Feeding			6	1	2	2	2	0	2
Drinking			1	0	0	0	0	0	1
<u>0-30 mins</u>									
Feeding	16	5	12	2	2	2	2	2	3
Drinking	1	0	0	0	2	0	0	0	0
<u>30-90 mins.</u>									
Feeding	15	14	12	2	3	2	3	3	2
Drinking	10	7	7	2	3	2	2	1	2
<u>0-90 mins</u>									
Feeding	16	14	12	3	3	3	3	3	3
Drinking	10	7	7	2	3	2	2	1	2
Total*	16	16	12	3	3	3	3	3	3
no. animals tested	**12**	**12**	**11**	**3**	**3**	**3**	**2**	**2**	**3**

<u>Table 35</u>: Number of animals Feeding and Drinking in each condition. (* Total = number of sites tested under each particular condition).

stimulation in the presence of the antagonists.

While it has been possible to examine the feeding elicited by the baseline conditions using statistical tests, such methods cannot be used to analyse the effectiveness of the cholinergic antagonists, as the enforced 'design' of the experiment has restricted the number of animals in each group to only three. However, the data presented in Tables 29, 30, 31, and 32 would appear to show that cholinergic antagonists did, in some way, affect the feeding response. To an extent, we can show that the feeding elicited following blockade of cholinergic receptors was different to that elicited when no blockade was present. This can be done by observing whether (i) the mean value of feeding in any 'antagonised' condition falls beyond the mean $\pm$ 1 standard deviation of the feeding induced by 5.0µg ACh and eserine and (ii) the median values of feeding in any 'antagonised' state falls beyond the 6.25 percentile point of the feeding induced by 5.0µg ACh and eserine. If values for any dose of atropine or mecamylamine fall bayond both of these, then it might be tentatively suggested that a blockade of the cholinergic response has been established.

Figure 18 shows, schematically, which mean "antagonised" values fall beyond 1 standard deviation from the mean of the baseline 5.0µg ACh and eserine response, and which median values fall beyond various percentile points, the values of which are given in Table 36. The following doses of antagonist appear to attenuate feeding on the criterion that their mean value (n=3) falls beyond both the mean minus 1 SD, and the median value falls beyond the 6.25 percentile point of the feeding induced by 5.0µg ACh and eserine.

Figure 18. Antagonism of feeding induced by 5.0µg ACh and eserine by various doses of either the muscarinic receptor antagonist atropine or the nicotinic receptor antagonist mecamylamine. Mean minus SD and 3 percentile points of the feeding response to 5.0µg ACh and eserine were calculated for separate periods following microinjection (0-30 min, 30-90 min. and 0-90 min.) and used as standards against which to compare the effects of receptor blockade. Mean or median values, as relevant, observed following microinjection of 0.5µl artificial CSF, 0.5µl artificial CSF followed 15 min. later by 5.0µg ACh and eserine or various doses of atropine or mecamylamine followed by 5.0µg ACh and eserine, were compared with these standards. ▲ indicates that the mean or median is greater than the standard value for 5.0µg ACh and eserine against which it is being compared; ▼ indicates that the mean or median is less than the standard against which it is being compared.

(see Table 36 for the data from which this figure is constructed).

	CSF	CSF	Atropine 0·5	Atropine 1·0	Atropine 5·0	Mecamylamine 0·5	Mecamylamine 1·0	Mecamylamine 5·0
		Followed by 5·0 μg ACh and Eserine						
0-30 minutes								
1SD below $\bar{x}$	▼	▲	▼	▲	▲	▲	▼	▲
25 percentile	▼	▼	▼	▼	▲	▼	▼	▼
12·5 "	▼	▲	▲	▼	▲	▼	▲	▼
6·25 "	▼	▲	▲	▼	▲	▼	▲	▲
30-90 minutes								
1SD below $\bar{x}$	▲	▲	▲	▲	▼	▲	▲	▲
25 percentile	▲	▲	▲	▲	▲	▼	▲	▲
12·5 "	▲	▲	▲	▲	▼	▲	▲	▲
6·25 "	▲	▲	▲	▲	▲	▲	▲	▲
0-90 minutes								
1SD below $\bar{x}$	▲	▲	▼	▲	▼	▲	▲	▲
25 percentile	▲	▲	▼	▲	▼	▲	▲	▲
12·5 "	▲	▲	▼	▲	▼	▲	▲	▲
6·25 "	▲	▲	▼	▲	▼	▲	▲	▲

Figure 18

	5.0µg ACh and eserine	0.5µl CSF	0.5µl CSF	Atropine Sulphate			Mecamylamine Hydrochloride		
				0.5µg	1.0µg	5.0µg	0.5µg	1.0µg	5.0µg
			← Followed by 5.0µg ACh and Eserine →						
30-90 mins									
$\bar{x}$	74.53	67.66	74.07	49.20	69.13	20.27	69.40	133.70*	64.17
SD	46.36								
$\bar{x}$ - SD	28.18								
median	65.45	77.30	74.25	60.90	75.80	11.90	35.60	118.40	83.00
25% ile	41.55								
12.5% ile	28.95								
6.25% ile	10.65								
0-30 mins									
$\bar{x}$	48.22	4.66	40.28	17.97	21.13	43.63	41.87	14.57	56.6
SD	27.55								
$\bar{x}$ - SD	20.67								
median	45.30	0	30.20	24.3	7.2	48.4	4.7	14.9	30.40
25% ile	31.84								
12.5% ile	17.45								
6.25% ile	16.00								
0-90 mins									
$\bar{x}$	122.75	72.26	114.34	67.17	90.27	63.90	111.27	148.33	120.77
SD	52.52								
$\bar{x}$ - SD	70.23								
median	116.25	85.95	120.80	60.90	83.00	48.90	142.50	118.40	121.40
25% ile	74.55								
12.5% ile	70.00								
6.25% ile	64.20								
n =	16(12)	16(12)	12(11)	3(3)	3(3)	3(3)	3(3)	3(2)	3(3)

Table 36: Mean, Standard Deviation, Median and Percentile values used in constructing Figure 18. (*This value lies beyond 1 SD above the mean, which is 120.89).

(n = no. of nigral sites examined. Figures in parentheses indicate no. of rats tested.)

0-30 minutes post-injection: none (though CSF alone, predictably, meets the criterion).

30-90 minutes post-injection: none

0-90 minutes post-injection: Atropine 0.5µg
Atropine 5.0µg

It is not possible to state with certainty that any significant effect has occurred as a result of cholinergic receptor antagonism. It is however interesting to note that two different doses of atropine fall outside the expected range of the scores. Why the third dose of atropine did not have this effect is not clear; the most we can say about this dose (1.0µg) is that in the first 30 minutes following administration of 5.0µg ACh and eserine, 1.0µg atropine antagonised the response sufficiently to cause the mean amount of feeding to fall beyond the 6.25 percentile, though not more than 1 SD from the mean.

Mecamylamine had no such antagonistic effects. In the first 30 minutes following cholinergic stimulation, 1.0µg mecamylamine appears to cause a fall in feeding greater than 1 SD but not the 6.25 percentile point. It is of more interest to note that in the subsequent hour, this dose appears to actually potentiate feeding: the mean falls more than one SD above the mean of cholinergic-induced feeding (1.0µg mecamylamine, $\bar{x}$ = 133.70; 5.0µg ACh and eserine, $\bar{x}$ + 1 SD = 120.89 cms.). This might represent a rebound effect from the suppression of feeding observed in the previous 30 minutes. (Indeed, the total (0-90 minutes feeding elicited by this dose shows no difference at all to the baseline.)

Only two other noteworthy points emerge from this data. First,

the latency of feeding in the first 30 minutes following cholinergic stimulation appears to be unaffected by receptor blocking agents, (Table 34). No dose of atropine induced feeding with a latency beyond the range of the mean ± SD of baseline (5.0µg ACh and eserine) feeding, although two doses of mecamylamine appear to be borderline. (5.0µg ACh and eserine, mean + SD = 23.51 minutes; 0.5µg mecamylamine, 24.00 minutes; 1.0µg mecamylamine 23.33 minutes) Statistically significant differences can be shown between the three baseline conditions, as would be expected. (ANOVA: $\chi_r^2 = 7.63$, df = 2, $p < .05$; Wilcoxon Tests: 5.0µg ACh and eserine vs. CSF, N = 16, T = 21, $p < .01$; 5.0µg ACh and eserine preceded by 0.5µl CSF vs. CSF, N = 12, T = 12, $p < .025$; both ACh and eserine conditions compared, N = 11, T = 15, not significant; all tests 1-tailed.) Duration of feeding was not tested, but mean (±SE) and median values appear in Table 34. Secondly, the antagonists alone, in the 15 minutes preceding administration of cholinergics, appear to have a variable effect on food intake, (Table 33), but no value lies outside the mean ± SD range of the CSF pre-injection, which serves here as a control.

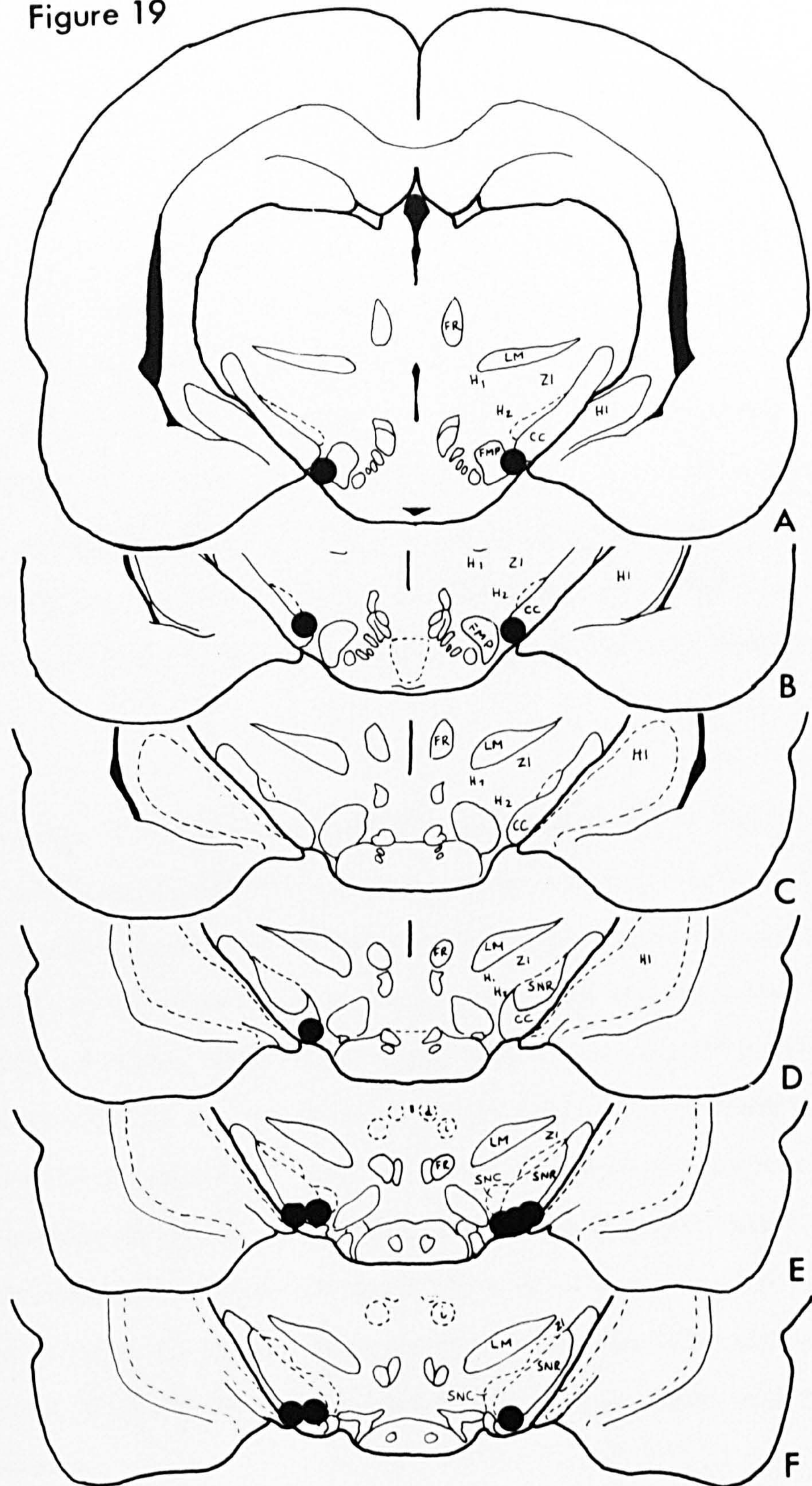
Figure 19 shows representative frontal sections of the brain, indicating the injection sites investigated. As is apparent by the clustering together of these sites, no consistent relationship between the behavioural responses, drugs tested and anatomical site could be discerned, using the techniques outlined in Experiment 3.

Conclusions

This experiment has very little to offer in terms of hard-and-fast conclusions. The demonstration that 5.0µg ACh and eserine elicits

Figure 19. Representative frontal sections of the rat brain, modified from the atlas of Konig and Klippell (1963) indicating the placement of effective injection sites (●) in Experiment 5. The sections are arranged from anterior (A) to posterior (F); the distance of each from the inter-aural line (zero reference point) is, in microns; A 3290, B 3180, C 2970, D 2790, E 2580, F 2420. Sites at which cholinergic-induced feeding was examined following pre-treatment with mecamylamine are shown on the left, those pre-treated with atropine on the right. (Abbreviations: cc, Crus cerebri; d, n. Darkschewitsch; FMP, Fasciculus medialis prosencephali; FR, Fasciculus retroflexus; HI, Hippocampus; H1, Forel's field H1; H2, Forel's field H2; i, n. interstitialis; LM, Lemniscus medialis; SNC, substantia nigra pars compacta; SNR, substantia nigra zona reticulata; zi, zona incerta.)

Figure 19



feeding behaviour in the first 30 minutes following microinjection has been repeated. As in Experiment 3, no drinking was found to be associated with this response. Unlike experiment 3, the feeding observed in ninety minutes following microinjection was not significant.

The most convincing demonstration of this experiment is probably the validation of the pre-injection technique. Microinjection of 0.5µl CSF into the substantia nigra 15 minutes before microinjection of 5.0µg ACh and eserine at the same site never affected the cholinergically induced response to a statistically significant degree, suggesting that repeated injections of this nature may be an appropriate method for examining such problems as receptor characterisation.

In terms of the purpose of this study, only tentative conclusions can be advanced. The observation that, on the criteria adopted, only doses of atropine affected the cholinergic response might be taken as suggesting the presence of muscarinic receptors mediating the cholinergic feeding, while on the other hand, the failure of mecamylamine might suggest an absence of nicotinic receptors. Previous localisation of muscarinic receptor binding to the substantia nigra might support this claim (De Montis, 1979). However, the failure of an intermediate dose of atropine to have any effect, and the curious effects of 1.0µg mecamylamine in inducing 'see-saw' effects within the 90 minute observation period tend to cast doubt over these suggestions.

Chapter 9: General Discussion

i. Summary of the Main Experimental Findings

a. 0.25 mg/kg d-amphetamine caused a statistically significant increase in feeding when systemically administered. Higher doses (1.00 and 4.00 mg/kg) caused a statistically significant decrease in feeding, but a significant increase in locomotion, rearing and stereotyped behaviours. Drinking, wood-chip gnawing and grooming were not potentiated by any dose of d-amphetamine used (0.125 - 4.000 mg/kg), although all were reduced by the highest dose (4.000 mg/kg) which elicited stereotyped sniffing and rearing.

b. Intra-striatal microinjections of d-amphetamine elicited dose-dependent feeding. 0.7 and 0.6 µg had no effect on food intake, while 2.0 µg caused a statistically significant increase in the amount of food consumed. However, the mean (±SE) latency of feeding was 52.78 ± 9.04 minutes. (Latency of feeding following saline control: 87.50 ± 6.72 minutes (mean ± SE)). Neither drinking nor locomotor activity was affected by intra-striatal d-amphetamine. Stereotyped behaviours were not observed.

c. An inverted U-shaped function with respect to feeding was found following cholinergic stimulation of the substantia nigra by solutions of artificial CSF containing equal quantities of ACh and eserine (2.5, 5.0 and 10.0 µg of each). Feeding appeared to be most strongly stimulated by 5.0 µg ACh and eserine within the first 30 minutes following microinjection. There was no significant cholinergic stimulation

of feeding in the 30-90 minute period following microinjection, but the early potentiation was sufficient to show a statistically significant effect when intake was measured over 90 minutes following microinjection. Drinking was not similarly potentiated. A statistically significant correlation between the intensity of drug-induced feeding and distance from the pars compacta was also found, indicating that the response became weaker with increasing distance from this area.

d. Microinjections of eserine sulphate alone into the substantia nigra were also found to be capable of potentiating feeding behaviour. In the first 30 minutes following microinjection a similar pattern of responding was seen as following ACh and eserine stimulation - feeding following an inverted U-shaped function with no drinking occurring. In the subsequent hour (30-90 minutes post-injection) 10.0 μg significantly potentiated feeding. Both 5.0 and 10.0 μg eserine sulphate potentiated feeding over 90 minutes following microinjection.

e. An attempt to characterise cholingeric receptors which might be mediating the feeding response was made. The technique of pre-injecting substances onto a site to be tested later appears to have been successful (in that 0.5 μl CSF, pre-injected into substantia nigra did not affect the feeding response to subsequent microinjections of 5.0 μg ACh and eserine). Few conclusive data were generated, it might be suggested that muscarinic rather than nicotinic receptors mediate the cholinergically induced feeding.

f. The latency of feeding following administration of 5.0 μg ACh and eserine was 15.00 ± 1.00 minutes (mean ± SE); that of 5.0 μg eserine

sulphate 14.11 ± 1.65 minutes (mean ± SE). The duration of the response (measured only within the first 30 minutes following microinjection), was, for 5.0 μg ACh and eserine, 13.89 ± 0.96 minutes (mean ± SE) and for 5.0 μg eserine sulphate, 11.44 ± 2.26 minutes (mean ± SE). The only difference observed between ACh and eserine and eserine sulphate alone occurred at the highest level, where 10.0 μg eserine sulphate had a greater stimulant effect on feeding (especially in the 30-90 minutes following microinjection) than 10.0 μg ACh and eserine (see Figure 17).

g. While feeding was apparently potentiated by cholinergic stimulation of substantia nigra, drinking remained unaffected. No relationship between the amount of food eaten and water drunk appears to exist, either in the same period or in a later period.

These data may be interpreted in the following manner. The effects of peripherally applied d-amphetamine appear to be rate dependent. Feeding is potentiated at low doses, but is depressed at high doses which potentiate rearing, locomotion and stereotyped behaviours. This is in agreement with the change in behaviour patterning and shortening of the duration of an act once initiated observed by Norton (1973) following d-amphetamine administration (0.25 - 1.00 mg/kg) and is also compatible with the general hypothesis of amphetamine action formulated by Lyon and Robbins (1975).

It has been suggested that low, peripherally applied doses of (+) amphetamine stimulate striatal DA turnover (but not concentration) (Costa et. al. 1972). As similar doses have been observed to elicit eating in satiated rats, it might be possible to suggest that the

feeding and increase in striatal DA turnover are in some way related. This possibility was examined in Experiment 2. A statistically significant increase in the amount of food consumed in 100 minutes following microinjection was found following administration of 2.0 µg d-amphetamine, but not 0.7 or 6.0 µg. While this appears to confirm the prediction of a striatal involvement with the d-amphetamine feeding response, the fact that feeding only occurred at a very long latency casts doubts over the interpretation of this experiment. Several behavioural and/or physiological explanations of this effect may be forwarded. (See pp. 123-125).In the light of this latency effect, the value of this study in examining the involvement of striatal DA with feeding is reduced.

It has been suggested that cholinergic substances within the substantia nigra stimulate nigro-striatal DA neurones. The DA neurones contain and release AChE (Butcher and Marchand, 1978; Greenfield et. al., 1980) which, it might be suggested, serves to inactivate a cholinergic input. Indeed, ACh (Jacobowitz and Goldberg, 1977) CAT (Kuczenski et. al. 1975) and muscarinic cholinergic receptors (De Montis et al., 1979) are all present within substantia nigra. Stereotyped behaviours have been observed following nigral stimulation by high doses of carbachol (Winn and Redgrave, 1979) suggesting an excitation of nigro-striatal DA neurones. The observations of experiments 3 and 4 - that low-level cholinergic stimulation could elicit feeding behaviour - might be thought to suggest that low-level nigro-striatal activation can support feeding. That feeding could be elicited by blockade of nigral AChE by eserine sulphate might suggest that endogenous ACh can fulfill the role of nigro-striatal stimulation. Differences between ACh and eserine stimulation and eserine alone emerged only at the higher doses,

suggesting that the higher levels of exogenous ACh had a depressant effect on responding. This might be achieved behaviourally or physiologically. That the lower doses of ACh had no additive effect with with stimulation achieved by eserine might be explained by hypothesising that either responding was constrained by a ceiling effect or that additional exogenous ACh was physiologically ineffective, having possibly been metabolised.

The failure of the receptor blocking experiment is disappointing, as it is of interest to know whether cholingeric receptors mediate these effects of ACh within substantia nigra. The results of the experiment might suggest either that atropine inhibits responding in the later periods of testing or that atropine had little blocking effect However, the low values of n. and poor design make the drawing of conclusions virtually impossible. While this particular experiment revealed no conclusive evidence for the presence of a cholinergic receptor on the DA neurones, other studies have been able to provide such evidence. Cross and Waddington (1980) show that ^{3H}QNB (muscarinic receptor) binding in substantia nigra is reduced by approximately 30% following 6-OHDA lesions, ^{3H}GABA binding being unaffected. This might be interpreted as suggesting that (i) muscarinic receptors exist on the DA neurones, (ii) there is no direct striato-nigral GABA feedback on to the DA neurones, and (iii) as binding of ^{3H}QNB fell by only 30%, another population of non-DA associated muscarinic receptors presumably exists. This is consistent with the data of De Montis et al. (1979) which suggests the presence of muscarinic receptors on zona reticulata neurones projecting to the thalamus and/or superior colliculus. It is also of interest to note that low doses of carbachol -a synthetic cholinergic with muscarinic receptor agonist properties - also appear

to stimulate feeding when injected into the substantia nigra (Winn and Redgrave, 1979).

The data in this thesis might thus be said to provide the following information:

i. confirmation that low doses of d-amphetamine elicit eating. (This is the first study to examine the entire range of d-amphetamine stimulated behaviour.)

ii. experiment 2 attempted to examine whether or not striatal DA stimulation could elicit feeding. While the amount of food consumed was potentiated dose-dependently the long latency of feeding makes interpretation of the data difficult.

iii. experiments 3 and 4 attempt to show that feeding can be elicited by low level cholinergic stimulation of the substantia nigra. There are indications in the literature that cholinergic stimulation of the pars compacta might stimulate nigro-striatal DA neurones. As d-amphetamine-like behaviour was elicited, it might be suggested that this DA stimulation was achieved. If so, this represents an important advance in our understanding of the regulation of para compacta DA neurones, and a novel method for achieving intra-cranial stimulation of nigro-striatal DA neurones.

ii. Dopamine and Feeding: Involvement of the Striatum and Striatal Efferents

It is well known that the nigro-striatal pathway liberates DA from terminals in the striatum. It is, moreover, known that lesions either of, or within, the striatum (Sorenson and Ellison, 1970; Sanberg and Fibiger, 1979), or of the nigro-striatal pathway (Ungerstedt, 1971d) either at the level of the LH (Anand and Brobeck, 1951) or its source in the substantia nigra (Marshall et. al., 1974), can induce a syndrome of aphagia and possibily adipsia. However, it has been argued that adipsia is not genuinely induced by LH or nigro-striatal lesion, but is instead either a test artifact or a consequence of additional zona incerta damage (Rowland et. al., 1979). Striatal lesions are also known to abolish the stereotypic effects of d-amphetamine (Creese and Iversen, 1975; Kelly et. al., 1975). If the consequences of striatal, or nigro-striatal pathway lesions are aphagia and an attenutation of d-amphetamine-induced stereotypy, it might be expected that potentiation of DA release from these fibres should result in both feeding and stereotyped behaviour. Moreover, it has been suggested that d-amphetamine acts to stimulate activity and that this activity will first of all be manifest as an increase in complex behaviour, amenable to performance at a moderate rate, and possibly including long inter-response intervals. As the dosage is increased behaviour will become less complex: the manifest activity has to be capable of ever-increasing rates of performance and ever-shorter inter-response times as the level of CNS activity generated by the drug increases.

The results of Experiment One show this trend quite clearly, (see

Table 9). At the lowest levels of stimulation feeding is observed, but as the dosage increases this gives way initially to wood-chip gnawing, (though this is neither statistically significant nor frequently observed), grooming, general locomotor activity, rearing on to the hind legs and finally, stereotyped sniffing. It is of particular interest that the initial behaviour potentiated by d-amphetamine should be feeding. This has been observed previously. Blundell and Latham (1978) found that 0.125 mg/kg potentiated eating even when measured over 24 hours. Total food intake increased by 20%: average meal size and the rate of eating both increased slightly, but Blundell and Latham concluded that the number of meals taken was the primary cause of the increase in intake. More interesting still is the observation by Costa et. al. (1972) that 0.3 mg/kg (+) amphetamine should affect the turnover of striatal DA leaving the turnover of tel-diencephalic NA unaffected. This enables us to gather together these separate lines of evidence which may suggest that low level stimulation of DA neurotransmission within the striatum potentiates feeding behaviour. These are (i) lesions of the nigro-striatal pathway induce aphagia (and possible adipsia), (ii) low levels of d-amphetamine potentiate feeding behaviour, and (iii) low levels of d-amphetamine influence the activity of striatal DA at doses which stimulate eating.

This picture is completed by adding that low levels of d-amphetamine microinjected into the striatum apparently potentiate food intake, as was found in Experiment 2. Other studies have previously shown that higher doses of d-amphetamine administered intra-striatally induce locomotion and have no effect on the food intake of deprived animals (Neill and Grossman, 1973; 1-10µg d-amphetamine, crystal implantation) or induce stereotyped sniffing (Costall et. al., 1972b; 25-400µg

d-amphetamine injected in 0.9% saline solution). As lesions within the striatum prevent the expression of amphetamine stereotypy, the demonstration by Costall et. al. of stimulation-induced stereotypy is not surprising. However, the suggestion of Neill and Grossman's work - that a complete spectrum of amphetamine effects including locomotor activity can be elicited by striatal stimulation - runs counter to the accepted hypothesis of n. accumbens mediation of locomotor activity. A single study investigating a complete range of doses microinjected at various striatal and accumbens sites would clearly aid our understanding of the issue.

There have also been suggestions recently that the reverse is also true, for n. accumbens involvement in the mediation of oral behaviour and feeding have been suggested. Jones and Mogenson (1979) report that spiroperidol injections into the n. accumbens but not the caudate n. attenuate oral-motor performance. This does indeed run counter to the traditional "striatum-oral stereotypy, n. accumbens - locomotor activity" scheme. However, Jones and Mogenson only looked at changes in lap volume and tongue extension in water-deprived rats, and the generalisation from this to oral-motor performance *per se* is too large. The studies reported here seem to suggest that stimulation of nigro-striatal systems will not necessarily induce drinking, but this is not to say that these pathways are not involved in oral-motor performance: feeding and drinking, both oral behaviours, appear dissociable.

A more recent paper however, suggests that food deprivation increases DA utilisation in the n. accumbens, posterior hypothalamus and the amygdala, but not in the corpus striatum (Heffner, Hartman

and Seiden, 1980). This, quite clearly, is not consistent with the predictions expected from the current data. That n. accumbens, hypothalamic or amygdala DA utilisation should change in response to food deprivation is not the primary point of concern; that striatal DA should not change, however, is. The prediction made using the present data concerning changes in striatal DA during food deprivation might be as follows:

i. Costa et. al. (1972) suggest that low level systemic d-amphetamine potentiates DA turnover in the striatum but does not affect DA concentration.
ii. Experiment 1 demonstrates that a similar dose of d-amphetamine potentiates feeding in satiated animals.
iii. We might therefore hypothesise that as low level amphetamine administration and food deprivation have the same behavioural effects (i.e. feeding) they might have equivalent neurochemical effects (i.e. an increased turnover of DA but no effect on concentration within the striatum).

Therefore, using the measures of Heffner et. al., (1980) we might predict an increase in the level of DOPAC (a DA metabolite) in the striatum, but no change in DA levels. Heffner et. al.in fact report no change in either striatal DA or DOPAC during deprivation. It is possible that the failure to observe changes in DOPAC is an artifact of the method used. Heffner et. al. report that they adopted the dissection method of Heffner and Seiden (1980), which involved:- (i) decapitation and brain removal, (ii) placing the brain in an aluminium cutting block kept cold on ice, (iii) sectioning the brain, (iv) dissecting out from the sections relevant brain areas, and (iv)

storing the tissue "frozen in liquid nitrogen for up to one week prior to assay". Importantly, no estimate of the time taken to perform all of these operations is given. Moleman et. al., (1977) report that DOPAC levels in the striatum change very rapidly following decapitation. They claim that: "The results presented show that no gross changes in DA, HVA and DOPAC concentrations occur from $2\frac{1}{2}$ to 5 min. after decapitation ..... However, when the period was extended beyond 5 minutes, considerable post-mortem effects were observed for DOPAC. Remarkably, striatal DOPAC levels sharply increased when the striata were 'incubated' at $0^{o}C$ for more than 5 min ...., while they decreased when the brain was 'incubated' as a whole."

That Heffner et. al. dissected at approximately $0^{o}C$ is probably correct; an aluminium block resting on ice would not actually freeze tissue, merely cool it. The critical question now becomes one of time, and as no indication of speed is given it might be surmised that the authors did not consider this an important variable, but instead took care in accurately dissecting out various brain regions, as Heffner and Seiden (1980) describe. It thus becomes quite possible that the whole brain was incubated at $0^{o}C$ for more than 5 min, leading to a decrease in striatal DOPAC levels. The level which Heffner et. al. (1980) subsequently report might thus be considered too low; as it was not significantly different to control levels, an increase would tend towards fulfilment of our prediction, though whether this would actually be so we cannot say.

While the results presented in this thesis in no way preclude the involvement of any brain region other than the striatum in the control or regulation of feeding behaviour, it does predict that,

specifically, the striatum is involved. While the work of Heffner Hartman and Seiden appears to cast doubt on this, we cannot accurately judge it without full details of the DOPAC estimation used. There would appear to be a strong suggestion of a methodological error, which, if so, might well lead to an accurate fulfilment of our predictions for striatal DA and DOPAC levels during food deprivation.

Moreover, the involvement of the n. accumbens with feeding has been investigated by Koob et. al. (1978), who found that 6OHDA lesions of this structure did not affect feeding behaviour when measured either in the home cage over 12 hours, or in prolonged tests in photo-cell cages. Amphetamine anorexia was similarly unaffected by n. accumbens lesions. However, these authors did observe an increase in the intake of dry food pellets or wet mash in food-deprived animals but ascribed this hyperphagia to an inability to switch from one behaviour to another. A general conclusion of this study might thus be that the n. accumbens is not involved in the normal regulation of food intake, contrary to the suggestions of Heffner, Hartman and Seiden (1980).

While we can claim that feeding is mediated by the low level activation of striatal DA, other studies have investigated the role of systems efferent to the DA terminals in mediating DA-stimulated behaviour. Figure 20 is a diagram of some supposed striatal efferent and afferent projections, showing details of how various "dopaminergic" behaviours may be elicited from several different points in the system. If DA is released in the striatum, one of the consequences appears to be an increase of activity in the striato-nigral GABA projection. This is thought to be brought about, in part at least, by modification

Figure 20. A schematic representation of some substantia nigra and striatal projections showing some hypothesised neurochemical consequences of dopaminergic stimulation within the striatum. The following outlines some behavioural evidence supporting these neurochemical hypotheses.

1. DA lesion: aphagia (Joyce, 1980; Ungerstedt, 1971d). Agonists: low doses enhance feeding (this thesis), higher doses induce stereotyped behaviours (Costall et. al., 1972b; Ernst and Smelik, 1966). Unilateral DA stimulation elicits contralateral rotation (see Pycock, 1980).

2. Intra-striatal atropine induces stereotyped behaviour (Zambo et. al., 1979).

3. Bilateral muscimol microinjections: low doses enhance feeding (Redgrave, 1980 unpublished observations); higher levels of GABA stimulation enhance and induce stereotyped behaviours (Koob et. al., 1978). Unilateral muscimol induces contralateral rotation (Arnt and Scheel-Krüger, 1979).

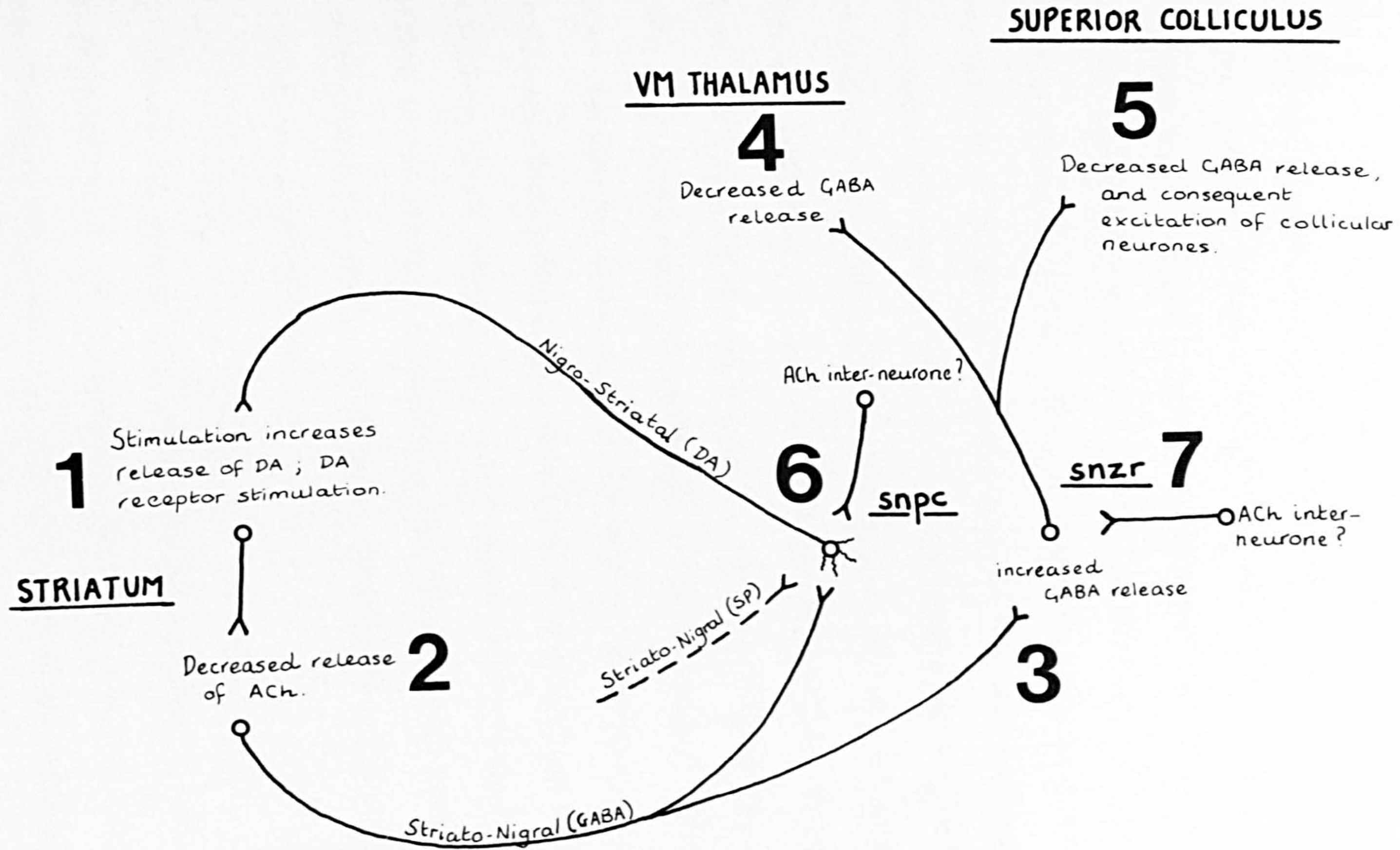
4. Electrolytic lesion: aphagia (Shumway and Lindholm, 1978). Bilateral muscimol elicits catalepsy while unilateral muscimol elicits ipsilateral rotation (Di Chiara et. al., 1979b).

5. Kainic acid lesions (deep and intermediate collicular layers): aphagia (Pope and Dean, 1979). Picrotoxin induces stereotyped gnawing (Dean et. al., 1980).

6. Cholinergic stimulation: low doses elicit feeding behaviour (this thesis, and Winn and Redgrave, 1979); higher doses elicit stereotyped behaviour (Winn and Redgrave, 1979; Decsi et. al., 1978; Smelik and Ernst, 1966).

7. Unilateral carbachol injections induce ipsilateral rotation (De Montis et. al., 1979).

Figure 20.



of the activity of cholinergic inter-neurones within the striatum, which are inhibited by nigro-striatal DA. The exact relationships between DA-ergic, cholinergic and GABA-ergic neurones is not clear; several possible formulations have been presented by Bartholini (1980). Once within the substantia nigra, the GABA-ergic striato-nigral pathway synapses in the zona reticulata on neurones which project to the ventro-medial thalamus (Somogyi et. al., 1979). These neurones are believed to be branched, axons also ascending to deep and intermediate layers of the superior colliculus (Bentivoglio et. al., 1979; Anderson and Yoshida, 1980). Both of these projections - thalamic and tectal - are thought to be GABA-ergic. As the GABA released from striato-nigral neurones is believed to have inhibitory properties, activation of this pathway should result in inhibition of activity in the zona reticulata efferents. These too are believed to utilise GABA to exert inhibitory control in the thalamus and tectum, which should, as a consequence of inhibition of the inhibitory pathways, show an increase in activity. This appears to be the case within the superior colliculus. While not themselves commenting upon it, Brown and Wolfson (1978) clearly show that apomorphine increases metabolic activity in the superior colliculus, (see Figure 1, p. 189), which is exactly the consequence of striatal DA stimulation predicted by the model we are presenting.

The striato-nigral GABA projection also appears to interact with the DA neurones of pars compacta. DA-sensitive adenylate cyclase has been localised to pre-synaptic elements of neurones arising in the globus pallidus and striatum; moreover, DA has been shown to selectively stimulate the release of GABA from descending nigral afferents, and not substance P (Reubi et. al., 1978) which would seem to indicate

an interaction between GABA and DA. Arbuthnott (1978) has shown that this relationship is not one of simple GABA-ergic feedback, for lesions which cause a fall in both GABA and GAD content of the substantia nigra do not alter DA turnover in the striatum. GABA does however appear able to inhibit the release of SP from peptidergic striato-nigral fibres (Jessell, 1977).

Although the precise localisation of cholinergic neurones within the nigra remains a mystery, ACh does appear to be active here. We have already discussed at length cholinergic-dopaminergic interactions within the nigra, and we have seen that ACh also appears to have a role within the zona reticulata, controlling posture in a manner opposite to GABA (De Montis et. al., 1979). As these GABA neurones appear to control posture by inhibiting zona reticulata efferent neurones, it might be presumed that cholinergic neurones have an excitatory influence on these.

Behavioural observations which provide evidence supporting this model of striatal output include:

a. <u>DA liberated from nigro-striatal neurones inhibits cholinergic inter-neurones</u>. Atropine, a muscarinic receptor antagonist, induces stereotyped behaviour when injected directly into the striatum (Zambo et. al., 1979); this is consistent with the hypothesis that a corollary of striatal DA stimulation is cholinergic inactivation. Scopolamine, another anti-muscarinic, suppressed the food intake of deprived rats when injected into the striatum (Neill and Grossman, 1973). This might, according to the authors, be a consequence of the dose being high enough to elicit stereotyped behaviour, for they observe that "it

appears ..... likely that they drug interfered with motor functions ... essential to the ingestion of dry foods".

b. <u>DA activity in the striatum leads to an increase in the activity of GABA-ergic striato-nigral neurones</u>. Animals are thought to turn away from the side of highest striatal DA activity following a unilateral manipulation - a positive correlation has been found between the rate of turning and striatal DA depletion (Thornburg and Moore, 1975). Therefore, animals should also turn away from the side of highest striato-nigral GABA activity, if striatal DA activation does lead to increased striato-nigral activation. This has been found to be so: muscimol, a GABA agonist induced strong contralateral rotation, resistant to DA receptor blockade, when injected into the zona reticulata (Arnt and Scheel-Krüger, 1979). These authors found ipsilateral body-posturing as a result of pars compacta muscimol injections, but this was converted to ipsilateral rotation by sub-cutaneously administered apomorphine: as Arnt and Scheel-Krüger observe, the result suggests "that the interaction between DA and nigral GABA is extremely complex". Intra-nigral and intra-thalamic injections of tetanus toxin -which is reported to block GABA release - are both reported to elicit a very powerful ipsilateral rotation (McGeer et. al., 1980). James and Collingridge (1979) also found ipsilateral rotation as a result of tetanus toxin injections into caudal substantia nigra (i.e. zona reticulata) but contralateral rotation if the GABA antagonist was injected into rostral substantia nigra (i.e. the area of the pars compacta) suggesting dual GABA effects within the substantia nigra. Stereotyped behaviour has also been elicited by potentiation of GABA transmission in the substantia nigra (Koob et. al., 1978; Matsui and Kamioka, 1978). Perhaps the most

striking result of all however, is that extremely low doses of muscimol (0.05ng) bilaterally microinjected into the substantiae nigrae, zona reticulata, elicit feeding behaviour, while higher doses (0.25, 0.50ng) induce stereotyped behaviour (Redgrave, 1980, unpublished). This is contrasted by findings which show that injections of the GABA agonist bicuculline into "the origin of the nigrostriatal DA neurones" suppresses intake of a liquid diet (Kelly et. al., 1977). Adjacent VTA injections have no such effect. This would seem to match the rotational data of James and Collingridge (1979) and it would thus appear that zona reticulata GABA activation might support the same range of behaviour as striatal DA activation.

c. <u>DA activity leads to a decrease in GABA release from nigro-thalamic and nigro-tectal neurones</u>. That this might be so is indicated by results which show first, that GABA antagonists applied to the superior colliculus elicit gnawing (Dean et. al., 1980) strikingly similar to that seen following intra-striatal apomorphine implantation (Ernst and Smelik, 1966), while GABA agonists applied to the ventro-medial thalamus induce ipsilateral asymmetry when applied unilaterally (Di Chiara et. al., 1979b). Lesions of the VM thalamus have also been found to induce aphagia. (Shumway and Lindholm, 1978). This might be expected if we consider that excitation (i.e. the absence of GABA inhibition) might be the consequence of either striatal DA or zona reticulata GABA stimulation. As either of these can induce feeding, so might VM thalamic excitation, and consequently, a lesion might result in aphagia. (This however, is rather speculative: aphagia might well result from a wide variety of causes not linked to the present model.)

It would thus appear that behavioural data can make at least

two contributions; first, it enables us to examine the functional effects of neurotransmitter activation which allows us to construct and validate a model of nigral and striatal connections; and second, it shows us that behaviour characteristic of dopaminergic activity, which might indeed be the initial product of specific DA stimulation, can also be mediated by efferent,non-dopaminergic, systems.

It is apparent that when peripherally applied drugs are used other neuroanatomical structures and neurochemical systems will be activated; mesolimbic and nigro-striatal DA neurones appear to be activated by high doses of peripherally applied d-amphetamine, and serotonergic systems are also implicated in the very high dose effects of this drug (Lees et. al., 1979). Such features of systemic administration of drugs provide one reason for using intra-cranial techniques in examining the involvement of particular neuroanatomical systems in eliciting particular behaviours. However, a question still to be answered is, how far do different parts of the model presented contribute to any particular observed behaviour? A great deal of evidence suggests that the behaviours supported by stimulation of the nigro-striatal DA system (feeding at low doses, through to stereotypy at high doses) can be elicited by the appropriate stimulation of other striatal afferents and efferents. Feeding may be induced by stimulation of pars compacta cholinergic systems, striatal DA neurones or zona reticulata GABA neurones; aphagia may be induced by nigro-striatal, striatal, superior colliculus or VM thalamic lesions. Stereotyped behaviour is elicited by intra-nigral carbachol, intra-striatal d-amphetamine, intra-nigral GABA potentiation and by intra-collicular picrotoxin.

These data suggest that the appropriate stimulation of points along a neuronal chain stretching from the pars compacta to the thalamus and tectum elicit behaviour in a similar dose-responsive manner. The observation of stereotypy following intra-striatal microinjection of d-amphetamine by Costall, Naylor and Olley (1972b) is particularly interesting, in that while these authors observed characteristic DA stereotypies, they did not observe high dose gnawing and biting. The authors comment on this, and the implication for the present discussion is clearly that structures other than striatal efferents are necessary for the expression of high-dose stereotypic behaviours. However, Fray et. al. (1980) in a very thorough examination of d-amphetamine and apomorphine stereotypies show that peripherally applied d-amphetamine in doses from 0.5 mg/kg to 15.0 mg/kg does not elicit gnawing;incontrast, high doses of apomorphine (1.0 and 5.0 mg/kg) do induce gnawing. On the basis of this evidence, striatal stimulation by d-amphetamine would not be expected to induce gnawing and biting.

Morelli et. al., (1980) claim to show that kainic acid lesions of the substantia nigra abolish only the high-dose stereotypic effects of apomorphine, a finding which might be used to suggest that intact striatal efferent neurones are necessary for the expression of such high-dose stereotypies as gnawing and biting. However, as no control data is presented, it is difficult to accurately assess the value of these results.

We might therefore claim that in general the available data show that characteristic DA-induced behaviours may also be demonstrated by the appropriate stimulation or inhibition of nigro-striatal pathway

afferents and efferents. Moreover, a range of dose-dependent behaviours appear to be elicited by stimulation of several of these afferent and efferent systems.

iii. Dopamine-Acetylcholine Interactions Within the Substantia Nigra

The main point of interest of this thesis has been the activity of ACh within the substantia nigra. Previous studies have clearly indicated the possibility of an ACh-DA interaction here. Observations that pars compactta DA cell dendrites contained AChE and released it under physiological stimulation clearly suggest the presence of a cholinergic system. Why else liberate AChE, if not to inactivate a cholinergic input? Electrophysiological studies generally suggest a cholinergic-dopaminergic interaction here, although clearer characterisation than this is difficult to gauge from such studies, for the reasons already discussed, (see Chapter 4, Section iiib), while behavioural studies have indicated that a cholinergic system excites nigro-striatal DA cells to elicit stereotyped behaviour (Winn and Redgrave, 1979; Decsi, Nagy and Zambo, 1978; Smelik and Ernst, 1966).

It is the behavioural approach which the present studies have used in an attempt to evaluate, functionally, whether or not nigro-striatal DA cells of the pars compacta are activated by a cholinergic system. To do this we have first of all attempted to establish that, striatal DA stimulation, at a low level, potentiates feeding behaviour, while a survey of the literature also reveals that the behavioural effects of striatal DA stimulation can be achieved by the appropriate

stimulation or inhibition of striatal efferent neurones (Figure 20). Experiment 3 reveals that cholinergic stimulation of the substantia nigra, at levels which did not induce behavioural stereotypy, also stimulated feeding behaviour. Moreover, this response became weaker with increasing distance from the pars compacta region, from where maximal responding to cholinergic stimulation was elicited. A strikingly similar response was elicited by inhibition of AChE activity, which suggests that endogenous ACh can also induce feeding. Data is also presented which might be taken as suggesting that muscarinic cholinergic receptors mediate this response.

We can therefore claim that a cholinergic system is active within substantia nigra. Several points suggest that this activity is directed towards stimulating pars compacta DA neurones.

i. While the presence of AChE is a poor indicator of the point of cholinoception, identification of the point of AChE release is most probably not. The accepted function of AChE is to inactivate ACh by hydrolysation: we might therefore assume that if AChE is released into extra-neuronal spaces, then ACh is most probably also present. As AChE is both stored within and released from pars compacta DA cell dendrites, it might realistically be assumed that ACh is normally also present within that area and binds to cholinergic receptors in an area within or proximal to the pars compacta, over which the DA cells have some control.

It has been demonstrated that cholinergic stimulation within the region of the pars compacta elicits feeding as does systemic or intra-striatally administered d-amphetamine; inhibition of AChE activity

can also achieve the same result, and cholinergic receptor blockade appears able to block the response. These results suggest that ACh has a functional role within substantia nigra, and facilitates the same behaviour as stimulation of DA release from striatal terminals. Moreover, it has been shown that low, non-sedative doses of haloperidol (0.4 mg/kg) can inhibit the feeding elicited by low doses of carbachol injected into the substantia nigra, or the stereotypy elicited by higher doses, strongly suggesting that the cholinergically-induced feeding is DA-dependent.

ii. The presence of a cholinergic system within the substantia nigra zona reticulata has already been discussed. It is thought unlikely that it is this sytem which is responsible for the observed feeding behaviour. A consequence of DA stimulation within the striatum appears to be activation of the striato-nigral GABA pathway, and consequent release of this inhibitory neurotransmitter within the zona reticulata. Thus, potentiation of GABA activity (by blockade of degradative enzymes) or mimicry of GABA activity (using muscimol) can elicit stereotyped behaviour (Koob et. al., 1978), contralateral rotation (Arnt and Scheel-Krüger, 1979) and, at very low levels of stimulation, feeding (Redgrave, 1980, unpublished). Injections of carbachol into the zona reticulata elicit ipsilateral rotation (De Montis et. al., 1979; James and Massey, 1978) - quite the reverse of GABA stimulation - and indicate that cholinergic activity here is excitatory (as in the pars compacta, if judged by the data presented here), and acts in a manner opposite to GABA (which is inhibitory). This GABA activity is believed to be a reflection of striatal DA activity, and as such, we may conclude that stimulation of excitatory cholinergic neurotransmission in the zona reticulata is

antagonistic to the effects of DA stimulation. As low-level striatal DA stimulation or low-level zona reticulata GABA activity are thought to potentiate eating, ACh activity here should inhibit feeding.

iv. Whether there are two anatomically distinct populations of cholinergic neurones is not clear. Results suggest however, that cholinergic neurones exert an excitatory influence in both the pars compacta and zona reticulata, but that the functional effects of this are different, compacta stimulation potentiating the effects of striatal DA and reticulata stimulation causing an inhibition of striatal effects relayed via efferent neurones.

In the preceding section we saw how the effects of striatal DA activation may be mediated not solely within the striatum but also by striatal efferents. The studies reported here using cholinergic stimulation would seem to indicate that "dopaminergic" behaviour cannot only be mimicked by the appropriate stimulation of efferent neurones, but also by stimulation of afferent systems. More questions must now be answered. As we noted in the introduction (Chapter 4, section iib), ACh has proved difficult to localise within the substantia nigra. That it is in fact present has been demonstrated (Jacobowitz and Goldberg, 1977), while localisation of AChE and muscarinic receptors, and demonstrations of a behavioural function confirm that it is active. Kuczenski et. al. (1975) have observed nigral CAT to be associated with cell bodies, while McGeer et. al. (1973) found no changes in CAT levels following hemitransections anterior or posterior to substantia nigra. These results suggest that ACh might be the neurotransmitter of the inter-neurones observed

by Gulley and Wood (1971). Only the results of Nagy et. al. (1977) fail to conform to this hypothesis, in that they observed no change in nigral CAT content following Kainic acid lesions, suggesting that it was contained neither in inter-neurones nor efferent fibres. If, as seems more likely on the basis of the evidence to date, ACh is released from inter-neurones rather than efferent neurones, which neurones are afferent to them? The only nigral efferent system with any cholinergic property appears to be that from the striatum, which some authors claim contains AChE (Olivier et. al., 1970; Kaiya et. al., 1979), although other authors dispute this (Lehmann et. al., 1979). If these neurones do contain AChE, it might suggest a complex GABA-ACh interaction within the zona reticulata. However, the question of afferents to ACh inter-neurones - if in fact they exist at all - must remain for the time being, open.

In respect of the nigro-striatal DA neurones, a picture seems to be emerging of cholinergic interaction within both the striatum and the substantia nigra. As has been previously noted (Chapter 4, section i), nigro-striatal neurones synapse within the striatum upon a dense population of cholinergic inter-neurones; most evidence suggests that DA inhibits these. We also noted that two classes of pre-synaptic cholinergic receptors have been found on the terminals of nigro-striatal neurones: excitatory nicotinic and inhibitory muscarinic cholinergic receptors (De Belleroche and Bradford, 1978). Pre-synaptic regulation of DA release by ACh appears to be dependent on the rate of firing of the DA neurones, the activating effects of ACh on DA release not being present when the nigro-striatal neurones were already activated (Giorguieff-Chesselet et. al., 1979). In the substantia nigra, an excitatory cholinergic influence on pars

compacta DA neurones has been suggested.

Clinically, these findings might prove to be of importance. Post-mortem studies of the brains of Parkinsonian patients have revealed that this disease is associated with large reductions (to 10% of control) in striatal and substantia nigra DA levels (Ehringer and Hornykiewicz, 1960; Hornykiewicz, 1966). In an attempt to restore DA function, L-DOPA, a pre-cursor of the neurotransmitter has been administered (Yahr, 1978). However, while treatment may be initially effective, a gradual decline in the efficacy of the drug almost always occurs, sometimes leading to a Parkinsonian state worse than that initially encountered. In addition, disturbing side-effects often become apparent during the treatment.

While L-DOPA therapy did not originate by accident - it is one of only few modern drugs to have been deliberately tried following theoretical consideration - there are questions to answer: where does the transformation of exogenous L-DOPA to DA take place? (Can it in fact occur in the area where it is required, which is DA-depleted as a result of neuronal degeneration?) Are all of its therapeutic actions mediated by DA? L-DOPA is after all, also a pre-cursor of noradrenaline. In the normal brain DA appears to interact with cholinergic neurones both in the striatum and substantia nigra, and any account of Parkinson's disease must take account both of these and other normal mechanisms of regulating nigro-striatal DA activity. It is of great relevance to note that before L-DOPA the most commonly used anti-Parkinsonian drug was atropine which enjoyed a limited success. As such a combined cholinergic-dopaminergic approach to Parkinson's disease might be

of value, in an attempt to re-establish the normal regulation and function of these neurones.

iv. What do Nigro-Striatal DA Neurones Do?

In the previous two sections we have discussed how DA-mediated behaviour can be stimulated indirectly by manipulation of neurones efferent to the striatum and by cholinergic activity within the substantia nigra. It is interesting to note that behaviour is reproducible at different points in a neuronal chain. Feeding may be potentiated by low-level stimulation of nigral cholinergic activity (pars compacta), striatal DA activity, and by GABA activity in the zona reticulata. Stereotyped behaviours have been elicited by high-level stimulation of all of these, as well as by striatal cholinergic systems, and by inhibition of superior colliculus neurones. Aphagia has been induced following nigro-striatal lesions (Ungerstedt, 1971d); destruction of DA terminals in the striatum (Joyce 1980; Neill et. al., 1975); superior colliculus lesions, (though these necessarily damaged the underlying tegmentum) (Pope and Dean, 1979); and by lesions of the VM thalamus (Shumway and Lindholm, 1978). Brook and Iversen (1975) note that "the more severe the .... (striatal) ... DA depletion, the more likely the animal is to show aphagia and adipsia", although these authors also note that this phenomenon does not always occur and that it may be associated with additional NA depletion. One recent report suggests that highly specific pars compacta 6OHDA lesions do not induce aphagia (Butcher and Hodge, 1979). As a large body of evidence strongly suggests that severe striatal DA depletion leads to aphagia, it is possible that sub-total DA lesions might not.

However, is there any way in which we can classify what nigro-striatal DA neurones do? Recent work has suggested that these neurones respond to sensory information. Unilateral stimulation of a forelimb paw increases DA acvitity in contralateral substantia nigra and reduces DA activity in contralateral caudate nucleus; on the ipsilateral side the reverse pattern is seen. The same pattern of activity was also seen following unilateral visual stimulation (Nieoullon et. al., 1977). Tail pressure, light flash, puffs of air to the snout and olfactory stimulation all alter the discharge rate of identified pars compacta DA neurones in anaesthetised rats (Chiodo et. al., 1980). Moreover, these authors identified, electro-physiologically, two types of pars compacta DA neurone: Type A increased their discharge rate to either tail-pinch, air puffs or olfactory stimuli, while Type B decreased discharge rates. Light flash increased discharge rates of both neuronal types.

In discussing their results Chiodo et. al. note that specific sensory stimuli are not likely to be transmitted, as both visual, tactile and olfactory stimulation altered discharge rates. Moreover, pain does not seem to be a common factor between the stimuli. These authors go on to suggest that such a common feature of these stimuli may be that, in the unanaesthetised animal, they all produce behavioural activation. It is thus of interest to note that stimuli which do not produce behavioural activation, but in fact immobilize animals - intense (800g) mechanical stimulation of the cervix - do not alter the activity of pars compacta neurones (Chiodo et. al., 1979). It is also pertinent to recall the studies noted previously (Chapter 4, section iiib) which show that d-amphetamine decreases striatal

firing in anaesthetised animals but increases firing in conscious, freely-moving rats (Hansen and McKenzie, 1979) and cats (Trulson and Jacobs, 1979) and that Hansen and McKenzie were quoted as saying that "... the discrepancy between data derived from freely-moving and immobilized animals suggests that striatal activation ... may depend on sensory feedback from behaviour". Perhaps we could thus say that nigro-striatal DA neurones are important for producing behavioural activation and necessary for the integration of multisensory inputs.

This is a concept which D.O. Hebb developed; "... We can now distinguish two quite different effects of a sensory event. One is the Cue Function guiding behaviour; the other, less obvious but no less important, is the Arousal or Vigilance Function. Without a foundation of arousal, the cue function cannot exist". (Hebb, 1955, p. 249.) It is Hebb's concept of arousal - an alerting or activating function - which can form the basis for a unified and sensible framework accounting for the behavioural consequences of nigro-striatal manipulation. Feeding, aphagia, sensory-motor neglect, tail-pinch induced feeding, stereotypy, rotation, catalepsy and even Parkinson's disease can all be accommodated within such a framework; a framework moreover which has the power to account for some previously paradoxical data.

In experiments where an increase in feeding has been seen following d-amphetamine stimulation, animals have always been tested when not food-deprived, and in an environment where food was a principal feature. This of course is especially so in the experiments reported here where spaghetti represented an unusual and highly palatable food. (See Tables 10, 13 and 19 for an indication of food intake before

microinjections were made. It is remarkable that animals consumed such amounts and then were able to eat in a dose-responsive fashion following stimulation.) Drinking was consistently not observed. Turning behaviour was also never observed, despite the unilateral nature of the stimulation, which was thought to specifically activate ipsilateral DA systems. The present hypothesis might suggest that animals did not show turning because of the quality of the sensory-motor stimuli from the environment. Examination of behaviour when either no environmental stimuli are available - as is often the case in turning experiments where a "rotometer" can be used -or when stimuli other than food are available, would be a useful means of discovering whether or not activation of this pathway mediates specific behaviours.

One of the paradoxical effects found when using dopaminergic stimulants is that when satiated rats are challenged with low doses of dopaminergic stimulants, their responses appear to depend on internal state. Eichler and Antelman have shown that apomorphine (0.20 mg/kg) stimulates feeding in satiated animals but inhibits feeding in deprived animals. Similarly, it is reported here than 0.25 mg/kg d-amphetamine potentiates feeding in satiated rats while Costa et. al. (1972) report that 0.3 mg/kg (iv) (+) amphetamine inhibits feeding in deprived animals. Simpson (1974) has also reported that while food deprivation alone does not potentiate motor activity, it does potentiate the locomotor stimulant effects of 1.0 mg/kg d-amphetamine by an amount which was roughly proportional to the duration of deprivation. These data therefore suggest that food deprivation enhances the activating properties of d-amphetamine. As such, low feeding-stimulant doses might be effectively enhanced when animals are deprived, giving rise to an effective locomotor- or stereotypy-inducing stimulus. While

food deprivation alone did not induce locomotor activity, we may assume that it would induce feeding behaviour - just as low doses of d-amphetamine would.

The concept of behavioural activation mediated by nigro-striatal neurones might also help explain why apomorphine in very low doses helps restore activity following nigral 60HDA lesions (Ljungberg and Ungerstedt, 1976), as do other activating stimuli. Wolgin and Teitelbaum (1978) in summarising the results of a study investigating activation and the LH syndrome say that "in addition to aphagia and adipsia, neurological examination revealed deficits suggestive of deficient endogenous arousal, including somnolence, catalepsy, akinesia and sensory neglect. Manipulations (tail-pinch and injection of amphetamine) that counteracted these deficits also restored feeding. During recovery from aphagia, feeding gradually became activated by sensory stimuli (sight, feel and smell) associated with food." The suggestion here is quite clearly that nigro-striatal damage causes aphagia and adipsia by removal of activation-mediating neurones, in exactly the same way as neuroleptics establish aphagia by neurochemically blocking DA receptors to prevent normal functioning. The recent observations that kainic acid lesions of the lateral hypothalamus (Grossman et. al., 1978; Stricker et. al., 1978) establish aphagia and adipsia, independently of DA neurone destruction, might suggest that there is a LH syndrome and a separate nigro-striatal ("activation depleting") syndrome.

The concept of nigro-striatal neurones as an activating system

allows for an explanation of tail-pinch induced feeding. It has been suggested that feeding relieves anxiety and stress in these supposedly stressful test situations. This hypothesis cannot however account for certain observations - for instance animals treated with chlordiazepoxide (CPZ), at doses which do not elicit feeding but which do supposedly reduce anxiety, show an increase in tail-pinch induced feeding (Robbins et. al., 1977). Robbins and Fray (1980) conclude that "eating is induced by stress but the eating does not act to reduce that stress. The eating response is learned in much the same way as eating in response to food deprivation. The activating effects of stress produce increased attention to external stimuli, many of which are likely to be food-related." It is this concept of stress-elicited activation that is compatible with the activation mediating concept of nigro-striatal neurones. Lesion of this pathway by 6OHDA has been reported to diminish tail-pinch induced feeding (by shortening duration rather than increasing latency); while feeding was absent, vocalisations by the stressed animals were still present indicating that sensory aspects of the pinch were still present (Antelman et. al., 1975). Neuroleptic drugs have also been reported to affect tail-pinch induced eating; acquisition was most reliably affected, although there were suggestions that motor performance was inhibited rather than any more specific mechanism

(Sahakian and Robbins, 1977).

Overall we might therefore suggest that stress-induced eating arises as a result of an arousal property (Hebb, 1955) of the stimulus. This would explain both why anxiety-reducing doses of CPZ increase tail-pinch induced eating, and why it appears to be amenable tc modification through the nigro-striatal pathway. It is of some interest to note that "the intensity of the eating response is related to the level of activation in a curvilinear fashion; low levels increase eating and high levels inhibit eating" (Robbins and Fray, 1980). This is exactly what injections of cholinergic substances into the substantia nigra do.

This brings us to stereotyped behaviour, which was discussed in the introduction (Chapter 5, section iiib). Clearly animals that exhibit stereotypy are in some sense activated, and as such, mediation of this process by nigro-striatal DA neurones is acceptable to the current hypothesis. What stereotypy seems to represent is the extreme of activation, where much sensory control has been lost. As the nervous system is so highly activated at such a constant level, activation never ceases but is forced into a repetitive high-rate, low inter-response time schedule, where no complex co-ordinated behaviour can be completed. Neurochemical stimulation can induce such a state, although it is doubtful that tail-pinch can. As tail-pinch becomes more severe, not only activation but also distress increases: it is quite possible that animals would be able to avoid entering a stereotypic state by an alternative process.

Of the remaining behaviours surveyed in Chapter 5, both rotation

and catalepsy can be accommodated within the "activation" framework. Catalepsy simply represents the extreme point of DA receptor blockade; no DA activity gives rise to no behavioural activity. Rotation on the other hand is a product of unilateral stimulation. As we previously saw, Nieoullon et. al. (1977) have shown that unilateral sensory stimulation - either tactile or visual - influence nigro-striatal DA activity in a unilateral manner, which presumably should orient animals to a particular side. This would suggest that animals might, if no other environmental factors intervene, display an asymmetric posture; this is, in part, the formulation adopted by Kelly and Moore (1976), who hypothesised that unilateral stimulation of nigro-striatal DA neurones was responsible for asymmetric posture while stimulation of mesolimbic DA neurones supplied an ambulatory component to convert asymmetric posture into rotation. While the first of these points is essentially in agreement with the suggestion derived from the data of Nieoullon et. al. (1977) (that unilateral stimulation of nigro-striatal DA neurones will orient animals to a particular side), it might however be argued that nigro-striatal neurones can also provide all or part of the motor component of rotation. Neill and Grossman (1973) have data which suggest that d-amphetamine-induced locomotion might be striatally mediated, as well as n. accumbens mediated. Whether this is so or not, it can be argued that unilateral nigro-striatal stimulation will orient animals asymmetrically unless other environmental cues intervene, and it is this which causes activation to take the form of rotation, which might therefore be considered as asymmetric locomotion.

The final aspect of nigro-striatal activity which might be examined in the light of the activation hypothesis is Parkinson's

Disease, which, as has already been noted, is associated with degeneration of nigro-striatal neurones; as such, L-DOPA has been used therapeutically in an attempt to restore depleted DA levels. (DA itself cannot be used because of a failure to penetrate beyond the blood-brain barrier.) In its most complete form, Parkinson's Disease induces an almost complete akinesia. Sacks (1976), in the book "Awakenings", presents case-histories of patients suffering from the most extreme forms of the disease, this having been caused by the virus encephalitis lethargica. One patient, Rolando P. "... would sit in his chair, with his head bowed forwards and very little spontaneous movement, for hours on end." "His voice was so soft as to be inaudible: sudden effort and excitement, however, rendered exclamatory speech possible for a few seconds. Thus, when I asked him whether his salivation disturbed him much, he exclaimed loudly: "You bet it does! It's one hell of a problem!" immediately afterwards relapsing into virtual aphonia." (Sacks, 1976, p. 148-149.) Rolando P. was treated with L-DOPA, and began to show improvements. But, his new-found "... activities were an accentuation of phenomena already present before L-DOPA was given ... Around 10 June a new symptom appeared, which could be described as a voracity, an oral mania, or a devouring urge. The taking of the first mouthful at any meal seemed to let loose an irresistible desire to grab, bite and devour food, as fast as possible .... In the third week of June (his dose of L-DOPA remaining unchanged), symptoms of a more disquieting nature appeared, with agitation, perseveration, and stereotypy as their hallmarks." (Sacks, 1976, p. 152-153.)

If we can see Parkinson's Disease as a loss of activating systems, the case of Rolando P. illustrates the activating effects

of L-DOPA therapy; moreover, Mr. P. showed a spontaneous remittance of his akinesia in response to activating stimuli such as music which would call for "... singing, 'conducting', and occasional dancing, and at this time... his symptoms are minimal" (p. 157) and, remarkably, Mr. P. was able to swim the length of a pool, and showed an amazing reduction of Parkinsonism when in the water. Rolando P's tragedy, like so many other Parkinsonian patients, was that the correct dose of L-DOPA could not be found, and as such he was caught between akinesia and hyperactivity, which soon gave way to stereotypies. This is a failure of the therapy, not of his personal treatment; it appears that the balance for L-DOPA therapy grows ever finer, until a dose is found which is ineffective while the minutest amount more gives rise to the gross overactivation problems. As such, this is a failure of our understanding of DA regulation. However, the case of Rolando P.shows us that loss of nigro-striatal DA leads to inactivity and that this can be restored (poorly) by L-DOPA therapy, and by sensory activation. It is an unpleasant fact of Parkinson's disease that sensory information is still present; what is absent is the power to turn this information into action, and this is in accord with our hypothesis that nigro-striatal DA neurones mediate behavioural activation.

Retrospective

This thesis has attempted to examine two questions. The first of these has been, as the title states, what are "the functional effects of cholinergic stimulation of the substantia nigra in the rat"? The second question which has been addressed - what are the behavioural consequences of dopaminergic stimulation within the striatum - is, in many respects a consequence of the first, for it has been suggested that ACh within substantia nigra can stimulate nigro-striatal DA neurones. Thus the predicted consequence of cholinergic stimulation of substantia nigra would be a release of DA within the striatum: the two forms of stimulation might thus be expected to produce similar functional effects.

As a first step, an examination of the behavioural effects of peripherally applied d-amphetamine was made, and at low doses feeding was observed. This declined as the dosage increased, locomotion, rearing and stereotyped behaviours becoming more dominant. This appears to be in accordance with the rate-dependent hypothesis of amphetamine action formulated by Lyon and Robbins (1975). Following this, an attempt was made to examine the role of the striatum in mediating the feeding response. While intra-striatal d-amphetamine increased, the amount of food consumed in a dose-responsive fashion, the latency of responding calls into question the conclusions which can be drawn. A more thorough investigation of striatal DA stimulation including microinjection at various striatal sites, and using a greater range of doses, concentrations and volumes might be appropriate for re-examining this question.

Cholinergic stimulation of substantia nigra appeared able to

stimulate feeding dose-dependently. Both ACh and eserine mixtures and eserine alone showed the effect, though as the only difference between the two forms of stimulation was the depressant effect of 10.0 µg exogenous ACh, it might be argued that both forms simply represent the facilitatory effect on feeding of eserine sulphate. However, as this acts to enhance activity of endogenous ACh, a true cholinergic effect is suggested. While it was not possible to demonstrate receptor blocking actions - a more careful examination of this is called for before meaningful conclusions can be drawn - the similar effect of intranigral carbachol in stimulating feeding (Winn and Redgrave, 1979) suggests that cholinergic receptors mediate the response. That it is DA dependent is suggested by several points: (i) behaviourally, intranigral cholinergic stimulation appears to facilitate similar activities to those mediated by DA (feeding at low doses, stereotypy at high doses (Winn and Redgrave, 1979)) (ii) non-sedative doses of haloperidol abolish both the feeding and stereotypy elicited by intranigral carbachol (Taha and Redgrave, 1980) (iii) cholinergic receptors exist on pars compacta DA neurones (Cross and Waddington, 1980), which also contain (Butcher and Marchand, 1978) and release AChE within the nigra (Greenfield et al., 1980).

However, clearly lacking is a direct demonstration that nigral ACh can cause a release of DA in the striatum. In vivo electrochemical detection, perfusate assay and measurement of lateral ventricular DA concentration following nigral injections of eserine would seem to be appropriate techniques for examining this critical question. While these, and further examination of the functional effects of striatal DA stimulation would seem to be of value in enhancing our understanding of the neurochemical and neuroanatomical processes at work, behavioural analysis of the feeding response might reveal more about the involvement

of nigro-striatal neurones in the regulation of eating. A comparison with food deprivation, and feeding in response to regulatory deficits, in conjunction with d-amphetamine or cholinergic stimulation might be of value, as might studies examining the consumption of less novel foods. A microanalysis of the structure of feeding comparing food deprived, normal, and cholinergic stimulated feeding might reveal to what extent the stimulated feeding resembles normally motivated behaviour.

This thesis tackles a problem that has been largely ignored: what systems within the brain stimulate nigro-striatal DA neurones at source? A functional approach to neuronal interactions has been adopted, in the hope of providing meaningful information as to how neurochemical and anatomical systems interact, and what the consequences are for behaviour. It might thus be hypothesised that low-level DA release may be involved in the control of feeding behaviour and that nigral ACh may stimulate DA neurones and facilitate such behaviour. Thus, the original contribution of this thesis is that nigral ACh appears to have the function of stimulating nigro-striatal DA neurones. This is of importance in our understanding of the normal regulation of DA within nigro-striatal neurones, and may eventually have therapeutic value in combating the loss of nigro-striatal DA associated with Parkinson's disease.

APPENDIX

Appendix

i. A Note on the Terms Neurotransmitter and Neuromodulator

The term 'Neurotransmitter' has no clear cut definition. In general the following definition appears to summarise the main featurss of a neurotransmitter. It is "...a substance endogenous to neurones that (a) is released from pre-synaptic loci into the adjacent synaptic cleft upon depoliarsation of the pre-synaptic region at physiologic levels of stimulation, (b) crosses the synaptic cleft via passive and/or active processes, and (c) combines with specialised portions of the post-synaptic membrane - the receptors - to induce excitatory or inhibitory post-synaptic potentials of characteristic amplitude and duration that can directly elicit or inhibit propagated electrical activity" (Butcher and Talbot, 1978b p. 4).

Earlier definitions, taking ACh as the only conclusively identified synaptic transmitter, included a wider range of criteria, which are presented in Table 37. These may be summarised as: (1) a non-uniform distribution in neurones and in the nervous system (2) presence of synthetic enzymes (3) storage in an inactive form (4) released upon relevant physiological stimulation (5) reacts with specific receptors on the post-synaptic membrane (6) presence of degradative enzymes (7) pharmacological agents should mimic endogenous processes. A neurochemical fulfilling all of these criteria could be considered a neurotransmitter.

While these criteria are still held to be valid by some authors,

FLOREY (1961)	PHILLIS (1966)
(not necessarily listed in order first published)	
1. Transmitter concentrated at nerve terminals, but it or its immediate precursor found everywhere in the neurone.	1. The substance must be present in those neurones from which it is released.
2. The axons and nerve endings contain the enzymes for synthesis of the transmitter.	2. The neurones must possess the necessary enzymic mechanisms for the manufacture of the transmitter and for its release.
3. The transmitter is stored in a physiologically inactive, bound form.	3. The presence of the various precursors and intermediates in the synthetic pathway should be demonstrable.
4. The transmitter is released from nerve terminals on arrival of a nerve impulse. The amount released is rather constant.	4. During stimulation the substance may be detectable in extra-cellular fluid collected from the region of the activated synapses.
5. The free transmitter diffuses across the synaptic gap.	
6. It reacts with specific receptor molecules in the sub-synaptic membrane to give the post-synaptic response.	5. When applied to the post-synaptic structure, the substance should mimic the action of the synaptically released transmitter.
7. Specific receptor sensitivity is found only on sub-synaptic patches of the post-synaptic membrane.	
8. An enzyme system in the synaptic region inactivates the transmitter.	6. There may be systems for the inactivation of the transmitter. These could include an enzyme system for the inactivation of the transmitter and a specific uptake mechanism for the re-absorption of the transmitter into the pre- or post-synaptic structure.
	7. Pharmacological agents which interact with the synaptically released transmitter should interact with the suspected transmitter in an identical manner

Table 37: Major criteria involved in the identification of a neurotransmitter as proposed by Florey (1961) and Phillis (1966). (From Davidson (1976), p. 2-3.)

many feel that these criteria may not be of value when attempting to establish whether or not new substances have neurotransmitter properties. Davidson (1976) argues that "it is evident ..... that at least the first six of the criteria listed by Phillis (1966) .... (Table 37) ... can be traced back to concepts of synaptic transmission current in 1961 or earlier, when acetylcholine was the sole molecule to have been properly identified as a synaptic transmitter. It does not necessarily follow that these concepts are equally acceptable for non-cholinergic synapses nor that all these criteria are applicable to non-cholinergic transmitter candidates ....." (Davidson 1976 p. 3). Furthermore, Davidson aruges, " ...... the criteria frequently referred to as necessary for identification of a neurotransmitter ..... (Table 37) .... are in fact relevant only in the case of a chemical synaptic transmitter at an axo-dendritic or an axo-somatic synapse, and may be neither sufficient nor necessary for identification of substances involved in other forms of chemical transmission between nerve cells ...." (Davidson, 1976 p. 4). In particular, recent advances in our understanding of dendro-axonic or dendro-dendritic connectivity come to mind here.

Werman (1966) considered that the standard criteria for identifying neurotransmitters were of value only if related to the biological function of the synapse. This view was developed and in 1972 Werman proposed that only two of the major criteria had to be satisfied: "identity of action" and "collectability of the transmitter". Myers (1974) discusses the concept of "identity of action".

"The criterion of identical actions is the basic one upon which

many of the studies employing chemical stimulation are based ..... One supposition inherent in the theory that embodies this approach is that a certain group of cells displays a special sensitivity to the compound while others are either insensitive or react to the artificial application of the endogenous chemical in some way other than depolarisation.

"Without prior biochemical information about the content, synthesis, metabolic turnover and mechanism of degradation of an endogenous compound the criterion of 'identity of action' can also be deceptive. If the criterion is considered in vacuo one could easily promulgate, by demonstration alone, the ludicrous concept that formaldehyde is a transmitter substance at the neuro-muscular junction" (Myers, 1974, p. 13-14).

Myers here appears to hit upon what seems to be the most sensible way of evaluating neurotransmitter status. He says that "Werman's conceptualization of 'identical actions' must be regarded as a powerful criterion with which to examine the local synaptic action of a compound. The potential of this criterion is enhanced further not only if the substance is present naturally, but if the enzyme systems for its manufacture and degradation are firmly established" (Myers, 1974, p. 13).

The views presented here can therefore be reconciled if we adopt the following scheme. Broadly, a neurotransmitter, as Butcher and Talbot (1978b) propose, is an endogenous substance, released from pre-synaptic loci, which crosses the synaptic cleft and combines with specific and specialised receptors on the post-

synaptic membrane. Werman's concept of identical actions appears useful in identifying substances which have this property, but the claims of such a substance are greatly enhanced if the 'classic' criteria of Florey (1961) and Phillis (1966) can also be met. Though Davidson (1976) and others have argued that these are based on specific conceptions of cholinergic transmission, with no necessary relevance to other classes of neurotransmitter, it remains true that ACh is an undisputed neurotransmitter, and that, by and large, these criteria, as well as the principle of identical actions, are met by other substances - for instance the monoamine neurotransmitters dopamine, noradrenaline and serotonin, and the amino acid neurotransmitter GABA. However, as there is no necessary relevance of a cholinergic system to other systems, and as our conceptions of chemical communication between neurones is still changing it would be prudent to adopt this scheme in a flexible and liberal manner, and not treat the term neurotransmitter as fixed.

Substances which are synthesised and stored in neurones and liberated during activation, but which do not interact with receptors, are not thought to be neurotransmitters. Such substances may be classified as neuromodulators. These substances may influence neurotransmission either pre-synaptically, post-synaptically or within the synaptic cleft but do not themselves possess neurotransmitter properties. Second Messengers may be classified as neuromodulators. These are substances such as adenylate cyclases (and other cyclic nucleotides) and certain ionophores which can transmit information across the lipid bilayer of the post-synaptic membrane. Neurotransmitters stimulate this passage by combining with the specialised receptor molecules present on the outer surface of the post-synaptic membrane.

REFERENCES

Adinolfi A.M. and Pappas G.D. The fine structure of the caudate nucleus of the cat. J. Comp. Neurol. (1968) 133 167-184.

Aghajanian G.K. and Bunney B.S. Dopaminergic and non-dopaminergic neurons of the substantia nigra: differential responses to putative neurotransmitters. In "Proc. 9th Int. Congr. of the Collegium Internationale Neuropsychopharmacologium" ed. J.R. Boissier, H. Hippius and P. Pichot. Excerpta Medica Foundation, Amsterdam 1974.

Almli C.R., Fisher R.S. and Hill D.L. Lateral hypothalamic destruction in infant rats produces consummatory deficits without sensory neglect or attenuated arousal. Exp. Neurol. (1979) 66 146-157.

Anand B.K. and Brobeck J.R. Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. (1951) 24 123-140.

Anden N-E., Carlsson A., Dahlström K., Fuxe K., Hillarp N-Å and Larsson K. Demonstration and mapping out of nigral-neostriatal dopamine neurons. Life Sciences (1964) 3 523-530.

Anden N-E., Dahlström A., Fuxe K. and Larsson K. Further evidence for the presence of nigro-neostriatal DA neurons in the cat. Amer. J. Anat. (1965) 116 329-334.

Anden N-E., Fuxe K., Hamberger B. and Hokfelt T. A quantitative study of the nigro-neostriatal dopamine neurons system in the rat. Acta Physiol. Scand. (1966) 67 306-312.

Anderson M.E. and Yoshida M. Axonal branching patterns and location of nigrothalamic and nigrocollicular neurons in the cat. J. Neurophysiol. (1980) 43 883-895.

Antelman S.M., Rowland N.E. and Fisher A.E. Stress related recovery from lateral hypothalamic aphagia. Brain Res. (1976) 102 346-350.

Antelman S.M., Rowland N.E. and Fisher A.E. Stimulation bound ingestive behaviour: a view from the tail. Physiol. Behav.(1976b) 17 743-748.

Antelman S.M., Szechtman H., Chin P. and Fisher A.E. Tail pinch induced eating, gnawing and licking behaviour in rats: dependence on the nigro-striatal dopamine system. Brain Res. (1975) 99 319-337.

Arbuthnott G.W. Studies on the interactions between the substantia nigra and the neostriatum. In "Dopamine" (Adv. in Biochem. Psychopharm. 19) ed. P.J.Roberts, G.N. Woodruff and L.L. Iversen. Raven Press N.Y. (1978) 235-248.

Arbuthnott G.W. and Crow T.J. Relation of contraversive turning to unilateral release of dopamine from the nigro-striatal pathway in rats. Exp. Neurol. (1971) 30 484-491.

Arnfred T. and Randrup A. Cholinergic mechanism in brain inhibiting amphetamine-induced stereotyped behaviour. Acta Pharmacol. Toxicol. (1968) 26 384-394.

Arnt J. and Scheel-Krüger J. Behavioural differences induced by muscimol selectively injected into pars compacta and pars reticulata of substantia nigra. Naunyn-Schmiedebergs Arch. Pharmacol. (1979) 310 43-51.

Arnt J. and Scheel-Krüger J. Intranigral GABA antagonists produce dopamine-independent biting in rats. Eur. J. Pharmacol. (1980) 62 51-61.

Bak I.J. Electron microscopic observations in the substantia nigra

of the mouse during reserpine administration. Experientia (1965) 21 568-570.

Bak I.J. The ultrastructure of the substantia nigra and caudate nucleus of the mouse and the cellular localisation of catecholamines. Exp. Brain Res. (1967) 3 40-57.

Bak I.J., Choi W.B., Hassler R., Usnoff K.G. and Wagner A. Fine structural synaptic organisation of the corpus striatum and substantia nigra in Rat and Cat. In Advances in Neurology, 9 D.E.Calne, T.N.Chase and A. Barbeau, eds., Raven Press N.Y. (1975).

Bartholini G. Interaction of striatal dopaminergic, cholinergic and GABA-ergic neurons: relation to extra-pyramidal function. Trends in Pharmacol. Sci. (1980) 1 (January) 138-141.

Baruk H. Précis de Psychiatrie. Masson, Paris (1950).

Ben-Ari Y., Pradelles P., Gros C. and Dray F. Identification of authentic substance P in striatonigral and amygdaloid nucleii using combined high performance liquid chromatography and radioimmunoassay. Brain Res. (1979) 173 360-363.

Bentall A.C.C. and Herberg L.J. Blockade of amphetamine-induced locomotor activity and stereotypy in rats by spiroperidol but not by an atypical neuroleptic, thioridazine. Neuropharmacol. (1980) 19 699-703.

Bentivoglio M., Van der Kooy D. and Kuypers H.G.J.M. The organisation of the efferent projections of the substantia nigra in the rat. A retrograde fluorescent double labelling study. Brain Res. (1979) 174 1-17.

Bergmann F., Chaimovitz M., Pasternak (Na'Or) V. and Ramu A. Compulsive gnawing in rats after implantation of drugs into the ventral

thalamus. A contribution to the mechanism of morphine action. Br. J. Pharmacol.(1974)51 197-205.

Bjorklund A. and Lindvall O. Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. Brain Res. (1975) 83 531-537.

Bleuler E. Textbook of Psychiatry. Macmillan N.Y. (1924).

Blocq P. and Marinesco G. Sur un cas de tremblement parkinsonien hemiplegique symptomatique d'une tumeur du pedoncult cerebral. C.R. Soc. Biol. (Paris) (1893) 5 105-111.

Bloom F.E. and Aghajanian G.K. An electron microscopic analysis of large granular vesicles of the brain in relation to monoamine content. J. Pharmacol. Exp. Therap.(1968) 159 261-273.

Bloom F.E., Costa E. and Salmoiraghi G.C. Anesthesia and the responsiveness of individual neurons of the caudate nucleus of the cat to acetylcholine, norepinephrine and dopamine administered by microelectrophoresis. J. Pharmacol. Exp. Therap. (1965) 150 244-252.

Bloom F.E. and Giarman N.J. Physiologic and pharmacologic considerations of biogenic amines in the nervous system. Ann. Rev. Pharmacol. (1968) 8 229-258.

Bloom F.E. Segal D.S., Ling N. and Guillemin R. Endorphins: profound behavioural effects in rats suggest new etiological factors in mental illness. Science (1976) 194 630-632.

Blundell J.E. and Latham C.J. Pharmacological manipulation of feeding behaviour: possible influences of serotonin and dopamine on food intake. In "Central Mechanisms of Anorectic Drugs" ed. S. Garattini and R. Samanin. Raven Press N.Y. (1978) 83-109.

Blundell J.E., Latham C.J. and Leshem M.B. Differences between the anorexic actions of amphetamine and fenfluramine - possible effects on hunger and satiety. J. Pharm. Pharmacol. (1976) 28 471-477.

Booth D.A. Amphetamine anorexia by direct action on the adrenergic feeding system of rat hypothalamus. Nature (1968) 217 869-870.

Brand S. A comparison of the distribution of acetylcholinesterase and muscarinic cholinergic receptors in the feline neostriatum. Neurosci. Letts. (1980) 17 113-117.

Brissaud E. Lecons sur les maladies nerveuses (Salpetriere, 1893-1894) recueillies et publiees par Henri Meige. Paris: Masson et Cie (1895).

Brook C. and Iversen S.D. Changed eating and locomotor behaviour in the rat after 6-hydroxydopamine lesions to the substantia nigra. Neuropharmacol. (1975) 14 95-105.

Brown L.L., Makman M.H., Wolfson L.I., Dvorkin B., Warner C. and Katzman R. A direct role of dopamine in the rat subthalamic nucleus and adjacent intrapeduncular area. Science (1979) 206 1416-1418.

Brown L.L. and Wolfson L.I. Apomorphine increases glucose utilisation in the substantia nigra, subthalamic nucleus and corpus striatum of rat. Brain Res. (1978) 140 188-193.

Bunney B.S. and Aghajanian G.K. The precise localisation of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res. (1976) 117 423-435.

Bunney B.S., Walters J.R., Roth R.H. and Aghajanian G.K. Dopaminergic neurons: effect of anti-psychotic drugs and amphetamine on single cell activity. J. Pharmacol. Exp.Therap.

(1973) 185 560-571.

Butcher L.L. and Bilezikjian L. Acetylcholinesterase-containing neurons in the neostriatum and substantia nigra revealed after punctate intracerebral injection of di-isopropyl-fluorophosphate. Eur. J. Pharmacol.(1975) 34 115-125.

Butcher L.L. and Hodge G.K. Selective bilateral lesions of pars compacta of the substantia nigra: effects on motor processes and ingestive behaviours.In Advances in Neurology (1979) 24 "The Extrapyramidal System and its Disorders". Eds. L.J. Poirier, T.L. Sourkes and P.J. Bedard. Raven Press N.Y..

Butcher L.L. and Marchand R. Dopamine neurons in pars compacta of the substantia nigra contain acetylcholinesterase: histochemical correlations on the same brain section. Eur. J. Pharmacol. (1978) 52 415-417.

Butcher L.L. and Talbot K. Acetylcholinesterase in rat nigro-neostrital neurons: experimental verification and evidence for cholinergic - dopaminergic interactions in the substantia nigra and caudate putamen complex.In "Cholinergic-Monoaminergic Interactions in the Brain" ed. L.L. Butcher. Academic Press (1978) 25-95.

Butcher L.L. and Talbot K. Chemical communication processes involving neurons: vocabulary and syntax. In "Cholinergic-Monoaminergic Interactions in the Brain" ed. L.L. Butcher. Academic Press (1978b) 3-22.

Butcher L.L., Talbot K. and Bilezikjian L. Acetylcholinesterase neurons in dopamine-containing regions of the brain. J. Neural. Transm. (1975) 37 127-153.

Butcher S.H. and Butcher L.L. Origin and modulation of acetylcholine activity in the neostriatum. Brain Res. (1974a) 71 167-171.

Butcher S.H. and Butcher L.L. Acetylcholine and choline levels in the rat corpus striatum after microwave irradiation. Proc. West. Pharmacol. Soc. (1974b) 17 37-39.

Butcher S.H., Butcher L.L. and Cho A.K. Modulation of neostriatal acetylcholine in the rat by dopamine and 5-hydroxytryptamine afferents. Life Sciences (1976) 18 733-744.

Cajal S.R. Textura del sistema nervioso del hombre y de los vertebrados Tomo II. Nicholas Moya, Madrid (1904) pp. 561-564.

Cajal S.R. Histologie du systeme nerveux de l'homme et des vertebres CSIC Madrid (1955) II 275-278.

Carey R.J. and Goodall E.B. Attenuation of amphetamine anorexia by unilateral nigro-striatal lesions. Neuropharmacol. (1975) 14 827-834.

Carlsson A. Receptor mediated control of dopamine metabolism. In "Pre- and Post-Synaptic Receptors" ed. E. Usdin and W.E. Bunney Jr., Marcel Dekker N.Y. (1975) 49-65.

Carlsson A., Falck B. and Hillarp N-Å. Histochemical localisation at the cellular level of hypothalamic noradrenaline. Acta Physiol. Scand. (1962a) 54 385-386.

Carlsson A., Falck B. and Hillarp N-Å. Celluar localisation of brain monoamines. Acta Physiol. Scand. (1962b) 56 suppl. 196, 1-28.

Carlsson A. and Lindqvist M. Effect of chlorpromazine on haloperidol in the formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol. Toxicol. (1963) 20 140-144.

Carpenter M.B. and Peter P. Nigro-striatal and nigro-thalamic fibres in the rhesus monkey. J. Comp. Neurol. (1972) 144 93-115.

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

Carter C.J. and Pycock C.J. Potentiation of haloperidol-induced catalepsy by dopamine agonists: possible involvement of central 5-hydroxytryptamine. Pharmacol. Biochem. Behav.(1979) 10 475-480.

Cheney D.L., Lefevre H.F. and Racagni G. Choline acetyltransferase activity and mass fragmentographic measurement of acetylcholine in specific nucleii and tracts of rat brain. Neuropharmacol. (1975) 14 801-809.

Cheramy A., Nieoullon A. and Glowinski J. Inhibition of dopamine release in the cat caudate nucleus by nigral application of glycine. Eur. J. Pharmacol.(1978) 47 141-147.

Cheramy A., Nieoullon A., Michelot R. and Glowinski J. Effects of intranigral application of dopamine and substance P on the in vivo release of newly synthesised (^{3}H)-dopamine in the ipsilateral caudate nucleus of the cat. Neurosci. Letts. (1977) 4 105-109.

Chiodo L.A., Antelman S.M., Caggiula A.R. and Lineberry C.G. Sensory stimuli alter the discharge rate of dopamine neurons: evidence for two functional types of DA cells in the substantia nigra. Brain Res. (1980) 189 544-549.

Chiodo L.A., Caggiula A.R., Antelman S.M. and Lineberry C.G. Reciprocal influences of activating and immobilizing stimulii on the activity of nigro-striatal dopamine neurones. Brain Res. (1979) 176 385-390.

Chiueh C.C. and Moore K.E. Relative potencies of d- and l-amphetamine in the release of Dopamine from cat brain _in vivo_. Res. Comms. Chem. Pathol. Pharmacol.(1974) _7_ 189-199.

Clavier R.M., Atmadja S. and Fibiger H.C. Nigrothalamic projections in the rat as demonstrated by orthograde and retrograde tracing techniques. Brain Res. Bull. (1976) _1_ 379-384.

Conrad L.C.A., Leonard C.M. and Pfaff D.W. Connections of the median and dorsal raphe nucleii in the rat: an autoradiographic and degeneration study. J. Comp. Neurol. (1974) _156_ 179-206.

Consolo S., Ladinsky H. and Bianchi S. Decrease in rat striatal acetylcholine levels by some direct- and indirect-acting dopaminergic antagonists. Eur. J. Pharmacol.(1975) _33_ 345-351.

Consolo S., Ladinsky H. and Garattini S. Effect of several dopaminergic drugs and trihexyphenidyl on cholinergic parameters in the rat striatum. J. Pharm. Pharmacol.(1974) _26_ 275-277.

Cools A.R. The function of dopamine and its antagonism in the caudate nucleus of cats in relation to the stereotyped behaviour. Arch. Int. Pharmacodyn. Ther. (1971) _194_ 259-269.

Cools A.R., Janssen H-J., Struyker Boudier H.A.J. and Van Rossum J.M. Interaction between antipsychotic drugs and catecholamine receptors. In Wenner-Gren Center International Symposium Series (1975) _5_ 73-87. Pergamon Press, Oxford.

Cools A.R. and Van Rossum J.M. Caudal dopamine and stereotyped behaviour of cats. Archs. Int. Pharmacodyn. Ther. (1970) _187_ 163-173.

Cools A.R. and Van Rossum J.M. Excitation-mediating and inhibition-mediating dopamine receptors: a new concept towards a better understanding of electrophysiological, biochemical, pharmacological

functional and clinical data.
Psychopharmacol.(1976) 45 243-254.

Costa E., Gropetti A. and Naimzada M.K. Effects of amphetamine on the turnover rate of brain catecholamines and motor activity. Br. J. Pharmacol.(1972) 44 742-751.

Costall B., Marsden C.D., Naylor R.J. and Pycock C.J. The relationship between striatal and mesolimbic dopamine dysfunction and the nature of the circling responses following 6-hydroxydopamine and electrolytic lesions of the ascending dopamine systems of rat brain. Brain Res. (1976) 118 87-113.

Costall B. and Naylor R.J. Specific asymmetric behaviour induced by the direct chemical stimulation of neostriatal dopaminergic mechanisms. Naunyn-Schmiedebergs' Arch. Pharmacol.(1974) 285 83-91.

Costall B., Naylor R.J. and Olley J.E. Catalepsy and circling behaviour after intracerebral injections of neuroleptic, cholinergic and anti-cholinergic agents into the caudate-putamen, globus pallidus and substantia nigra of rat brain. Neuropharmacol. (1972a) 11 645-663.

Costall B., Naylor R.J. and Olley J.E. Stereotypic and anti-cataleptic activities of amphetamine after intracerebral injections. Eur. J. Pharmacol. (1972b) 18 83-94.

Cragg B.G. The density of synapses and neurons in normal, mentally defective and ageing human brains. Brain (1975) 98 81-90.

Creese I.and Iversen S.D. The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Res. (1975) 83 419-436.

* Crossman A.R. and Sambrook M.A. The neurological basis of motor assymetry

* Cross, A.J. and Waddington, J.L. ^{3}H Quinuclidinyl benzylate and ^{3}H GABA receptor binding in rat substantia nigra after 6-hydroxydopamine lesions. Neurosci. Letts. (1980) 17 271-275.

following unilateral nigro-striatal lesions in the rat: the effect of secondary superior colliculus lesions. Brain Res. (1978) 159 211-213.

Dahlström A. and Fuxe K. Evidence for the existence of monoamine containing neurones in the central nervous system. Acta Physiol. Scand. (1964) 62 suppl. 232.

Dahlström A. and Fuxe K. Evidence for the existence of monoamine containing neurons in the central nervous system. 1. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiol. Scand. (1965) 62 suppl. 232 1-55.

Davidson N. Neurotransmitter Amino Acids Academic Press (London) (1976).

Dean P., Redgrave P., Souki W. and Lewis G. Behavioural effects of microinjections of picrotoxin into rat superior colliculus and the modulation by intranigral 6OHDA. Br.J. Pharmacol.(1980) in press.

De Belleroche J. and Bradford H.F. Biochemical evidence for the presence of pre-synaptic receptors on dopaminergic nerve terminals. Brain Res. (1978) 142 53-68.

Decsi L., Nagy J. and Zambo K. Stereotyped behaviour after cholinergic but not dopaminergic stimulation of the substantia nigra in rats. Life Sciences(1978) 22 1873-1878.

Decsi L.,Zambo K., Nagy J. and Gacs E. Atropine stereotypy and its inhibition by intra-caudate triperidol in the rat. Pol. J. Pharmacol. Pharm. (1979) 31 277-281.

De Montis G.M., Olianas, M.C., Serra G., Tagliamonte A. and Scheel-Krüger J. Evidence that a nigral GABA-ergic - cholinergic balance controls posture. Eur. J. Pharmacol. (1979) 53 181-190.

Deniau J.M., Chevalier G. and Feger J. Electrophysiological study of the nigro-tectal pathway in the rat. Neurosci. Letts. (1978a) 10 215-220.

Deniau J.M., Hammond C., Riszk A. and Feger J. Electrophysiological properties of identified output neurons of the rat substantia nigra (Pars Compacta and Pars Reticulata): Evidence for the existence of branched neurons. Exp. Brain Res. (1978b) 32 409-422.

Dews P.B. Studies on behaviour: iv. Stimulant actions of methamphetamine. J. Pharmacol. Exp. Therap.(1958) 122 137-147.

Di Chiara G., Morelli M., Porceddu M.L. and Gessa G.L. Role of thalamic γ-aminobutyrate in motor functions: catalepsy and ipsiversive turning after intrathalamic muscimol. Neuroscience (1979b) 4 1453-1465.

DiChiara G., Olianas M., Del Fiacco M., Spano P.F. and Tagliamonte A. Intranigral kainic acid is evidence that nigral non-dopaminergic neurones control posture. Nature (1977) 268 743-745.

DiChiara G., Porceddu M.L., Morelli M., Mulas M.L. and Gessa G.L. Evidence for a GABAergic projection from the substantia nigra to the ventromedial thalamus and to the superior colliculus of the rat. Brain Res. (1979a) 176 273-284.

DiChiara G., Porceddu M.L., Morelli M., Mulas M.L. and Gessa G.L. Striato-nigral and nigro-thalamic GABA-ergic neurons as output pathways for striatal responses. In "GABA neurotransmitters, Pharmacochemical Biochemical and Pharmacological Aspects" (eds) P. Krogsgaard-Larson, J. Scheel-Krüger and H. Kofod. (1979c) 465-481. Alfred Benzon Symposium, 12, Munksgaard, Copenhagen.

Dill R.E., Nickey W.M. and Little M.D. Dyskinesia in rats following chemical stimulation of the neostriatum. Tex. Rep. Biol.

Med. (1968) 26 101-106.

Dobrzanski S. and Doggett N.S. The effects of (+) amphetamine and fenfluramine on feeding in starved and satiated mice. Psychopharmacol. (1976) 48 283-286.

Dray A. and Straughn D.W. Synaptic mechanisms in the substantia nigra. J. Pharm. Pharmacol.(1976) 28 400-405.

Ehringer H. and Hornykiewicz O. Verteilung von noradrenalin und dopamin (3-hydroxytyramin) im gehirn der menschen und ihr verbalten bei erkrankunger des extrapyramidalen systems. Klin. Wochenschr (1960) 38 1236-1239.

Eichler A.J. and Antelman S.M. Apomorphine: feeding or anorexia depending on internal state. Comms. Psychopharmacol.(1977) 1 533-540.

Epstein A.N. and Teitelbaum P. Severe and persistent deficits in thirst produced by lateral hypothalamic damage. In "Thirst in the Regulation of Body Water" ed M.J. Wayner. Macmillan N.Y. (1964).

Epstein A.N. and Teitelbaum P. Specific loss of the hypoglycemic control of feeding in recovered lateral rats. Am. J. Physiol. (1967) 213 1159-1167.

Ernst A.M. and Smelik P.G. Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. Experientia (1966) XXII 837-838.

Falck B. and Hillarp N-Å. On the cellular localisation of catecholamines in the brain. Acta Anat. (1959) 38 277-279.

Fallon J.H., Riley J.N. and Moore R.Y. Substantia nigra dopamine neurons: separate populations project to neostriatum and allocortex. Neurosci. Letts. (1978) 7 157-162.

Faull R.L.M. and Carman J.B. Ascending projections of the substantia

nigra in the rat. J. Comp. Neurol. (1968) 132 73-92.

Faull R.L.M. and Mehler W.R. The cells of origin of nigrotectal, nigrothalamic and nigro-striatal projections in the rat. Neuroscience (1978) 3 989-1002.

Feeney D.M. and Wier C.S. Sensory neglect after lesions of substantia nigra or lateral hypothalamus: differential severity and recovery of function. Brain Res. (1979) 178 329-346.

Ferrier D. Experimental researches in cerebral physiology and pathology. West Riding Lunatic Asylum Med.Rep. (1873), ed. J. Crichton-Browne. Vol. III pp. 30-96.

Fibiger H.C. and Miller J.J. An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. Neuroscience (1977) 2 975-987.

Fleisher L.N. and Glick S.D. A telencephalic lesion site for d-amphetamine-induced contralateral rotation in rats. Brain Res. (1975) 96 413-417.

Florey E. Comparative physiology: transmitter substances. Ann. Rev. Physiol. (1961) 23 501-528.

Fog R., Randrup A. and Pakkenberg H. Lesions in corpus striatum and cortex of rat brains and the effect on pharmacologically induced stereotyped aggressive and cataleptic behaviour. Psychopharmacol (1970) 18 346-356.

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

Franklin K.B.J. and Herberg L.J. Amphetamine induces anorexia even after inhibition of noradrenaline synthesis. Neuropharmacol. (1976) 15 1-2.

Fray P.J., Sahakian B.J., Robbins T.W., Koob G.F. and Iversen, S.D. An observational method for quantifying the behavioural effects of dopamine agonists: contrasting effects of d-amphetamine and apomorphine. Psychopharmacol.(1980) 69 253-259.

Fuxe K. Evidence for the existence of monoamine neurons in the central nervous system. iv. Distribution of monoamine nerve terminals in the central nervous system. Acta Physiol. Scand. (1965) 64 suppl. 247.

Gale K., Costa E., Toffano G., Hong J-S. and Guidotti A. Evidence for a role of nigral GABA and substance P in the haloperidol-induced activation of striatal tyrosine hydroxylase. J. Pharmacol. Exp. Therap.(1978) 206 29-37.

Gale K., Guidotti A. and Costa E. Dopamine-sensitive adenylate cyclase: location in substantia nigra. Science (1977) 195 503-505.

Gale K., Moroni F., Kumakura K. and Guidotti A. Opiate receptors in substantia nigra: role in the regulation of striatal tyrosine hydroxylase activity. Neuropharmacol. (1979) 18 427-430.

Geffen L.B., Jessel T.M., Cuello A.C. and Iversen L.L. Release of dopamine from dendrites in rat substantia nigra. Nature (1976) 260 258-260.

Geyer M.A., Puerto A., Dawsey W.J., Knapp, S., Bollard, W.S. and Mandell A.J. Histologic and enzymatic studies of the mesolimbic and mesostriatal serotonergic pathways. Brain Res. (1976) 106 241-256.

Gillilan L.A. The nuclear pattern of non-tectal portions of the midbrain and isthmus in rodents. J. Comp. Neurol. (1943) 78 213-251.

Giorguieff M.F., Le Floc'h M.L., Glowinski J. and Besson M.J. Involvement of cholinergic presynaptic receptors of nicotinic and muscarinic types in the control of the spontaneous release of dopamine from striatal dopaminergic terminals in the rat. J. Pharmacol. Exp. Therap. (1977) 200 535-544.

Giorguieff-Chesselet M.F., Kemel M.L., and Glowinski J. The pre-synaptic stimulating effect of acetylcholine on dopamine release is suppressed during activation of nigro-striatal dopaminergic neurons in the cat. Neurosci. Letts. (1979) 14 177-182.

Glick S.D., Jerussi T.P., Waters D.H. and Green J.P. Amphetamine induced changes in striatal dopamine and acetylcholine levels and relationship to rotation (circling behaviour) in rats. Biochem. Pharmacol. (1974) 23 3223-3225.

Goldman P.S. and Nauta W.J.H. An intricately patterned prefronto-caudate projection in the rhesus monkey. J. Comp. Neurol. (1977) 171 369-385.

Gray E.G. Axo-somatic and axo-dendritic synapses of the cerebral cortex: An electron microscope study. J.Anat. (Lond.) (1959) 93 420-433.

Greenfield S., Cheramy A., Leviel V. and Glowinski J. In vivo release of acetylcholinesterase in cat substantia nigra and caudate nucleus. Nature (1980) 284 355-357.

Groppetti A., Parenti M., Cattabeni F., Maggi A., De Angelis L., Monduzzi M. and Racagni G.* In "Dopamine" (Adv. in Biochem. Psychopharm. 19) ed. P.J.Roberts, G.N. Woodruff and L.L. Iversen. Raven Press N.Y. (1978) 363-372.

Grossman S.P., Dacey D., Halaris A.E., Collier T. and Routtenberg, A. Aphagia and adipsia after preferential destruction of nerve cell bodies in hypothalamus. Science (1978) 202 537-539.

* Dopaminergic receptor activity in substantia nigra in the mechanisms of autoregulation of striatal dopamine release

Groves P.M., Rebec G.V. and Harvey J.A. Alteration of the effects of (+) amphetamine on neuronal activity in the striatum following lesions of the nigro-striatal bundle. Neuropharmacol. (1975b) 14 369-376.

Groves P.M. Rebec G.V. and Segal D.A. The action of d-amphetamine on spontaneous activity in the caudate nucleus and reticular formation of the rat. J. Behav. Biol. (1974) 11 33-47.

Groves P.M., Wilson C.J., Young S.J. and Rebec G.V. Self-inhibition by dopaminergic neurons. Science (1975a) 190 522-529.

Gulley R.L. and Smithberg M. Synapses in the rat substantia nigra. Tissue and Cell (1971) 3 691-700.

Gulley R.L. and Wood R.L. The fine structure of the neurons in the rat substantia nigra. Tissue and Cell (1971) 3 675-690.

Guyenet R.G., Agid Y., Javoy F., Beaujouan J.C., Rossier J. and Glowinski J. Effects of Dopaminergic receptor agonists and antagonists on the activity of the neo-striatal cholinergic system. Brain Res. (1975) 84 227-244.

Guyenet P.G. and Aghajanian G.K. Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra. Brain Res. (1978) 150 69-84.

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

Hanaway J., McConnell J.A. and Netsky M.G. Cytoarchitecture of the substantia nigra in the rat. Amer. J.Anat. (1970) 129 417-438.

Hansen E.L. and McKenzie G.M. Dexamphetamine increases striatal neuronal firing in freely moving rats. Neuropharmacol.

(1979) 18 547-552.

Hassler R. Pathologie der paralysis agitans und des postencephalitischen Parkinsonismus. J. Psychol. Neurol. (Lpz) (1938) 48 387-476.

Hassler R. The pathological and pathophysiological basis of tremor and Parkinsonism. Proc. 2 Internat. Congr. Neuropathol., London (1955) vol. I 29-48.

Hattori T., Fibiger H.C. and McGeer P.L. Demonstration of a pallidonigral projection innervating dopaminergic neurons. J. Comp. Neurol. (1975) 162 487-504.

Hattori T., Singh V.K. and McGeer P.L. Immunohistochemical localisation of choline acetyl-transferase containing neostriatal neurons and their relationship with dopaminergic synapses. Brain Res. (1976) 102 164-173.

Hebb D.O. Drives and the CNS (Conceptual Nervous System). Psychol. Rev. (1955) 62 243-254.

Hedreen J.C. Separate demonstration of dopaminergic and non-dopaminergic projections of substantia nigra in the rat. Anat. Record (1971) 169 338.

Heffner T.G., Hartman, J.A. and Seiden L.S. Feeding increases Dopamine metabolism in the rat brain. Science (1980) 208 1168-1170.

Heffner T.G. and Seiden L.S. Synthesis of catecholamines from ^{3}H tyrosine in brain during the performance of operant behaviour. Brain Res. (1980) 183 403-419.

Heffner T.G., Zigmond M.J. and Stricker E.M. Effects of dopaminergic agonists and antagonists on feeding in intact and 6-hydroxydopamine-treated animals. J. Pharmacol. Exp. Therap. (1977) 201 386-399.

Hefti F. and Lichtensteiger W. Dendritic dopamine: studies on the release of endogenous dopamine from subcellular particles derived from dendrites of rat nigro-striatal neurons. Neurosci. Letts. (1978a) 10 65-70.

Hefti F. and Lichtensteiger W. Subcellular distribution of dopamine in substantia nigra of the rat brain: effects of γ-butyro-lactone and destruction of noradrenergic afferents suggest formation of particles from dendrites. J. Neurochem. (1978b) 30 1217-1230.

Herz A., Albus K., Metys J., Schubert P. and Teschemacher H.J. On the central sites for the antinociceptive action of morphine and fentanyl. Neuropharmacol. (1970) 9 539-551.

Hökfelt T. and Ungerstedt U. Electron and fluorescence microscopical studies on the nucleus caudatus putamen of the rat after unilateral lesions of ascending nigro-neostriatal dopamine neurons. Acta Physiol. Scand. (1969) 76 415-426.

Holtzman S.G. Behavioural effects of separate and combined administration of naloxone and d-amphetamine. J. Pharmacol. Exp. Therap. (1974) 189 51-60.

Hong J.S., Yang H-T.Y., Racagni G. and Costa E. Projections of substance P containing neurones from neostriatum to substantia nigra. Brain Res. (1977) 122 541-544.

Hornykiewicz O. Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Revs. (1966) 18 925-962.

Hubel D.H. The Brain. Scientific American (1979) 241 39-47.

Huff R., Adams R.N. and Rutledge C.O. Amphetamine dose-dependent changes of in vivo electrochemical signals in rat caudate. Brain Res. (1979) 173 369-372.

Iwamoto E.T. and Way E.L. Circling behaviour and stereotypy induced by intra-nigral opiate microinjections. J. Pharmacol. Exp. Therap. (1977) 203 247-359.

Jacobowitz D.M. and Goldberg A.M. Determination of acetylcholine in discrete regions of the rat brain. Brain Res. (1977) 122 575-577.

Jacobowitz D.M. and Palkovits M. Topographic atlas of catecholamine and Acetylcholinesterase-containing neurons in the Rat Brain. I. Forebrain (Telencephalon, Diencephalon). J.Comp. Neurol. (1974) 157 13-28.

James T.A. and Collingridge G.L. Rapid behavioural and biochemical effects of tetanus toxin microinjected into the substantia nigra: a dual role for GABA? Neurosci. Letts. (1979) 11 205-208.

James T.A. and Massey S. Evidence for a possible dopaminergic link in the action of acetylcholine in the rat substantia nigra. Neuropharmacol. (1978) 17 687-690.

Javoy F., Agid Y., Bouvet D. and Glowinski J. Changes in neostriatal DA metabolism after carbachol or atropine microinjections into the substantia nigra. Brain Res. (1974) 68 253-260.

Jeffrey P.L. and Austin L. Axoplasmic transport. Prog. Neurobiol. (1973) 2 205-255.

Jerussi T.P. and Glick S.D. Amphetamine-induced rotation in rats without lesions. Neuropharmacol. (1974) 13 283-286.

Jerussi T.P. and Glick S.D. Apomorphine-induced rotation in normal rats and interaction with unilateral caudate lesions. Psychopharmacol. (1975) 40 329-334.

Jerussi T.P. and Glick S.D. Drug-induced rotation in rats without lesions: behavioural and neurochemical indices of a normal

asymmetry in nigro-striatal function. Psychopharmacol. (1976) 47 249-260.

Jerussi T.P., Glick S.D. and Johnson C.L. Reciprocity of pre- and post-synaptic mechanisms involved in rotation as revealed by dopamine metabolism and adenylate cyclase stimulation. Brain Res. (1977) 129 385-388.

Jessell T.M. Inhibition of substance P release from the isolated rat substantia nigra by GABA. Br. J. Pharmacol. (1977) 59 486P.

Jones D.L. and Mogenson G.J. Oral motor performance following central dopamine receptor blockade. Eur. J. Pharmacol.(1979) 59 11-21.

Jones E.G., Coulter J.D., Burton H. and Porter R. Cells of origin and terminal distribution of cortico-striatal fibres arising in the sensory-motor cortex of monkeys. J.Comp. Neurol. (1977) 173 53-80.

Joyce E.M. On the nature and function of the mesotelencephalic dopamine neurone system. Unpublished Ph.D. Thesis, University of Cambridge (1980).

Jurna I. Striatal monoamines and reserpine and chlorpromazine rigidity. Pharmac. Ther. (1976) B2 113-128.

Kaiya H., Okinaga M., Namba M., Shoumura K. and Watanabe S. Ultra-structure of acetylcholinesterase containing striato-nigral neurons transporting HRP retrogradely. Neurosci. Letts. (1979) 14 7-11.

Kanazawa I., Emson P.C. and Cuello A.C. Evidence for the existence of substance P containing fibres in striato-nigral and pallido-nigral pathways in rat brain. Brain Res. (1977) 119 447-453.

Kanazawa I., Marhsall G.R. and Kelly J.S. Afferents to the rat substantia nigra studied with horseradish peroxidase, with

special reference to fibres from the subthalamic nucleus. Brain Res. (1976) 115 485-491.

Kebabian J.W. and Saavedra J.M. Dopamine-sensitive adenylate cyclase occurs in a region of substantia nigra containing dopaminergic dendrites. Science (1976) 193 683-685.

Kebabian J.W. and Calne D.B. Multiple receptors for Dopamine. Nature (1979) 277 93-96.

Keesey R.E., Boyle P.C. and Storlien L.H. Food intake and utilization in lateral hypothalamically lesioned rats. Physiol. Behav. (1978) 21 265-268.

Kelley A.E. and Iversen S.D. Behavioural response to bilateral injections of substance P into the substantia nigra of the rat. Brain Res. (1978) 158 474-478.

Kelly J., Alheid G.F., Newberg A. and Grossman S.P. GABA stimulation and blockade in the hypothalamus and midbrain: effects on feeding and locomotor activity. Pharmacol. Biochem. Behav. (1977) 7 537-541.

Kelly P.H. Unilateral 6-hydroxydopamine lesions of nigro-striatal or mesolimbic dopamine-containing terminals and the drug induced rotation of rats. Brain Res. (1975) 100 163-169.

Kelly P.H. and Moore K.E. Mesolimbic dopaminergic neurones in the rotational model of nigro-striatal function. Nature (1976) 263 695-696.

Kelly P.H., Seviour P.W. and Iversen S.D. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. (1975) 94 507-522.

Keren O. and Korczyn A.D. Asymmetry of turning behaviour in rats induced by amphetamine and apomorphine. Experientia (1978) 34 616.

Kissileff H.R. Food associated drinking in the rat. J. Comp. Physiol. Psychol. (1969) 67 284-300.

Kitai S.T., Kocsis J.D. and Sugimori M. Action of dopamine in the Caudate Nucleus. In "Cholinergic-Monoaminergic Interactions in the Brain". ed. L.L. Butcher. Academic Press (1978) 169-176.

Kitai S.T., Sugimori M. and Kocsis J.D. Excitatory nature of dopamine in the nigro-caudate pathway. Exp. Brain Res. (1976) 24 351-363.

Kizer J.S., Palkovits M. and Brownstein M.J. The projections of the A8, A9 and A10 dopaminergic cell bodies: evidence for a nigral-hypothalamic-median eminence dopaminergic pathway. Brain Res. (1976) 108 363-370.

Kobayashi R.M., Brownstein M., Saavedra J.M. and Palkovits M. Choline acetyltransferase content in discrete regions of the rat brain stem. J. Neurochem. (1975) 24 637-640.

Kobayashi R.M., Palkovitz M., Hruska R.E., Rothschild R. and Yamamura H.I. Regional distribution of muscarinic cholinergic receptors in rat brain. Brain Res. (1978) 154 13-23.

Koffer K.B., Berney S. and Hornykiewicz O. The role of the corpus striatum in neuroleptic- and narcotic-induced catalepsy. Eur. J. Pharmacol.(1978) 47 81-86.

Konig J.F.R. and Klippel R.A. The Rat Brain. William and Wilkins (Baltimore)(1963).

Koob G.F., Del Fiacco M. and Iversen S.D. Spontaneous and amphetamine-induced behaviour after bilateral injection of ethanolamine-O-sulphate into the substantia nigra. Brain Res. (1978) 146 313-323.

Koob G.F., Riley S.J., Smith S.C. and Robbins T.W. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and

olfactory tubercle on feeding, locomotor activity and amphetamine anorexia in the rat. J. Comp. Physiol. Psychol. (1978) 92 917-927.

Korczyn A.D. and Eshel Y. Dopaminergic and non-dopaminergic circling activity of mice. Neuroscience (1979) 4 1085-1088.

Kuczenski R., Segal D.S. and Mandel A.J. Regional and subcellular distribution and kinetic properties of rat brain choline acetyltransferase - some functional considerations. J. Neurochem. (1975) 24 39-45.

Kuhar M.J., Aghajanian G.K. and Roth R.H. 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. (1972) 44 165-176.

Kuhar M.J. and Yamamura H.I. Light autoradiographic localisation of cholinergic muscarinic receptors in rat brain by specific binding of a potent agonist. Nature (1975) 253 560-561.

Kuhar M.J. and Yamamura H.I. Localisation of cholinergic muscarinic receptors in rat brain by light microscopic radio autography. Brain Res.(1976) 110 229-243.

Kultas-Ilinsky K., Ilinsky I.A., Massopust L.C., Young P.A. and Smith K.R. Nigro-thalamic pathway in the cat demonstrated by autoradiography and electron microscopy. Exp. Brain Res. (1978) 33 481-492.

Kuypers H.G.J.M., Kievit J. and Groen-Klevant A.C. Retrograde axonal transport of horseradish peroxidase in rat's forebrain. Brain Res. (1974) 67 211-218.

Laatsch R.H. and Cowan W.M. Electron microscopic studies of the dentate gyrus of the rat. I. Normal structure with special

reference to synaptic organisation. J.Comp. Neurol. (1966) 128 359-397.

Lashley K.S. In search of the engram. Symposia of the Soc. for Exp. Biol. (1950) 4 454-482.

Lees A.J., Fernando J.C.R. and Curzon G. Serotonergic involvement in behavioural responses to amphetamine at high dosage. Neuropharmacol. (1979) 18 143-158.

Lehmann J. and Fibiger H.C. Acetylcholinesterase in the substantia nigra and caudate-putamen of the rat: properties and localisation in dopaminergic neurons. J. Neurochem. (1978) 30 615-624.

Lehmann J., Fibiger H.C. and Butcher L.L. The localisation of acetylcholinesterase in the corpus striatum and substantia nigra of the rat following kainic acid lesion of the corpus striatum: a biochemical and histochemical study. Neuroscience (1979) 4 217-255.

Leibowitz S.F. Amphetamine: possible site and mode of action for producing anorexia in the rat. Brain Res. (1975) 84 160-167.

Leviel V., Cheramy A. and Glowinski J. Role of the dendritic release of dopamine in the reciprocal control of the two nigro-striatal dopaminergic pathways. Nature (1979a) 280 236-239.

Leviel V., Cheramy A., Nieoullon A. and Glowinski J. Symmetric bilateral changes in dopamine release from the caudate nucleii of the cat induced by unilateral nigral application of glycine and GABA related compounds. Brain Res. (1979b) 175 259-270.

Lichtensteiger W., Felix D., Lienhart R. and Hefti F. A quantitative correlation between single unit activity and fluorescence intensity of dopamine neurones in zona compacta of substantia nigra, as demonstrated under the influence of nicotine and physostigmine. Brain Res. (1976) 117 85-103.

Lindvall O. and Bjorklund A. The organisation of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiol. Scand. (1974) Suppl. 412, 1-48.

Lindvall O. and Bjorklund A. Anatomy of the dopaminergic neuron systems in the rat brain. In "Dopamine" (Adv. in Biochem. Psychopharmacol. vol.19) ed. P.J.Roberts, G.N. Woodruff and L.L. Iversen.Raven Press N.Y.(1978) 1-23.

Ljungberg T. and Ungerstedt U. Reinstatement of eating by dopamine agonists in aphagic dopamine denervated rats. Physiol. Behav. (1976) 16 277-283.

Ljungberg T. and Ungerstedt U. Classification of neuroleptic drugs according to their ability to inhibit apomorphine induced locomotion and gnawing: evidence for two different mechanisms of action. Psychopharmacol. (1978) 56 239-247.

Llinas R. Physiology and Pathology of Dendrites. Adv. in Neurology 12 ed. G.W. Kreutzberg. Raven Press N.Y. (1975).

Lyon M. and Robbins T.W. The action of central nervous system stimulant drugs: a general theory concerning amphetamine effects. In "Current Developments in Psychopharmacology" 2 ed.W.B. Essman and L. Valzelli. Spectrum Pub. Inc. (1975) 79-163.

Lyon M. and Randrup A. The dose-response effect of amphetamine upon avoidance behaviour in the rat seen as a function of increasing stereotypy. Psychopharmacol. (1972) 23 334-347.

Malone E.F. Uber de Kerneddes menschlichen Diencephalan. Abh. Konigl. Wiss. Berlin Phys-math.(1910).

Malone E.F. Recognition of members of the somatic motor chain of nerve cells by means of a fundamental type of cell structure,

and the distribution of such cells in certain regions of the mammalian brain. Anat. Rec. (1913) 7 67-82.

Marin-Padilla M. Cortical axo-spinodendritic synapses in man: a Golgi study. Brain Res. (1968) 8 196-200.

Marques D.M., Fisher A.E., Okrutny M.S. and Rowland N.E. Tail pinch induced fluid ingestion: interactions of taste and deprivation. Physiol. Behav. (1979) 22 37-41.

Marshall J.F. Somatosensory inattention after dopamine-depleting intracerebral 6-OHDA injections: spontaneous recovery and pharmacological control . Brain Res. (1979) 177 311-324.

Marshall J.F. and Berrios N. Movement disorders of aged rats: reversal by dopamine receptor stimulation. Science (1979) 206 477-479.

Marshall J.F., Levitan D. and Stricker E.M. Activation-induced restoration of sensorimotor functions in rats with dopamine depleting brain lesions. J. Comp. Physiol. Psychol. (1976) 90 536-546.

Marshall J.F., Richardson J.S. and Teitelbaum P. Nigro-striatal bundle damage and the lateral hypothalamic syndrome. J. Comp. Physiol. Psychol. (1974) 87 808-830.

Marshall J.F. and Teitelbaum P. Further analysis of sensory inattention following lateral hypothalamic damage in rats. J. Comp. Physiol. Psychol. (1974) 86 375-395.

Matsui Y. and Kamioka T. The effects of elevating γ amino butyrate in the substantia nigra on the behaviour of rats. Eur. J. Pharmacol. (1978) 50 243-251.

Matthews R.T. and Chiou C.Y. Effects of acute and chronic injections of carbachol in the rat caudate nucleus. Neuropharmacol. (1979) 18 291-294.

McDermott L.J., Alheid G.F., Kelly J., Halaris A.E. and Grossman S.P. Regulatory deficits after surgical transections of three components of the MFB: correlation with regional amine depletions. Pharmacol. Biochem. Behav. (1977) 6 397-407.

McGeer E.G., Fibiger H.C., McGeer P.L. and Brooke S. Temporal changes in amine synthesising enzymes of rat extrapyramidal structures after hemi-transections or 6-hydroxydopamine administration. Brain Res. (1973) 52 289-300.

McGeer E.G. and McGeer P.L. Some factors influencing the neurotoxicity of intrastriatal injections of kainic acid. Neurochem. Res. (1978) 3 501-517.

McGeer P.L., Fibiger H.C., Maler L., Hattori T. and McGeer E.G. Evidence for descending pallido-nigral GABA-containing neurons. Advances in Neurology (1974) 5 153-160.

McGeer P.L., McGeer E.G. and Campbell J.J.R. Rotatory effects of intracerebral tetanus toxin injections. Exp. Neurol. (1980) 67 363-367.

McGeer P.L., McGeer E.G. and Innanen V.T. Dendro-axonic transmission: Evidence from receptor binding of dopaminergic and cholinergic agents. Brain Res. (1979) 169 433-441.

McKenzie G.M., Gordon R.J. and Viik K. Some biochemical and behavioural correlates of a possible animal model of human hyperkinetic syndromes. Brain Res. (1972) 47 439-456.

McLennan H. and Hicks T.P. Pharmacological characterisation of the excitatory cholinergic receptors of rat central neurones. Neuropharmacol. (1978) 17 329-334.

Meibach R.C. and Katzman R. Catecholaminergic innervation of the subthalamic nucleus: evidence for a rostral continuation of the A9 (substantia nigra) dopaminergic cell group. Brain Res.

(1979) 173 364-368.

Meibach R.C. and Weaver L.M. Histochemical identification of acetylcholinesterase in dopaminergic nigrostriatal neurons. J. Neural Transm. (1979) 44 87-96.

Michelot R., Leviel V., Giorguieff-Chesselet M.F., Cheramy A. and Glowinski J. Effects of the unilateral nigral modulation of substance P transmission on the activity of the two nigrostriatal dopaminergic pathways. Life Sciences (1979) 24 715-724.

Miller N.E. Chemical coding of behaviour in the brain. Science (1965) 148 328-338.

Moleman P., Bruinvels J. and Westerink B.H.C. Rapid post-mortem changes in 3,4 dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite in rat striatum. J. Neurochem. (1977) 29 175-177.

Moore R.Y., Bhatnagar R.K. and Heller A. Anatomical and chemical studies of a nigro-striatal projection in the cat. Brain Res. (1971) 30 119-135.

Morelli M., Porceddu M.L. and Di Chiara G. Lesions of substantia nigra by kainic acid: effects on a morphine induced stereotyped behaviour. Brain Res. (1980) 191 67-78.

Morgan L.O. The Corpus Striatum. Arch. Neurol. Psychiat. (1927) 18 495-549.

Morley B.J., Lorden J.F., Brown G.B., Kemp G.E. and Bradley R.J. Regional distribution of nicotinic acetylcholine receptor in rat brain. Brain Res. (1977) 134 161-166.

Mroz E.A., Brownstein M.J. and Leeman S.E. Evidence for substance P in the striato-nigral tract. Brain Res. (1977) 125 305-311.

Mumford L., Teixeira A.R. and Kumar R. Sources of variation in locomotor activity and stereotypy in rats treated with d-amphetamine. Psychopharmacol. (1979) 62 241-245.

Myers R.D. General Laboratory Procedures. In "Methods in Psychobiology" vol. 1 ed. R.D. Myers. Academic Press, London and N.Y. (1971) p. 65.

Myers R.D. Handbook of drug and chemical stimulation of the Brain. Van Nostrand Reinhold Company (1974).

Myers R.D. and Hoch D.B. ^{14}C-Dopamine microinjected into the brain-stem of the rat: dispersion kinetics, site content and functional dose. Brain Res. Bull. (1978) 3 601-609.

Nagy J.I., Vincent S.R., Lehmann J., Fibiger H.C. and McGeer E.G. The use of kainic acid in the localisation of enzymes in the substantia nigra. Brain Res. (1978) 149 431-441.

Nauta W.J.H. Afferents to the rat caudato-putamen studied with horseradish peroxidase. An evaluation of a retrograde neuro-anatomical research method. Brain Res. (1974) 67 219-238.

Nauta W.J.H. and Mehler W.R. Fiber connections of the basal ganglia. In "Psychotropic drugs and dysfunctions of the Basal Ganglia. A Multidisciplinary Workshop" ed. G.E. Crane and R. Gardner Jr. (1969) 68-74.

Nauta W.J.H., Smith G.P., Faull, R.L.M. and Domesick V.B. Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience (1978) 3 385-401.

Neill D.B. Frontal-striatal control of behavioural inhibition in the rat. Brain Res. (1976) 105 89-103.

Neill D.B., Boggan W.O. and Grossman S.P. Behavioural effects of amphetamine in rats with lesions in the corpus striatum. J.Comp. Physiol. Psychol. (1974) 86 1019-1030.

Neill D.B. and Grossman S.P. Effects of intrastriatal injections of scopolamine on appetitive behaviour. Pharmacol. Biochem. Behav.

(1973) 1 313-318.

Neill D.B. and Herndon J.G. Jr. Anatomical specificity within rat neostriatum for the dopaminergic modulation of DRL responding and activity. Brain Res. (1978) 153 529-538.

Neill D.B. and Linn C.L. Deficits in consummatory responses to regulatory challenges following basal ganglia lesions in rats. Physiol. Behav (1975) 14 617-624.

Neill D.B., Parker S.D. and Gold M.S. Striatal dopaminergic modulation of lateral hypothalamic self-stimulation. Pharmacol. Biochem. Behav. (1975) 3 485-491.

Neill D.B., Peay L.A. and Gold M.S. Identification of a subregion within rat neostriatum for the dopaminergic modulation of lateral hypothalamic self stimulation. Brain Res. (1978) 153 515-528.

Neill D.B., Ross J.F. and Grossman S.P. Comparison of the effects of frontal, striatal and septal lesions in paradigms thought to measure incentive motivation or behavioural inhibition. Physiol. Behav. (1974b) 13 297-305.

Nieoullon A., Cheramy A. and Glowinski J. Nigral and striatal DA release under sensory stimuli. Nature (1977) 269 340-342.

Nieoullon A., Cheramy A. and Glowinski J. Role of Dopamine in substantia nigra in the regulation of nigro-striatal dopaminergic neuron activity. Adv. in Neurology 24 ed. L.J. Poirier, T.L. Sourkes and P.J. Bedard. Raven Press N.Y. (1979).

Norcross K. and Spehlmann R. Selective blockade of excitatory caudate responses to nigral stimulation by microiontophoretic application of dopamine antagonists. Neurosci. Letts. (1977) 6 323-328.

Norcross K. and Spehlmann R. A quantitative analysis of the excitatory and depressant effects of dopamine on the firing of caudatal neurons: electrophysiological support for the existence of two distinct dopamine-sensitive receptors. Brain Res. (1978) 156 168-174.

Norton S. Amphetamine as a model for hyperactivity in the rat. Physiol. Behav.(1973) 11 181-186.

Olianas M.C., De Montis G.M., Concu A., Tagliamonte A. and Di Chiara G. Intranigral kainic acid: evidence for nigral non-dopaminergic neurons controlling posture and behaviour in a manner opposite to the dopaminergic ones. Eur. J. Pharmacol. (1978a) 49 223-232.

Olianas M.C., De Montis G.M., Mulas G. and Tagliamonte A. The striatal dopaminergic function is mediated by the inhibition of a nigral, non-dopaminergic neuronal system via a strio-nigral GABA-ergic pathway. Eur. J. Pharmacol.(1978b) 49 233-241.

Olivier A., Parent A., Simard H. and Poirier L.J. Cholinesterasic striatopallidal and striatonigral efferents in the cat and the monkey. Brain Res. (1970) 18 273-282.

Oltmans G.A. and Harvey J.A. Lateral hypothalamic syndrome in rats: a comparison of the behavioural and neurochemical effects of lesions placed in the lateral hypothalamus and nigro-striatal bundle. J. Comp. Physiol. Psychol.(1976) 90 1051-1062.

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

Pasquier D.A., Kemper T.L., Forbes W.B. and Morgane P.J. Dorsal raphe, substantia nigra and locus coeruleus: interconnections

with each other and the neostriatum. Brain Res. Bull. (1977) 2 323-339.

Pechnick R., Janowsky D.S. and Judd L. Differential effects of methylphenidate and d-amphetamine on stereotyped behaviour in the rat. Psychopharmacol. (1979) 65 311-315.

Pellegrino L.J. and Cushman A.J. A stereotaxic atlas of the rat brain Appleton-Century-Crofts (1967).

Perry T.L., Berry K., Hansen S., Diamond S. and Mox C. Regional distribution of amino acids in human brain obtained at autopsy. J. Neurochem. (1971) 18 513-519.

Peters A. and Palay S.L. The morphology of laminae A and A_1 of the dorsal nucleus of the lateral geniculate body of the cat. J. Anat. (Lond.) (1966) 100 451-486.

Petrovicky P. Ascending pathways from the substantia nigra to the striatum. Folia Morphologica (1972) 20 156-158.

Phillips K.C. Cholinergic modification of amphetamine-induced stereotyped behaviour in rats. IRCS (1974) 2 p. 1517.

Phillipson O.T. and Horn A.S. Substantia nigra of the rat contains a dopamine sensitive adenylate cyclase. Nature (1976) 261 418-420.

Phillis J.W. The Pharmacology of Synapses. Pergamon Press (London) (1966).

Pijnenburg A.J.J., Honig W.M.M., Van Der Heyden J.A.M. and Van Rossum J.M. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur. J. Pharmacol. (1976) 35 45-58.

Pijnenburg A.J.J., Honig W.M.M. and Van Rossum J.M. Inhibition of d-amphetamine-induced locomotor activity by injection of

haloperidol into the nucleus accumbens of the rat. Psychopharmacol. (1975) 41 87-95.

Pijnenburg A.J.J. and Van Rossum J.M. Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. J. Pharm. Pharmacol. (1973) 25 1003-1005.

Pijnenburg A.J.J., Woodruff G.N. and Van Rossum J.M. Ergometrine induced locomotor activity following intracerebral injection into the nucleus accumbens. Brain Res. (1973) 59 289-302.

Pollard H., Llorens C., Schwartz J.C., Gros C. and Dray F. Localisation of opiate receptors and enkephalins in the rat striatum in relationship with the nigro-striatal dopaminergic system: lesion studies. Brain Res. (1978) 151 392-398.

Polz-Tejera G., Schmidt J. and Karten H.J. Autoradiographic localisation of α-bungarotoxin binding sites in the central nervous system. Nature (1975) 258 349-351.

Pope S.G. and Dean P. Hyperactivity, Aphagia and Motor disturbance following lesions of superior colliculus and underlying tegmentum in rats. Behav. Neural Biol. (1979) 27 433-453.

Powley J.L. and Keesey R.E. Relationship of body weight to the lateral hypothalamic feeding syndrome. J. Comp. Physiol. Psychol. (1970) 25-36.

Pycock C.J. Turning behaviour in animals. Neuroscience (1980) 5 461-514.

Racagni G., Cheney D.L., Trabucchi M and Costa E. In vivo actions of clozapine and haloperidol on the turnover rate of acetylcholine in rat striatum. J. Pharmacol. Exp. Therap. (1976) 196 323-332.

Randrup A. and Munkvad I. Behavioural stereotypies induced by pharmacological agents. Pharmakopsychiat. Neuro-psychopharmak. (1968) 1 18-26.

Reavill C., Leigh N., Jenner P. and Marsden C.D. Dopamine-mediated circling behaviour does not involve the nigro-tectal pathway. Exp. Brain Res. (1979) 37 309-316.

Rebec G.V. and Groves P.M. Apparent feedback from the caudate nucleus to the substantia nigra following amphetamine administration. Neuropharmacol.(1975) 14 275-282.

Redgrave P. A modified push-pull system for the localised perfusion of brain tissue. Pharmacol.Biochem. Behav. (1977) 6 471-474.

Redgrave P. and Taha E. Neuroleptic suppression of feeding and stereotyped behaviour induced by injections of carbachol into substantia nigra. Neurosci. Letts. (1980) in press.

Reubi J-C., Iversen L.L. and Jessell T.M. Dopamine selectively increases 3H GABA release from slices of rat substantia nigra *in vitro*. Nature (1977) 268 652-654.

Reubi J-C., Iversen L.L. and Jessell T.M. Regulation of GABA release by dopamine in the rat substantia nigra.In "Dopamine" (Adv. in Biochem. Psychopharm. 19) ed. P.J.Roberts, G.N. Woodruff and L.L. Iversen. Raven Press N.Y. (1978) 401-404.

Reubi J-C. and Sandri C. Ultrastructural observations on inter-cellular contacts of nigral dendrites. Neurosci. Letts. (1979) 13 183-188.

Rinvik E. and Grofova I. Observations on the fine structure of the substantia nigra in the cat. Exp. Brain Res. (1970) 11 229-248.

Rioch D. Studies on the diencephalon of carnivora. II.Certain nuclear configurations and fiber connections of the subthalamus and midbrain of the dog and cat. J. Comp. Neurol. (1929) 49 121-154.

Robbins T.W., Everitt B.J. and Sahakian B.J. Stress induced eating

in animals. In "The body weight regulatory system: Normal and Abnormal aspects". Symposium in Capri, October 1980. Ed. W. James, T. Van Itallie and L. Cioffi. Raven Press N.Y. (1980).

Robbins T.W. and Fray P.J. Stress-induced eating: fact, fiction or misunderstanding? Appetite (1980) 1 103-133.

Robbins T.W., Phillips A.G. and Sahakian B.J. Effects of chlordiazepoxide on tail-pinch induced eating in rats. Pharmacol. Biochem. Behav.(1977) 6 297-302.

Robbins T.W. and Sahakian B.J. "Paradoxical" effects of psychomotor stimulant drugs in hyperactive children from the stand-point of behavioural pharmacology. Neuropharmacol. (1979) 18 931-950.

Rolls E.T., Rolls B.J., Kelly P.H., Shaw S.G., Wood R.J. and Dale R. The relative attenuation of self-stimulation, eating and drinking produced by dopamine-receptor blockade. Psychopharmacol. (1974) 38 219-230.

Rose S.P. Can the neurosciences explain the mind? Trends in Neurosciences (1980) 3 I-IV.

Rosenzweig M.R., Krech D. and Bennett E.L. Brain chemistry and adaptive behaviour. In "Biological and Biochemical Bases of Behaviour" ed. H.F. Harlow and C.N. Woolsey. Madison Univ. Wisconsin Press (1958) 367-400.

Rowland N. Transection of fibers crossing the lateral border of the lateral hypothalamus: fragmented spontaneous ingestive patterns and reanalysis of "regulatory deficits" in rats with sagittal knife cuts. Physiol. Behav.(1979) 22 1061-1066.

Rowland N.E., Grossman S.P. and Grossman L. Zona Incerta lesions: regulatory drinking deficits to intravenous NaCl, Angiotensin, but not to salt in the food. Physiol. Behav.(1979) 23 745-750.

Rowland N. and Engle D.J. Feeding and drinking interactions after acute butyrophenone administration. Pharmacol. Biochem. Behav. (1977) 7 295-301.

Russek M., Mogenson G.J. and Stevenson J.A.F. Calorigenic, hyperglycemic and anorexigenic effects of adrenaline and noradrenaline. Physiol. Behav. (1967) 2 429-433.

Sacks O.W. Awakenings Pelican Books (Penguin, Harmondsworth) (1976)

Sahakian B.J., Growdon J.H., Millington W.R., Barr J.K. and Wurtman R.J. The effects of cholinergic agonists on apomorphine-induced stereotyped behaviour in the rat. Comms. Psychopharmacol. (1978) 2 255-262.

Sahakian B.J. and Robbins T.W. Isolation-rearing enhances tail pinch-induced oral behaviour in rats. Physiol. Behav. (1977) 18 53-58.

Salvaterra P.M., Mahler H.R. and Moore W.J. Subcellular and regional distribution of ^{125}I-labelled α bungarotoxin binding in rat brain and its relationship to acetylcholinesterase and choline acetyltransferase. J. Biol. Chem. (1975) 250 6469-6475.

Sanberg P.R. and Fibiger H.C. Body weight, feeding and drinking behaviours in rats with kainic acid-induced lesions of striatal neurons - with a note on body weight symptomatology in Huntington's disease. Exp. Neurol. (1979) 66 444-466.

Schiørring E. An open field study of stereotyped locomotor activity in amphetamine treated rats. Psychopharmacol. (1979) 66 281-287.

Scheel-Krüger J. Some aspects of mechanism of action of various stimulant amphetamine analogues. Psychiat. Neurol. Neurochem. (Amst.) (1972) 75 179-192.

Schwartz W.J. A role for the dopaminergic nigro-striatal bundle in the pathogenesis of altered brain glucose consumption after lateral hypothalamic lesions. Evidence using the ^{14}C-labelled deoxyglucose technique. Brain Res. (1978) 158 129-147.

Segal D., Bloom F.E., Ling N. and Guillemin R. β-endorphin: endogenous opiate or neuroleptic? Science (1977) 198 411-413.

Sethy V.H. Regulation of striatal acetylcholine concentration by D_2-dopamine receptors. Eur. J. Pharmacol.(1979) 60 397-398.

Sethy V.H. and Van Woert M.H. Regulation of striatal acetylcholine concentration by dopamine receptors. Nature (1974) 251 529.

Sherman K.A., Hanin I. and Zigmond M.J. The effect of neuroleptics on acetylcholine concentration and choline uptake in striatum: implications for regulation of acetylcholine metabolism. J. Pharmacol. Exp. Therap.(1978) 206 677-686.

Shimizu N. and Onishi S. Demonstration of nigro-neostriatal tract by degeneration silver method. Exp. Brain Res. (1973) 17 133-138.

Shumway G.S. and Lindholm E. Weight loss in rats following lateral hypothalamic or thalamic lesions. Physiol. Psychol. (1978) 6 325-331.

Shute C.C.D. and Lewis P.R. Cholinesterase-containing systems of the brain of the rat. Nature (1963) 199 1160-1164.

Shute C.C.D. and Lewis P.R. The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections. Brain (1967) 90 497-520.

Siegel S. Non-parametric statistics for the Behavioural Sciences. McGraw-Hill Kogakusha Ltd. (1956).

Simpson L.L. A study of the interaction between amphetamine and food deprivation. Psychopharmacol.(1974) 38 279-286.

Smelik P.G. and Ernst A.M. Role of nigro-neostriatal dopaminergic fibres in compulsive gnawing behaviour in rats. Life Sciences (1966) 5 1485-1488.

Soemmering S.T. Hirnlehre und Nervenlehre. Frankfurt: Yarrentrapp (1791).

Sokoloff L., Reivich M., Kennedy C., Des Rosiers M.H., Patlac C.S., Pettigrew K.D., Sakurada O. and Shinohara M. The (^{14}C) deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anaesthetised albino rat. J. Neurochem. (1977) 28 897-916.

Somogyi P., Hodgson A.J. and Smith A.D. An approach to tracing neuron 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 (1979) 4 1805-1852.

Sorenson C.A. and Ellison G.D. Striatal organisation of feeding behaviour in the decorticate rat. Exp. Neurol. (1970) 29 162-174.

Sotelo C. The fine structural localisation of norepinephrine-^{3}H in the substantia nigra and area postrema of the rat. J. Ultrastruct. Res. (1971) 36 824-841.

Spano P.F., Trabucchi M. and Di Chiara G. Localisation of nigral dopamine-sensitive adenylate cyclase on neurons originating from the corpus striatum. Science (1977) 196 1343-1345.

Speth R.C., Chen F.M., Lindstrom J.M., Kobayashi R.M. and Yamamura H.I. Nicotinic cholinergic receptors in rat brain identified by (^{125}I) Naja Naja siamensis α toxin binding. Brain Res. (1977) 131 350-355.

Starr M.S. Investigation of possible interactions between substance P and transmitter mechanisms in the substantia nigra and corpus striatum of the rat. J. Pharm. Pharmacol. (1978) 30 359-363.

Starr M.S. GABA mediated potentiation of amine release from nigro-striatal DA neurones in vitro. Eur. J. Pharmacol. (1979) 53 215-216.

Steg G.B. and Ungerstedt U. A method for mechanographical recording of muscle tone in the rat: the effect of some antiparkinsonian drugs on rigidity induced by reserpine. Brain Res. (1978) 140 177-181.

Stricker E.M., Drinking by rats after lateral hypothalamic lesions: a new look at the lateral hypothalamic syndrome. J.Comp. Physiol. Psychol. (1976) 90 127-143.

Stricker E.M., Swerdloff A.F. and Zigmond M.J. Intrahypothalamic injections of kainic acid produce feeding and drinking deficits in rats. Brain Res. (1978) 158 470-473.

Taber E. The cytoarchitecture of the brain stem of the cat. I. Brain stem nucleii of cat. J. Comp. Neurol. (1961) 116 27-69.

Taber Pierce E., Foote W.E. and Hobson J.A. The efferent connections of the nucleus raphe dorsalis. Brain Res. (1976) 107 137-144.

Teitelbaum P. and Epstein A.N. The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions. Psychol. Rev. (1962) 69 74-90.

Teitelbaum P. and Stellar E. Recovery from the failure to eat produced by hypothalamic lesions. Science (1965) 120 894-895.

Ternaux J.P., Hery F., Bourgoin S., Adrien J., Glowinski J. and Hamon M. The topographical distribution of serotonergic terminals in the neostriatum of the rat and the caudate

nucleus of the cat. Brain Res. (1977) 121 311-326.

Thiebot M-H. and Soubrie P. Amphetamine-induced circling behaviour in rats: effects of unilateral microinjections of GABA and GABA-related drugs into substantia nigra. Brain Res. (1979) 167 307-322.

Thornburg J.E. and Moore K.E. Supersensitivity to dopamine agonists following unilateral 6-hydroxydopamine-induced striatal lesions in mice. J. Pharmacol. Exp Therap.(1975) 192 42-49.

Tindall R.S.A., Kent M., Baskin F. and Rosenberg R.N. Nicotonic acetylcholine receptor of mammalian brain: naja toxin binding to subcellular fractions of rat brain. J. Neurochem. (1978) 30 859-863.

Tretiakoff C. Contribution a l'etude de l'anatomie pathologique du locus niger de Soemmering avec quelques deductions relatives a la pathogenie des troubles du tonus musculaire et de le maladie de Parkinson. These no. 293, Paris (1919).

Trabucchi M., Cheney D., Racagni G. and Costa E. Involvement of brain cholinergic mechanisms in the action of chlorpromazine Nature (1974) 249 664-666.

Trabucchi M., Cheney D.L. Racagni G. and Costa E. *In vivo* inhibition of striatal acetylcholine turnover by L-DOPA, apomorphine and (+)-amphetamine. Brain Res. (1975) 85 130-134.

Trulson M.E. and Jacobs B.L. Effects of d-amphetamine on striatal unit activity and behaviour in freely moving cats. Neuropharmacol. (1979) 18 735-738.

Ungerstedt U. 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system in the rat brain. Eur. J. Pharmacol. (1968) 5 107-110.

Ungerstedt U. Stereotaxic mapping of the Monoamine pathways in the rat brain. Acta Physiol. Scand. (1971a) 82 suppl. 367 1-48.

Ungerstedt U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. Acta Physiol. Scand. (1971b) 82 suppl. 367 49-68.

Ungerstedt U. Post-synaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system in the rat brain. Acta Physiol. Scand. (1971c) 82 suppl. 367 69-93.

Ungerstedt U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. (1971d) 82 suppl. 367 95-122.

Ungerstedt U. and Arbuthnott G.W. Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigro-striatal dopamine system. Brain Res. (1970) 24 485-493.

Valenstein E.S., Cox V.C. and Kakolewski J.W. Re-examination of the role of the hypothalamus in motivation. Psychol. Rev. (1970) 77 16-31.

Van der Kooy D. The organisation of the thalamic, nigral and raphe cells projecting to the medial vs. lateral caudate-putamen in rat. A fluorescent retrograde double labelling study. Brain Res. (1979) 169 381-387.

Vicq d'Azyr F. Traite d'anatomie et de physiologie. Paris (1786).

Vincent S.R., Hattori T. and McGeer E.G. The nigro-tectal projection: a biochemical and ultrastructural characterisation. Brain Res. (1978) 151 159-164.

Waldmeier P.C. and Delini-Stula A.A., Serotonin-dopamine interactions in the nigro-striatal system. Eur. J. Pharmacol. (1979) 55 363-373.

Waldmeier P.C., Kam R. and Stocklin K. Increased dopamine metabolism in rat striatum after infusions of substance P into substantia nigra. Brain Res. (1978) 159 223-227.

Wechsler L.R., Savaki H.E. and Sokoloff L. Effects of d- and l-amphetamine on local cerebral glucose utilisation in the conscious rat. J. Neurochem. (1979) 32 15-22.

Werman, R. A review - criteria for identification of a central nervous system transmitter. Comp. Biochem. Physiol. (1966) 18 745-766.

Werman R. CNS cellular level: membranes. Ann. Rev. Physiol. (1972) 34 337-374.

Wilson C.J., Groves P.M. and Fifkova E. Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra. Exp. Brain Res. (1977) 30 161-174.

Winn P. and Redgrave P. Feeding following microinjection of cholinergic substances into substantia nigra. Life Sciences (1979) 25 333-338.

Winocur G. Functional dissociation within the caudate nucleus of rats. J. Comp. Physiol. Psychol. (1974) 86 432-439.

Winterkorn J.M.S. and Meikle T.H., Jr. Lesions of the tecto-spinal tract of the cat do not produce compulsive circling. Brain Res. (1980) 190 597-600.

Wolfson L.I., Brown L., Makman M., Gardner E., Dvorkin B. and Katzman R. Evidence of dopamine receptors in subthalamic nucleus in rats. Proc. 6th Int. Meet. Int. Soc. Neurochem. (1977) p. 453.

Wolgin D.L. and Teitelbaum P. Role of activation and sensory stimuli in recovery from lateral hypothalamic damage in the cat. J. Comp. Physiol. Psychol. (1978) 92 474-500.

Wuerthele S.M., Friedle N.M. and Moore K.E. Effects of intra-nigral administration of dopamine agonists and antagonists and baclofen on concentrations of DOPAC and dopamine in the striatum and substantia nigra of the rat. J. Neural Transm., (1979) 45 117-127.

Yahr M.D. Overview of present day treatment of Parkinson's Disease. J. Neural Transm. (1978) 43 227-238.

Yang H-Y.T., Hong J-S., Fratta W. and Costa E. Rat brain enkephalins: distribution and biosynthesis. Adv. Biochem. Psychopharmacol. (1978) 18 149-159.

Zambo K., Decsi L. and Nagy J. Stereotypy after intracaudate injection of atropine in the rat. Neuropharmacol. (1979) 18 727-730.

Zigmond M.J. and Stricker E.M. Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions. Science (1973) 182 717-720.

Zis A.P. and Fibiger H.C. Neuroleptic-induced deficits in food and water regulation: similarities to the lateral hypothalamic syndrome. Psychopharmacol. (1975) 43 63-68.