BEHAVIOURAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF THE NIGRO-STRIATAL DOPAMINE SYSTEM

by

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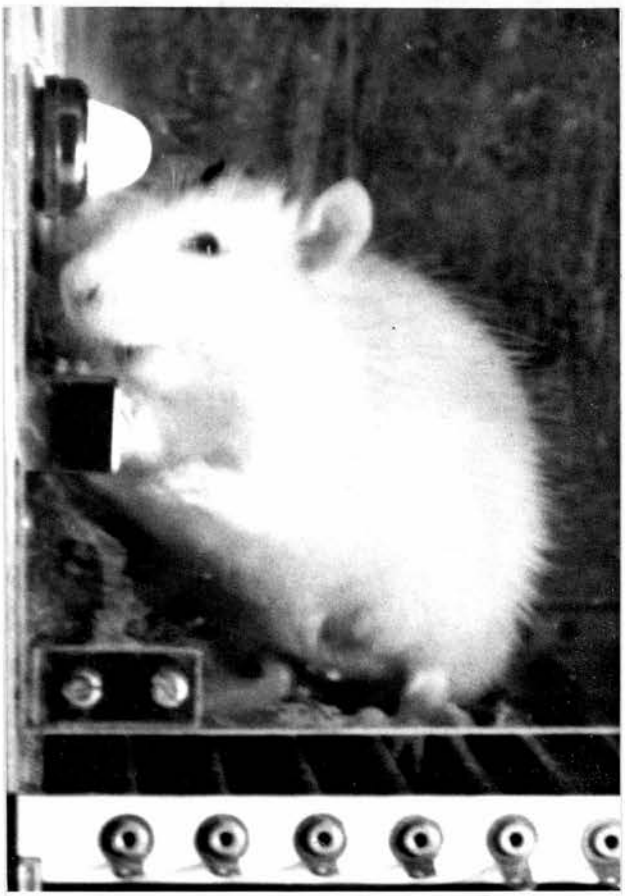

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THE UNIVERSITY *of* EDINBURGH

CONTENTS

LIST OF ABBREVIATIONS

ACh	Acetylcholine
ACH	Autocorrelation histogram
BDH	British Drug Houses
BSA	Bovine serum albumin
CAT	Choline acetyltransferase
CM	Centromedian nucleus of the thalamus
CPZ	Chlorpromazine
DA	Dopamine
DA-AC	Dopamine sensitive adenyl-cyclase
DRL	Differential reinforcement of low rates
DRN	Dorsal raphe nucleus
EDTA	Ethylene-diamine tetraacetic acid
EPSP	Excitatory post-synaptic potential
FET	Field effect transistor
FLH	Feedback loop hypothesis
GABA	γ-amino-butyric acid
GAD	Glutamic acid decarboxylase
GDU	Graphics display unit
GHB	γ-hydroxybutyrate
HRP	Horse-radish peroxidase
5-HT	5-hydroxytryptamine
ICSS	Intracranial self-stimulation
IH	Interval histogram
IPSP	Inhibitory post-synaptic potential
KA	Kainic acid
LH	Lateral hypothalamus
MFB	Medial forebrain bundle
MRN	Median raphe nucleus
NA	Noradrenaline
NMA	N-methyl aspartate (racemate)
NMDA	N-methyl d-aspartate
NMLA	N-methyl l-aspartate
6-OHDA	6-hydroxydopamine

PF	Parafascicular nucleus of the thalamus
PPH	Post-pulse histogram
PSTH	Post-stimulus histogram
SN	Substantia nigra
SNC	Substantia nigra pars compacta
SNL	Substantia nigra pars lateralis
SNR	Substantia nigra pars reticulata
SP	Substance P
TOH	Tyrosine hydrolylase
TTX	Tetrodotoxin
VTA	Ventral tegmental area, A_{10}

All other abbreviations are those in current scientific usage.

Statement in terms of regulations 2.4.15 and 2.4.11 of the Post Graduate Board of Study Programme of the University of Edinburgh

I declare that I composed this Thesis myself and that all the experimental work has been done by myself with the exception of that noted below:

a) The 6-OHDA lesions described in Section 2.2 and used for the experiments, the results of which are given in Section 3.3, and

b) the estimation of dopamine in the striata of some of the above animals, described in Chapter 3.3,

were performed for me by Mrs. Ann Wright.

c) All experiments in relation to the KA results described in Section 3.3 were done by Walter Muir as part of his Honours B.Sc. course, and were supervised by myself.

The following publications are based on work described in this Thesis:

1. Hamilton, M.H. and Arbuthnott, G.W. Effects of antidromic driving of nigral dopamine cells. Neurosci. Lett. Suppl. 7, S477 (1981).

2. Hamilton, M.H., Garcia-Munoz, M., Muir, W.J. and Arbuthnott, G.W. Separation of motor and other effects of unilateral 6-OHDA lesions. Neurosci. Lett. Suppl. 7, S179 (1981).

ABSTRACT

Operant behaviour techniques were used to show that rats have a preferred paw and to support the existence of a motor function plus another function of DA in the nigro-striatal pathway. Other lesions to motor systems in the brain (neurotoxic lesions of the striatum and electrolytic lesions of the striato-nigral pathway in the crus cerebri), will mimic the motor effects of a unilateral 6-OHDA lesion on paw use, but not its effects on performance. Similarly, peripheral paralysis of the preferred forelimb results in a change in paw use, with no performance deficit. Training rats to use the "other" paw does not result in a performance deficit. However, it is possible the 6-OHDA induced deficit in performance results from depletion of limbic DA which is affected by the 6-OHDA injection, but not the other lesions. The 6-OHDA specific component may be involved in the motivation for or initiation of movements.

Electrophysiological evidence is presented suggesting the existence of two mechanisms controlling the activity of DA cells. One involves the striato-nigral pathway and the other involves the release of DA from the dendrites in the SN. Evidence for this comes mainly from experiments showing that the effects of haloperidol and picrotoxin on cell firing rate are additive. Antidromic and orthodromic effects on the activity of DA cells resulting from striatal stimulation resulted in complex sequences of events. Lesions of the striato-nigral pathway seemed to have little effect on the results of striatal stimulation and so it has been suggested that all the observed effects may in fact not be mediated by the striato-nigral pathway, but by some other means. The lesions do cause an increase in the spontaneous firing rate of the DA cells, attributed to a removal of a tonic inhibition on the DA cells.

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CHAPTER I

General Introduction

1.1 Introduction

The nigro-striatal dopamine (DA) system, originating in the pars compacta of the substantia nigra (SNC), has been the subject of intensive study for the past few decades (150,151,481). It is thought to be of real importance in the integration of the sensory motor functioning of an animal. The key position of the pathway in relation to other brain areas related to sensory pathways and motor control supports such a role, as does the behavioural evidence resulting from interference with the system. The nigro-striatal pathway and its target nucleus the striatum are also of importance in several clinical disorders - such as Parkinson's Disease and Tardive Dyskinesia, therefore the system is of interest to medicine. The nigro-striatal DA system may also have a role in mediating the reward value of certain stimuli.

Two aspects of the nigro-striatal system have been investigated in this thesis. The first of these, described in Chapter III, studies the system at the level of the whole animal. Here the components of a behavioural deficit caused by 6-hydroxydopamine (6-OHDA) injected into the nigro-striatal path are dissected using operant behaviour techniques. The second, described in Chapter IV, looks at the functioning of the DA neurons at a cellular level, using electrophysiological methods. The effects of lesions of the striato-nigral pathway and of some dopaminergic and GABA-ergic drugs are studied on the spontaneous firing of these neurones and in response to striatal stimulation.

This chapter will consider the anatomical position of the system within the brain. The more specific background material for Chapters III and IV is dealt with at the start of each of these chapters. Overlap

between this chapter and these has been kept to a minimum, but for the sake of clarity some has been unavoidable.

General methods used in both Chapters III and IV are dealt with in Chapter II but, again, those used specifically in one of these chapters only are covered in the relevant chapter. Also the results from each section are discussed in that section, but a final general conclusion is given in Chapter V.

From the details given in the rest of this chapter, it is clear that the connections of the substantia nigra (SN) and the striatum are extremely complex. As there are also numerous connections between the regions that the SN and striatum project to and receive afferents from (see review by Dray, 151, for details), it is difficult to envisage how the nuclei interact in their functioning.

The large degree of topography in the projections is worth noting. It is this that enables the system to co-ordinate movements of all parts of the body. Also the frequency of reciprocal connections is interesting. The reason for this is not entirely clear but it must be related to the control of the activity of the projections.

Connections exist that would allow interaction between the SN and the striatum and the relay nuclei in the thalamus and the sensory-motor processing area in the cortex. Connections also exist that allow the flow of information between the extrapyramidal motor system and the limbic system. These may be involved in the emotional control of movement. Areas of cortex other than the sensorimotor cortex also project to the striatum. These may be involved in the process of turning thought into action and in the responses to various environmental and internal stimuli.

Due to the complexity of the interconnections, it is difficult to analyse the systems in relation to their function as far as the whole animal is concerned, and so not much is known about what each system does in the behaviour of the animal.

There now follows an anatomical review of the systems related to the SN and the striatum. These are summarised in Figures 1 and 2 at the end of Section 1.5.

1.2 Interrelations between the substantia nigra and the striatum

The substantia nigra

The SN was so named because of the dark pigment seen in the nucleus in primates. This pigment is not seen in other groups of animals. It is situated bilaterally in the midbrain, between the cerebral peduncles and the tegmentum. The nucleus is divided into three parts: the pars compacta (SNC), the pars reticulata (SNR) and the pars lateralis (SNL) (194,248,255,305,501,541). The SNR lies beneath the SNC and is larger. It has less densely packed, but more highly organised cells, which are large, medium or small in size. The SNC lies dorsal to the SNR and is relatively rich in cells, which are mostly medium or small in size (305). The SNL has been relatively little studied in its own right, although it is phylogenetically the oldest part of the nucleus. It is an elliptical mass of cells lateral to the SNR and SNC (255,305) and has a similar cytological structure to the rest of the nucleus. It forms connections with the SNR through the dendrites of medium and large cells. The actual size of these cells depends to some extent on the particular study and on the species. It seems that in the cat the smallest cells are about 19 μm in diameter and in the rat they are about 8 μm. The largest cells in the cat are of the order of 74 μm and in the rat, 40 μm (248,255,305).

The smaller cells presumed to be interneurones are present in all the three areas (194,246,305,541).

The cells of the SNC send aspiny dendrites into the SNR, whereas the dendrites of the SNR tend to stay within the SNR, but extend throughout it. From this it can be seen that there is extensive overlap between the SNC and the SNR (541).

Recent anatomical studies have suggested that the SNR may be further split into two layers (305). One region is just below the SNC and contains the dendrites of the SNC cells. These dendrites run dorsoventrally and rostrocaudally. The other region is below this and is next to the peduncle. The dendrites in this region run parallel to the peduncle.

The largest number of synapses in the SN are on the dendrites in the SNR and relatively few are on the soma in either division of the nucleus (501). Many "en passage" synapses are seen allowing several points of connection with the same dendrite. As well as axo-dendritic and axosomatic synapses, axoaxonic synapses are also seen on the axon hillock or terminals of other cells (240,247).

The striatum

The striatum, situated in the telencephalon, consists of the caudate nucleus and the putamen, separated in primates by the internal capsule. It is the largest of the basal ganglia (see reviews by Carpenter, 67, and Niewenhuys, 429, and Fox and Rafols, 192). It has in the past been considered as a homogeneous entity with respect to cellular organisation, the caudate nucleus and the putamen having the same, densely packed cells, with no special arrangement (326). However, some groupings of cells and synapses can be seen within the structures forming various shapes (325,396,529,586). Using certain

treatments, patterns can be seen. For example, when stained for acetylcholinesterase mosaic-like patterns can be seen (238,272). It is likely that these patterns are related to the topographic inputs from other brain areas (229,520).

Fibres within the striatum are generally fine (0.3-1.6 μm) and poorly myelinated. Intrinsic and other fibres form a close-packed network around the cells in the nucleus. The cells can simply be divided into two groups - small, pale cells, with larger multipolar cells scattered through them. However, Golgi staining suggests a further division into spiny and aspiny neurones, in as many as six groups (145,258,325). The spiny types of cells may constitute as many as 95% of the cells. Originally these were thought only to have short axons not projecting out of the striatum (325) but it is now known that 50% or even as much as 60-70% of the cells do project out of the striatum (46,55,145,239) and that at least some of the medium-sized spiny neurones do project to the SN (556,558,559). It is possible that the Golgi method failed to demonstrate the fine axons of these cells.

There appears to be two types of aspiny neurone (326). One is large with myelinated axons projecting out of the striatum and was originally thought to be the output neurones,having collaterals in the striatum. The other type is smaller and has smooth radiating dendrites leading to their name: spidery cells. Axons from these cells could not be seen in the Golgi preparation.

Synapses are seen making contacts between axons and soma and dendritic shafts, spines or the initial segments of other axons (258). Dendrodendritic synapses are rare. Some "en passage" synapses are occasionally seen (586).

The nigro-striatal pathway

Early studies using retrograde degeneration techniques have suggested the existence of a nigro-striatal pathway (28,70,265,276,339, 513,597), despite the fact that other studies using the Nauta method (415) have failed to do so (4,71,172). Some of these have suggested a nigro-pallidal system (68,338,399) and the difference may arise from methodological problems.

However, more recent studies have confirmed the existence of the pathway. Injection of anterogradely transported radiolabelled amino acids into the SNC shows the presence of radioactivity in autoradiographs of the neostriatum and hence supports the existence of a nigrostriatal pathway. Similarly, the injection of retrogradely transported proteins [e.g. horse radish peroxidase (HRP) or tetanus toxin] into the neostriatum leads to the labelling of cells in the SNC, indicating that axons travel from there to the injection site in the striatum.

From lesions at various sites in the pathway, a route for the fibres has been worked out. The fibres leave the SNC, pass through the ventral tegmental area and gather in the lateral hypothalamus, just medial and dorsal to the ventral tip of the crus cerebri (the medial forebrain bundle, MFB). Then, at the level of the median eminence, it enters the ventral crus cerebri and travels rostrally and dorsally in the internal capsule to innervate the neostriatum (12,193,456,471,597).

From electron microscope work it seems that the terminals of the SNC DA cells terminate on the dendritic spines and cell bodies of spiny neurones and the cell bodies of spidery neurones in the striatum (274, 322,405).

Morphologically in rats the projection appears to be uniform to all of the striatum (122,322) but in some systems, for example the adult

rat treated with alpha-methyl paratyrosine (450) and the developing cat (237,238), there appear to be islands of dopamine as shown by fluorescence histochemistry. These islands have also been seen in autoradiographic studies looking at the input from the SN (638). The islands may be functionally significant in relation to other transmitter systems.

From selective lesion studies, the general topography seems to be mediolateral and anteroposterior, that is medioanterior sites in the SNC project to ventral and anterior sites in the striatum and lateral and posterior sites project to posterior striatum. There is also an inverse dorso-ventral relationship (28,70,166,250,582,591).

The information from these studies is supported by work on the transmitter involved. DA has been confirmed as the transmitter in the nigro-striatal pathway by biochemical, fluorescence and electrophysiological work. DA levels fall in the neostriatum after lesions of the SNC (16,173,265,471,537) often in proportion to the extent of the lesion (268,404,470). Fluorescence histochemistry has shown the presence of DA in the SNC cell bodies (122) and in the striatum (12) which disappears on lesioning the nigro-striatal pathway (268,365,591). Electron microscope studies have even shown that DA is contained in the terminals and that the type of terminal containing DA falls in number after lesions of the SNC (202,274). Stimulation of the SNC results in the release of DA in the neostriatum (16,325,423) and causes changes in cell firing that can be mimicked, at least in some cases, by the iontophoretic application of DA (92,93), and is blocked by DA antagonists (177,469,550).

Some recent work has suggested the possibility that the SNC is divided into two layers. A ventral layer has large pyramidal cells,

with varicose dendrites reaching into the SNR. This ventral layer of the SNC has a mediolateral and antero-posterior topography in the ipsilateral striatum. The dorsal layer consists of fusiform cells, whose dendrites stay within the SNC and whose axons project to the olfactory tubercle and amygdala (167).

A sparse contralateral projection to the striatum from the SN has also been proposed. Evidence for this comes from studies with the retrograde transport of dyes in rats and cats (171,521).

There is also the suggestion that not all the cells are dopaminergic. Studies using 6-OHDA lesions which specifically destroy DA cells combined with fluorescence studies using retrogradely transported dyes and immunohistochemistry have suggested that as much as 20% of the projection may be non-DA (265,369), although it may be as little as 5% (251,602,606). The 20% value comes from lesion studies combined with fluorescence and the 5% from retrograde labelling combined with fluorescence. This methodological difference may account for the differences in value.

It has been suggested that these non-DA nigro-striatal cells may take a different route than the DA ones (267) and that their origin may be in the SNR. These could be collaterals from other nigral efferents such as the nigro-thalamic ones (254).

The striato-nigral pathway

A striato-nigral pathway had been described several times during the first half of this century (454,523,609,630), but had not been described in detail until the 1960's with the work of Voneida (611) and Szabo (579,580,581). These studies used degeneration methods to demonstrate the existence and topography of this pathway. The head of the caudate nucleus projects to the rostral third of the substantia

nigra with a mediolateral correspondence. The putamen projects to the caudal two-thirds of the substantia nigra, dorsal parts going to lateral SN and ventral parts to medial SN. In the rat there is also an antero-posterior topography, with the tail of the striatum going to the more lateral parts. The early studies mentioned above, in the cat and monkey, along with others (240,323,541) showed all afferents ending in the SNR although it appears that in the rodent the whole SN is innervated (both the SNC and SNR) (55,87,620). This topography was later confirmed by biochemical and immunohistochemical techniques (591). Both axosomatic and axodendritic synapses exist, but the axodendritic ones are more common in cats (259) and in the rat the axosomatic type is more common (252).

The striato-nigral path is one of the two largest output pathways from the striatum (the other being the striato-pallidal system). Both of these were at first thought to originate from only 5% of the striatal cells (192,326) but recent work has suggested that far more cells do project out of the striatum, possibly as many as 30-50% (23,55,239,309, 345) or even as much as 70% (46). The low values were obtained with Golgi staining and this may not have shown the full length of the axons. The later studies used the retrograde transport of dyes to the striatum from the target nuclei of the striatal output and have been able to show projections out of the striatum.

A significant proportion of the cells projecting to the SN are the medium-sized spiny neurones (23,345,559) once thought to be inter-neurones.

The fibres leave the striatum and pass through the globus pallidus to enter the internal capsule in which they travel through the midbrain tegmentum to the SN which they enter from the ventral side.

Two candidates have been proposed as transmitters in the striato-nigral pathway: an inhibitory one and an excitatory one. The former is probably gamma-amino-butyric acid (GABA) and the other may be substance P (SP).

There is strong evidence for the inhibitory transmitter being GABA. The SN has one of the highest concentrations of this compound in the central nervous system (164,165,336,442), which along with the existence of glutamic acid decarboxylase (GAD), the synthesising enzyme, in the terminals in the area (190,497) is good support for a transmitter role for GABA.

Lesions of the striatum, or the striato-nigral pathway lead to a drop of GABA and GAD in the SN (190,262,312,389). Also electrical and K^+ stimulation of SN slices will release GABA by a Ca^{++} dependent process (441,494), and a reuptake mechanism for GABA exists in the SN (441,574). *In vivo* stimulation studies where the striatum is stimulated show an increased release of GABA in the SN (346,578) and an increased rate of loss of prelabelled GABA after inhibition of GAD (33,376). Striatal stimulation also leads to a decrease in the cell firing rate or inhibitory evoked potentials in the SN (650) and this can be blocked with bicuculline or picrotoxin, both thought to be GABA antagonists (87,111,153,651). These drugs also affect the responses to iontophoretic GABA (111). Tetanus toxin, an agent thought to block the presynaptic release of GABA in the central nervous system blocks the effects of striatal stimulation on the SN cells but not the effects of iontophoretic GABA (125).

All the above pieces of evidence support the existence of GABA as the inhibitory transmitter in the striato-nigral pathway.

As well as GABA of striatal origin in the SN there is also other GABA (190,262,313,411,574). Some of this may be from the globus pallidus and some of it may be intrinsic or from other GABA ergic output cells (156,411).

The evidence for the excitatory transmitter is not as good. It does not seem to be an excitatory amino acid or acetylcholine as alpha-amino adipate (86) or atropine (310) do not seem to block the effects of striatal stimulation. The best candidate is SP (156). Like GABA the levels of SP are extremely high in the SN (51,86,117,210,404,473), mostly in the rostral SNC and caudal SNR where it would be closest to the DA cells and dendrites (119,370). Monoclonal antibody studies have shown that SP is contained in terminals in the SN and that these synapse mainly on dendritic processes (116).

SP also has a synaptic localisation in subcellular fractions of SN homogenates (117,158,430,494). It can also be released from the SN on stimulation (295).

SP applied iontophoretically will excite the SN cells, both in the SNC and SNR (87,123,468,615), however, a SP antagonist has not yet been developed to block this pharmacologically, and to compare this with the effect on striatal stimulation. One compound, Lioresal, was thought to be a specific antagonist (525) but was later shown to be non-specific (123). Lesions of the striato-nigral path reduce the amount of SP in the SN especially the more anterior lesions in striatum (277,296,308, 407,458).

All these facts also support a role for SP as a transmitter in the striato-nigral pathway. Lesion studies show that the two transmitters exist in different neurones, as anterior lesions reduce SP levels far more than they reduce GABA levels (51,117,204), suggesting an anterior

localisation for SP and a more caudal one for GABA (412) although GABA does exist throughout the striatum. This distribution has been confirmed using KA, to specifically kill cell bodies, rather than electrolytic lesions (204) and is also supported by immunological studies (308).

The striato-pallidal and pallido-nigral pathways

The second main output from the striatum is the striato-pallidal pathway (75,192,296,323) and in fact this seems to be even larger than the striato-nigral one (598,608). Along with the striato-nigral projection this pathway was supposed to originate from only 5% of the striatal cells, but recent work has shown that this figure is in fact much higher (46,55,239).

The projection is topographic to both segments of the globus pallidus, projecting like the spokes of a wheel with the head of the caudate nucleus going to the dorsal and rostral pallidus and the putamen going to the ventral and caudal pallidus (454). In the monkey the lateral cuadate goes to the external pallidal segment and the medial caudate to the internal one (579). The dorsal putamen projects to be dorsomedial pallidus and the ventral putamen to ventral pallidus (580). The body of the caudate goes to the dorsomedial third of the globus pallidus with an anteroposterior and mediolateral topography (581).

In cats, stimulation of the caudate nucleus leads predominately to an inhibition in the entopeduncular nucleus (equivalent to the internal segment of the primate globus pallidus), with a few excitatory responses (375,480,652,653). From stimulation studies it has been suggested that the inhibitory responses may in part come from collaterals of the striato-nigral GABA cells as they pass through this nucleus (192, 314,652,653). The inhibitory transmitter is most likely to be GABA,

especially if the fibres are collaterals of the striato-nigral fibres (438, 498). Only GABA mimicked the effect of caudate stimulation on the pallidal cells (438).

The excitatory transmitter may be a peptide as in the striato-nigral pathway. Recent work using lesions combined with immunocytochemistry has shown the possible existence of a leu-enkephalin pathway from the striatum to the globus pallidus of rats (116,120).

A pathway also goes from the globus pallidus to the SN (296) although positive confirmation of this was more difficult than for the striato-nigral pathway due to the fact that the striato-nigral fibres pass through this region and any effects in SN could be attributed to lesions of these.

Early studies did suggest the existence of such a pathway using degeneration techniques, but these were inconclusive because of the problem of lesioning the striato-nigral fibres as well (190,300,389,417, 496). Stimulation studies have the same problems as the lesioning ones, in that there are inevitably complications from the activation of the striato-nigral fibres.

Confirmation of the existence of the pathway had to wait until the development of retrograde tracing techniques. These have shown that such a pathway does exist (55,73,239,296). GABA is also indicated as the transmitter in this pathway from immunocytochemical work, combined with lesions (296,496,498). There may also be an excitatory transmitter in this pathway, and this is likely to be SP as SP immunoreactive cells have been seen in the globus pallidus (308).

It has been suggested that the pallido-nigral cells may be innervated by the DA cells from the SNC (260,366), possibly by collaterals from the nigro-striatal pathway (653).

1.3 Afferent connections of the substantia nigra

The most important input to the SN comes from the ipsilateral striatum and globus pallidus (see Section 1.2), but the SN does also receive several other inputs, including those from raphe nuclei, possibly the cortex, subthalamic nucleus, the locus coeruleus, the cerebellum, the nucleus accumbens and the reticular formation.

The raphe-nigral pathway

The projection from the raphe nuclei appears to have a medio-lateral topography, and to come from both the median and dorsal raphe nuclei (MRN and DRN) (45,55,94,122,152,154,464). However, some studies with retrograde tracing techniques have failed to show the projection from the MRN (55,182). Many of the fibres from the DRN are collaterals of the projection from the DRN to the striatum (605).

The effect of the raphe-nigral projection on SN neurones appears to be mainly inhibitory (152,154) and is monosynaptic in nature (152). Some excitatory responses from intracellular studies have also been reported (312), and it has been suggested that the inhibitions seen in extracellular studies are due to polysnaptic mechanisms (312). This is certainly conceivable in view of the collaterals to the striatum.

The transmitter in this path is likely to be 5-hydroxytryptamine (5-HT). Autoradiography with ^{3}H-5-HT has demonstrated the pathway (575) and fluorescence studies have shown the presence of 5-HT in the raphe nuclei (122,591). Lesions of the raphe nuclei lead to decreases in the levels of 5-HT and in uptake of 5-HT in the SN (453,493).

Iontophoresis of 5-HT onto the cells in the SNC has little effect, but it will consistantly block the excitatory effects of glutamate (7). The effect on the SNR cells is variable, resulting in inhibitions, excitations and no effect (7).

One study suggests that the projection is densest to the SNC (152), while another appears to show a greater projection to the SNR (493). The discrepancies are probably due to the fact that the fibres pass through the SNR on their way to the SNC and some studies might be detecting these. Also the dendrites of the SNC cells go into the SNR and so synapses in this area could affect the DA cells in the SNC.

The cortico-nigral pathway

This pathway was first suggested on the evidence from early degeneration studies (398,609), but its existence was later questioned by improved silver staining techniques, which suggested the degeneration seen was due to fibres of passage in the SN (3,503). However, recently improved methods have again raised the question of the existence of a small cortico-nigral path. Retrograde tracing techniques have suggested the existence of a small projection (55) and degeneration studies using electron microscopy have shown degenerating terminals in the SNC and SNL (3,499), and only a few in the SNR.

Electrophysiology has shown variable latency excitations, with either single spike responses or occasionally 2-4 spikes in response to cortical stimulation (232). However, it is possible that it is a polysynaptic pathway (232). One possibility is via the subthalamus. This nucleus is excited by cortical stimulation and does send projections to the SN as can be shown by antidromic driving (137).

The possible transmitter is not known, but it might be glutamate in view of the fact that KA lesions of the frontal cortex reduce glutamate uptake in the SN (72).

Other nigral afferents

Other relatively minor and unstudied projections to the SN come from the subthalamic nucleus, the locus coeruleus, the cerebellum, the nucleus accumbens and the reticular formation.

HRP studies have shown an input to the SN from the subthalamic nucleus (308), with possibly as many as 90% of the fibres sending axon collaterals to the globus pallidus or the entopeduncular nucleus (134,264,604). Electrophysiology shows that the pathway is excitatory (254). It has also been suggested that the subthalamic nucleus is a continuation of the DA cells of the SNC (395).

The pathway from the locus coeruleus has not been demonstrated by anatomical means but has been inferred from other types of experiment. Noradrenaline (NA) is present in the SN (168), and nigral cells will respond to iontophoretic application of NA with either excitations or inhibitions (112,156). NA uptake in slices of SN is Ca^{++} dependent suggesting a transmitter role and it can also be released by K^{+}. On stimulation of the locus coeruleus, an excitation followed by an inhibition is seen in the SNC (88).

The cerebellar afferents come from the contralateral cerebeller nuclei and have been demonstrated by HRP in conjunction with silver impregnation (555). Stimulation of the cerebellar nucleus results in the decrease of DA from the SN dendrites (426) by an as yet unknown mechanism.

The projection from the nucleus accumbens to the SN is ipsilateral and projects topographically onto both the SNC and SNR (95,418,474, 577,626), and is apparently monosynaptic (557). Stimulation of the nucleus results in mixed excitatory and inhibitory responses in the SN (155,201). The response is blocked by bicuculline (201), suggesting

that the transmitter may be GABA, although lesions have been reported not to decrease levels of GAD in the SN (155). This could, however, be due to a masking effect of the large amount of GABA from other sources in the SN.

Connections from the midbrain reticular formation to the SN have been shown by electrophysiological methods (455). The input is thought to be excitatory (455). Cells have also been antidromically activated from the SN (647). Little else is known.

1.4 Efferent connections of the substantia nigra

The main projections from the SN go to the striatum (see Section 1.2), the thalamus and the brainstem, but other projections exist to the amygdala, the cerebellum, locus coeruleus, raphe nuclei, cortex and possibly spinal cord.

The nigro-thalamic pathway

The nigro-thalamic pathway seems to come exclusively from the SNR (67,69,70,73,83,174,183,272,500), more specifically a longitudinal band of cells in central and lateral SN (174). It projects to the VA/VL (VM in rats) thalamus and also to the dorsomedial thalamus. Some of the cells also give off collaterals to the striatum or the superior colliculus. This is demonstrated both by stimulation studies (13,136,196) and by double retrograde labelling (32,568,569). There also appear to be short axon collaterals, arborising within the SN, sometimes not outwith the dendritic field of the parent cell (133). There may be a few bilateral fibres (637).

The pathway is inhibitory and monosynaptic to the thalamic cells (13,138,250,254,589) but again excitations as well as inhibitions have been reported on SN stimulation. However, it seems likely that the

excitations result from activation of cortico-thalamic fibres passing near to or through the SN, as the short latency excitation disappears if the cortex is lesioned (138).

The transmitter in the pathway is most likely to be GABA. Lesions of the SN lead to a decrease in GAD in the thalamus (143, 335) and GABA antagonists block the inhibition caused by SN stimulation (393,649).

Nigral connections with the brainstem

Degeneration and tracing techniques have shown the existence of a pathway from the SN to the midbrain reticular formation (278, 502,636), the tectum (deep layers of the superior colliculus) (174,236, 278,290,502,636) and the periaquaductal grey (276,290). This projection also seems to be exclusively from the SNR, from a longitudinal ventral band (174) and the projection to the superior colliculus is bilateral (135,636,637), with some cells projecting both ipsilaterally and bilaterally (135). The medial superior colliculus receives a bilateral input, whereas the lateral portion receives only ipsilateral innervation from the SN (135,290).

Some nigro-tectal cells also send collaterals to the thalamus (13,32,569).

The effect of nigral stimulation is inhibition of the brainstem neurones with a few reported excitations (647). As with the nigro-thalamic path the excitations are likely to be from activation of cortico-fugal fibres (80,647). The transmitter is probably GABA. Iontophoretic GABA mimics the effects of nigral stimulation on the superior colliculus cells (81) and GAD levels fall in the superior colliculus on lesioning the SN (610).

Similarly, the nigro-reticular path is inhibitory and monosynaptic, with excitations resulting from the activation of corticofugal fibres in the SN (231,455).

Other nigral efferents

The SN also sends efferents to the cerebellum (see review by Dray, 151), the amygdala, the locus coeruleus, possibly the spinal cord, the cortex and the raphe nuclei.

The projection to the amygdala appears to come from the SNC (167) and as DA inhibits cell firing in the basolateral amygdala, this may be the transmitter (29). The projection goes to the lateral and central amygdala (167,306,307).

The path to the locus coeruleus is uncrossed and has been shown by retrograde transport of HRP (526). That to the spinal cord has only been suggested from biochemistry. Lesions of the SN lead to a drop in DA levels in the spinal cord (91). The nigro-cortical pathway comes from the SNC and therefore may be DA (19). The projection from the raphe nuclei to the SN has been shown electrophysiologically (570) and by retrograde transport of HRP (457). It appears to be mainly inhibitory with a few excitations (570), again possibly from the activation of corticofugal fibres.

1.5 Afferent and efferent connections of the striatum

In addition to the input from the SN (see Section 1.2), the striatum receives other inputs, the major ones being the cortical and thalamic ones. There are also several other less extensive inputs. These other striatal afferents are now discussed.

The cortico-striatal pathway

The striatum receives an extensive topographic input from all parts of the cerebral cortex (66,72,99,148,209,218,229,303,324,356, 362,363,364,583,623). It has been estimated that, in cats, 13% of the synapses in the caudate nucleus, come from the cortex, whereas 33% of the synapses in the putamen are with terminals from the cortex (258). The projection is ipsilateral, with some areas, e.g. sensori-motor cortex (66), projecting bilaterally. There is evidence that suggests that at least some of the cortico-striatal fibres are collaterals from other corticofugal fibres, given off as the internal capsule passes the nucleus. Both degeneration studies (218,622,623) and studies using intracellular injections of HRP have suggested this. However, some electrophysiological work has suggested that some fibres are not collaterals (43,341), by showing that there was no activity in the striatum on stimulation of the pyramids.

The cortico-striatal fibres consist of fine non-myelinated axons (218,622) and are thought to terminate on the dendritic spines of the spiny striatal cells (1,99,327,328). The projections from the different cortical areas are not homogeneously distributed in the striatum. They appear as intricate patterns, the projection from the prefrontal cortex being described as consisting of clusters surrounding unlabelled cores (229) and that from the motor cortex as consisting of patches or clusters (583). In the monkey the projection from the sensory-motor cortex goes to restricted areas of the striatum and it was suggested that other areas of cortex might supply the "spaces", leading to a high degree of overlap between the different cortical projections (303).

Electrophysiology suggests a reverse topography in the cortico-striatal projection, that is medial cortex projecting to lateral striatum,

and anterior cortex to posterior striatum (43,209,362,364). As well as being topographic, the projection from the sensorimotor cortex appears to be somatotopic (364).

The projections from the cortex have been shown to converge with those from the SN and the thalamus by means of autoradiography (26) and electrophysiology (53,281,345,382). Electrophysiology has also shown the pathway to be monosynaptic (340,345,510), as has the retrograde transport of HRP (340). In at least some cases the input is onto striatal output cells as shown by anatomical (556) or by electrophysiological techniques (382).

Activating the cortico-striatal pathway leads to an excitation of the striatal cells. This has been shown both by extracellular (363,534, 510,561) and intracellular recording (53,340,345). In fact cortical stimulation has been used as a means of activating the striatal cells which normally have a very low spontaneous firing rate (534). Although not proven, it seems likely that the transmitter in the pathway is an excitatory amino acid, most probably glutamate. The glutamate antagonist glutamic acid diethyl ester when applied iontophoretically blocks the effect of cortical stimulation (562). Lesions of the cortex lead to reduced striatal levels of glutamate (337). A high affinity uptake system for glutamate is present in the striatum (146) and this is also reduced by cortical lesions (72,388). Despite the evidence for glutamate as the transmitter, aspartate cannot be entirely ruled out as the transmitter. Glutamate receptors, shown by KA binding, do exist in the striatum (552) and after KA lesions of the striatum, cortico-striatal terminals are seen synapsing with degenerating cells four times as often as with other cells (263).

Initially it was thought that the projection ended on striatal interneurones rather than on output cells, due to the lack of cells driven from the cortex and antidromically activated from the output area of the nucleus (199,345,363). However, more recent work has shown that far more cells than were originally thought do project out of the striatum, and that the cortical afferents do terminate on striato-nigral cells (47,199,363,556,558).

The cortex may also influence the striatum polysynaptically via the thalamus. If the thalamus is lesioned, the mean latency of response to cortical stimulation in the striatum falls, and the response variability is reduced (79).

The thalamo-striatal pathway

The largest projection to the striatum comes from the ipsilateral thalamus and this mainly arises in the intralaminar nuclei especially the centromedianum-parafasicular complex (CM-PF) (67,104,258,304,316, 322,327,328,475,476,520,521,529). It has been estimated that 35% of the input to the caudate nucleus comes from the thalamus, whereas 16% of those in the putamen do so (258).

The projection traverses the internal capsule and projects topographically onto the striatum (476). Autoradiography shows the terminals ending in patches, bands or semicircular configurations (520), mainly on the dendritic spines of the spiny neurones (327,328). The CM projection is denser than that from the PF area and both appear to have a dorsoventral topography (520).

Retrograde degeneration studies show that the CM-PF complex in monkeys preferentially projects to the putamen, whereas other intralaminar nuclei project to the caudate nucleus (476). Also in the rabbit and rat degeneration studies show a projection from the midline

and intralaminar nuclei (104,475), with the midline nuclei and CM area going to the caudate and the PF nucleus to the putamen in the rabbit (104).

Retrograde transport studies, using HRP, support the projections seen with the degeneration techniques: the intralaminar nuclei and CM-PF complex project to the striatum (304,529) and also send a few axons to the cortex. HRP studies also show some nuclei outside the intralaminar areas also project to the striatum: the ventroanterior and mediodorsal and centrolateral areas (521,529).

The thalamo-striatal projection has also been demonstrated electrophysiologically and these studies suggest that the CM-PF complex projects to the putamen, while the CL area probably goes to the cortex and fibres are activated on the way through the striatum (355,487).

The thalamo-striatal pathway is excitatory in nature, and the input converges with those of the SN and the cortex (53,345,382,406). The transmitter is thought to be acetylcholine (ACh) as release increases on stimulation of the thalamus (390) and levels fall in the striatum on lesioning the thalamus (551). Also iontophoretic application of ACh mimics the effects of thalamic stimulation and the effects of both the iontophoretic application of ACh and thalamic stimulation are blocked by atropine (391).

Raphe-striatal projection

Antero- and retrograde tracing techniques have both shown the existence of a pathway from the DRN to the striatum, and only a minimal projection, if any, from the MRN to the striatum (19,20,45,94, 285,401,521). As many as 70-80% of these fibres also give off collaterals to the SN (603,605), as they pass in the MFB. The densest projection is to the caudal caudate nucleus and appears to be primarily ipsilateral, although a few crossed fibres may exist (19,45,94,285).

Activation of the DRN leads to a long-lasting monosynaptic inhibition in the striatum (124,401,447) although a few facilitatory responses have been reported from intracellular studies (607).

Only very few cells respond to stimulation of the median raphe nucleus (447) in support of the very sparse projection seen in the anatomical studies. However, the possibility exists that even the few responses seen were due to current spread to the DRN.

The transmitter appears to be 5-HT, 5-HT fluorescence has been shown in the pathway from the DRN to the striatum (122,591) and the 5-HT fluorescence in the striatum disappears on lesioning of the DRN (354). Tryptophan hydroxylase levels fall, as does 5-HT uptake in the striatum after lesions of the DRN (48,354). 5-HT levels in cerebro-spinal fluid from the lateral ventricle increases on stimulation of the DRN and at least some of this is presumed to come from the striatum (275).

Other striatal afferents

Other afferents to the striatum incude projections from the midbrain reticular formation, the amygdaloid complex, the nucleus accumbens, the locus coeruleus and the globus pallidus. None of these projections have yet been extensively studied and so little is known about them, apart from their existence.

The projection from the reticular formation is diffuse and relatively minor (373,507,508), and has been demonstrated by retrograde transport of HRP and by electrophysiology (373,508). It is possibly part of the ascending reticular activating system (507).

The projection from the amygdala has been demonstrated both by retrograde degeneration and retrograde tracing techniques (414,521), and by electrophysiology (228,436). This pathway may be a means by which the limbic system can affect the extrapyramidal system.

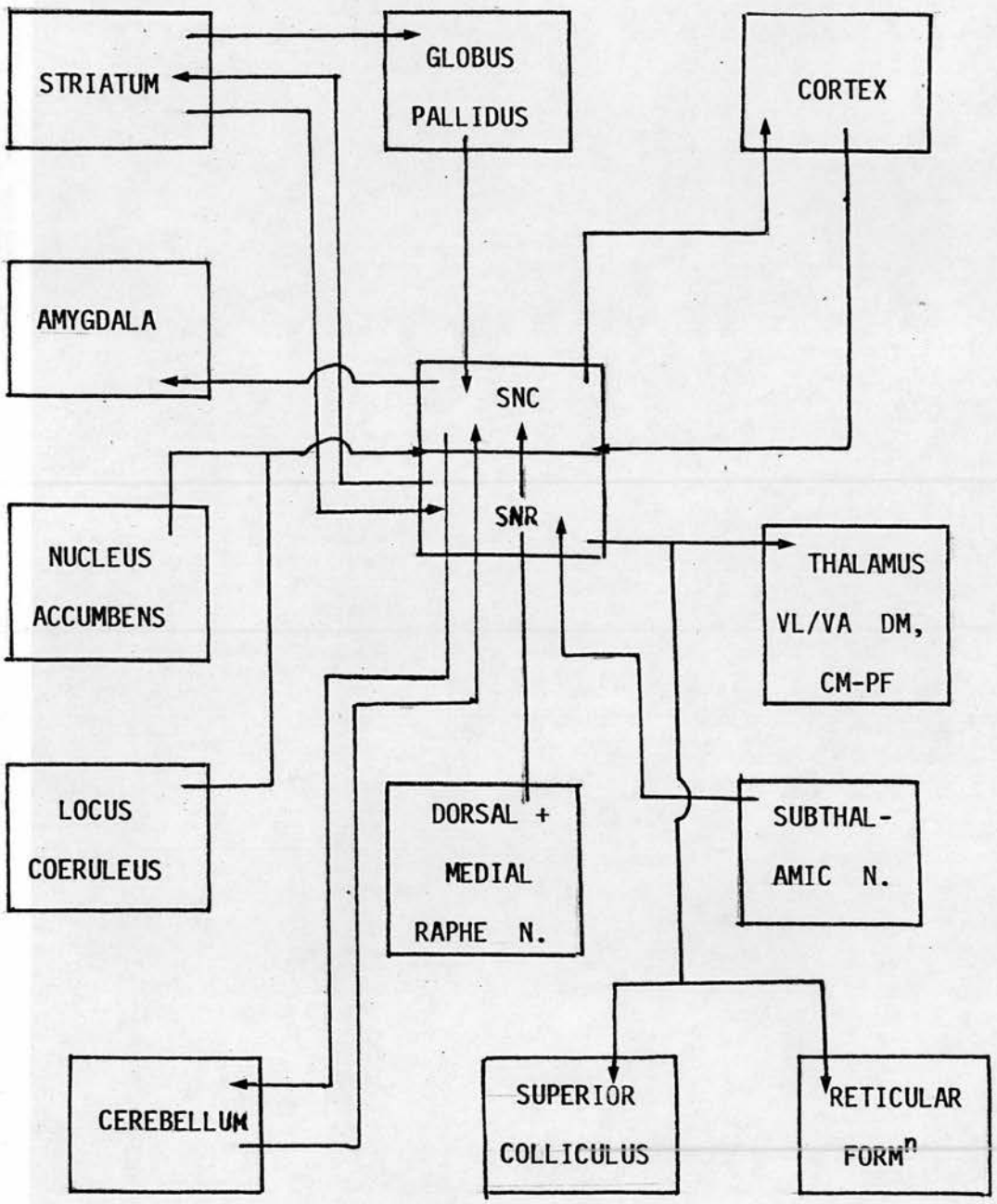


FIGURE 1 Afferent and efferent connections of the substantia nigra

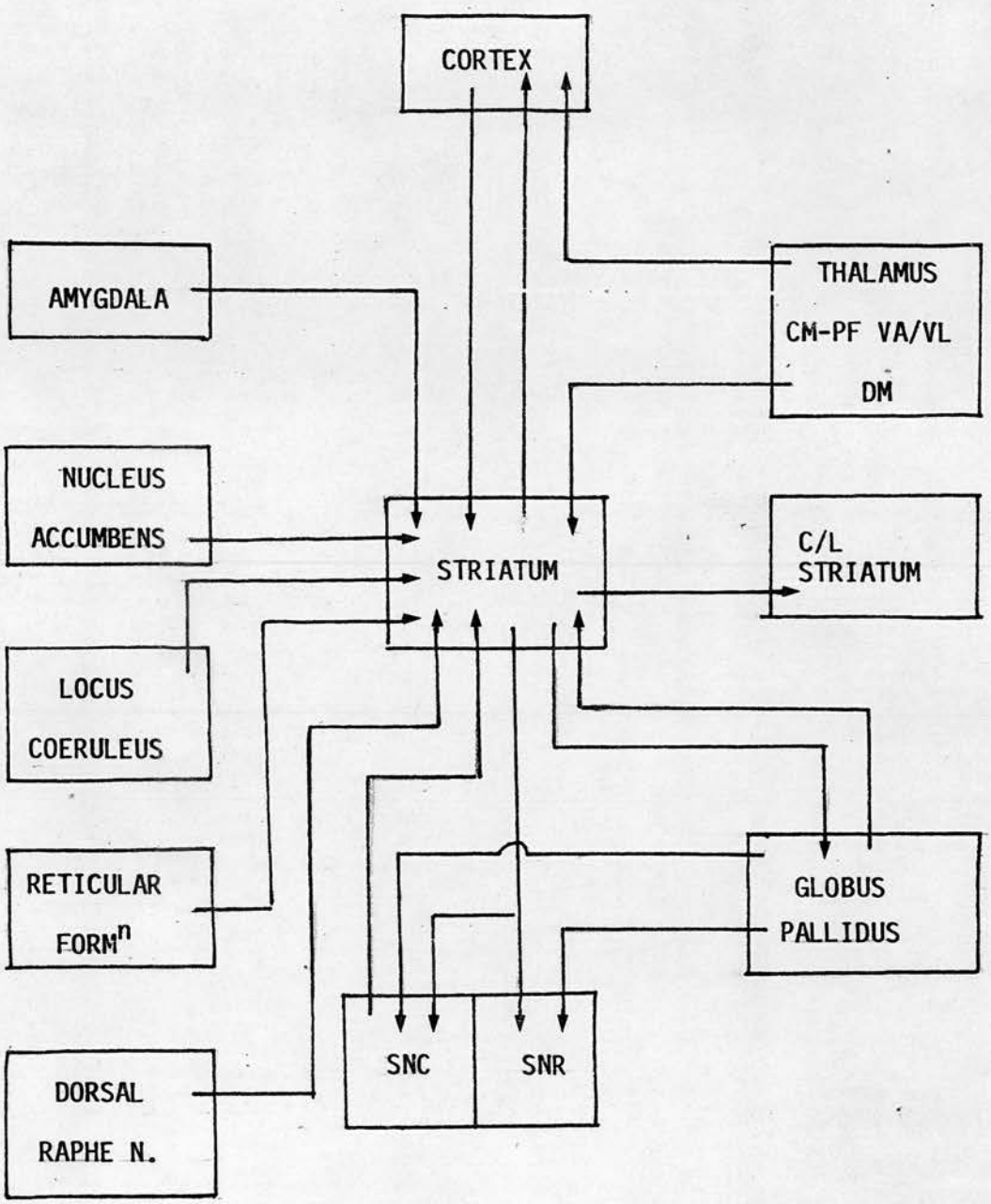


FIGURE 1 Afferent and efferent connections of the striatum

A small projection from the nucleus accumbens has been shown by autoradiography and degeneration studies to go to the anterior tip of the striatum (95,474).

A very small projection to the striatum comes from the locus coeruleus and has been shown by catecholaminergic fluorescence studies (122) and by autoradiography (39). This projection is supported by the existence of small amounts of NA in the striatum (168).

Finally, very recently a pathway has been shown to go from the globus pallidus to the striatum (564). This could not be shown until the development of the combined HRP- wheat germ agglutinin technique which allows much more localised injections.

Efferent connections of the striatum

There are very few efferents from the striatum other than those to the SN and the globus pallidus. However, HRP work has suggested the existence of a striato-cortical pathway (289) and degeneration studies have suggested the existence of a small projection to the contralateral striatum (397).

1.6 Kainic acid and n-methyl aspartate lesions

The neurotoxic lesions described later were made with KA or n-methyl aspartate (NMA), both analogues of the central excitant glutamate (621). The structures of these compounds are shown in Figure 3. KA has been reported to be from 8-80 times as potent as glutamate in exciting cells in the spinal cord (37,301) and the d-isomer of NMA (NMDA) is from 7-18 times as potent in similar experiments (121,301). The l-isomer (NMLA) is similar to glutamate in its excitatory potency in the spinal cord (121). The excitotoxic nature of these drugs, as well as that of glutamate itself has been discussed by Olney in 1978 (444)

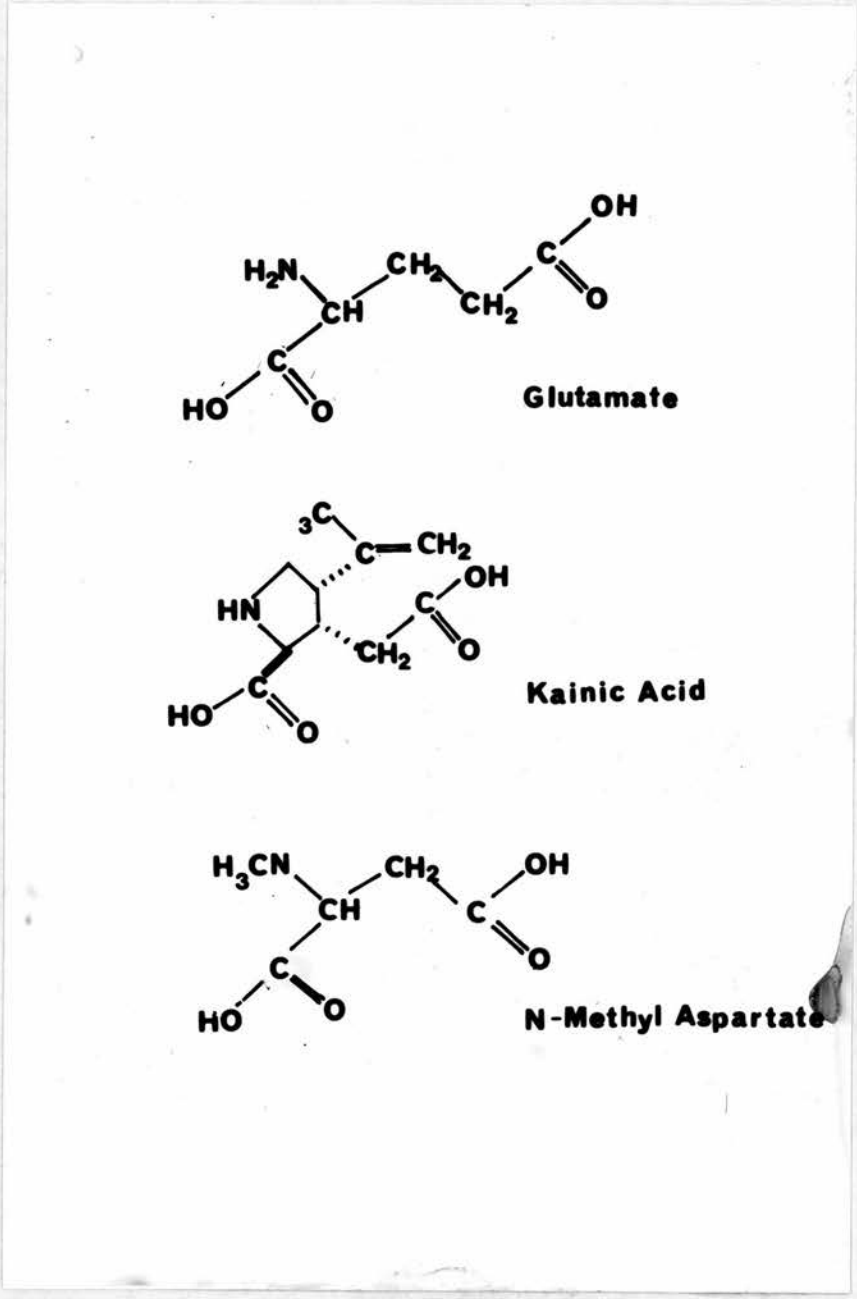


FIGURE 3: Chemical structures of glutamate, kainic acid and n-methyl aspartate, showing the similarities in structure.

and in 1979 by Nadler (410). Systemic application of these agents will cause neurotoxic reactions in a wide range of species and brain nuclei. The neurotoxic potency seems to be related to the excitatory potency, hence the term excitotoxic. When injected directly into the striatum, KA is more potent than NMA which is in turn more potent than glutamate itself. This corresponds to the order of potency seen when these agents are injected systemically (446).

The mechanism of cell death is still unclear, but it has been postulated (444,445) that excitotoxic agents lead to a continuous state of depolarisation and increase in plasma membrane permeability. This would lead to a strain on the cell's energy stores as the cell attempted to restore the ionic balance and death would result when the energy stores were depleted or the ionic environment of the cell became lethal. A variation on this idea is that the analogues do not act directly on the glutamate receptors on the affected cells but that they act presynaptically on the glutamate terminals to block uptake or increase release of glutamate (387). This could lead to excessive amounts of glutamate in the synaptic cleft, and a continuous, toxic depolarisation. This might explain why intrastriatal KA seems to be more effective in the presence of the cortico-striatal pathway than in its absence (38,385). This observation, however, could be explained by a removal of a tonic excitatory drive to the nucleus on removal of the cortico-striatal pathway, meaning that a greater amount of KA will be needed to cause the same amount of excitation as when the drive was present (387).

KA has widely been used as an agent that will kill the cell bodies in a brain area while sparing the axons of passage and terminals. This is supported by light and electron microscope studies (108,147, 261,444,445). Up to 48 hours after the lesion progressive chromatolysis,

shrinkage of perikarya, clumping of the nuclear chromatin and finally disruption of the nuclear membrane occurs. From 1-3 weeks after the injection of the KA, proliferation of the astrocytes is seen where the neurones used to be. Histofluorescence shows that the DA axon and terminals seem to be relatively undamaged. Electron microscopy of the striatum after the injection of KA has shown that the corticofugal fibres that traverse the striatum are undamaged, as are the terminals of afferents to the striatum. These often have remnants of post synaptic membranes attached.

Despite the above evidence for an axon sparing action of KA, there is other evidence that indicates the action of KA might not be quite as specific for cell bodies as has been claimed. Damage to the fibre tracks passing through injected areas has been seen (639) and this seems to affect primarily the thicker myelinated axons, leaving the fine unmyelinated axons intact (353). It also seems that in some cases certain striatal cells seem to escape damage while others are destroyed. The resistant cells appear to be the large fusiform ones that may project to the SN (353). Reduced levels of tyrosine hydroxylase (TOH) have also been reported after KA lesions (98), and could be evidence for a non-specific, toxic action of KA. However, this could represent the dying back of axons resulting from the lack of any innervating tissue (444).

Many of the reports that indicate non-specific damage use higher doses and injection volumes than the other studies and this may be the reason for the non-specific damage.

The striatum has received a lot of attention in experiments with KA, possibly because it is an easily studied structure from many points: behaviourally it mediates known effects, it is easily dissected and cut for

biochemistry and histology. Also KA lesions of the striatum are thought to resemble the pathology of the striatum in Huntingdons' Chorea (107,109,179,279,384,527,528,536).

Intrastriatal KA leads to large depressions in several parameters of striatal activity. Levels of choline acetyltransferase (CAT) and ACh, choline uptake and ACh release are all reduced (195,384,537). GAD and GABA levels and GABA uptake and release are all affected by KA lesions in the striatum (195,384,421,537,538). Dopamine-sensitive adenyl cyclase (DA-AC) is also greatly reduced (537). Inconsistent results have been obtained in relation to TOH levels. They seem to increase (384), decrease (98) or show no change (179). These inconsistent changes could be due to the changing morphology after the lesion. Histofluorescence work has shown that DA increases near to the lesion, but it decreases further away from the lesion, presumably due to the "bunching" of the remaining tissue in the area (232). This would mean that results from biochemistry might depend on the method of tissue sampling. However, DA release seems to be relatively unaffected (195). DA binding changes in a manner consistent with the loss of the DA-AC (186). 5-HT release is also relatively unaffected although binding is reduced (195,536). The levels of glutamate and aspartate in the nucleus also decrease. GAD and GABA fall in the SN after striatal KA lesions (141,549,612).

Longer term studies after KA indicate that care ought to be taken in interpreting the results of biochemical experiments. Even months after the lesion was made, pre and post synaptic elements were seen in electron micrographs of the area. This means that binding studies may pick up sites that are on the lesioned cells, although they are not functional (654).

Rats lesioned with KA acid also show some behavioural changes, and changes in body weight, as do 6-OHDA lesions of the SN. These are discussed in the introduction to Chapter III.

Chronic unilateral lesions with KA will lead to turning behaviour in response to apomorphine (540). The acute effects of this lesion result in ipsilateral circling for about 2 hours after the injection, then this is followed by a period of from 10 to 36 hours of violent contralateral circling (293). These effects of KA are dependent on the presence of the cortico-striatal pathway and the ipsilateral phase is prolonged by the prior removal of the striato-nigral pathway. This is supported by the increase in SNR and SNC cell firing seen on acute application of KA (49,288).

NMA has been much less used than KA, but NMDA has recently been reported to cause similar postoperative changes in behaviour (585), although the contralateral running phase does not occur. Photographs of KA and NMA lesions are shown in Section 3.3.

As well as the neurotoxic lesions of the striatum, electrolytic lesions in the crus cerebri are also used to interupt striatal output. The position of this lesion is at a point where the striato-nigral pathway has been shown to run separately from the nigro-striatal fibres (206, 588). These lesions lead to ipsilateral turning when the animals are challenged with 2 mg/kg of apomorphine. The apomorphine activates the receptors on the intact side of the brain rather than preferentially activating those that have become supersensitive on the lesioned side as happens after 6-OHDA. The lack of supersensitivity also explains why a much higher dose of apomorphine is needed to cause turning in all the striato-nigral lesioned animals than in the 6-OHDA lesioned ones (2 mg/kg cf 0.3 mg/kg).

CHAPTER II

General Methods

In this chapter, all the methods that are used in both Chapters III and IV are described. These methods include all the lesion procedures: electrolytic lesions in the crus cerebri, KA lesions and NMA lesions in the striatum, and 6-OHDA lesions in the medial forebrain bundle. The 6-OHDA lesions are only used in Chapter III. They also include details of the histological procedures used as well as the biochemical ones for CAT and GAD and for the protein estimation. All other more specific methods are dealt with in the relevant chapters.

2.1 Histology

All tissues were stained by a modification of the luxol fast blue and cresyl violet method. The brains were frozen and then cut into 40 µm sections on the cryostat (Dittes). The sections were cut in the coronal plane and on cutting these were melted onto washed microscope slides (Chance Propper Ltd) and allowed to dry.

The slides marked with pontamine blue dye (Section 4.2) were left overnight in formalin vapour (BDH) to fix the dye, before staining. A list of the solutions and the times used is given below:

95% ethanol	30 min
0.1% luxol fast blue (BDH) in 95% ethanol + 0.5 ml 10% acetic acid per 100 ml	30 min (or longer if fixed in formalin)
70% ethanol/0.05% $LiCO_3$	10 s alternately in each, until the myelin (blue) and grey matter (unstained) were nicely differentiated
Distilled H_2O	5 min
Cresyl Violet (0.1%) (Fluka)	4 min
95% ethanol	5 min
100% ethanol	20 min or until blue and pink staining were both clearly seen
Xylene (BDH)	5 min or until mounted.

On removal from the xylene the slides were covered with cover-slips mounted in D.P.X. (BDH).

2.2 Lesioning procedures

Four types of lesions were performed: KA and NMA injections into the striatum, 6-OHDA injections into the nigro-striatal pathway in the MFB and electrolytic lesions of the striato-nigral pathway in the crus cerebri. All types of lesion were used in the behavioural experiments but only the KA, NMA, and the electrolytic ones in the electrophyisology experiments. After the experiments, the lesions were verified by one or more of the following methods: behavioural (turning), biochemical (GAD and CAT levels) or histological.

General surgical procedure

All operations were performed under halothane anaesthesia (Fluothane, ICI Ltd) with a 4% mixture in medical air, and maintaned at 1.5% through a rubber nose-cuff. After induction, the rats were placed in the small animal stereotactic frame (David Kopf), with blunt earbars, tip angle 45 degrees. The toothbar was set at 2.4 mm below the earbar zero. The nose of the animal was placed in a rubber nose-cuff, supplied with the anaesthetic and attached to a suction pump for removal of the expired anaesthetic.

A midline incision was made and the skull surface exposed to reveal the suture lines. A 2 mm burr hole was drilled with a dental drill at the points determined by the coordinates for the particular operation. Bregma was used as a reference point in all cases. In the cases where an injection was being made through a needle, the orientation of the needle tip was considered as this affected the position of the sphere of the ejected liquid.

The needle through which the injection was made and the lesioning electrode were both inserted through these burr holes. After the removal of the needle or the electrode, the hole was filled with bone wax (Ethicon Ltd) and the wound sprayed with antibiotic and fungicidal spray (Rikospray, Rikolaboratories Ltd) and the wound stitched together. The animal was then placed in a recovery cage to regain consciousness. Supplementary feeding (bread and milk) was given to aid the recovery process.

6-OHDA injections

The animals were pretreated with 50 mg/kg pargyline (Sigma) and 25 mg/kg desmethylimipramine (Geigy Pharmaceuticals) for 30 minutes before being anaesthetised and placed in the frame.

8 µg of 6-OHDA base were injected into the brain in a volume of 4 µl over 5 minutes into the lateral hypothalamic area in the region of the MFB. The injection was made from an Agla syringe, by a perfusion pump. The needle was left in place for 5 minutes after the end of the injection to prevent reflux up the needle track. The coordinates for the injection site were:

3.6 mm posterior to bregma
1.0 mm lateral to bregma
8.5 mm ventral from the skull surface

Kainic acid injections

2 µg of kainic acid (Sigma) were injected into the striatum in 0.2 µl of saline, the pH being adjusted to 7.4. The injection was made over 4 minutes from a Hamilton syringe (1 µl), attached to a 30-guage needle, and the needle was left in place for another 5 minutes to prevent reflux. The coordinates used for the injection were as follows:

0.5 mm anterior to bregma
2.5 mm lateral to bregma
4.5 mm ventral from the brain surface

After regaining consciousness the animals were further anaesthetised for 6-10 hours with pentobarbitone (Sagatal, May and Baker Ltd). This was to prevent the extensive convulsive activity seen in these animals for several hours after the injection.

In the animals used for the behavioural experiments, the side of the lesion, left or right, depended on the preferred paw of the animal (see Section 3.2).

N-methyl aspartate injections

Initially, a series of injections was given to determine the best doses of the neurotoxin to use. Those tried were 2.5, 5, 10 and 20 µg injected from a 1 µl Hamilton syringe through a 30-gauge needle, in volumes of 0.2, 0.4 and 0.8 µl of saline. Again, the pH was adjusted to 7.4. The injection was made at a rate of 0.1 µl/min and the needle left in position for a further 5 or 10 minutes to prevent reflux.

In initial experiments the same coordinates as were used for the KA injections were used, but it was later seen that the following led to a more effective lesion:

1.0 mm anterior to bregma
3.0 mm lateral to bregma
4.5 mm ventral from the brain surface.

Electrolytic lesions

Lesioning electrodes were made from twisted teflon coated stainless steel wire (0.16 mm diameter, Pheonix Wire Inc.), bared only at the tip. These were monopolar and were used as the cathode in the

circuit outlined in Figure 4. The lesion was made by giving 6 mC of current either once or twice. If the latter, the electrode was moved slightly before the second current application. Two sets of co-ordinates were used for this lesion, both placing the lesion in the same place, but using different approaches. The first approach used the following coordinates, putting the electrode into the brain at an angle of 46° to the vertical:

3.8 mm posterior to bregma
7.7 mm lateral oblique to bregma
6.0 mm
and ventral oblique from the surface of the brain
7.0 mm

The term "oblique" is used to allow for the angle of the electrode. The second injection was placed further down on the same penetration. The second approach was to put the electrode in vertically, at the following coordinates:

3.8 mm posterior to bregma
2.1 mm
and lateral from bregma
2.4 mm
7.8 mm ventral from the brain surface

In this case to make two lesions the electrode must be removed and replaced at the second lateral position.

Behavioural assessment of the lesions

Seven or 14 days after the operations, the animals were challenged with apomorphine (Macfarlane Smith Ltd) to determine the turning response to the drug. This was done by placing the rats in a hemispherical plastic bowl, 18 in. in diameter, and attaching them to a spring by a length of bandage. The spring was attached to automatic counters

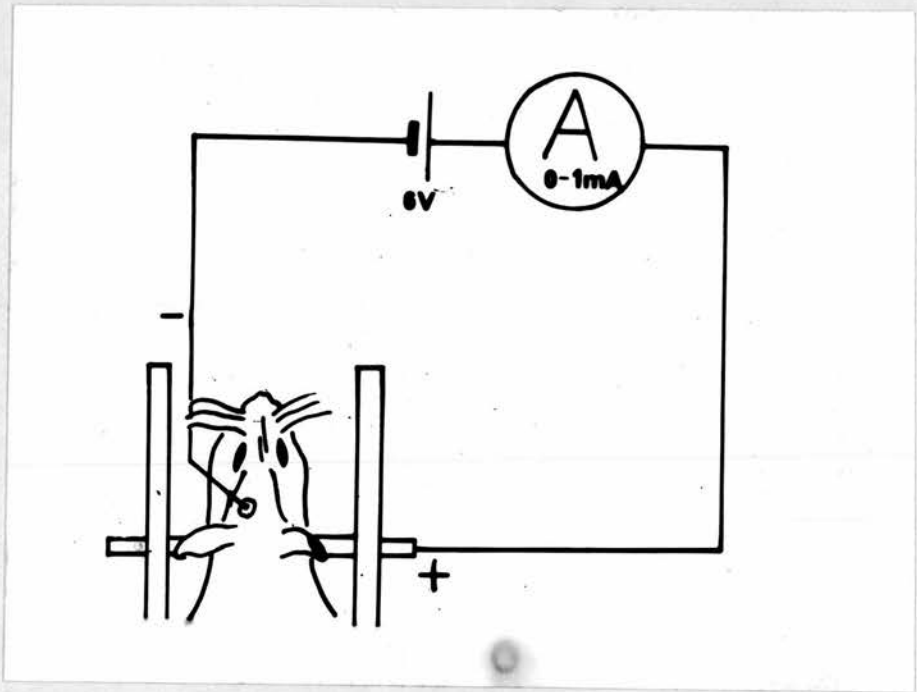


FIGURE 4: Diagram of the circuit used for making electrolytic lesions in the crus cerebri. The electrode is the cathode and the circuit is completed via the ear bar.

(Rat rotometers), which, by using photocells, count the number of revolutions made by the rat in each direction in a given time. The rats were observed frequently to ensure they were turning as slight inaccuracies could result if a rat "oscillated" about the counting point of the system.

The 6-OHDA lesioned animals were given 0.3 mg/kg apomorphine, dissolved in saline with ascorbic acid, intraperitoneally. A response of about 200 or more turns in one direction in 45 minutes was taken to be indicative of a lesion. The other lesioned animals were given 2 mg/kg of the apomorphine and about 100 turns in 30 minutes was taken to suggest a lesion.

Histological assessment of the lesions

The lesions in the crus cerebri and the striatum were examined histologically. In the case of the KA lesions, only the posterior part of the lesion was studied as the anterior portion (in front of the injection track) was used for biochemistry.

The procedure used to prepare and stain the tissue is described in Section 2.1.

Photographs of the lesions are shown in Section 3.3.

2.3 Biochemistry procedures

The biochemical effect of the KA lesions was determined by measuring the levels of GAD and CAT in the portion of the striatum just anterior to the injection site of the neurotoxin. The posterior portion was kept for histology (see Section 2.1). The NMA lesions were estimated by measuring the GAD in the substantia nigra ipsilateral to the lesion, and histology was done on the whole striatum. In each case the GAD assay was the same.

All brains were removed from the animals immediately on death which was caused by decapitation after stunning by a strong blow on the thorax. The samples were dissected out on ice as shown in Figure 5. After dissection the samples were wrapped in aluminium foil and dropped into liquid nitrogen where they were stored until the assay was performed. Each side of the brain of each animal was assayed individually.

In each case the results were expressed as nmol/mg protein/hour so the amount of protein in the samples was also measured.

CAT assay

The reaction catalysed by the enzyme is as follows:

$$1\text{-}^{14}\text{C-acetyl CoA} + \text{choline} \rightarrow {}^{14}\text{C-ACh} + \text{CoASH}$$

The method used was modified from that of Fonnum (189). The product, labelled ^{14}C-acetylcholine is bound to tetraphenylboron, in the organic phase of a two-phase solvent system. The organic phase is also the scintillant phase of the system and so the labelled product is in contact with the scintillant while the labelled precursor, which stays in the aqueous phase, is not. CAT activity is expressed as nmol acetylcholine produced per hour per mg of protein.

- *Reagents:*

1. Homogenisation Buffer:

   1 mM ethelenediamine tetra-acetic acid (EDTA), (BDH) pH 7.0, containing 1 µl Triton X 100/100 ml (BDH).

2. Incubation Medium:

   a) 100 mM EDTA (BDH), pH 7.4
   b) 200 mM choline chloride (Sigma)
   c) 3M NaCl (BDH)

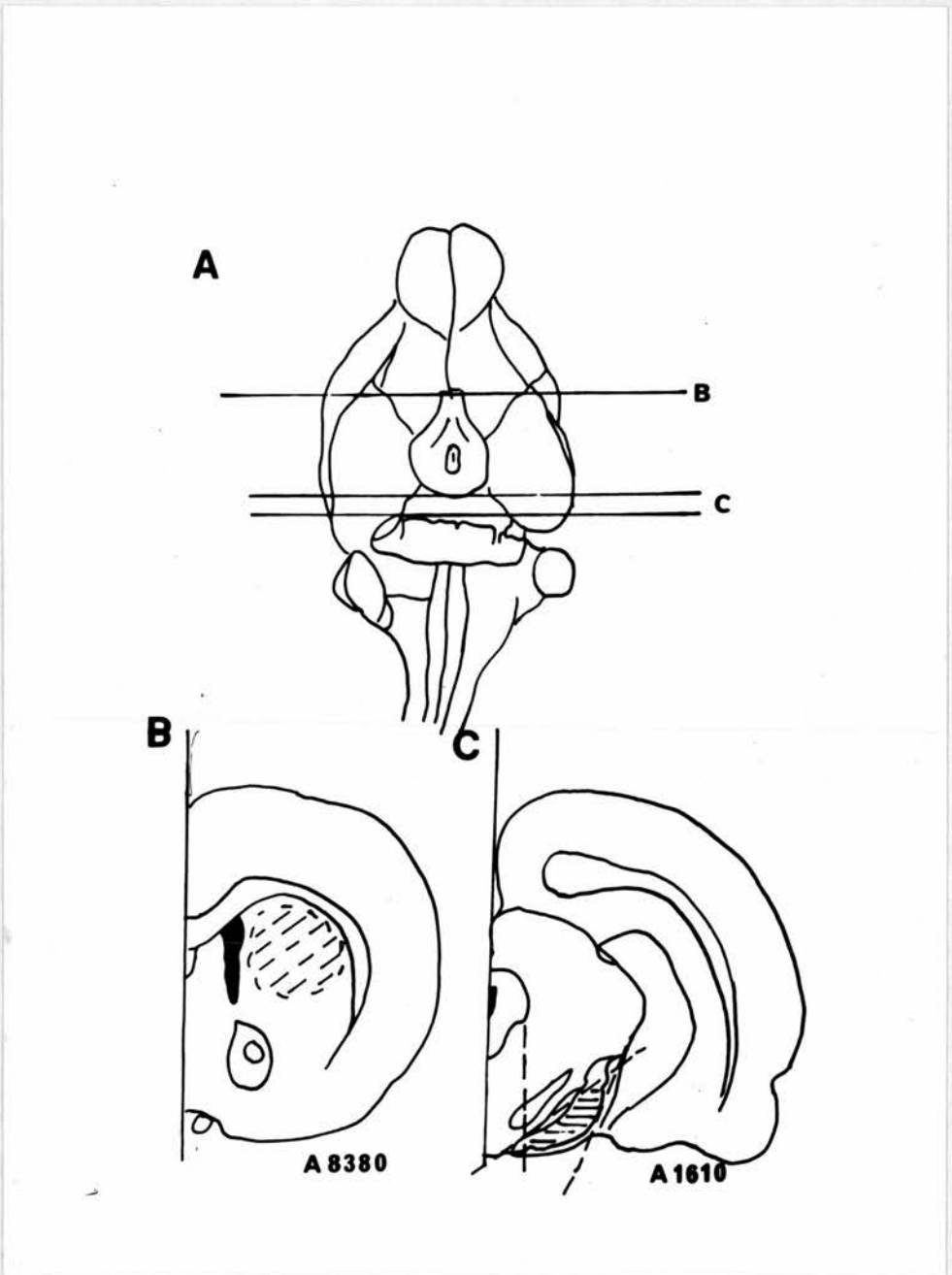


FIGURE 5: Dissection of the striatum and substantia nigra for biochemistry.

A. Base of the brain, showing the position of the cuts.
B. Dissection of striatal tissue: the area outlined with the dotted line was scooped out with a pair of small curved forceps.
C. Dissection of nigral tissue: the SN was isolated by scalpel cuts along the dotted lines and then removed with forceps.

d) 500 mM NaH_2PO_4 buffer, pH 7.4 (BDH)
e) 2 mM eserine salycilate (Sigma)
f) 9 mg/ml bovine serum albumin (BSA) (Sigma)
g) (1-^{14}C) acetyl CoA (Amersham Radiochemical Centre); solid (50 dpm/nmol) taken up in 2 mM unlabelled acetyl CoA to give 18,500 dpm/nmol.

Each 100 µl of medium was prepared as follows:

20 µl 100 mM EDTA
10 µl 200 mM choline chloride
20 µl 3M NaCl
20 µl 0.5M NaH_2PO_4 buffer, pH 7.4
10 µl 2 mM eserine salycilate
10 µl 9 mg/ml BSA
10 µl acetyl (1-^{14}C) CoA

3. 10 mM NaH_2PO_4 buffer, pH 7.4, containing 18 mg unlabelled ACh in each 500 ml.

4. 186.9 mg sodium tetraphenylboron (Sigma-important) dissolved in each 20 ml of acetonitrile (Koch Light Laboratories).

5. Scintillant: 8.5 g 2,5-diphenyloxazole (PPO) in 2 l toluene (BDH).

6. Internal Quench Standard (^{14}C-Me)-toluene, 2µCi (Amersham Radiochemicals Ltd). Toluene is diluted approximately 1 in 4 with cold toluene (BDH), to give a solution of around 20,000 dpm/80 µl. Volumes were determined by weight for accuracy. The density of unlabelled toluene is 0.8645 g/ml at 20°C.

- *Procedure:*

1. Tissue was homogenised in 50 µl/mg of the homogenisation buffer in an Eppendorf tube. If the required volume was more than 200 µl, the homogenisation was done in 200 µl and then diluted later. This prevented overflow from the tube during homogenisation. This homogenate was then further diluted tenfold, to make a 0.2% homogenate. This was then used for the assay and the undiluted

homogenate kept for the protein estimation. The homogenates were kept on ice until needed.

2. The incubation medium was made up on ice.

3. 2 ml of the tetraphenylboron/acetonitrile mix, 5 ml of the 10 mM NaH_2PO_4 and acetyl choline and 10 ml of the scintillant were added to the required number of scintillation vials.

4. 10 µl of diluted homogenate and 10 µl of incubation medium were added together in an uncapped Eppendorf tube and incubated for 20 min in an Eppendorf Dri-bloc at 37°C.

5. The reaction was stopped by shaking for at least 5 s in the scintillation vial, ensuring the fluid penetrated the Eppendorf tube. Care was also taken not to shake too vigorously as this prevented the phases separating cleanly.

6. The vials were then left in the scintillation counter for at least an hour before counting to allow chemiluminescence to settle. After this the vials were counted for 4 min each.

Each sample was assayed in duplicate, as were the blanks. Extra blanks were included to allow for quench correction. For this, 80 µl ^{14}C toluene was added to the vials along with 10 µl of a dilute homogenate, before placing in the counter.

- *Calculation:*

The results were calculated as follows:

$$\text{nmol/hr/mg protein} = \frac{\text{cpm (sample) - cpm (blank)}}{\text{dpm added in 80 µl toluene/cpm (blk+tol) - cpm (blk)}} \times \frac{1}{18500} \times \frac{1000}{\text{mg protein}} \times 3$$

(1/18500 (dpm to nmoles); 1000/mg protein (from µg to mg); 3 (up to 1 hour))

GAD assay

The reaction catalysed by this enzyme is:

$$1\text{-}\ ^{14}\text{C-glutamate} \rightarrow \text{GABA} + {}^{14}CO_2$$

The method was adapted from that of Drummond and Phillips (157). The radiolabelled $^{14}CO_2$ is taken up in Protosol, mixed with the scintillant and then counted. The results are expressed as nmol carbon dioxide produced per hour per mg protein.

- *Reagents:*

1. Homogenisation Buffer:

   100 µl of 10% Triton X-100 (BDH) and
   100 µl of 1 mM KPO_4 buffer, pH 6.5.

   The buffer is made up of 300 ml of solution A (below) with approximately 100 ml of solution B - as much as is needed to obtain the correct pH.

   A: 54.4 g/400 ml KH_2PO_4 (BDH)
   B: 34.84 g/200 ml K_2HPO_4 (BDH).

2. Incubation Medium:

   a) 1M KPO_4 buffer (made as for 1 mM, but using gram quantities).
   b) 10 mM pyridoxal phosphate (Sigma) made up daily.
   c) 10 mM dithiothreitol (Sigma) made up daily.
   d) 10 mM sodium arsenite (BDH) made up daily.
   e) 100 mM sodium glutamate (Sigma) made up daily.
   f) 1% Triton X-100 (BDH).
   g) (1-^{14}C) glutamic acid, 55 mCi/mmol, 50 µCi/ml (Amersham Radiochemical Centre).

3. 6N H_2SO_4 for stopping the reaction.

4. Scintillant NE260 (Nuclear Enterprises Ltd).

- *Procedure:*

1. The tissue was removed from the liquid nitrogen, placed in an Eppendorf tube and homogenised in 20 µl of homogenisation buffer per mg of tissue, giving a 5% homogenate.

2. The incubation medium was then made up on ice.

3. 10 µl of the incubation medium was placed in the required number of Eppendorf tubes placed inside insert vials in scintillation vials containing 250 µl Protosol. This whole procedure was carried out on ice. 10 µl of the homogenates was then added to the incubation medium and the vials were placed in a shaking water bath at 37°C for 20 min.

4. After the 20 min, the reaction was stopped by adding 100 µl of 6N H_2SO_4 through the rubber seal. The vials were then left for another hour in the water bath to ensure that all the CO_2 had been taken up by the Protosol.

5. The seals were then removed and the outside of the insert vials washed with 2 ml ethanol to remove any Protosol/CO_2. The ethanol was collected in the vial.

6. 10 ml of scintillant was then added and the vials placed in the counter and counted for 4 min each.

The results were calculated as follows:

nmol/hr/mg protein = cpm - cpm (blank)

x 3 (to hours)
x 1.23 (correct for 83% efficiency)
x 1000/µg protein in 10 µl homog. (to mg)
x 1/552.5 (cpm to nmol)

Protein estimation

The method of Lowry *et al* (372) was used to estimate the amount of protein in each homogenate. This method consists of reacting the protein with copper and then quantifying the protein-copper complex spectrophotometrically.

- *Reagents:*

1. 2% Na_2CO_3 in 0.1N NaOH.
2. a) 1% $CuSO_4.5H_2O$
   b) 2% Na.K.Tartarate
3. 50 parts 1 + 1 part 2 (equal parts of a and b).
4. Folin Ciocalteau reagent (BDH).

- *Standards:*

Standards for the range 15-60 µg protein were used and more than covered the range in the experimental samples. 2.5 ml/ml BSA was used as the standard stock solution. 100-400 µl of this were used to obtain a standard curve. This volume was diluted to 5 ml and 0.3 ml was used for the estimation.

- *Method:*

1. 10 µl of the homogenate used for the GAD assay and 50 µl of the undiluted homogenate for the CAT assay were diluted to 0.3 ml with distilled water.
2. 3 ml of reagent 3 were added, mixed and allowed to stand for 15 min.
3. 0.3 ml of reagent 4 was added, mixed and allowed to stand for 45 min.
4. The amount of product was then read on a Gilson Spectrophotometer at 750 nm.

The results were calculated by averaging the readings at different amounts of standard on the linear part of the curve. Above this range it becomes non linear, and values must be read from the graph. A reading for 1 µg of protein was calculated and this was then used to estimate the amount of protein used in each sample.

Dopamine estimation

The measurement of DA in Chapter III was done for me, using a radio-enzyme assay, a modification of the methods of Coyle and Henny (106) and Palkovits *et al* (452).

CHAPTER III

The Function of the Nigro-Striatal Pathway Studied by Operant Behaviour Techniques

In the introduction to this Chapter, the background detail to the experiments is given, followed by the experimental rational and the methods not already given. After this the results are presented and discussed.

3.1 Introduction

Lateral hypothalamic syndrome and nigro-striatal lesions

It has long been known that lesions in the lateral hypothalamic (LH) area cause severe aphagia and adipsia in the lesioned animals. This leads to death more rapidly than food or water deprivation would (379). If given food and water intragastrically, the LH lesioned animals will eventually begin to eat and drink spontaneously again, but not in a normal fashion. They do not eat in the heat and do not respond normally to cellular dehydration. Also, they will not drink at all in the absence of food and will not eat in response to lowered insulin levels as control rats will. However, they will overeat in a cold environment. The LH lesioned rats also show taste preference abnormalities: they will refuse water made bitter with quinine at much lower levels than that refused by control rats (379). It was later seen that the aphagia and adipsia caused by these lesions could be mimicked by damage to the ascending DA systems resulting either from injection of 6-OHDA into the LH, SN or ventral tegmental area (VTA), or from electrocoagulation of the SN (21,184,379,594). This suggested that the effects of LH lesions are mediated by the DA fibres passing through that area, in the MFB, rather than by cells with cell bodies in that area. KA injections, thought to destroy cell bodies and leave passing axons intact, do affect ingestatory behaviour and so possibly not all effects of LH lesions are caused by the lack of DA fibres (576). Animals lesioned electrolytically in the LH show different food preferences after

the lesion from animals with depletions of DA only (379). However, despite this there is a good case for the involvement of DA in the LH syndrome.

As further evidence for the involvement of DA in causing the aphagic and adipsic effects of the lesions, these can also be produced by neuroleptic administration (657) and by electrolytic (419,532) and KA lesions (459,527,528) in the striatum. The electrolytic striatal lesions are similar to the LH ones in that the DA terminals are destroyed. However, in the KA acid lesioned animals, the DA terminals are intact, but the cells they synapse with are destroyed and so the effects might be expected to be the same. However it appears that the feeding behaviour after KA lesions in the striatum is different from that seen after the LH lesions. The KA lesioned animals show a prolonged reduction in body weight when compared with controls, but when food and water ingested are monitored there appears to be no difference between lesioned and control animals. These animals have been reported to show increased locomotion at night (527) and so they may have higher calorific requirements than controls, and the lower body weight is explained by a reduced "set point" as has been suggested for the LH lesioned animals. The KA acid animals will also increase their water consumption if they are deprived of food (non-prandial drinking) unlike the LH animals which only take water to help digest dry food pellets. Amphetamine induced anorexia is increased in the KA lesioned animals whereas it is decreased in the LH lesioned animals (184). The former ones also show increased stereotypy, unlike the LH ones which show decreased stereotypy in response to amphetamine (50a). The increased anorexia may be due to increased competition between the two activities, eating and stereotypy.

The DA systems passing through the LH are also thought to be involved in the mediation of intracranial self-stimulation (ICSS), where rats will learn to lever press to give themselves electric current in certain brain regions. This process is thought to activate a reward mechanism in the animals (114,180). Rats will also learn to lever press for food reward and for intravenous administration of amphetamine or apomorphine (127), as well as for ICSS, further supporting a rewarding role for DA in this system.

The anatomical localisation of sites in the brain which will support ICSS corresponds well, but not exclusively, with the DA distribution in the brain (113,180,632), some of the most effective sites being in the LH, near to where lesions produce the LH syndrome symptoms. The area of importance in the striatum for this behaviour is possibly the ventral, anterior striatum, as injections of 6-OHDA in this area affect ICSS, as do crystalline DA implants (418a,420). Studies of fluorescence build up of DA after the LH lesions shows that DA builds up in the SN and VTA DA neurons (85). The involvement of NA in the rewarding effects of ICSS and neuroleptic administration has also been suggested (113,632) but the extent of this involvement has been questioned (180). It is possible another, different, reinforcement mechanism exists using NA (114).

The interpretation of the LH syndrome and lever pressing experiments is complicated by the close involvement of DA in the motor behaviour of the animals. As well as being aphagic and adipsic, animals suffering from the LH syndrome do not show much spontaneous movement and have a strongly depressed response to sensory stimulation. It is possible that this may contribute to the eating and drinking deficits. However, these animals do display consistent regulatory changes in body

weight and motivational changes in feeding "preferences" once they have started feeding spontaneously again (379). This fact is difficult to explain on the basis of a purely sensorimotor deficit, although the possibility of this being the case has been suggested because of the lack of movement shown by these animals.

Bilaterally LH lesioned animals are difficult to design experiments for, as they are so akinetic. Also it is difficult to differentiate between an animal that cannot perform or learn a task, and one that simply does not want to.

Manipulation of lever pressing behaviour is a much easier model to use than that of the bilaterally LH lesioned animal. DA antagonists will cause an attenuation of lever pressing for ICSS (656,632), as will removal of the reward on pressing (191,634,635). This led to the formation of the "anhedonia" hypothesis which states that neuroleptics reduce the performance in lever pressing tasks by reducing the rewarding value of the reinforcer (634). More support for this involvement comes from experiments with 6-OHDA : lesions of the MFB with 6-OHDA also lead to reduced pressing rates for ICSS (181). Lever pressing to self-administer drugs is also attenuated by DA antagonists (644), but not by NA blockers. Neuroleptics will also reduce lever pressing for food reward, and will do so in similar doses to those that reduced the other pressing behaviours (512).

Very low doses of neuroleptics induce a higher rate of pressing throughout a session when pressing for amphetamine (504,644,495). This increases the total amount of drug given, and argues strongly along with all the above data in favour of neuroleptics decreasing the rewarding effect of the reinforcers. The animal will increase drug injection itself until the previous reward level has been reattained.

It has been suggested that the reduction in lever pressing on the administration of a neuroleptic is mediated purely by sensori-motor deficits rather than by a reduction in reward (181). In one study, it was shown that the rats did have the motivation to feed, as shown by free feeding measurements (181), in the presence of a dose of neuroleptic that reduced lever pressing. Also, using a variable interval schedule (so that ICSS and food rewarded pressing rates were the same), there was no increase in response rate before the fall in rate, as happened when the reward was stopped on execution of the behaviour (181). The same happened with 6-OHDA lesioned animals. Evidence against the effect of neuroleptics being caused by a block in the rewarding impact of the result of lever pressing also comes from experiments that show that the effects of pimozide and extinction are additive (381,461). If the action was due to the removal of reward, there would be expected to be no further effect of pimozide on top of extinction. Also the effects of pimozide treatment fail to transfer to the extinction case as also would be expected if it was acting by reduced reward (381). However, another study has in fact shown this transfer effect (634). Using a differential reinforcement of low rates paradigm, pimozide failed to cause a decrease in responding (381). This was taken as evidence that the rats could press slowly, but because of the deficit in performance caused by the drug they could not press quickly. The fact that rats can press slowly, but not quickly, means that the effect of the drug could no longer be seen at low pressing rates. Therefore reduction in performance would not be seen in studies with low responding rates throughout (461). In one such study, although the deficits in rate were not so clear, the fact that there was a reduction in rate before the first reward was given supports a motor deficit

involvement (461). It also argues against a reduced reward effect in the presence of the neuroleptic, as the rate falls before the reduction in reward could be detected by the animals. In reviewing the literature in the discussion of this paper the author concludes that the deficit cannot be only in reward, nor can it be a single motor fault. The explanation given is that the deficits in performance are due to a reduced ability to initiate movements (461).

A certain amount of confusion arises in the literature over what constitutes a motor deficit. In the paper quoted advocating an impaired motor ability, the definition of a deficit uses the concept that the affected animals cannot *maintain* a high pressing rate (461). The other studies mentioned, advocating the involvement of some other mechanism, defines the motor deficit as an inability to perform at a high rate at all (190a,635). This is obviously going to lead to differences in interpretation.

From all the above data it is clear that unresolved conflict has arisen from the results of the lever pressing experiments as to the reason for the reduction in pressing rate caused by neuroleptics and, by inference, what the role of DA is in the nigro-striatal pathway. The evidence for and against a reduction in the reward value of the reinforcers was reviewed in 1978 by Wise (631) and again more recently (633). In these reviews, Wise clearly favours a block of reward rather than a motor deficit and states that a difference in interpretations could come from the different nature of the reinforcers used. Food and amphetamine or apomorphine, when used as a reinforcer, are temporarily satiating, that is, the reinforcer temporarily saturates the rewarding mechanisms, and the animals will not respond again until the mechanism is no longer saturated. ICSS, however, is not satiating

in that the rewarding effect ceases immediately the stimulus stops and therefore the rat can reach much higher, or even maximal, pressing rates, this would lead to an almost immediate fall in pressing rate when reward is withheld or a neuroleptic given (assuming it did act by a reward reduction mechanism). This difference accounts for the increase in rate of pressing for drug self-administration on treatment with a neuroleptic (641,642) and the lack of increase in some ICSS experiments (191). However, in most of the ICSS experiments, the response rate is usually initially normal until the rat "realises" that the rewarding effect of the particular behaviour is no longer present. There are exceptions to this too, however: in one experiment, using a variable ratio design, the pressing rate was seen to fall before the first response had been given (461).

The problem of different reward values in the different experiments can partly be overcome by making the animals run along an alley before pressing for ICSS (190a). When rats are given neuroleptics in this experiment, no impairment of the running before reaching the lever was seen.

In the recent review (633) Wise has put forward a revised form of the "anhedonia" hypothesis already mentioned (634). This hypothesis suggests that:

> "The most subtle and interesting effect of neuroleptics is a selective attenuation of motivational arousal which is a) critical for goal directed behaviour, b) normally induced by reinforcers and associated environmental stimuli, and c) normally accompanied by the subjective experience of pleasure."

Because of the conflicting evidence and opinions on the role of DA and neuroleptics in the nigro-striatal pathway, it is clear that other experiments than the ones already described must be considered. Evidence from some of these is now discussed.

Neuroleptics cause greater disruption of ICSS than they do of open field exploratory behaviour (511). This suggests a specific role for neuroleptics in blocking ICSS but not for a general inhibition of movement. Neuroleptics also interfere with the acquisition of some tasks. Pimozide will cause rats to learn to press more slowly for food than in the control state (633). Rats will not learn the best way to escape from an underwater Y-maze if they are treated with neuroleptics or if they have bilateral 6-OHDA lesions of the DA system (484,486). This evidence has been used to suggest a learning deficit in the animals, as the motor problems have been overcome by forcing the animals to swim. Using a conditioned stimulus to train rats to lever press it was shown that rats which were given haloperidol during the conditioning period did not learn to press for the conditioned stimulus. This must be an effect of reduced reward value of the reinforcer or learning as no motor response was demanded from the animals during the conditioning period and so a motor deficit caused by the neuroleptic cannot be involved. It seemed that the effectiveness of the primary reinforcer, amphetamine, in rewarding the rat was reduced by the haloperidol (126). Acquisition of other classically conditioned responses is also affected by neuroleptics (30,31,185). For example, neuroleptics interfere with the learning of foot shock avoidance, but do not interfere with foot shock escape (185). Also, although the rats failed to learn the task while under the influence of the neuroleptic, if the animals were tested after the effects of the drug had worn off, they were no different from controls that had had saline for the same time that the others had had neuroleptics for (185). This suggests that the rats had leanred the task and that the deficit was an inability to initiate movements, rather than a reward or a simple motor deficit or a learning deficit. Amphetamine

enhances passive avoidance behaviour after acquisition and increases extinction time on removal of the stimulus and haloperidol has the opposite effect (352). These effects were noted at doses well below those that caused a deficit in exploratory behaviour, again arguing against a motor deficit. As with the operant behaviour experiments, the effects of extinction and neuroleptics were additive and indicates that it is possible some factor other than a block of reward is involved, but as the exploratory behaviour was not affected, a gross motor abnormality could not have existed.

Rats lesioned with KA also show behavioural changes. Rats lesioned bilaterally with KA show an increase in locomotory behaviour, but only in the dark (179). Apomorphine stereotypy is not enhanced, but amphetamine stereotypy is (179). Using a passive avoidance test, KA lesioned animals have been shown to take longer to learn and they show less retention than controls. They showed no deficit in locomotory behaviour, so they were unlikely to be suffering from a gross motor deficit (179,528). The learning deficit may have resulted from an inability to suppress the step down response rather than a learning or retention problem and so the experimental design is possibly not ideal for investigating the cause of the deficit caused by the lesion. In a lever pressing task for food, the controls and lesioned animals learned at the same rate, but the KA lesioned animals responded more during extinction trials. This again could reflect an inability to suppress certain behaviours.

Electrolytic lesions of the anterior striatum impair delayed alternation but does not affect visual discrimination (418a). KA lesions also cause these changes (147). KA acid lesions of the anterodorsal striatum also impair the ability of the rats to acquire a spatial alternation task (179).

As the KA lesions destroy the cells post-synaptic to the nigro-striatal ones, it would be expected that they would mimic the effects of the 6-OHDA lesions. From the above it does seem that this is the case and supports a role for the DA pathway in these behaviours.

In summary, the data seem to support a role for neuroleptics in blocking the rewarding effect of the reinforcers and indirectly for a role for DA in reward. However, there is also evidence in favour of a motor deficit and a learning deficit, and it is highly probable that DA has multiple effects in the brain. It also seems that at least some of the effects of DA on behaviour are mediated by the nigro-striatal pathway.

Induced or inherent nigro-striatal asymmetry

Unilateral damage to the nigro-striatal system is not nearly so debilitating to the animal as a bilateral lesion, but does still cause changes in behaviour. These are easier to study because of the less akinetic behaviour of the animals.

Animals with a LH or SN lesion, either electrolytic or 6-OHDA, show a strong sensory neglect on the side of the body contralateral to the lesion (368,380). This neglect gradually recovers from about 1 week after the lesion onwards. It seems that the lack of orientation to sensory stimulation in the lesioned animals is not due to a motor deficit as the different sensory modalities recover at different rates, tactile stimuli never fully recovering (175,368). During recovery the rat will orientate to one stimulus type but not to another. The sensory inattention, produced by a 6-OHDA lesion, can be reversed by apomorphine and reproduced again by administration of neuroleptics (378). In one study of sensory inattention, some animals showed no recovery of response to touch after 4 months whereas some recovered after about

1 month (377). The extent of recovery was correlated with the extent of the DA depletion measured at the end of the experiment. In the spontaneously recovered animals, the neglect could be reinstated by giving the animals α-methyl-p-tyrosine. Partial recovery of the asymmetries caused by unilateral 6-OHDA lesions of the nigro-striatal path could also be achieved by giving the animals a transplant of embryonic SN tissue (41,42,159), or by injecting a DA cell suspension into the striatum (161). These procedures cause a reinnervation of the cells by DA axons and these seem to function in some way similarly to the removed DA pathway even although there is no actual input from the SN.

Experiments have also shown that the sensory neglect can, to some extent, be reversed by conditioning (545). The arousing effects of a noxious tail stimulus on the EEG of the animals is removed by the lesioning of the DA pathway (544). If the stimulus is applied along with a conditioned stimulus, the arousing effect is again seen despite the lesion. It seems therefore that conditioning can amplify sensory signals and cause a response to the signal to go via a route not involving the SN DA system (545).

As well as causing sensory neglect, unilateral lesions of the nigro-striatal DA pathway result in a strong rotatory response to apomorphine (contralateral) or amphetamine (ipsilateral) (592,593,595). The former is due to a greater action of the drug on the lesioned side which has become supersensitive as a result of the lesion. The latter is suggested to be due to the release of DA from the intact terminals. This is evidence in favour of a motor function of the nigro-striatal system.

Unilateral lesions also lead to changes in operant behaviour. Recent experiments in this laboratory have shown that the assumption that the effect of a lesion on behaviour will be the same, whichever side of the brain it is given on, is not necessarily correct (590). It was shown that a 6-OHDA lesion on the side of the brain that was ipsilateral to the paw the animals chose to use most often in the pressing behaviour, was far less disruptive to the lever pressing than a lesion contralateral to the rats "preferred paw", that is the one the rat chose to use most often. Figure 6 shows the results of these experiments (adapted from 590). The group operated contralaterally to the initially preferred paw show a decrease in total performance in each 30 min session and they also have a tendency to use the initially "unpreferred" paw more than the other one. This is not seen in animals operated on the side ipsilateral to the preferred paw or in controls. Eight weeks later, the performance level has returned to normal, but the paw preference remains changed.

Several other studies have recently used unilateral 6-OHDA lesions to study ICSS (84, 348,460). In these the lesions are placed ipsilateral to the ICSS electrode in experimental animals and contralateral to it in the control animals. These studies produced results that to some extent were conflicting. In one, only transient deficits in pressing were seen when the lesion was contralateral to the electrode, whereas longer disruption was observed when the lesion was on the same side as the electrode (348). This experiment supports a role for the DA system in reward processes. Another of these studies observed equal disruption when the lesion was both ipsi- and contralateral to the ICSS electrode and concluded that DA was not involved in reward and that the attenuation must be due to a non-specific motor deficit caused

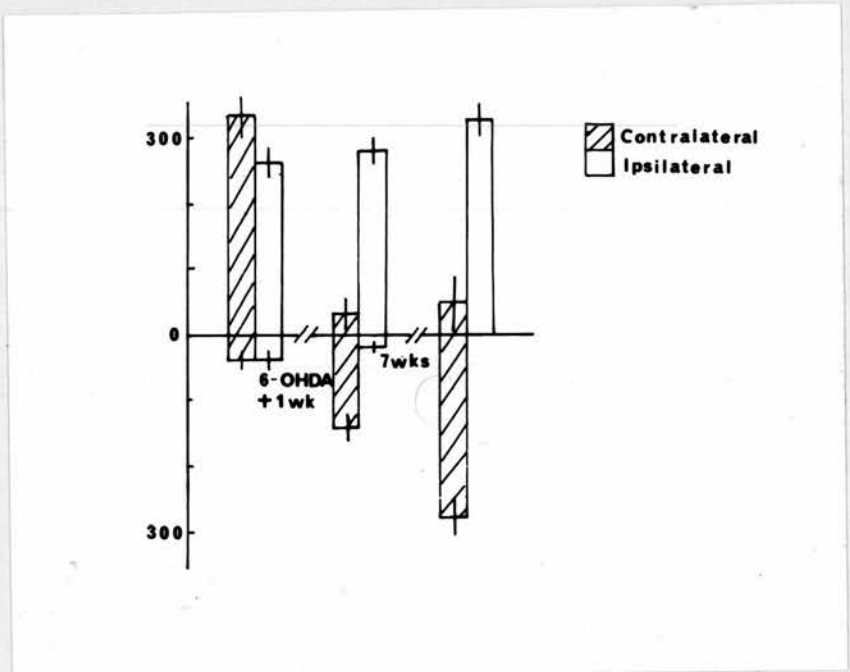


FIGURE 6: Effect of ipsilateral and contralteral 6-OHDA lesions in the MFB on pressing behaviour.

The vertical axis represents the number of presses totalled over two sessions at each of the three times. Presses with the preferred paw are shown on the upward axis and those with the other paw are shown on the downward one. Presses not with an individual paw are not shown.

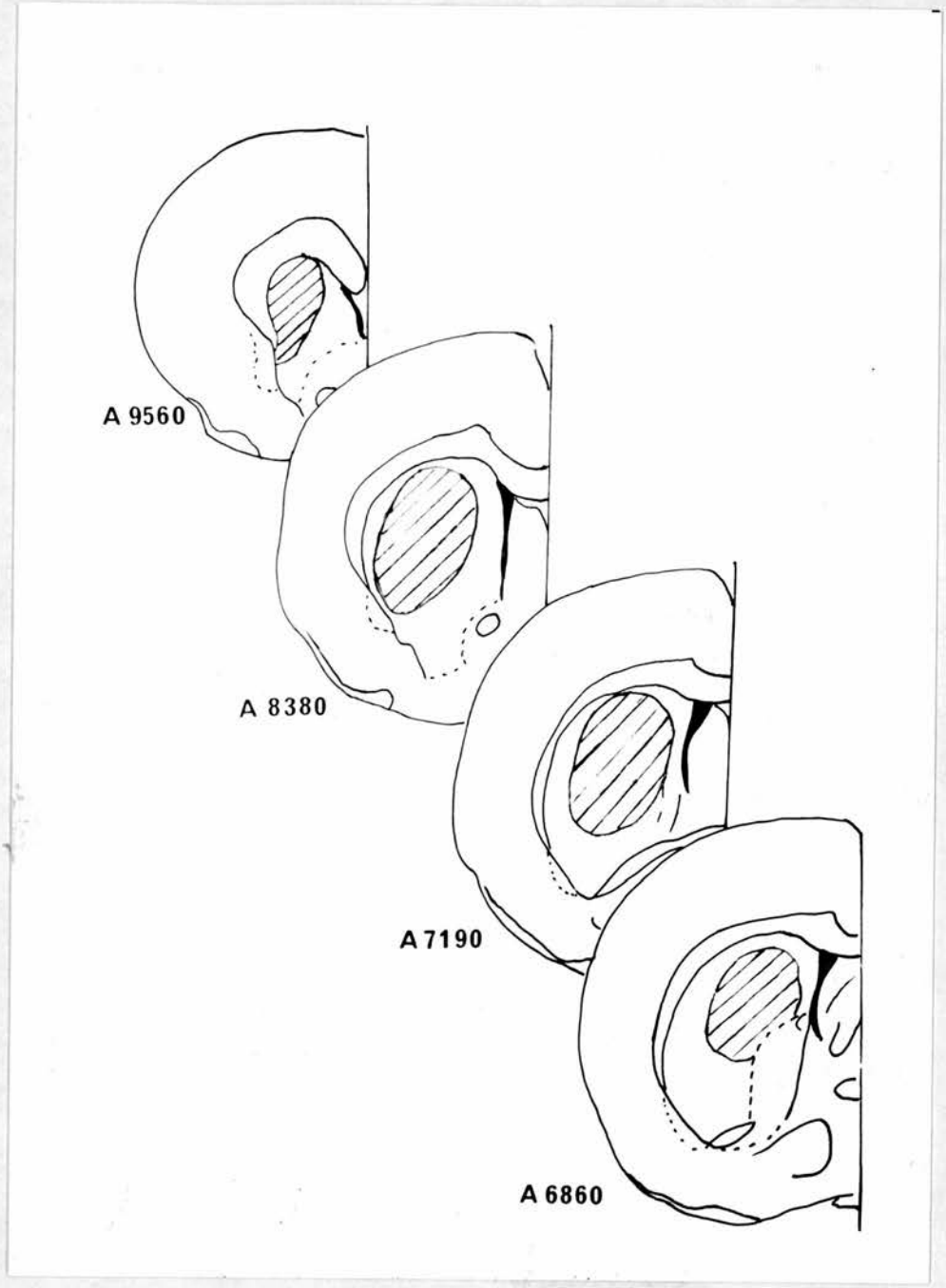


FIGURE 7: Diagrammatic representation of a typical NMA lesion that resulted in a change in paw use and turning in response to apomorphine. Some lesions were larger than this, but this example indicates the area of damage common to all the effective lesions. The more posterior part of the lesion seemed to be needed for the turning response, whereas the anterior part was also necessary for the change in paw use.

by the lesion (460). The third study found transient disruptions when the lesion was made on either side, and these recovered in 8-10 days (84). These also concluded that DA could not be important in mediating the effects of ICSS. The differences could possibly be explained if these lesions were made contralaterally or ipsilaterally to an inherent preference of the animals.

That rats do have inherent discrepancies between their DA systems has been extensively reported by Glick and coworkers (219-227, 297-299,656). Rats normally have a 10-15% difference in the levels of DA in the two striata (656) and this difference is correlated with the side preference of the animals in a T-maze, passive avoidance test. The higher DA levels correlate with the side contralateral to the preferred side. Rats sometimes show spontaneous turning behaviour when tested in a rotameter (225) although the levels are far lower than those induced by apomorphine in the lesioned animals. Amphetamine increases the asymmetry in DA levels (223) and it will cause circling in the rotameter in a direction contralateral to the higher DA level (219,220,222,227,297). Apomorphine will also cause rotation in rats and cats (298,299). Glick's laboratory has shown that out of 602 rats there appeared to be a right sided population bias (226).

In a differential reinforcement of low rates design (DRL), better performance is related to a greater side preference (220) as was the time taken to learn a passive avoidance task (519).

When unilateral caudate lesions are made, it appears that the rotation in response to the drugs is reduced or reversed if the lesion is contralateral to the initial direction of turning, but will enhance it if the lesion was ipsilateral to the initial direction of turning (298). Similarly lesions ipsilaterally to the rats preferred side in a T-maze

will enhance the side preference and facilitate passive avoidance learning (519).

Although Glick's work has concentrated on side preference, he has also considered paw-preference. In a bar pressing task for water reinforcement, most rats showed a consistent side preference (222). High pressing rates were correlated with greater side preference and there was a correlation between side and paw preference: 75% used the same paw as lever (224). In relation to preference, subtle differences appeared on different reinforcement schedules, depending to some extent on the relation between paw and side preference (220,222). This is discussed in detail in the discussion.

Stimulation of the caudate nucleus during the performance of a task will also cause a change in side preference, leading to a "strong overattention" to the opposite side to the stimulation (655). This supports the hypothesis that asymmetry is due to the overactivation of one striatum.

Another group, has also studied paw preference in rats. They have shown that an injection of 6-OHDA into the side of the brain ipsilateral to the side the animals preferentially reached for food with, caused little effect on the reaching behaviour. On the other hand, a lesion contralateral to the preferred limb caused a decrease in responding and also a tendency to use the other limb (546). This is similar to the experiments on paw-preference already described from this laboratory (590).

If the initial acquisition of the task was made 1 month after the lesion, all the animals learned to reach for food with the ipsilateral paw (546).

Experimental rationale

In this chapter the performance deficit seen in Uguru-Okori and Arbuthnott's experiments (590) is further investigated, with a view to further elucidating the nature of the deficit. The results described already were repeated and extended, and other disruptive procedures were administered to the animals in an attempt to reproduce the performance deficits seen in unilaterally 6-OHDA lesioned animals.

The first hypothesis tested was that a motor deficit on one side was sufficiently disruptive to cause a performance deficit as the animals started to use the other paw. In order to do this various motor lesions were given to the animals. These were neurotoxic lesions (NMA and KA) in the striatum, electrolytic lesions of the striato-nigral pathway in the crus cerebri and temporary, peripheral paralysis of the preferred forelimb with a local anaesthetic. The effects of these procedures on performance and preference were studied after each procedure.

The second hypothesis tested was that, because of the motor impairment of the preferred limb, the rats had to relearn the task with the other paw and that this was what caused the drop in pressing rate while the rats were in the process of changing paw use. To test this, the rats were trained to use the initially unpreferred paw and the pressing rate was monitored while the rats were swapping.

As well as the above, the effect of unilateral 6-OHDA lesions on the initial acquisition of the task was investigated, as was the effect of an ipsilateral lesion on forcing the rats to use the initially unpreferred paw. Further analysis of the relation between paw and lever preference was also carried out.

The results presented here show that neither of the hypotheses are valid and that none of the procedures used mimicked the effect of

6-OHDA in reducing total pressing rate although they all (except the ipsilateral procedures) caused a change in the paw preferentially used. 6-OHDA lesioned rats all learned to press with the paw ipsilateral to the lesion, and the animals lesioned before training could not be retrained to use the contralateral paw after the initial training had been completed. Some of these results have already been presented (253).

3.2 Behavioural methods

In most experiments, rats were pretrained to press for food in the standard Skinner box. The experimental procedures were then carried out, the animals replaced in the box and retested. In some of the 6-OHDA experiments, the operation was carried out before any training. In the case of the animals trained to swap paws the procedure was, of course, carried out in the box. The effects of the various procedures on learning or performance were then determined.

Initial training procedure

Male albino Wistar rats weighing 150-200 g were used in all experiments except where stated. The size was chosen so that the animals would be the correct weight (200 g) for the coordinates when they were ready for the operation. Rats to be given no pretraining were started at 180-200 g.

All animals were daylight reversed for at least 10 days before the start of the pretraining sessions. They were kept 14 hours in daylight and 10 in the dark, and all training was done early in the dark period. Also they were food deprived for 24 hours followed by a short feeding period, 1 hour, at the start of the next 24 hours, at the end of which the training procedure was started. This stopped the animals

from being too hungry to learn the task properly when they were placed in the box, yet hungry enough to maintain interest throughout the entire first session.

The training procedure consisted of placing food pellets (45 mg, Campden Instruments Ltd) in the food hopper and crushed food pellets on the two identical levers of a Skinner behaviour box (Campden Instruments Ltd). The rats were placed in the box and manually shaped until they had learned to press for the pellets. The shaping consisted of giving the rat pellets by remote control while it had its head in or near to the food hopper, until it learned to associate the noise of the pellet dropping with the actual arrival of food. Once this association was made, the emphasis in the shaping was gradually changed from giving pellets when the rat's head was in the hopper, to when the rat approached the lever with his paw. Eventually the rat pressed the lever and earned the pellets. Criterion was said to have been reached when 20 presses by the rat were immediately followed by food retrieval in an associating manner.

Criterion was reached within 2 or 3 hours in the box. Initial session length varied from 30 minutes to 1 hour, so this was in three or four sessions. Occasionally a rat would learn to press in less than 30 minutes. After acquisition of the task, four 30-minute sessions were recorded. Note was taken of the total number of successful presses and how these presses were divided between the two paws, both paws or "other" presses such as with the nose.

No other feeding was given other than in the training sessions unless the weight of the rat fell below 80% of its control weight. This only very rarely occurred, but when it did the rat was allowed to feed freely for 30 minutes after the training session.

Experimental procedures

- *Lesions:*

The methods for making all the lesions described are given in the general methods section, only the specific points relevant to the behaviour are mentioned here.

In all cases where lesions of the central nervous system were made, the animals were allowed to feed freely for 24-48 hours before the operation. They were also allowed at least 1 week to recover from the acute effects of surgery before being food deprived again.

In all cases where there was pretraining, the side of the brain which was operated, depended on the paw preferentially used by the rat. The lesion was then performed either ipsilaterally or contralaterally to this paw, depending on the experiment.

The retesting procedure involved placing the rat in the Skinner box and observing its performance as in the initial four sessions. Three or four sessions were recorded.

In one group of 6-OHDA lesioned animals the operations were performed before any pretraining. In this group the animals were left for 4 weeks before the initial training procedure was given. This meant that this group was larger in size (250-300 g) than the others. In another group of 6-OHDA lesioned animals, operated ipsilaterally to their preferred paw, they were retested for three sessions before the experimental procedure was carried out, in this case the injection of local anaesthetic to the preferred forepaw (see later).

The contralateral 6-OHDA lesioned group was also retested 4 and 8 weeks later to observe any change in performance. At 4 weeks three sessions were recorded, and at 8 weeks five sessions were recorded.

- *Local anaesthetic injections:*

At an interval after the pretraining sessions, the rats were injected with 0.3 ml lignocaine hydrochloride (Xylocaine, Astra Pharmaceuticals Ltd) in a 2% solution. This was put into the flexor muscle of the preferred forelimb. The animal was then returned to its home cage until it could not support its weight on that paw and the withdrawal reflex on pinching had disappeared. This generally took from 3-5 min. The animals were then retested, and the procedure repeated three or four times, one session per day. After the last session the animals were tested for full recovery. Some of the animals were tested again 8 weeks later to see if the original preference had returned.

- *Retraining to use the other paw:*

Two days or 1 week after pretraining one group of rats was replaced in the Skinner box and retrained to use the initially unpreferred paw. This was done by not rewarding the rat on use of the preferred paw and removing the lever just as the rat was about to press. For this experiment, the automatic reinforcing mechanism was disconnected and all pellets were given by remote control. This allowed for more operator bias in this experiment than the others. In order to be able to do this the Skinner boxes were modified to have retractable levers. Reward, however, was given on use of either both paws or the other paw. Reinforcement on both paws was needed to prevent an extinction response. Five sessions were recorded here. Lever withdrawals were counted rather than preferred presses in this case.

Some of this group were also tested 8 weeks later, to observe whether or not the original preference had returned.

- *Controls:*

One group of animals was pretrained and then left for a week before being retested. This was to assess the effect of a gap on the performance. Another group was pretrained as described, but the initial weight was higher, 250-300 g, to act as controls for the larger 6-OHDA lesioned group.

Analysis

As the pressing rates are not normally distributed it was decided to use non-parametric statistics. The most usual tests were the Wilcoxon Rank Sum test and Spearman Rank Correlation test. The tests were always done on the total of the specified type of press over the relevant time period, for each rat. In some instances a paired t-test was used. Although the numbers themselves are not normally distributed, the differences nearly always are, thus allowing the use of the paired t-test. When more than two sets of data were being compared with each other, a Kruskal-Wallis one-way analysis of variance was used, along with a critical range method for multiple comparisons to tell which pairs did differ (see 90). Further details of which test was used in a given case is reported in the Results section (Section 3.3).

3.3 Results

Before the results from the experiments are given, the development of the NMA lesion is described, as are the details of the other lesions used. The results from the lever pressing experiments are then presented in the following order: firstly, the results from the initial training procedure are covered, followed by the effects of the various experimental procedures on the pressing behaviour. Finally, a comparison of the different procedures is given.

Development and description of the NMA lesion

Although NMA has been reported to cause similar neurotoxic lesions to KA (see Section 3.1), details of the lesion making procedure were not available. Therefore in order to achieve a successful lesion, several combinations of doses and injection volumes were tried before the lever pressing experiments were started. NMA has been reported as being less potent than KA, and so the first dose attempted was just greater than that used for the KA lesions (2.5 µg) and injected in the same volume (0.2 µl). This dose caused virtually no damage to the brain apart from the injection track itself. The other doses tried at a preliminary stage were 5 µg and 10 µg in volumes of 0.4 µl and 0.8 µl all at a rate of 0.1 µl/min. 5 µg in either volume caused a small lesion, close to the injection site, although in one case there was more widespread damage, including nearly half the striatum. 10 µg in either volume caused a satisfactory lesion. 0.4 µl gave a greater extent of gliosis and cell loss than 0.8 µl, but it was not quite as extensive. The 0.8 µl volume spread further than the 0.4 µl one, but did not cause as severe damage, presumably due to the lower concentration. 10 µg in 0.4 µl was chosen as the first dose to try in the lever pressing experiments, as it caused widespread damage and extensive cell loss within the area of the lesion. It also caused the rotation of the animals in response to 2 mg/kg apomorphine in a reasonable number of cases. In a number of these test animals, as well as in all the animals used for the pressing experiments, GAD was measured in both SN to see if the lesion had caused a depletion in the lesioned side. However, when SN from both sides of the brain in control animals were assayed, the variation between the two sides was great (see Table 1). Because of this it was decided not to use the biochemistry as a diagnostic parameter of the lesion, but only to use it in support of other evidence.

GAD in substantia nigrae (nmol/mg protein/hr)	
Left side	Right side
322.3	305.0
127.2	315.4
339.5	109.9
118.6	148.8
181.6	135.1

TABLE 1: Levels of GAD in the SN of control rats.

The results from the first batch of rats used in the pressing experiments were not very consistent with respect to the histology, the turning behaviour and the pressing behaviour. Some of the lesions seemed small on examination and it was decided to use double the dose (20 µg in 0.8 µl) for the later set of experiments. Also as some rats swapped gradually, it was decided to leave the rats for 2 weeks after the operation before retesting to see if this made any difference. This did lead to more consistent results. However, the extent of damage caused by the different doses and recovery times did overlap to some degree and the results of the experiments are compared with the extent of histological damage rather than the dose used or time before retesting. A description of the histology, behaviour and biochemistry is given below and an attempt is made to correlate all the data in a meaningful manner.

In total, 48 rats were injected with NMA. In 34 GAD was measured in both SN and in 27 of these, the paw preference of the rats in the Skinner box was tested before and after the lesion. In the animals that were tested for paw preference, the injections were all given on the contralateral side to the animals' preferred paw. In all, 23 rats turned more than 75 times in response to 2 mg/kg apomorphine, and another 2 turned only 40 times but had a marked asymmetrical posture. All but 2 of these 25 turning rats had the same histological profile after the lesion. This consisted of a large lesion, covering about half or more of the nucleus and based medially and posteriorly in the nucleus, with a possible ventral tendency. The minimum extent of the lesion was from the anterior commisure or just anterior to it, right back, almost to the globus pallidus. The medial part of the striatum was always damaged, but the lateral rim was not always affected. One exception to

the above was one of the rats given the lowest dose of NMA, and virtually no damage was seen in the nucleus apart from the needle track.

Four rats with a similar profile to the above did not turn in response to the apomorphine. Two of these, however, did swap paws after the lesion and 1 responded inconsistently to the apomorphine, and was therefore not included in any further analysis. In response to the first challenge with apomorphine, it turned about 50 times ipsilaterally to the lesion, however at the next test session, it turned similarly in the other direction. The last of these 4 rats possibly did not have quite as posterior a lesion as the others.

In all, 16 rats changed the preferred paw in the Skinner box after the lesion. Ten of these swapped immediately on retesting, and 6 swapped more gradually over two or more sessions in the box. All the rats that changed the paw preferentially used for pressing immediately had a similar lesion. This was similar to that described above for the "turning rats", but they also had a considerable antero-lateral component, extending right to the front of the nucleus. Moving posteriorly in the brain the lesion appears to shift medially, leaving an outer rim, continuous with the tail of the striatum. This lesion is shown diagrammatically in Figure 7. The rats that took longer to swap paws also had a lesion like this, but it did not extend quite as far anterolaterally as the others.

All but 3 of the rats that swapped paws also turned in response to the apomorphine. Two of the exceptions showed the same histological lesion as described for the turners and swappers, but the third showed extensive septal damage, and it appeared that the injection had gone through the lateral ventricle. This animal was excluded because of this despite the fact that it did swap paws and had a drop in nigral GAD on

the lesioned side. The relationship between turning response to apomorphine and paw swapping is shown in Table 2.

Of the rats that turned, only 5 did not show a decrease in GAD in the SN from the lesioned side, when compared with control. 4 of these had comparable histology to those that did show a decrease, and 2 of this 4 did swap paws. One was not tested for preference.

On the other hand, 5 rats showed a decrease in GAD in the SN ipsilateral to the lesion, but did not turn. Three of those, however, swapped paws and the other 2 were not tested for preference. Of the 2 rats not tested, 1 had a small pallidal lesion, possibly counting for the drop in GAD in SN, and the other had a dorsal, slightly anterior lesion.

Three rats with decreased GAD neither turned nor swapped paws. One of these belonged to the group with comparable histology as the turners, and another showed contradictory responses to apomorphine as has already been mentioned. This leaves only 1 rat with a drop in GAD and no other asymmetry, except the lesion. However, in view of the variable control biochemistry this is not necessarily important. Despite the variability of the biochemistry, most animals with extensive lesions do show a decrease in GAD, although some did show a decrease with little evidence of histological damage. Changes in GAD levels are shown in Table 3, along with details of turning and swapping.

Of the rats that did not turn only the 4 already mentioned resembled the turning group. All the others had small or misplaced lesions which did not result in a change in pressing behaviour. It is interesting that the one pallidal lesion did not result in turning or swapping. Electrolytic lesions of the globus pallidus have been reported to cause a block in the use of the contralateral paw (359). This is possibly due to damage of DA fibres passing through the area (see Section 3.4). Even quite a large

	Turners	Non-turners* a	b
Swapped immediately	9	1	0
Swapped gradually	4	1	0
Did not swap	3	2	6

TABLE 2: Relationship between rats that turned in response to 2 mg/kg apomorphine and those that swapped paws after an injection of NMA into the striatum contralateral to the initially preferred paw.

* a) did not turn, but showed "turning" histology;
b) did not turn, and did not swap.

GAD (nmol/mg protein/hr)		GAD on lesioned side as % control	Turning behaviour (2 mg/kg apomorphine) ipsi/contralateral	Swap?	
Intact side	Lesioned side				
194	220	113	52/39	-	(c)
353	110	31	14/13	-	(c)
187	210	112	142/11	-	(c)
212	70	33	160/32	-	(a)
149	80	54	167/21	-	(b)
260	144	55	3/17	-	(c)
144	144	100	110/0	No	-
190	102	54	129/0	Gradually	(b)
365	72	20	198/6	Gradually	(b)
43	46	107	10/16	No	(c)
165	75	46	(303/37)	Immediately	(b)
196	153	78	8/0	No	(c)
221	189	86	198/2	Gradually	(b)
154	180	117	176/6	No	(a)
140	164	117	77/7	No	(a)
131	135	103	43/13*	Immediately	(b)
113	184	162	38/7	No	(c)
264	55	21	11/12	Gradually †	(c)
216	161	75	6/23	Immediately	(b)
217	229	106	27/12	No	(c)
173	32	18	54/0 or 0/74	No	(a)
237	231	97	0/54 or 45/1	No	(a)
244	128	57	23/8	No	(c)
194	61	31	191/1	Immediately	(b)
78	44	56	153/0	Immediately	(b)
132	115	87	21/9	No	(c)
240	118	49	132/0	Immediately	(b)
92	35	38	181/3	Immediately	(b)
322	177	55	40/0*	Gradually	(b)
212	92	43	178/2	Immediately	(b)
230	133	58	19/4	Gradually	(b)
174	138	79	79/0	Immediately	(b)
231	237	103	124/1	Immediately	(b)

TABLE 3: GAD levels in the SN, turning behaviour and swapping behaviour in all rats measured after an injection of NMA into the striatum contralateral to the initially preferred paw. For details see text. The figure in brackets is artificially high due to "oscillations" about the counting point in the rotometer.

(a) "turning only" histology; (b) "swapping" histology; (c) other histology.

† This rat was excluded as the injection appeared to be into the lateral ventricle. - Implies the information was not available. * Low turning rates, but with a distinct asymmetric posture.

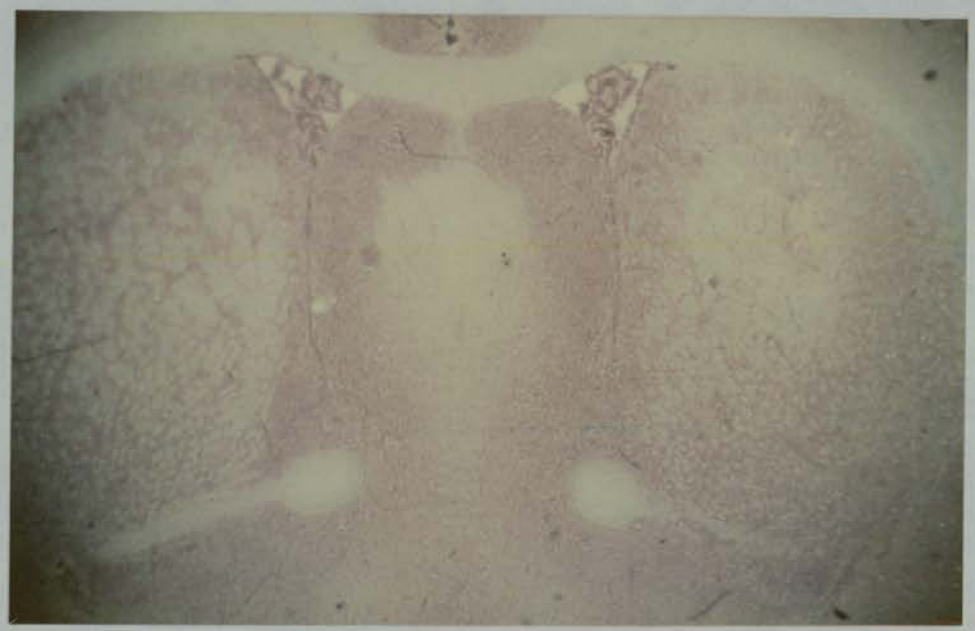
lesion in the front of the striatum, not extending behind the anterior commisure, resulted in neither turning nor paw preference changes. Also an animal with anterior lesion which was placed medially with no lateral damage did not swap paws.

It seems therefore that, in order for a rat to turn in response to apomorphine, the lesion must be placed in the posterior two-thirds of the nucleus more medially than laterally. For the paw preference to alter, this lesion must continue forward, moving to the lateral anterior striatum.

The results from the paw preference experiments in NMA lesioned animals are shown in Figure 19. All rats which swapped paws except the rat with the septal lesion are included.

NMA is much less potent than KA and also much less eleptogenic, yet it seems able to produce very similar lesions. The animals are much less ill after NMA injections than they are after KA injections. A complicated sequence of motor behaviour results on recovery from the halothane anaesthesia. The rats tend to circle gently, with the lesion on the inside, from time to time. This is interspersed with periods of normal behaviour and also, small forelimb twitches and occasional convulsions. The twitches were on the contralateral side to the lesion, and during the convulsions, the rats twisted violently in a contralateral direction. After the convulsion the rat would go back to circling gently ipsilaterally. This contrasts with the behaviour of the KA rats which run in violent contralateral circles and have violent convulsions leading to unconsciousness The NMA rats do not lose consciousness and are relatively normal between twitches or convulsions. The KA rats are unwell for several days, often with bleeding from the nose and urinary tract. However, the NMA rats recover well from the operation.

FIGURE 8: The photograph shows possible damage to the internal capsule fibres near to the injection site of an NMA lesioned striatum. An apparent loss of myelin has occurred in the bundles, as shown by reduced blue staining. There is possibly a slight shape change in the bundles as well. Fibres are normal in front of and behind this point as far as the luxol fast blue, cresyl violet staining method can tell.



B A

FIGURE 9: Histology of an NMA lesioned striatum (A) stained by the luxol fast blue and cresyl violet method, compared with a control striatum (B).

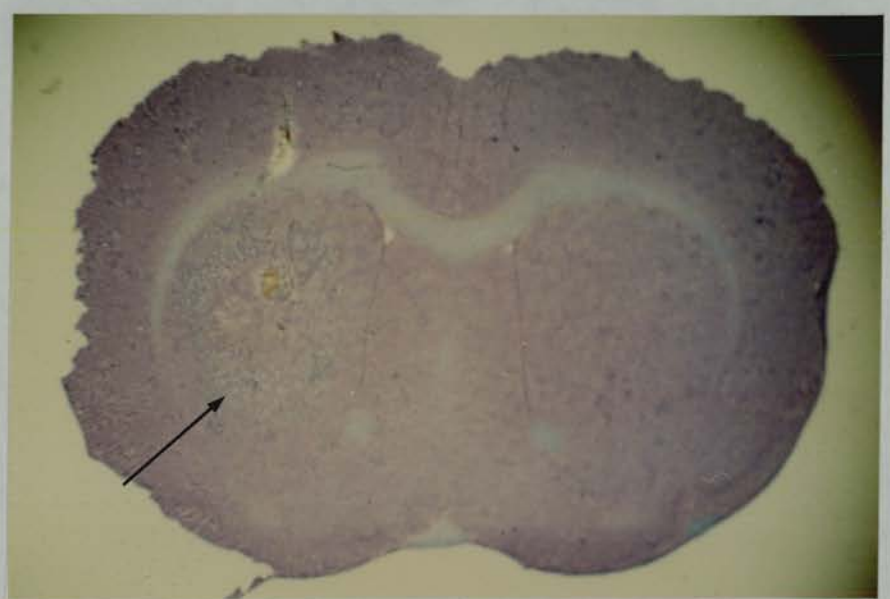


FIGURE 10: Illustration of the blue "halo" effect seen in the striata of rats injected with NMA and stained with the luxol fast blue, cresyl violet method. ~~Cell loss can be seen in this area, but there is less gliosis than in the centre of the lesion~~.

Associated with the reduced level of convulsions, the NMA lesioned rats do not have the non-specific damage resulting from the convulsions that the KA rats often have.

Histologically, the NMA lesion is similar to the KA lesion, with gliosis, cell loss and shrinkage of the striatum. Also, in most cases the internal capsule fibres are spared although in one case interesting changes in these were noted. The fibres appeared to change in shape and the blue staining was much less. This was only seen near the injection site, and the fibres are normal just behind this, showing the fibres are still there. This is shown in Figure 8 and discussed later. In many cases a blue "halo" was seen round the edge of the lesion. The blue was darker than that of the myelin and in this area, although cell loss was apparent, gliosis was not marked either. Figure 9 shows a NMA lesion and the control side of the brain, and Figure 10 shows an example of the halo.

Description of the other lesions

- *Kainic acid lesions:*

Histologically these lesions resemble those already described for the NMA treated rats, and those already described in the literature (see Section 1.6). Only the posterior portion of the striatum was available in these rats as the anterior portion had been used for GAD and CAT estimation. Ideally, the brain was divided down the injection track, but due to shape changes and drying on freezing, not all the posterior half of the lesion was examinable. Therefore in some animals little or none of the lesion could be examined histologically. Because of this, the type of correlation between all the measurements done in the case of the NMA lesioned rats could not be done with the KA ones. However, only one rat showed any differences from the NMA group. All

the others could well have been similar to the NMA ones. The exception swapped paws without turning and without the "turning" histology as mentioned in the previous section. This might imply that the posterior, medial lesion may not in fact be essential for the reversal of paw preference, and the fact that all those in the NMA group did show this as well as the anterior lesion was purely fortuitous.

The levels of turning in these rats was much lower than the NMA group. This may have been due to the fact that these rats were less healthy. Despite this one rat turned at a phenomenal rate and was stopped after 5 minutes with an injection of haloperidol as it was going to damage itself. In the 5 min the rat spun round 164 times. This animal was killed as it was extremely jumpy and was virtually impossible to handle. It would not settle in the box for starting at the tiniest movement or sound, and would not lever press. Histology was examined from this animal and both ventricles were seen to be enlarged. Non-specific damage from convulsions was clearly seen in the lateral and ventral cortex, as well as the specific KA lesion in the striatum.

The details of the behaviour, biochemistry and histology for the animals used are given in Table 4. Of the animals that swapped paw after the operation, only one turned well. Another three had similar histology to the one that did, but the other one did not. The lesion in this rat was purely anterior, and may mean, as commented on already, that the turning profile is not essential for swapping paws. The biochemistry on the striatal tissue was much more consistent than that on the SN and so is more useful in describing the lesions. The levels in both striata of control animals are given in Table 5.

Figure 11 shows an example of a KA lesion and its control side. This is not from an animal used in the paw preference experiments as

	GAD nmol/mg protein/hr		% of intact side	CAT nmol/mg protein/hr		% of intact side	Turning behaviour (2 mg/kg apomorphine) ipsi/contra	Histology
	Intact side	Lesioned side		Intact side	Lesioned side			
KA contralateral to initially preferred paw	130	86	66	218	169	78	5/4	✓*
	116	11	9	357	48	13	16/0	✓
	104	65	62	157	60	38	46/0	✓
	90	18	20	168	0	0	9/5	?
	63	7	11	433	22	5	121/0	✓
KA ipsilateral to initially preferred paw	107	68	63	157	94	60	30/0	?
	85	59	69	280	81	30	60/0	✓
	99	61	62	143	35	24	103/16	?
	92	19	21	206	8	4	16/0	?
	85	3	4	575	0	0	161/10	✓

TABLE 4: Levels of GAD + CAT, turning behaviour + histology in the striata of rats injected with KA in one striatum.

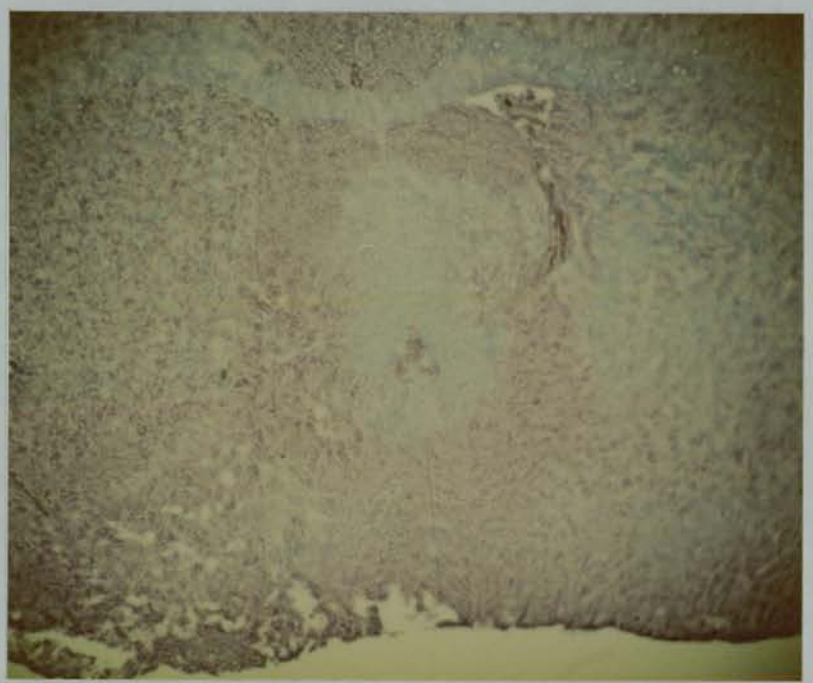
✓ histology does not disagree with NMA description (see text);

? not enough information available from histology;

* has anterior "swapping" lesion, without posterior medial "turning" lesion (see text).

GAD in striatum (nmol/mg protein/hr)		CAT in striatum (nmol/mg protein/hr)	
Left side	Right side	Left side	Right side
77.4	239.7	279.7	460.1
70.6	77.3	442.6	162.2
95.0	75.0	385.5	131.4
71.8	97.3	-	-
69.0	126.6	282.4	367.9
52.1	77.5	146.0	217.9
107.9	79.7	154.5	136.3
95.8	185.0	137.1	94.3
85.3	77.8	176.3	127.6
73.1	100.9	94.3	148.2
109.6	134.6	335.8	266.1
56.9	173.9	318.4	433.5

TABLE 5: Levels of GAD + CAT in the striata of control rats.



B A

FIGURE 11: Histology of a lesion caused by KA and stained with luxol fast blue and cresyl violet (A) compared to that of a control striatum (B).

DA µg/g net weight/hr		Turning behaviour (0.3 mg/kg apomorphine)	
intact side	lesioned side	contra/ipsilateral	
7.78	0.07	263/0	
5.57	0.29	167/12	
7.35	0.08	219/13	Successful
8.12	0.37	381/1	6-OHDA lesions
10.15	0.16	341/5	
8.81	0.33	131/11	
7.84	6.75	0/0	
8.56	2.00	0/0	Unsuccessful
8.65	9.76	0/0	6-OHDA lesions
10.01	8.88	0/0	(used as control)
11.07	10.32	0/0	

TABLE 6: DA levels in the striata and turning behaviour of rats after unilateral injections of 6-OHDA into the MFB ipsilateral to their initially preferred paw.

Turns in response to 0.3 mg/kg apomorphine:	
contralateral	ipsilateral
287	5
458	37
153	0
219	19
234	6
530	1

TABLE 7: Number of turns in response to 0.3 mg/kg in 6-OHDA lesioned rats. Swapping behaviour for these rats is shown in Figure 15.

some of the nucleus was used for biochemistry in all of these animals. This photograph comes from an animal destined for electrophysiology, but that had to be killed 4 days after the operation as it had broken a leg turning in the cage. Full degeneration may not have occurred in 4 days, but significant gliosis is seen.

- *Electrolytic lesions in the crus cerebri:*

These lesions were described only on the basis of histology and turning. A typical lesion is shown in Figure 12. All these rats turned 100 times or thereabouts in 30 minutes to 2 mg/kg apomorphine.

- *6-hydroxydopamine lesions*

All 6-OHDA lesioned animals turned more than 150 times in 45 min to 0.3 mg/kg apomorphine, some much more. These rats turned contralaterally in contrast to the other lesions all of which turned ipsilaterally to the operated side.

The group operated ipsilaterally to the preferred paw also had levels of DA measured in the striatum. The values for this group and those for the control group used in this experiment are given in Table 6. The turning figures, for the other rats used in the pretraining experiments, are given in Table 7.

Results from the initial training procedures

In total 131 rats were trained to lever press for food. All the rats used learned to press and all but three did so within 3 hours in the Skinner box and a few in as little as 30 min in the box. Of the 3 rats that took longer than this, the longest time was 5 hours.

The total number of presses in each 30 min varied a lot, tending to increase with each session. The number varied from 50 (in 2 rats only)

FIGURE 12: Histology of an electrolytic lesion of the striato-nigral pathway in the crus cerebri, stained with the luxol fast blue, cresyl violet method. The dotted line indicates the extent of the lesion.

to as many as 250, or occasionally more. Most sessions were in the 100 to 225 range. In the later sessions rats often pressed faster than this if the experimental procedure applied did not affect the total rate. The maximum in any session was 330.

The rats needed no supplementary feeding during the week. They all ate enough during the training sessions to maintain 80% body weight during one week of training. It seems likely that the rats had nearly reached the maximum pressing rate by the end of the initial training period. It would seem that an animal having eaten 200-250 pellets is quite full and unlikely to press for much more food. Despite this, very little drop in pressing rate was seen after the first few minutes in the box. 30 min therefore seems to have been the ideal duration for the test sessions.

All but 4 of the 131 rats showed a "paw preference" in the lever pressing behaviour. That is they used one paw consistently more than the other. The 4 exceptions pressed often with both paws or showed no real preference for one or other paw. Three other rats changed preference during the pretraining procedure, or showed signs of doing so. Similarly, the rats showed a lever preference. Only 3 rats did not have a strong preference, and another 6 tended to change which lever they used more often during the training sessions.

Eighty out of 131 rats used their left paw rather than the right and 51 used the right rather than the left. Seventy rats tended to use the left lever and 61 used the right one. Rats were assigned to the above groups using the total number of presses in the last four sessions with the preferred paw or with any other combination of presses. Those without a strong preference were included although their degree of preference was very low. Seventy-three rats pressed with the same lever

as their preferred paw, and 58 used the opposite lever to the preferred paw.

Using a Students paired t-test, it was shown that the rats do have a significant lever preference and a significant paw preference ($P < 0.001$). It was also shown, using a Spearman Rank Correlation test, that there was a significant correlation between the lever preference and the paw preference ($P < 0.01$). To do this the number of presses with the right paw was compared with the number on the right lever, and similarly for the left side. Therefore each rat had two comparisons made on its behaviour. The correlation coefficient was $R = 0.19$ which is very low and although this was significant due to the large number (262), it might suggest a low correlation level. This is supported by a plot of all the figures used in the correlation, shown in Figure 13, where a large number of points are not on the straight line. The left and right values are both plotted on the same axes in the graph.

It was also shown using a chi-squared goodness of fit test that the sample of rats had a slight left bias in paw preference ($P < 0.025$), but no bias in which lever was preferentially used ($P > 0.1$). The expected values used in the chi-squared test were those that would have resulted if the sample had been divided equally between the right and left hand side.

For the experimental procedures, no rats were used that did not show a clear paw preference at the start of the procedure.

- *Initial training of 6-hydroxydopamine lesioned rats:*

These animals were given the initial training procedure 4 weeks after an injection of 6-OHDA into the MFB. All 7 successfully lesioned animals, as determined by their response to 0.3 mg/kg apomorphine, learned to press for food. However, when compared with weight-matched

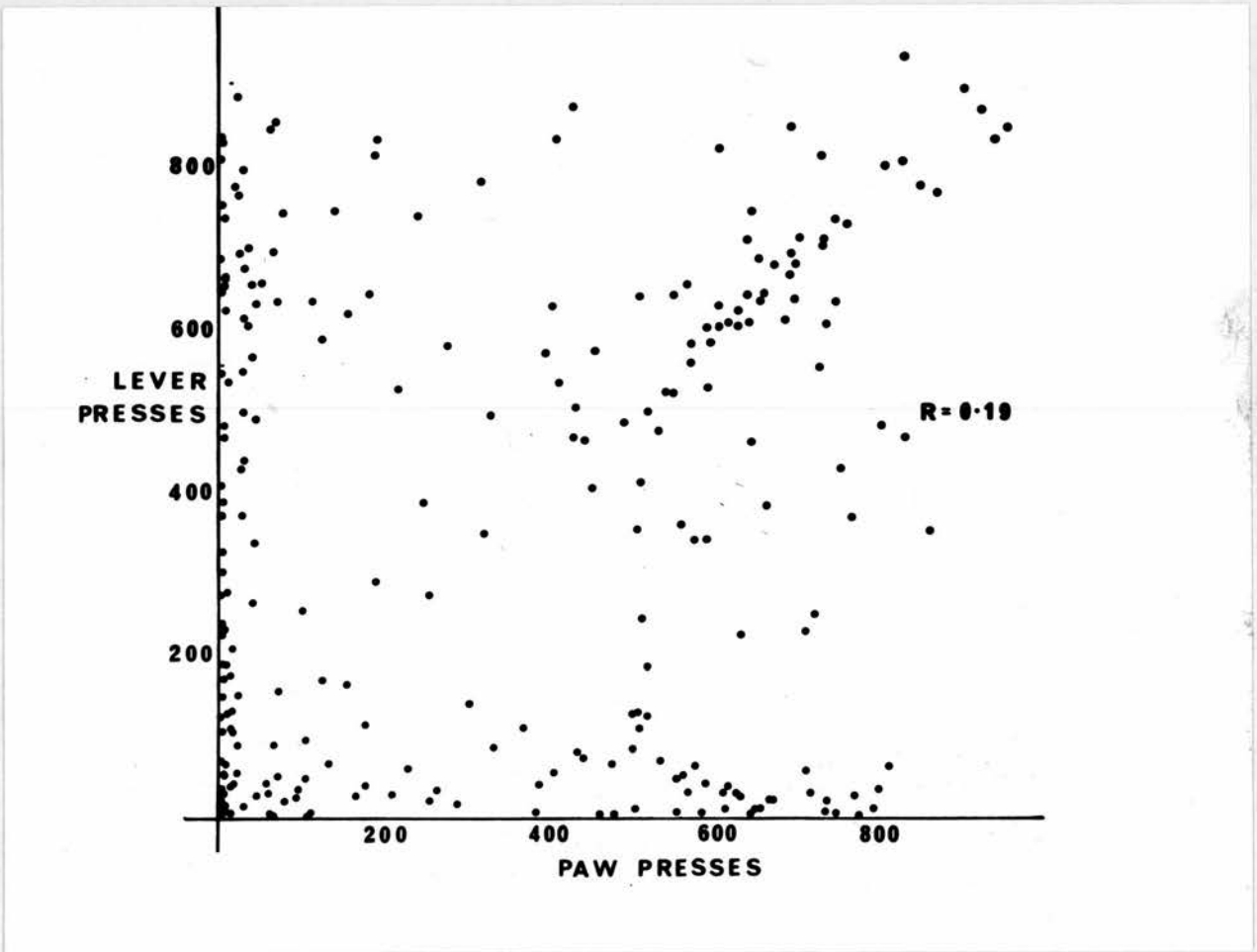


FIGURE 13: Scatter diagram showing the correlation between lever and paw preference. Two points are plotted for each rat: one for the number of presses with the right paw on the right lever and one for the number of presses with the left paw on the left lever. There is a highly significant correlation between the two preferences ($P < 0.01$).

controls the rate of learning proved to be significantly slower ($P < 0.01$ on a Wilcoxon Rank Sum test). The times taken to reach the criterion level of 20 consecutive presses followed by immediate food retrieval, is shown for lesioned and controls in Table 8, and a plot of the number of presses in each 30-minute session is shown in Figure 14. In this, sessions at the start which were not 30 min (these were either 60 min or 15 min) are corrected arithmetically to be equivalent o a 30-minute session. As well as being slower to criterion, the rats also appeared to attain a lower maximum pressing rate, although this did not reach significance. The mean control maximum was 210.4 and that of the lesioned group was 186.4. The lesioned rat that has values among the control ones did take a longer time to criterion that the controls. This does not show well in the graph due to the correction procedure. However, this lesioned rat did seem to learn faster than the other ones and reached a higher level.

The rats rotated spontaneously in a direction ipsilateral to the lesion while they were in the Skinner box, especially in the earlier sessions or after being disturbed. This may have resulted in the slower learning rate. These animals were also noted to have a high negative correlation between paw use and lever use ($P < 0.02$) whereas the overall level for control rats was a positive correlation. The respective correlation coefficients are +0.19 for the controls and -0.71 for the lesioned animals. There would therefore appear to be a much stronger correlation in the lesioned group. It certainly seemed so watching the animals. The rats tended to use the lever contralateral to their preferred paw (and the lesion) because of the tendency to circle: once the rat had learned to press, it moved very quickly to the food hopper, this movement was easier to perform in the direction that the rat was tending to circle in

Unilaterally 6-OHDA lesioned rats	Control rats
150	75
150	60
120	75
105	45
225	45
180	45
150	30
	45

TABLE 8: Time taken to reach criterion (20 consecutive presses followed by food retrieval) in minutes, to the nearest 15 minutes. The two groups are significantly different ($P < 0.01$) on a Wilcoxon Rank Sum test.

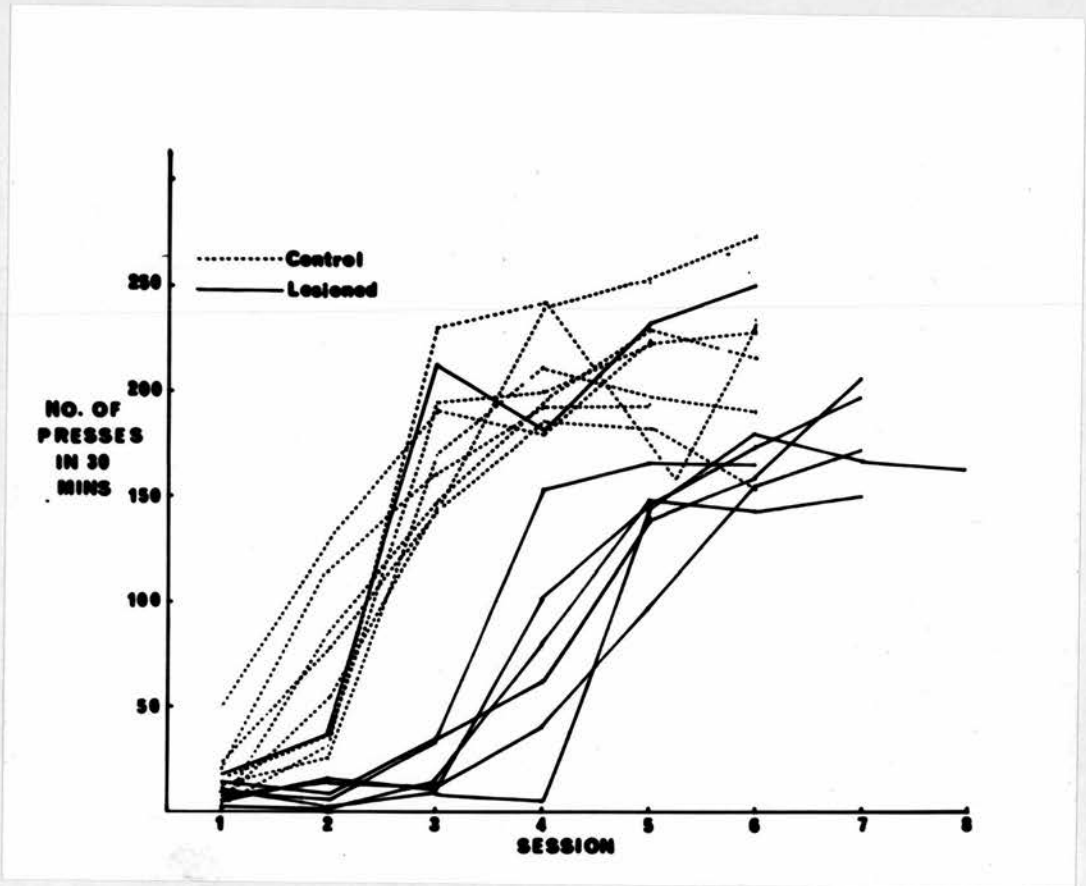


FIGURE 14: Graph showing the rates of acquisition of the lever pressing task in rats lesioned 4 weeks previously with 6-OHDA in the MFB and in weight matched controls. The lesioned rats were significantly slower to reach criterion (20 consecutive presses in 30 min) than controls ($P < 0.01$).

anyway than against it. The rats appeared to have no problems in going against their tendency to circle when they moved to press the lever.

No such correlation was seen in the NMA treated animals or in the LA treated group at 1 week or at 8 weeks.

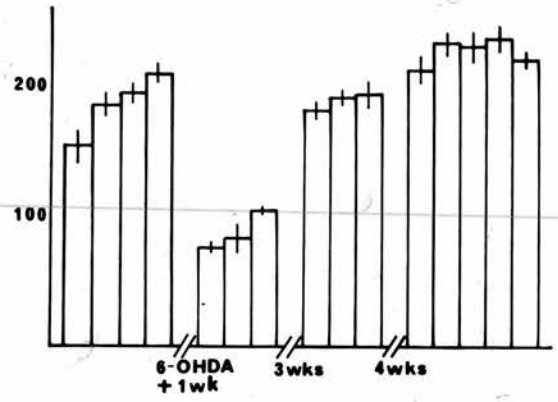
Effects of experimental procedures administered contralaterally to the initially preferred paw

Figures 15 to 21, situated on the following pages, show the effects on pressing behaviour of the various procedures. In these diagrams the vertical axis represents the number of presses in each session and the horizontal axis represents the sessions. In the preference graphs, the positive vertical axis represents presses with the preferred paw and the negative axis, all other presses. In each case the statistics are performed on the total number of the relevant type of press in the appropriate number of sessions, for each rat. The tests are made on the same number of sessions before and after the procedures, using the required number immediately before and after the procedure if uneven numbers of sessions are plotted in the graphs. The effects are described in detail in the figure legends and summarised at the end of this section. In order, the figures represent the following groups of animals:

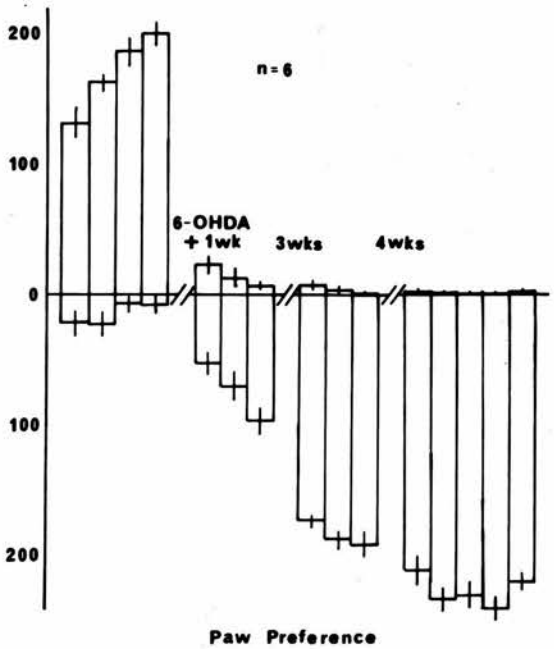
Figure	
15	6-OHDA lesions
16	Control animals
17	Local anaesthetic injections
18	KA lesions
19a	NMA lesions - immediate swappers
19b	NMA lesions - gradual swappers
20	Crus cerebri lesions
21	Training controls

FIGURE 17: Effect of local anaesthetic injections into the preferred paw on pressing behaviour.

Using a Kruskall-Wallis one way analysis of variance, it is shown that the total pressing rate does vary in the three groups of testing sessions ($P < 0.0005$). Using the critical range method for multiple comparisons it is shown that the total rate during local anaesthesia is not different from control, but that the total rate 8 weeks later is increased both from control and the local anaesthetic level ($P < 0.01$). Using the same statistics, the number of presses with the preferred paw is significantly different in the 3 groups ($P < 0.001$). The use of the initially preferred paw is reduced in the local anaesthetic sessions, and that of other presses is increased (both $P < 0.01$). The number of presses with the preferred paw is not different from control during the retesting sessions at 8 weeks, indicating a reversal of paw use. However the use of the initially unpreferred paw at this time is not significantly different from the local anaesthetic level, indicating the reversal to original paw use is not complete. Only 9 rats were tested at the 8 week time and as the multiple comparison test needs equal n the statistics were only done on those 9 rats throughout. However, another 5 rats are included in the graph at the 2 earlier times. In summary, the local anaesthetic injections result in a change in paw preference, that partially reverses with time. There is no corresponding decrease in total pressing rate.



Total Presses



Paw Preference

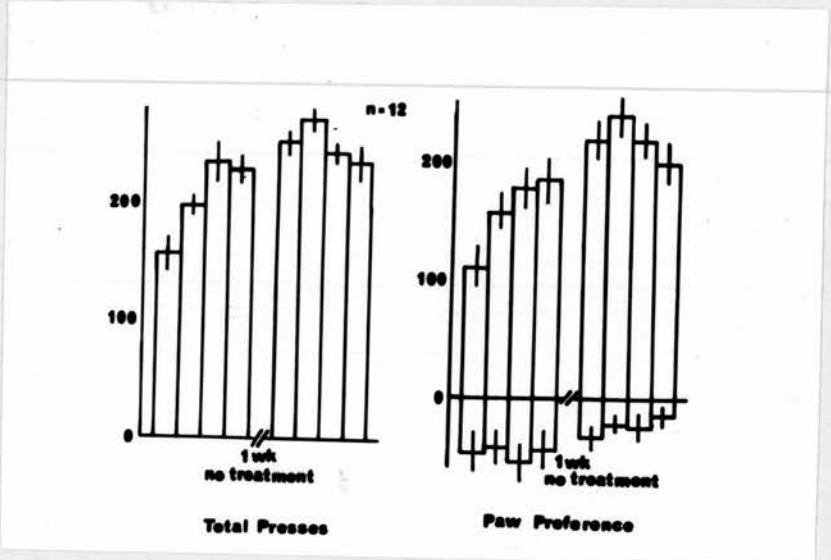


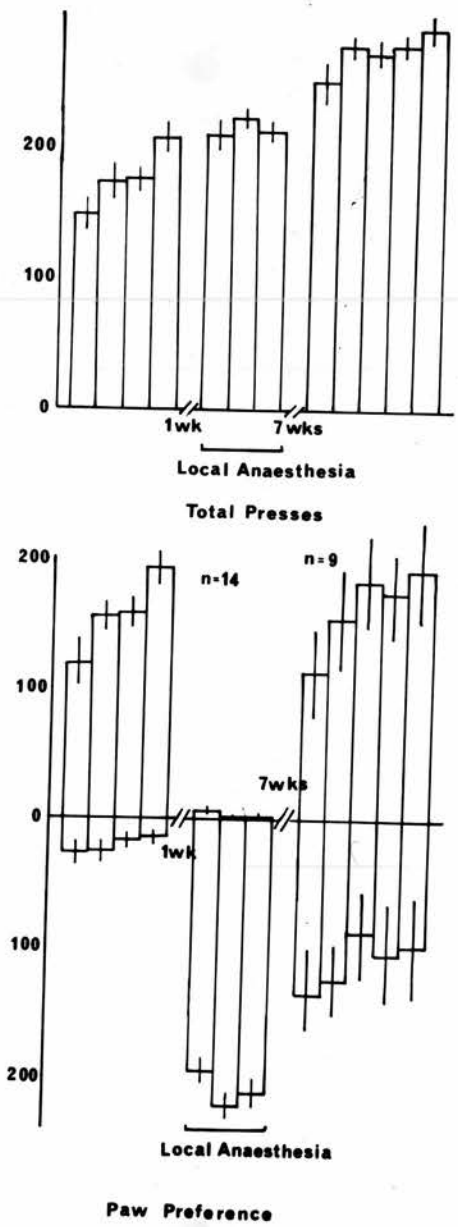
FIGURE 16: Effect of a 1 week break in training on pressing behaviour.

A 1 week gap does not impair the pressing behaviour at all. In fact, using a paired t-test it can be shown that the rate is greater after the week's break than before it ($P < 0.001$). Also, the number of presses with the preferred paw increases with no increase in the number of other presses. This indicates an increased preference.

FIGURE 19: Effect of NMA lesions contralateral to the preferred paw on pressing.

A. Rats that swap paws immediately. These rats do not show any change in total pressing rate on a paired t-test. However, the number with the initially preferred paw decreases and other presses increases ($P < 0.001$).

B. Rats swapping paws gradually. Using a paired t-test before and after, it can be seen that there is no change in total pressing, and very little change in the number of presses with the initially preferred paw ($0.1 > P > 0.05$). However there is a significant change in the number of "other" presses ($P < 0.001$).


200
100
0
1wk
7wks
Local Anaesthesia
Total Presses
200
100
0
100
200
n-14
n=9
7wks
1wk
Local Anaesthesia
Paw Preference

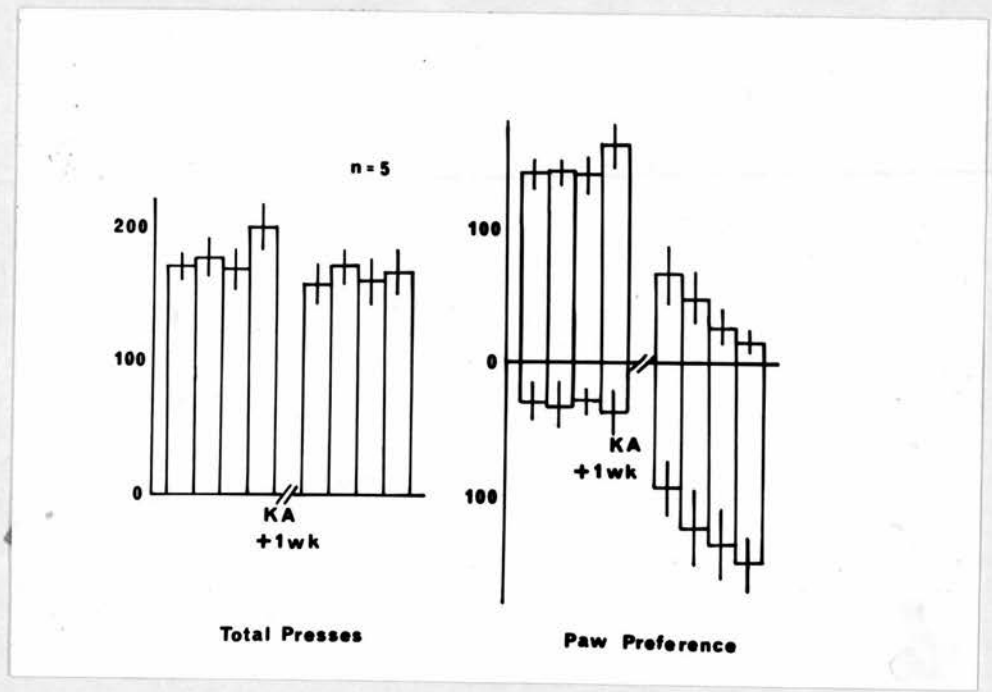


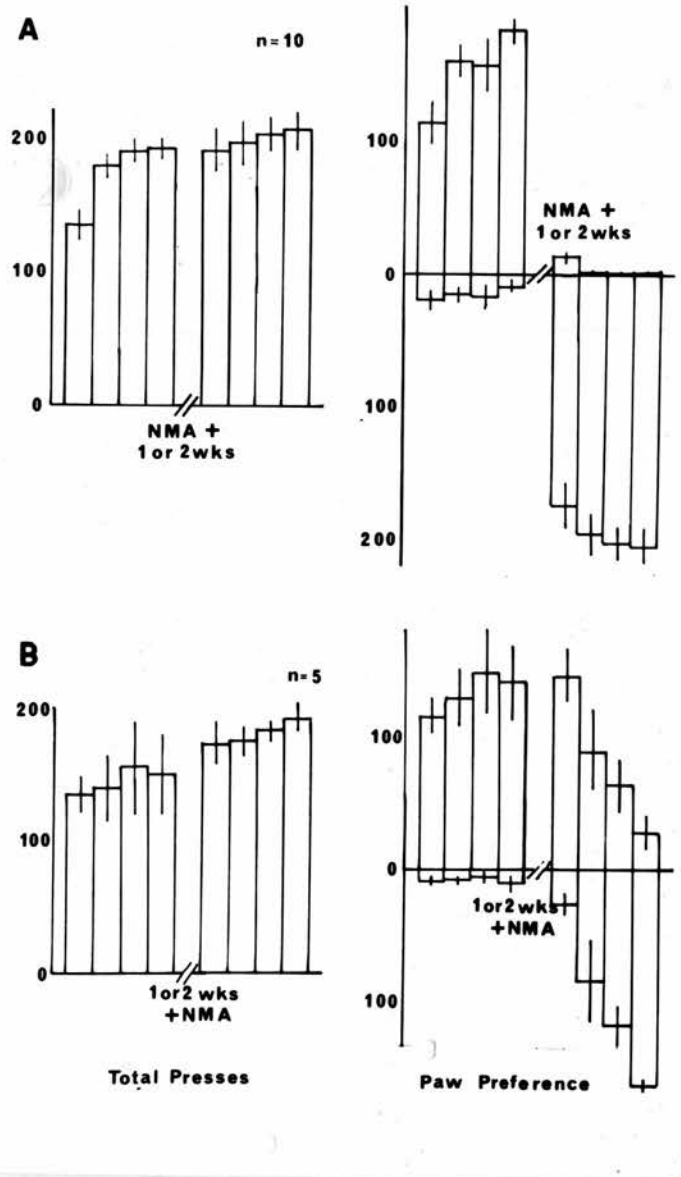
FIGURE 18: Effect of KA lesions contralateral to the preferred paw on the pressing behaviour.

These lesions cause a swap in paw preference, almost but not quite immediately after the operation. Using a paired t-test, the total pressing rate is not affected, but there is a decrease in the number of presses with the preferred paw ($P < 0.01$) and an increase in other presses ($P < 0.02$).

FIGURE 19: Effect of NMA lesions contralateral to the preferred paw on pressing.

A. Rats that swap paws immediately. These rats do not show any change in total pressing rate on a paired t-test. However, the number with the initially preferred paw decreases and other presses increases ($P < 0.001$).

B. Rats swapping paws gradually. Using a paired t-test before and after, it can be seen that there is no change in total pressing, and very little change in the number of presses with the initially preferred paw ($0.1 > P > 0.05$). However there is a significant change in the number of "other" presses ($P < 0.001$).


A
n=10
200
100
0
NMA +
1 or 2wks
100
0
100
200
NMA +
1 or 2wks
B
n=5
200
100
0
1or2 wks
+NMA
100
0
100
1or2 wks
+NMA
Total Presses
Paw Preference

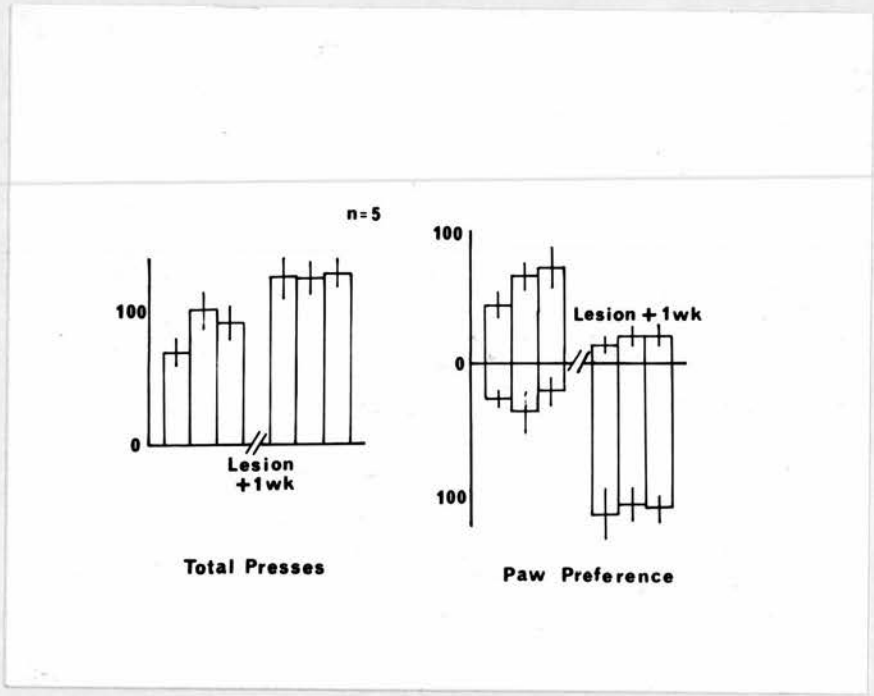
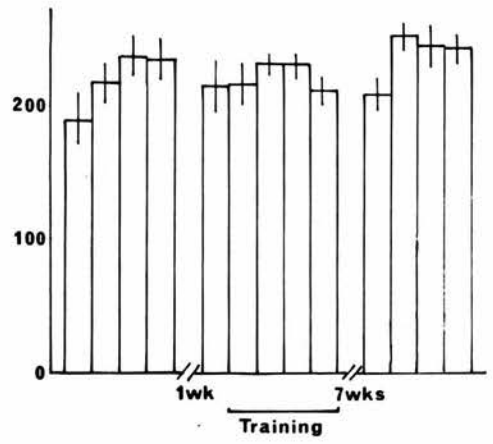


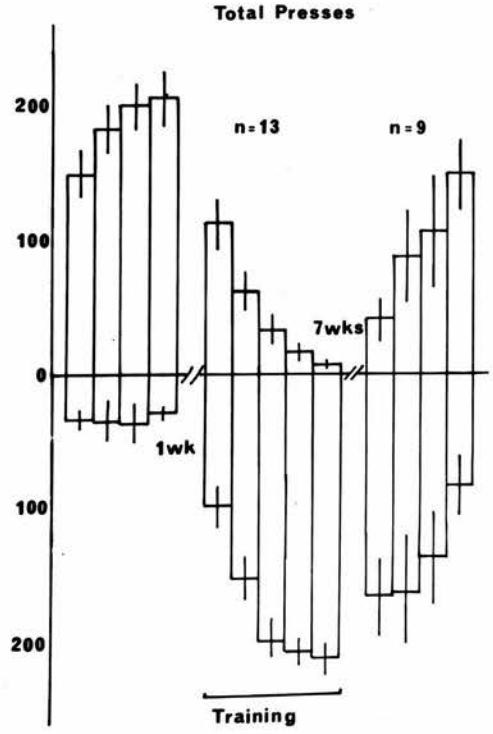
FIGURE 20: Effect of electrolytic lesions in the crus cerebri, contralateral to the preferred paw on pressing behaviour. There is a significant increase in the total pressing rate after the operation (paired t, $P < 0.01$) and also an increase in the number of "other" presses ($P < 0.001$), and associated with this a decrease in the number of presses with the initially preferred paw. The total overall rate of this group is lower than any of the other groups (see text for discussion on this point). These results have already been reported elsewhere (206).

FIGURE 21: Effect of training the rats to use the initially unpreferred paw.

The rats show no change in total pressing rate, either during the retraining sessions or 8 weeks later when they are retested (Kruskal-Wallis one way analysis of variance.) However, using the same test it is shown that the preference is different between the different groups. Using the critical range method for multiple comparisons, it was seen that the number of presses with the initially preferred paw was significantly reduced during the retraining period when compared with control ($P < 0.01$). The number at 8 weeks was not different from either control or the retraining period, indicating partial recovery. Similarly, the number of other presses is increased during the retraining procedure ($P < 0.01$), but here the number at 8 weeks is still different from control ($P < 0.05$), but not from that during the retraining. In summary, retraining causes a change in paw use during the procedure, with no change in total pressing rate. The change in paw use has partly reverted by 8 weeks after the retraining. The statistics are only performed on the rats which were tested at 8 weeks (see Figure 17 for explanation).



Total Presses



Paw Preference

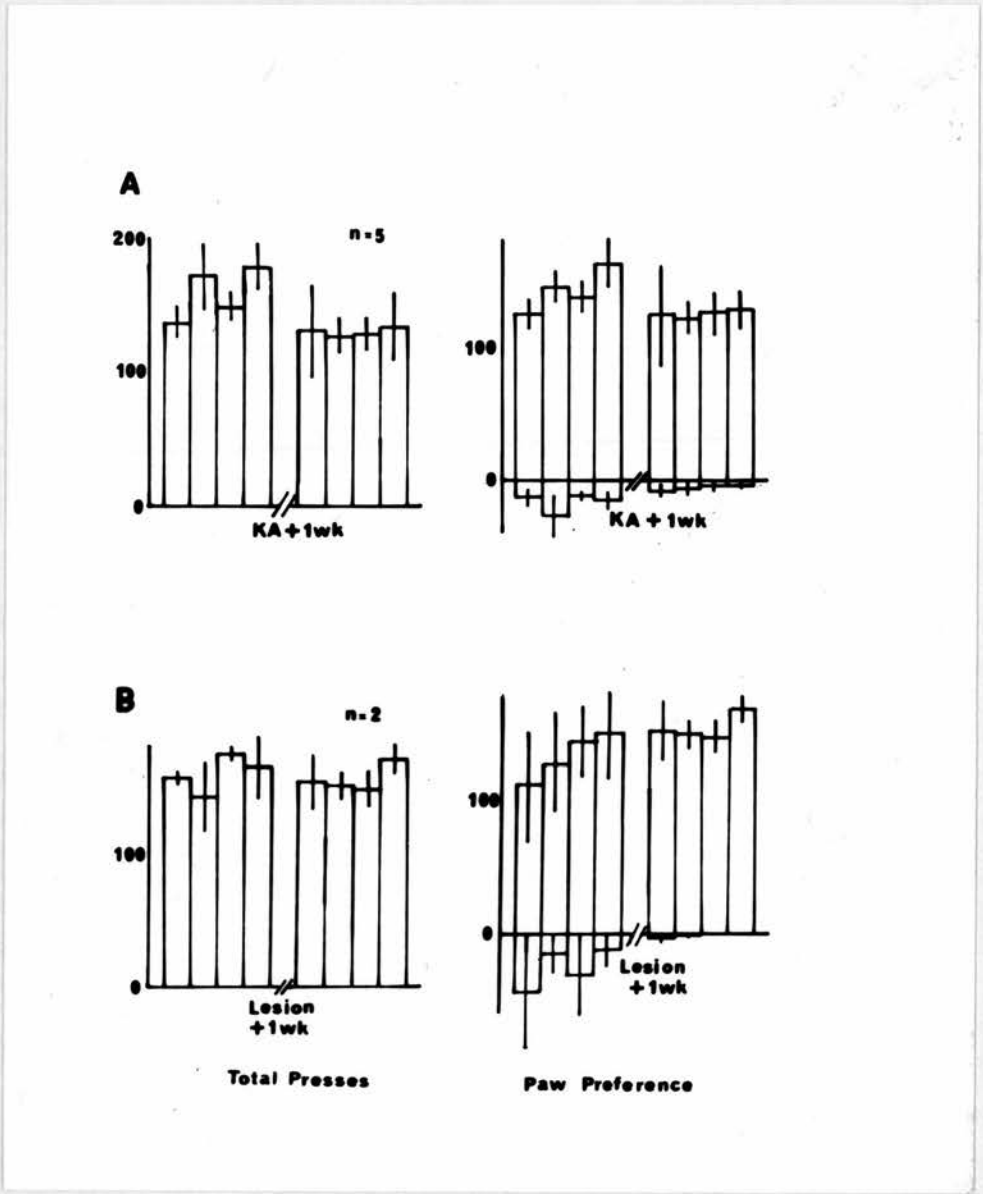


FIGURE 22: Effect of lesions ipsilateral to the preferred paw on pressing behaviour.

A. KA lesions.

B. Electrolytic lesions of the crus cerebri.

There is no effect on the pressing behaviour in either case.

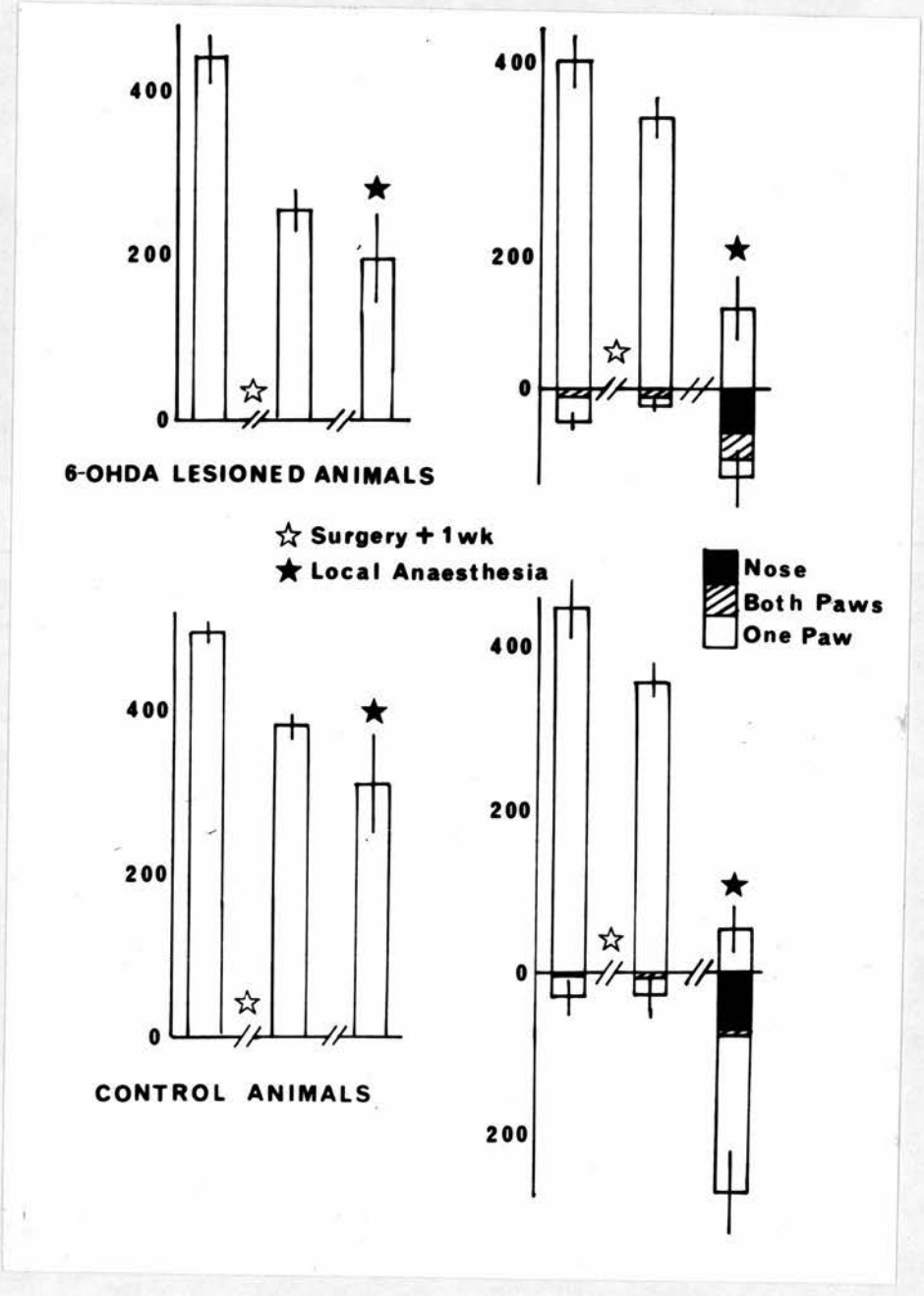


FIGURE 23: Effects of ipsilateral and unsuccessful 6-OHDA lesions on pressing behaviour. In the lesioned animals the lesion itself had no effect on the pressing behaviour. When local anaesthetic was given, however, the rats did not change to the other paw. Other combinations of pressing were used: both paws, paralysed paw and nose. However, the unsuccessfully lesioned animals did swap paws as those in Figure 17 did.

Each column represents the total presses over two days.

- *Summary of the results from the contralateral experiments:*

From the Figures on the previous pages, it is apparent that the only group of rats with a deficit in total performance are the 6-OHDA lesioned group. All the other experimental groups that swap paws do so without an accompanying drop in performance. It seems that motor disruption on one side is enough to cause a rat to change its preferred paw, but will not affect it enough to reduce total rate. If anything, there is an increased rate and preference in control animals. This increase is not seen in any of the other groups except the contralaterally operated crus cerebri lesioned animals. The crus lesioned animals were given a slightly different training procedure, in that they were allowed 1 hour free feeding after the training sessions and therefore may not have been as hungry. This may have led to the much reduced pressing rate in these animals, which was slightly less evident after the lesion. Apart from this the learning curves seem to be disrupted by the experimental procedures.

Effects of experimental procedures administered ipsilaterally to the initially preferred paw

A few animals were operated ipsilaterally to the initially preferred paw: 5 KA lesions and 2 crus cerebri lesions. The effects of these procedures are shown in Figure 22. In order to do a paired t-test, the 7 rats were combined and it could be shown that there was no significant effect on the pressing of these animals, either in total rate or preference. This is important as it shows that the operation itself, or the injection of KA or application of current is not sufficient on its own to cause a change in behaviour.

Results of training or forcing animals lesioned ipsilaterally to the initially preferred paw, to swap paws

Attempts were made to retrain the 6-OHDA lesioned animals that learned to press after the operation to use the paw contralateral to the lesion (and the paw they had learned to use). This proved an impossible task. When no reward was given on the use of the paw ipsilateral to the lesion, the rats occasionally attempted to use the contralateral paw. These attempts were usually unsuccessful, with the rat often completely missing the lever. They could just manage to press if they approached the lever with the contralateral side of the body pressed against the wall, possibly using it for support. It was also impossible to count lever withdrawals (corresponding to "wrong" presses) accurately and presses with the other paw. This was mainly due to human limitations. As food was being given and levers withdrawn by hand, the operator had to watch the rat closely to ensure the correct reinforcement was given, and to give the reinforcement or to remove the lever. It was difficult to note the number of presses made as well. The only clear observation was that no, or very few, presses with the contralateral paw were successful. The rats would continue to attempt to use the ipsilateral paw even though reward was being withheld. The noise of the food dispenser may have acted as a secondary reinforcer preventing extinction.

Another group of rats was operated ipsilaterally to the preferred paw after pretraining. After recovery these rats were retested for two sessions to ensure they were still pressing as before. They were then given an injection of local anaesthetic into the preferred forelimb. As with the retraining of the 6-OHDA lesioned animals, not one was persuaded to use the paw contralateral to the lesion. Some stopped

pressing, and some continued to try to press, generally unsuccessfully, with the paralysed paw or some other part of the body such as shoulder or nose. These animals were compared with controls which had had unsuccessful injections of 6-OHDA, as determined by the turning response and DA levels in the striatum at the end of the experiment. These rats did swap paws as expected. The results of the turning and biochemistry is shown in Table 5 earlier in this Chapter, and the pressing behaviour is shown in Figure 23.

3.4 Discussion

Review of results

Rats were shown to have a preference for which lever and which paw they used in a lever pressing task in the Skinner box. The two preferences were positively correlated.

6-OHDA lesions in the MFB on the side of the brain contralateral to the initially preferred paw of the animal led to a change in which paw was used. The change in preference remained stable over an 8-week period. A drop in performance which had recovered by 8 weeks after the lesion was also noted.

None of the other procedures used led to a drop in performance, but all the contralateral procedures led to change in paw use. The ipsilateral procedures did not affect the pressing behaviour. After 8 weeks the rats given local anaesthetic in the preferred paw and those trained to swap paws had partially reverted to the original paw use.

Rats lesioned with 6-OHDA before the initial training procedure took longer to learn the task than did weight matched controls, but the total rate was not significantly lower than in the controls. All learned with the ipsilateral paw. Rats given ipsilateral 6-OHDA lesions could not

be persuaded to change paws by a local anaesthetic injection into the preferred limb.

NMA was shown to be as effective as KA in producing neurotoxic lesions of the striatum. It is less potent than KA, but also far less toxic to the animals and therefore higher doses can be used to obtain enough damage. Using these lesions, an area of the striatum that must be destroyed for a swap in paw use to occur has been delineated.

Paw and lever preference

- *Control animals:*

Inherent asymmetry in operant behaviour has been extensively studied by Glick and co-workers (220,222,224, Section 3.1). Both paw and lever (i.e. side or spatial) preferences were studied as were the effects of amphetamine on these preferences. A fixed interval (15 s) schedule (FI 15) resulted in animals with both a paw and a lever preference, however a fixed ratio (30) schedule (FR 30) led to rats with a lever preference, but that used both paws alternately on the levers, enabling a higher pressing rate and therefore higher reward (222). In a DRL schedule all rats had a consistant lever preference and 8 out of 12 had a preferred paw (220). The degree of lever preference was correlated with the pressing rate, rats with a high lever preference having the highest pressing rates (222). In the FI 15 and FR 30 schedules, if the lever preference was the same as the paw preference, amphetamine administration led to an increase in the degree of preference for the lever. However, if the two preferences were different, the preference was reduced or even reversed. The total rate was reduced by amphetamine in both cases. These rats were also seen to rotate to amphetamine in a direction related to the lever preference (222). In other studies, amphetamine has been shown to cause rotation in a direction correlated

with DA levels in the striatum, and to the side preference in a T-maze (223,299,656). The asymmetry in the DA content of the striata of the rats has been used to explain the existence of the side preferences (222,224). It can also be used to explain the differential effects of amphetamine: DA release caused by the amphetamine will amplify the asymmetry and increase the preference when the paw and side preferences are the same. If the two preferences are different, more presses are generated with the lever corresponding to the paw preference.

Glick and Jerrussi (222) point out that the lever preference is more stable if it is linked to the paw preference and also that the paw preference is retained with and without the drug. This implies, along with the decreased preference on amphetamine administration in animals with a differential preference, that it is the paw preference rather than the lever preference that is determined by the DA asymmetry, and that the side preference is less dependent on the DA balance. If this is the case it is odd that a correlation between DA levels and side preferences and rotation was obtained. However, it is difficult to explain the differential effect of amphetamine in animals with opposite paw and lever preferences if this is not the case.

The experiments described in Section 3.3 resemble the FI 15 group of rats rather than the FR 30 group, despite the fact that the animals were trained on continuous reinforcement schedule. As food pellets are used as reward in these experiments the pressing itself is not actually rate limiting: a combination of eating and pressing is. Both paw and lever preferences were seen in these rats as with the FI 15 (222) and DRL (220) schedules. The reason that a paw preference is not seen in the FR 30 animals is probably because a higher rate can be achieved pressing with alternate paws and in this type of schedule higher pressing

rates lead to greater reward. In the schedules requiring lower rates, it does not really affect the reward if they press slightly slower and so the animal will use the paw it finds easier to use.

In the experiments described in Section 3.3 there are far fewer rats without a preference than in the other studies quoted. Here only 4 out of 131 do not have a preference, but in these studies 1 out of 12 (222) and 4 out of 12 (220) are seen. This may have been due to the criteria used to decide what the paw preference of a rat is. These papers give no details of this. In some of the rats described in this study it was very difficult to see which paw the rat was using and constant shifting of position of the observer was necessary. This may not have been possible when the rats were observed by videotape. Also, in some cases the first paw to touch the lever did not necessarily activate the food dispenser and food was not given until the rat pressed more strongly with the other paw. Also after one paw had initiated the food dispensing mechanism the other was often placed on the lever. In each case the paw that caused the activation of the food dispensing mechanism was designated as the paw used.

Glick and co-workers' studies (220,222) do not have enough numbers to allow an extensive correlation of the paw and lever preferences to be done. This study has correlated the two preferences in 131 rats. There is a significant correlation between the two. However, in a large number of individual cases, there is not a correlation (see Figure 13). The overall correlation of paw and lever preference (but with differences in individual cases) possibly explains why a correlation between side preference and DA levels was seen by Glick and co-workers, although it seems to be the paw preference that is primarily determined by the DA imbalance.

- *The effect of lesions on lever and paw preference*:

Electrolytic lesions of the caudate nucleus led to a change in the side preference in a T-maze (519) and in the paw preference of rats in a lever pressing task for sucrose reward (256). In the former experiments, paw preference was not mentioned and in the later experiments conditions were manipulated to obtain a set number of responses with each lever. When rats were only allowed to use the left paw on the left lever and the right paw on the right lever (359), electrolytic lesions of the globus pallidus led to a blockade of lever pressing with the paw contralateral to the lesion. In these experiments lever access was restricted as it had previously been shown that which lever was used did not affect the effects on pressing (256). Further evidence for the fact that it is the paw preference that is primarily determined by the DA imbalance comes from the fact that it did not seem to matter which lever was used in these experiments. Another experiment has shown that lesions in the entopeduncular nucleus will also affect laterality (360). In this experiment pallidal and LH lesions had no effect.

Ablations of cortex have shown that the effects of electrolytic caudate lesions are not caused by damage to corticofugal fibres as these lesions have no effect on paw use (256,359). However, a more recent experiment has shown that cortical ablations lead to a reduced strength and rate of pressing, especially in the contralateral paw (479). This might be expected as the pyramidal motor system will have been extensively damaged. Although the deficit was most pronounced on the contralateral side there is an effect on the other side too; these rats will therefore not swap paws. The rats lesioned in the crus cerebri in the experiments described in Section 3.3 do not show a drop in performance as might have been expected. However, they do swap paws,

presumably due to damage to the striato-nigral pathway. The lack of effect on performance may mean that the pyramidal motor system is not of vital importance in the behaviour of rats, or that the lesions did not affect the corticofugal fibres.

Glick (226) has suggested that there is a frontal cortical influence on striatal asymmetry and suggests this as a reason for the increase in asymmetry higher up the phylogenetic scale.

The electrolytic lesions of the caudate nucleus, the globus pallidus and entopeduncular nucleus could have damaged the DA fibres as they travel from the SN to the striatum. If this were the case, 6-OHDA lesions would be expected to cause the same effects. Such lesions in the SN or striatum block reaching behaviour with the contralateral paw (546). These rats also show a relative increase in the number of reaches with the ipsilateral paw. Similarly, in this laboratory, 6-OHDA lesions in the MFB have been shown to lead to a large drop in the use of the contralateral paw even as long as 8 weeks after the lesion (590). There is a slight increase in the use of the ipsilateral paw immediately after the lesion (there is an overall reduction in pressing rate at this time) and a complete swap of paw use by 8 weeks after the lesion (when the overall rate has returned to the control level). These experiments do mimic the effects of the electrolytic lesions in as far as there is a block of pressing with the contralateral paw. However, in these experiments (546,590) there also appears to be an increase with the ipsilateral paw. In the electrolytic lesion experiments, the experimental design restricts the swapping over of paw use as pressing is restricted to right paw on right lever and left paw on left lever. There is no opportunity to use the other paw on the lever. It is possible therefore that the effect of the electrolytic lesions in blocking the use of the contralateral paw is due

to the loss of DA from one side and the slight differences result from differing extents of DA depletion.

The experiments described in Section 3.3 also show the swap in paw use after 6-OHDA lesions in the MFB. Also all rats that were initially trained after the lesions learned to press with the ipsilateral paw. These results can also be explained by a reduction in the DA content of the contralateral striatum.

It was noted that in the 6-OHDA lesioned animals after they have recovered from the deficit in total pressing rate there is a strong negative correlation between paw use and lever use; that is, all rats operated contralaterally on the left side pressed on the right lever with the left paw and vice versa. This difference in paw and side preference cannot be explained on the basis of DA imbalances. However, a rather teleological explanation can be given from observation of the rats. The shaping process involves the animals learning to associate the noise of the food dispenser with the arrival of food pellets. Even after the task is well learned, the rats will still respond to the noise. The response seems almost reflexive: the rat rushes to the dispenser immediately on hearing the noise. To some extent this occurs when the rat presses the lever normally. The 6-OHDA lesioned animals tend to circle ipsilaterally (with the lesion on the inside) in the Skinner box, especially at the start of a session. Whenever the lesioned rat presses the lever, and "reflexively" dashes to the food hopper, it will move in this ipsilateral direction. Therefore, if it presses the ipsilateral lever it needs to turn a full circle to reach the centrally placed food hopper. The rat soon learns that this takes a long time and begins to use the contralateral lever from which there is only a short distance to the centrally placed food hopper. The rats appear to have no difficulty in

moving against the tendency to circle in order to use the contralateral lever.

Specific lesions of striatal cell bodies with the neurotoxin NMA and temporary lesions of the preferred forelimb (during anaesthesia and 8 weeks after the paralysis) do not show this strong negative correlation between paw and lever preferences. However, these rats do change the paw preferentially used. This indicates that the use of the initially unpreferred paw does not necessarily lead to a change in the relation between paw and lever use. The negative correlation seen in the 6-OHDA lesioned animals seems to be related to the strong tendency for spontaneous circling, not seen in the other groups of animals.

The NMA lesions and peripheral paralysis, along with KA lesions of the striatum, electrolytic lesions of the crus cerebri and training experiments designed to cause a change in paw use all lead to a change in the preferred paw when given contralaterally to the initially preferred paw. Ipsilateral procedures had no effect on the paw pressing behaviour. This indicates that all these procedures (excluding the training experiments) lead to a motor deficit on the side contralateral to the lesion. This will be further discussed later in this section.

The above discussion strongly indicates that the function of the nigro-striatal system is not bilaterally symmetrical and that the effect of unilateral lesions to the system will depend on which side of the brain is operated. Also, it is clear that not all rats show the same direction of asymmetry. Glick has shown a population bias towards the right side (226), whereas the experiments described in Section 3.3 suggest a left bias. It seems that the paw preference is the stronger indicator of this asymmetry and that the side preference is secondary although it does correlate with the DA asymmetry in the control animals. The

divergence of paw and lever preference after 6-OHDA lesions in the MFB might provide an insight into the behavioural mechanisms involved in these tasks.

Performance and the effect of lesions

Neuroleptics have been reported to cause performance deficits as have bilateral 6-OHDA lesions. Most studies using unilateral lesions to study performance have not taken the inherent asymmetry of the animals into consideration (see Section 3.1). The only experiment reported that has done so is that by Uguru-Okori and Arbuthnott (590). This study shows a large performance deficit in animals lesioned with 6-OHDA contralateral to their initially preferred paw. Animals lesioned ipsilaterally did not have this deficit. At 8 weeks after the lesion the total pressing rate had returned to normal, but the paw reversal remained.

The 6-OHDA experiments described in Section 3.3 also show this deficit at 1 week after the lesion. By 4 weeks after, the rate has partially recovered and by 8 weeks it has fully returned to the control value. The reversal in paw use is also seen. None of the other procedures carried out mimicked this effect on performance although all the contralateral procedures led to a swapping of paws. These procedures included lesioning the cell bodies in the striatum with NMA or KA, electrolytically lesioning the striato-nigral pathway in the crus cerebri and peripheral lesions with local anaesthetic. Training the rats to use the other paw also lead to the reversal of paw use with no drop in total rate.

The lack of effect of all these procedures, except the contralateral 6-OHDA lesions on total performance implies that DA depletion leads to more than a motor deficit in the lesioned animals. That there is a motor

deficit in these animals is implied by the fact that they change their paw use at all and also by the tendency to circle spontaneously and in response to DA agonists. That the deficit is not just motor and that there is likely to be another component to the effect of the lesion is shown by the fact that the other procedures affecting the motor system and leading to a change in paw use do not affect the total rate of performance whereas the 6-OHDA lesions do.

From Figure 23 it seems that animals operated on the side ipsilaterally to the initially preferred paw might have a deficit in performance after the lesion. This is due to the training conditions during this experiment. The experiment was done as part of a teaching course, but the initial training was done beforehand by a single observer. Most of the post-operative sessions were observed by the class of students. Although the design randomised the division of rats into those trained by students and those not, it was clear that the rats trained in the presence of the class had a lower rate than the others. Because of this less emphasis was placed on the absolute number of presses and attention focused on the proportion of the various types of presses.

Despite the problems of being part of a teaching course, this experiment does show that these lesioned animals could not be persuaded to use the contralateral paw, even when the ipsilateral one was paralysed with local anaesthetic. The control rats press most often with the unanaesthetised paw whereas the lesioned rats still press most often with the anaesthetised one and sometimes with both paws or the nose. They rarely press with the paw contralateral to the lesion. This implies that the lesion is more disruptive to the animal than the local anaesthetic is. The significance of this is considered later.

Learning and 6-OHDA lesions

Although there has been difficulty in designing experiments to study learning in bilaterally 6-OHDA animals due to the lack of spontaneous movements, one experiment has suggested that these lesioned animals do have difficulty in learning tasks. A lack of acquisition in a brightness task was shown to exist in experiments which forced the animals to move by placing them in an underwater Y-maze (484). Bilaterally, lesioned animals did not seem able to learn the task, even although they did swim through it. This was used as evidence for a learning deficit.

The experiments described in Section 3.3 also suggest that these animals have a reduced learning capacity. These animals were trained 4 weeks after the lesion and so had partially recovered from the performance deficit observed immediately after the lesion. These rats did learn to press for food, but did so more slowly than controls. Also, all learned the task with the paw ipsilateral to the lesion. The reduced learning rate may have been related to the high degree of spontaneous turning exhibited by these animals in the Skinner box. In this case, the decreased rate of learning would be due to the motor problems that are present in these animals. Alternately there may indeed be a learning deficit.

Rats trained to use the initially unpreferred paw, learn to use it with no deficit in total performance. If the rats needed to learn to use this paw after a motor lesion, a learning deficit might result in the drop in performance seen.

The nature of the performance deficit caused by unilateral 6-OHDA lesions

The nature of the deficit in performance caused by 6-OHDA lesions of the nigro-striatal system will give an insight into the role DA plays in that pathway. Various possibilities have been suggested for the role of DA in the system. It has been suggested that DA in this system only mediates motor behaviour. It has also been suggested that DA in this pathway is involved in the motivational state of the animal, and that motor deficits are only secondary to this. The third possibility is that DA serves a role in learning behaviour. The arguments for all these possibilities are considered in Section 3.1.

The working hypotheses used in the experiments described in Sections 3.2 and 3.3 are (i) that a motor deficit in the animals on one side is sufficient disruption to lead to an overall pressing deficit and (ii) that after motor disruption on one side the animals must relearn the task with the other paw and that this results in a drop in total pressing rate.

The first hypothesis was tested by giving the rats lesions to the motor system contralateral to the initially preferred paw and then comparing the effects of this to those of the 6-OHDA lesions. As discussed in Section 3.3 these motor disruptions lead to a change in paw usage, but there is no drop in performance as is seen in the 6-OHDA lesioned rats. This indicates that a lesion that paralyses one side, does not of itself lead to a drop in performance as the rat will continue to press at the same rate but with the other paw. However, as the rats do swap paws in both the 6-OHDA and other experimental cases, it would seem that the lesions do make it difficult for the rats to use the contralteral paw, and this is in all probability due to a motor deficit

especially when considered along with the rotation occurring after unilateral 6-OHDA lesions. However, it is clear that there is another component to the deficit caused by the lesion as well as the motor one. This is because of the drop in performance seen after 6-OHDA that is not seen after the other disruptions of the motor system.

To test the second hypothesis, rats were retrained to press the lever with the other paw. They managed to learn the variation in the task with no difficulty and there was no deficit in the pressing rate. As the 6-OHDA lesioned rats did show a deficit, it might be that there is a deficit in learning. A learning deficit is supported by the fact that rats lesioned before the initial training procedure learn to press more slowly than controls. However, this could be explained by the high levels of spontaneous turning distracting the rats from concentrating on the task.

Another possibility is that a mechanism for generalisation has been lost and that previously acquired knowledge cannot be applied to other situations easily. Rats given local anaesthetic into the preferred forelimb will change the paw used with no sign of having to relearn the task, they appear to be able to apply knowledge already learned. This would support the loss of a generalisation mechanism rather than a learning deficit.

The final suggestion for the role of DA in the nigro-striatal pathway is that it is involved in motivational or reward behaviour. This postulation is less easy to investigate than the others and this thesis does not shed much light on the problem. It does seem, however, that there is another component to the deficits as well as the motor one, and although this could be a learning or generalisation problem, as suggested above, it could be a deficit in the motivational drive of the animals.

There is strong evidence that neuroleptics block reward behaviour and this would support a motivational deficit caused by DA depletion. This explanation is clear enough for a bilateral lesion, but an overall deficit resulting from a unilateral DA deficit is more complicated. However, the DA asymmetry reflected in the existence of side preferences could also be reflected in other asymmetries, or more correctly other behavioural asymmetries could be reflected in the DA asymmetry. The side of the brain with most DA, may well have developed in relation to motivation in such a way that the other side of the brain is unnecessary and so becomes redundant. When the system with the higher DA is removed and the position is reversed, the other side of the brain takes time to assume its role in motivating the animals. In support of this it is seen that the effects of 6-OHDA lesions do recover with time - both the performance deficit seen in this study and the severe aphagia, adipsia and akinesia seen in the LH syndrome. However, the motor deficit does not seem to recover as the paw preference remains and the rats still turn in response to agonists for long periods of time. This is further evidence for separate motor and other behavioural deficits.

This type of lateralised function is known to exist for example the dominance of one hemisphere for speech in humans (257). Also in humans a condition of unilateral neglect exists that may resemble the sensory neglect seen in LH lesioned rats (270). Therefore the concept of lateralisation of ~~abstract~~ psychological functions is not completely new.

It is strange that rats lesioned ipsilaterally to the preferred paw still will not use the paw contralteral to the lesion when the ipsilateral paw is paralysed with local anaesthetic. This might imply that the deficits caused by the 6-OHDA on the contralateral side of the body are more disruptive than complete paralysis of the forelimb. If the deficit

had two components, a peripheral motor paralysis might be expected to be less disruptive than a lesion causing a motor deficit as well as another deficit. If motivation is supported by one side of the brain, a lesion ipsilateral to the preferred paw should not affect this aspect of the functioning of the system. However, the rats still cannot be persuaded to use the paw contralteral to the lesion by using local anaesthetic. It is possible that the central lesion to the motor system causes more damage than a peripheral one, for example by affecting a greater degree of musculature, and this accounts for the failure of these rats to swap paws. Alternately there might be a some "other" function on this side of the brain. This is very possible as lateralisation of function is reported never to be complete (257).

The differences in effect of the KA and NMA on one hand and of 6-OHDA on the other, must also be discussed. As the KA and NMA remove most of the cell bodies in the area the nigro-striatal pathway projects to, the KA and NMA lesions would be expected to mimic the effects of 6-OHDA as DA released in the striatum will not be able to have any effect. This does not happen: the deficit in pressing rate is not seen after the neurotoxic lesions of the striatum. There could be two explanations for this. Firstly, the KA and NMA might not destroy a sufficient portion of the striatum to cause the deficit in performance, but enough to cause a change in paw use. The other explanation is that the deficit is not caused by the destruction of the DA terminals in the striatum but of those in the nucleus accumbens. The fibres in the MFB that are destroyed by the 6-OHDA also project to this nucleus as well as to the striatum. It is possible that the motor components of the deficit caused by 6-OHDA are caused by loss of the DA in the striatum, but that the drop in performance seen is due to DA loss in the nucleus

accumbens. This agrees with the hypothesis put forward by Iversen in 1977 (283). Here Iversen proposed that the mesolimbic DA system provided motivational arousal and that the striatum provided motor arousal.

The evidence for this comes from the fact that the two areas have different functions with respect to DA. Because of the similarity of the two areas, the differences will arise from the different connections of the two systems. The nucleus accumbens is connected to areas of brain that are supposedly involved with "emotive" behaviours whereas the striatum is part of the exptrapyramidal system and as such is involved in the processing and execution of movements. Because of this Iversen (283) suggested that the nucleus accumbens was involved in the motivational arousal necessary for movement to occur and that the striatum is involved in the carrying out of the movements.

The difference in function in the two areas is that DA in the nucleus accumbens mediates locomotion, whereas the striatum mediates the stereotypy caused by amphetamine. The evidence for this comes from experiments using lesions along with direct injections of drugs into the brain. Amphetamine which releases DA from the terminals, results in increased locomotion and in a pattern, of stereotyped behaviour, including biting, gnawing and licking (283,321). The hyperactivity seen on the administration of amphetamine appears to be mediated by the nucleus accumbens as 6-OHDA lesions of this area block this component of the response to amphetamine, but not the stereotypy. Lesions of the striatum, however, abolish the stereotypy, but do not affect the hyperactivity (283,319,320). Injection of DA into the nucleus accumbens will lead to increased locomotion (465,467), and injection of haloperidol into the nucleus accumbens blocks the effects of systemic d-amphetamine

on locomotion (466). These experiments suggest that the nucleus accumbens is involved in motor behaviour to some extent, although Kelly (319) suggests that the locomotor activity is not really a motor phenomenon, but an indicator of an increased state of arousal of the animals. The fact that manipulations of this nucleus can affect 6-OHDA induced turning supports this. Turning induced by lesions of the caudate nucleus is blocked by further lesions (with 6-OHDA) in the nucleus accumbens (320,482), and it has been suggested that the mesolimbic DA system might in fact amplify the striatal asymmetry (320). The mesolimbic and mesocortical DA systems certainly seem to be necessary for the normal exploratory behaviour, as 6-OHDA lesions of these will reduce exploration in a novel environment, and this effect can be reversed by apomorphine (187). The authors of this study concluded that mesolimbic and mesocortical DA was necessary for normal exploratory behaviour and that the system might facilitate optimal sensorimotor integrations.

It seems from the above that DA in the nucleus accumbens is to some extent involved in the initiation or expression of motor behaviour. It could be that the deficit caused by removal of limbic DA is responsible for the drop in pressing rate seen after 6-OHDA but not after KA or NMA in the striatum.

In conclusion it seems that there is a motor deficit in rats given 6-OHDA into the MFB. There also appears to be another deficit, possibly in the learning ability of the rats, but also possibly in the motivational state of the animals or of the generalisation ability of the rats. The second aspect of the deficit is most likely to be mediated via the lack of mesolimbic DA rather than striatal DA whereas the motor deficit is mediated by striatal DA.

NMA and KA lesions of the striatum

The lesions caused by NMA described in Section 3.3 resemble the KA lesions. The histological profile is similar although the NMA diffuses less through the brain tissue than the KA does. This may contribute to the reduced toxicity of the compound when compared with KA. Although extensive lesions were observed with NMA, the motor abnormalities were not as severe as those seen after KA injection. Bleeding from the nose and the urinary tract was never observed in NMA lesioned animals, whereas it was quite common in the KA lesioned animals. Also none of the NMA lesioned animals died from the effects of the neurotoxin, whereas several of the KA rats did, or had to be killed because they were so unwell. The lower level of toxicity of the NMA suggests that it is a much better tool to use for lesioning cell bodies than KA is.

The differences in motor behaviour seen on recovery from the anaesthesia is very noticeable. KA injected rats, after a period of ipsilateral circling, will run contralaterally for long periods after the injection of KA (239,288 and Section 4.3), having full convulsions every so often. These convulsions involve full body twists and rearing in the direction contralteral to the side of the lesion. After these convulsions the rats are unconscious for a few minutes. On regaining consciousness the rats start running in contralateral circles again. The NMA lesioned rats tend to circle ipsilaterally most of the time, but more slowly and under control than the KA lesioned animals. From time to time these rats also show signs of convulsive activity. However, these are minor and often only consist of contralateral forepaw twitches or a few body twists in the contralateral direction. The NMA injected rats do not lose consciousness after these convulsions. It is interesting

that the convulsive activity is expressed in a contralateral direction, but the increased locomotion is ipsilateral. In the KA rats, the ipsilateral and contralateral phases are completely separated. Taylor *et al* (585) have reported that rats injected with NMA only rotate ipsilaterally, and not contralaterally at all. The explanations given for this is that the different glutamate analogues may have differential actions on different glutamate receptors. However, it may possibly reflect the action of the KA on the cell firing patterns in the striatum and SN.

James and Taylor (288) have shown that ipsilateral circling produced by KA is associated with a decrease in the firing of SNC cells and an increase in SNR cell firing. Conversely, the contralateral rotation was associated with an increase in SNC cell firing, and a decrease in SNR cell firing. Braszko *et al* (49) have shown that 1 hour after KA injection, the firing rate of the DA cells had increased. This time corresponds to the time when James and Taylor (288) see ipsilateral rotation and a decrease in DA cell firing. Twelve hours later, when James and Taylor see contralteral circling, and an increase in SNC firing rate, Braszko *et al* see fewer cells firing; Braszko *et al* also show that at this stage GABA will cause the SNC cells to start firing, but that glutamate will not. The authors suggest that the excitatory input from the striatum has increased the firing of the DA cells so much that they have entered depolarisation block (see Section 4.4). The differences between these two studies (49 and 288) is difficult to explain. At a time when one study shows an increase in DA cell firing, the other shows a decrease. The dose used in each case is similar (2.13 µg in 288 and 2.5 µg in 49) but the volumes may be different. Braszko *et al* (49) used a volume of 1 µl while James and Taylor do not report what volume was used. As a larger volume will spread further in the brain and it

might have different effects than a lower volume. The more widespread the area of action, the greater the area excited by the neurotoxin. However, if it does not spread far, there will be greater excitation in a more limited area. These differences could lead to different time courses and extents of effects seen. It is possible that in the case of Braszko *et al* (49), the cells are entering depolarisation block sooner than in the other case and that the acute effects wear off sooner leading to increased firing at the later time.

It is not clear whether changes in firing rate of the DA cells will be reflected in the circling behaviour of the animals. If the input to the SNR from the striatum is being maximally stimulated, the DA input to the striatum would not be expected to influence the output, if the striato-nigral pathway only mediates the output from the striatum. However, if this pathway also mediates the feedback, or part of the feedback control of DA cell activity (see Section 4.1) it might be expected to affect the output from the striatum and hence the motor behaviour of the animals. An oscillation in the system might explain the alternation from ipsilateral to contralteral in the NMA lesioned animals. The large excitatory effect of the NMA in the striatum leads to activation of the DA cells which then reduces activity in the striatum, and also the circling would reduce. The oscillation might also result from phases of depolarisation block in the cells. When the DA cells stop firing the inhibitory influence they exert will also be stopped. This could lead to the convulsive contralateral activity. Once the cells recover, they start firing and replace the inhibition in the striatum and stop the convulsive phase of rotation. Also it is possible that the more potent KA will excite the output so much that the effect of the DA input is not seen, whereas NMA which is less excitotoxic, might allow the expression of changes in DA cell activity.

Functional subdivision of the striatum

Various reports have suggested that there is a functional division of the striatum. Injection of the DA agonist, 2-(N,N-dipropyl)-amino-5,6-dihydroxytetralin at various sites in the guinea pig striatum produces different behavioural responses (102). Hyperactivity could be obtained without biting in lateral positions, the more dorsal the placement the better. Biting was obtained from ventromedial regions where the hyperactivity response was more variable. In rostral areas only weak biting occurred, but hyperactivity resulted. Lesions in anterior and medial positions have been reported to impair delayed alternation retention (147), but to leave visual discrimination unaffected.

Local application of crystalline DA or amphetamine onto the ventral anterior striatum, decreased the efficiency in a DRL task (418a) and affected LH self-stimulation (420). It was suggested that this area of striatum was involved in the modulation of behavioural arousal and as such functioned similarly to the nucleus accumbens and differently from the rest of the striatum. Although the authors say it is not the case, this may be due to spread to the nucleus accumbens as the ventral anterior striatum is very near to this nucleus. The lesion that was effective in producing a change in paw use in Section 3.3, seemed to include this region although it was possibly more lateral and dorsal as well. No deficit in performance in the lever pressing task used here was seen. The difference may be that the DA implants were bilateral and so a swap of paw use would not be possible and a deficit in performance would occur. It is worth noting that here it is an excess of DA that is resulting in the performance deficit and not a lack of it. This emphasises the fact that a correct DA balance is necessary for normal function, not just the presence of DA.

The problem of separating motor and motivational or other effects after bilateral lesions of the striatum again make interpretation difficult.

The existence of an area that must be damaged to result in a change of paw use supports the conclusion that the striatum is functionally divided, as does the existence of an area that must be lesioned for turning in response to apomorphine to occur. Because of this subdivision, care must be taken in interpreting data from lesions of this area. Details of which areas are affected and how great the extent of the lesion is, are important.

Lesions and turning behaviour

Unilateral destruction of the nigro-striatal DA pathway leads to spontaneous ipsilateral rotation in rats, which is much amplified by amphetamine. Apomorphine causes a contralateral rotation (592,593). The turning is attributed to the imbalance in the DA systems. The turning behaviour is mediated by the striato-nigral pathway and then by one or more of the ventromedial thalamus, the midbrain reticular formation or the superior colliculus (see Section 4.1). It is reasonable to assume that lesions of these pathways should affect the rotation in 6-OHDA lesioned animals as the effects of DA are mediated by these pathways. Also in control animals these lesions should cause rotation when the system is challenged with apomorphine. This has been reported in several studies (206,208,540). Rotation in KA lesioned animals has been reported and suggested as a model for studying intact DA receptors (540). Animals with lesions in the crus cerebri have also been reported to turn in response to apomorphine, and also these lesions, made ipsilaterally to a 6-OHDA lesion, will block the turning effect of that lesion (206). KA lesions of the ventromedial thalamus will reduce the turning

to apomorphine after 6-OHDA lesions (208) and lesions of the midbrain reticular formation and superior colliculus will result in turning to apomorphine (208).

The turning rates in the 6-OHDA lesioned rats tends to be higher and turning is elicited by a much lower dose of apomorphine than the other lesions (0.3 mg/kg cf 2 mg/kg). Also all the 6-OHDA lesioned rats exhibit high rates, whereas only some of the other lesioned ones do. The turning in response to apomorphine was shorter in duration in the striato-nigral lesioned animals and so the testing sessions were 30 min instead of 45 min. The different behaviour of the 6-OHDA lesioned rats in response to apomorphine reflects the supersensitivity that has developed to DA after the lesion (540). This does not occur in the case of the striatal lesions. The reason for the less consistent turning in these animals may be that the lesion has not affected a large enough portion of the area of striatum involved in turning (see above). The lesions affecting the input will get most of the striatum and therefore will be sure to have reduced the DA input to this area.

As well as being elicited at a lower dose, the turning behaviour emitted by the 6-OHDA lesioned rats is much more compulsive than that of the other lesions, and is observed spontaneously, long after the lesion. Spontaneous rotation is not seen in the crus lesioned rats and is not seen after the acute effects of the neurotoxins have worn off. The compulsive element of the turning seen in the 6-OHDA lesioned animals may be due to the limbic component. The asymmetry is provided by the lesioned striatum but the increased locomotion leading to the continual circling results from the imbalance in the nucleus accumbens. That the turning response seen in 6-OHDA lesioned animals is a two component system has been suggested in the literature by Pycock and Marsden (482).

These authors suggest that as well as a striatal imbalance, mesolimbic stimulation is necessary for rotation to occur.

CHAPTER IV

An Electrophysiological Study into the Functioning of the Nigro-Striatal Pathway

This Chapter is divided into four main sections. The first is an introduction covering the relevant background information and the rational behind the experimental designs. The second section describes the methods used, the third gives the results and finally the fourth section discusses these results.

4.1 Introduction

The SNC contains DA cells which send projections to the ipsilateral striatum (see Section 1.2). The electrical activity has been recorded from these cells in several species (62,132,153,156,243,250). The cells are spontaneously active with a characteristic action potential shape (6,235,250).

DA released in the striatum as a result of the firing of the DA cells, leads to a response in the post-synaptic striatal cells. There is some controversy about the nature of this response. Many results have shown a predominant inhibition in response to iontophoretic DA (29,44,93,124,163,178,231,383,392) or nigral stimulation (92,93,178,231). In some studies, however, comparison of the effects of nigral stimulation with those of iontophoretic DA are conflicting (93,392). In some studies there are also excitatory responses (35,36,44,92,93,178,392) and in others, especially those using intracellular recording show only excitatory responses, or at least EPSP-IPSP sequences, to nigral stimulation (280,281,342,344,345,562) or iontophoretic DA (342,562). The effect of iontophoretic DA and nigral stimulation is blocked by chlorpromazine (CPZ) (646). One suggestion has been that the two types of response were due to existence of an excitatory and an inhibitory receptor for DA in the striatum (431). There is a lot of evidence for the existence of more than one receptor for DA (see reviews by Kebabian and Calne,

315, and Seeman, 543), but the explanation for the excitatory and inhibitory effects of DA are not likely to be as straightforward as this. More likely explanations involve faster conducting non-DA pathways (see Section 1.2) or polysynaptic or collateral pathways involving stimulus spread to other brain regions (627).

The evidence in support of multiple DA receptors suggests there is a DA-AC receptor (or D1 receptor) on the post-synaptic cells and another type or types (D2) situated on the pre-synaptic terminals, not only of the DA neurones, but also of other afferents to the area, e.g. from the motor cortex (50,409,539). The D1 type is thought to mediate the effects of released DA on the post-synaptic cells and the D2 type possibly modulates transmitter release from the terminal it is situated on. However, recent reports have suggested that the D2 receptors may be situated on the same, or other, post-synaptic cells as the D1 ones and may also mediate a post-synaptic effect (294,587).

Feedback control of DA turnover?

The activity of the DA cells is under a tight feedback control: any alteration in the DA equilibrium will result in a change in activity designed to offset the disturbance. This effect was first described in 1963 by Carlsson and Lindqvist (65). They showed that, after monoamine oxidase inhibition, the accumulation of O-methylated metabolites seen was enhanced by the neuroleptic drugs CPZ and haloperidol. The suggested explanation was that blockade of the DA receptors resulted in a compensatory activation of the DA neurones themselves. They also suggested that the increase in neuronal activity did not result in a depletion of transmitter levels because the increase in activity was accompanied by an increase in synthesis. At this stage, no further details were given on a possible mechanism for this effect.

The convenient existence of the striato-nigral pathway immediately led to its being proposed as a mediator for the proposed feedback effect. It was suggested that the activation of post-synaptic receptors by DA led to the alteration of activity in the DA cell bodies by way of the striato-nigral fibres.

Since the idea was first proposed a large amount of data has accumulated in favour of the feedback loop hypothesis (FLH). However, in recent years, mostly since 1975, data has also been accumulating that would suggest that the FLH might only be a small part of the mechanism involved in regulating the metabolism of the DA cells. Firstly, the evidence in favour of the hypothesis will be covered and then the evidence against it will be dealt with.

- *Evidence in favour of the feedback loop hypothesis:*

The relation between an increased DA synthesis and increased impulse flow does seem, at first sight, to exist. The activity of the SN DA system is reflected in both the cell firing rate and the turnover of the transmitter. Increased impulse flow in the DA axons leads to an increased activity of TOH, the rate limiting enzyme in the synthetic pathway for DA, possibly by affecting the affinity of the enzyme for its substrate or its cofactor (518).

Stimulation of the SN leads to an increase in DA levels and turnover in the striatum (25,77,349,424,433,533). Similarly, drugs that increase DA turnover in the striatum, such as the neuroleptics haloperidol and CPZ (10,34,241,432,433,434,436,625) also increase the firing rate of the DA cells (5,60,62,624). Conversely, those drugs reducing turnover in the striatum such as amphetamine and apomorphine (62,436,517) also decrease firing rate at a comparable dose (5,54,56,57,59,62,614). The effect did seem to be mediated by the nigro-striatal pathway as an intact DA system seemed to be needed for the effect to occur (11,435).

The effects of stimulating the striato-nigral pathway also seemed to agree with a role for that pathway in the mediation of the feedback control of the DA cell activity. Stimulating the striatum led to an inhibition of firing rate in the DA cells (87,153,448) as does the local application of GABA or GABA agonists such as muscimol (71,448). Stimulation of the post-synaptic DA receptors in the striatum with apomorphine also leads to a reduction in cell firing in the SNC. The GABA antagonists picrotoxin and bicuculline reduce the effects of stimulation (87,153,448) and GABA agonists (448) on DA cell firing.

Biochemically it has been shown that intra-nigral muscimol blocks the effect of haloperidol on striatal TOH and that this effect is antagonist by bicuculine (244) and that GABA antagonists can cause an increase in DA metabolites in the striatum (248).

Lesions of the striato-nigral pathway have been reported to reduce the effects of the DA agents on DA turnover in the striatum (318) or the effect on cell firing rate (56,347).

As well as the straightforward effects of GABA just described, some paradoxical effects have been obtained in response to GABA-ergic agents. These have been seen to cause increases in the DA cell firing rate (234,374,437,616,619). Also there appears to be a lack of direct reciprocal relations between the nigro-striatal and the striato-nigral pathways (198,343). An attempt to reconcile these observations with the FLH suggests that an inhibitory GABA interneurone might exist - the existence of interneurones has been proposed in the SN (194,246, 305,541) - that is itself inhibited by GABA released from the striato-nigral pathway, or by exogenous GABA placed in the SN. This "double inhibition" leads to the paradoxical excitations of the DA cells (234,619). There is evidence that GABA does inhibit SN cells in general

(111,112,172,197,232,394,648,650,651), although in these experiments the type of cell recorded from was often not specified.

For a FLH involving striato-nigral GABA to be feasible the postulation of an interneurone is essential at some point. In order for DA receptor stimulation which results in the inhibition of the post-synaptic cell, to lead to an inhibition of DA cell activity, the inhibitory striato-nigral path would have to be excited if only two neurones were involved. The postulation of a nigral GABA interneurone simplifies this problem. Another approach to this problem has been to propose a striatal interneurone. One candidate is ACh. DA agents are known to affect the ACh systems in the striatum: DA agonists inhibit ACh cells, and so, by reducing release, increase the levels of ACh (25,76,77,547, 548,563). This decreased release of ACh could in turn lead to decreased activity in the GABA output neurones from the striatum. DA does reduce GABA release in the striatum (74) and this could be a reflection of reduced GABA release from collaterals of the output cells or of other cholinoceptive cells in the striatum.

Figure 24 shows diagrammatically how a feedback loop might act to cause a post-synaptic receptor mediated effect via the SN pathway.

However, if SP, also thought to exist in the striato-nigral pathway (see Section 1.2), were involved in the feedback, the DA cells would be excited and therefore no interneurone need be postulated. However, the evidence that it is the GABA-ergic component in the pathway that is important is so great it seems unlikely that SP is involved.

- *Evidence against the feedback loop hypothesis:*

No evidence against the FLH was produced until 1971 when it was shown that CPZ still had an effect on turnover in striatal slices. The amount of ^{3}H-DA released on field stimulation of the slices was increased

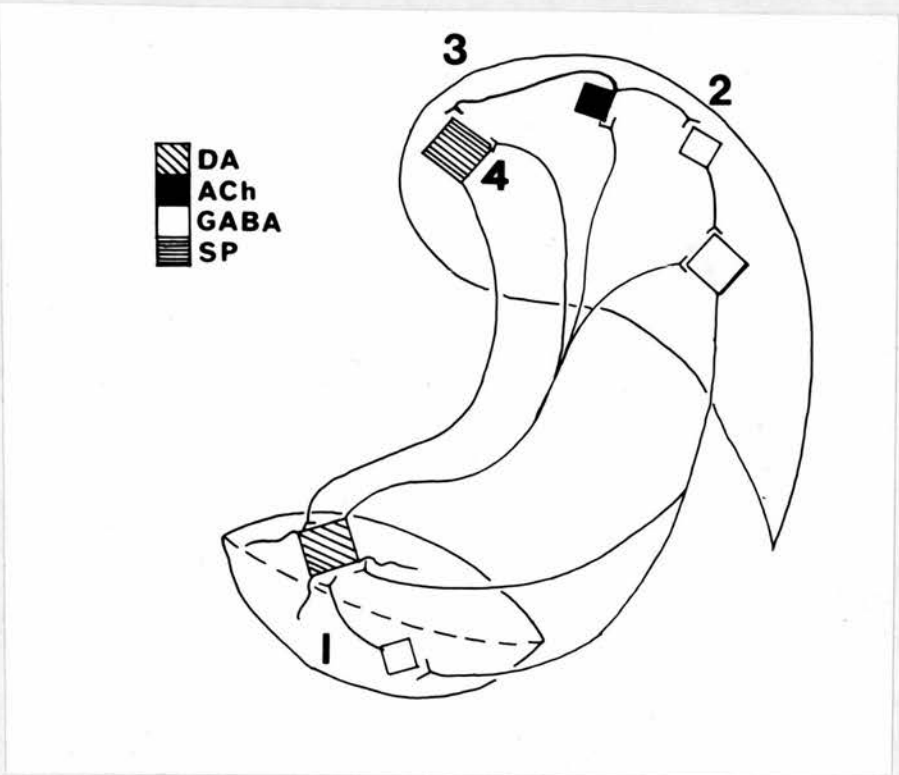


FIGURE 24: Diagrammatic representation of possible mechanisms for the feedback control of DA turnover, involving the striato-nigral pathway.

1. A loop involving a striatal ACh interneurone, a striato-nigral GABA cell and a nigral interneurone, probably containing GABA.
2. A loop involving a striatal ACh cell and a striatal GABA cell along with a striato-nigral GABA cell.
3. A loop involving a striatal ACh cell, and a striato-nigral SP cell.
4. A loop involving direct action of a DA cell on a striato-nigral SP cell.

by CPZ (169). This implied that a local factor might be involved in the regulation of DA release. Then in 1973 it was postulated that the SNC DA cells might also have DA receptors of their own (54). Iontophoretic DA into the SN was shown to reduce the firing rate of the DA cells. This suggested that an intra-nigral mechanism might also be involved. At this stage the local mechanisms were not thought to be a threat to the FLH. A more direct challenge to the FLH was made by Bedard and Larochelle (27). These workers cut the striato-nigral fibres and noted that there was no reduction in the effect of neuroleptics on HVA in the striatum. Therefore, they postulated, the striato-nigral pathway could not be involved in the feedback control of DA turnover. Later, this was supported by the work of Garcia-Munoz *et al* (207) who lesioned the striato-nigral pathway and showed that there was no difference in the effect of haloperidol on DA turnover. The DA receptors on the DA cells themselves have since been christened "autoreceptors" by Carlsson in 1975 (64) although he was more specifically referring to the ones on the pre-synaptic terminals in the striatum.

Since these early reports a large body of evidence has accumulated to suggest that local mechanisms involving only the striatum or only the SN may be involved in the regulation of the DA activity. This evidence will now be discussed.

Although lesions of the nigro-striatal pathway abolished the effects of DA agonists and antagonists on DA turnover, acute axotomy itself increased greatly the turnover of DA (9,618). Inhibition of impulse flow with γ-hydroxybutyrate (GHB), a general CNS depressant (554) or its precursor γ-butyrolactone, both of which have a specific action on the DA system (514) also leads to an acute enhancement of turnover in the striatum (212,449,517,618), but not in the SNC (458a). The

inhibitory action of GBH on cell firing possibly involves its conversion to GABA, especially as it is inhibited by picrotoxin (516). It may also have an endogenous inhibitory function as GBH is found naturally in the brain (515). The reason for this response to GBH is unclear.

This paradoxical response to axotomy and GBH implies that DA release in the striatum is not completely impulse dependent as earlier results may have suggested. In fact it may have been because of the increase in basal firing and release that the effects of dopaminergic agents were no longer seen after lesions of the nigro-striatal path. The increase in DA synthesis occurring on the cessation of impulse flow seems to result from a decreased sensitivity of the enzyme to the end product inhibitor, DA (64). The mechanism causing the change must exist in the striatum as the nigro-striatal connection has been destroyed. Other evidence also suggests the existence of an intra-striatal mechanism for the regulation of DA synthesis.

Firstly, the effect of neuroleptics (169,170,284) and DA agonists (169,170,284,400,565) on release is still present in slices. Other agents can also affect the release of DA in slices. ACh will still cause an increase in DA release (216), as will GABA (162,214,567,572) or a GABA-transaminase inhibitor (573) and glutamate (505). The glutamate effect is blocked by intra-striatal KA and so implies an involvement of the striatal cells in the response. However, other workers have shown that the effect of glutamate is not blocked by tetrodotoxin (TTX), an agent blocking action potentials, suggesting that the effect is pre-synaptic (213). The explanation may be that the KA lesions had some non-specific damage to terminals. ACh will also increase DA release from striatal synaptosomes, but GABA has no effect on synaptosomes. This implies that ACh may act directly on the terminals to affect DA

DA release. These pre-synaptic ACh receptors are thought to be nicotinic (215,217), although both muscarinic and nicitonic receptors have been shown to decrease in the striatum after 6-OHDA in the SN (130).

The fact that ACh and GABA are still effective in slices and ACh in synaptosomes suggests that their role in a FLH may not be quite as outlined in Figure 24. Also as the effects of DA drugs are still seen in animals with KA lesions of the striatum (24,142,144) or brain transections leaving DA paths largely intact, but severely reducing the GAD levels in nigra (640) and that cell firing is still altered by haloperidol and amphetamine in animals with small lesions in the crus cerebri (206) implies that the striato-nigral pathway may not be necessary for a feedback control of DA turnover. Also ACh or GABA interneurones in the striatum may not be necessary, and the mechanism of action for the DA drugs may be a purely pre-synaptic one. This is supported by work with TTX in which the effects of apomorphine and haloperidol are not affected, implying that an action potential is not needed for the effect on release (284). However, the effect of GABA on DA release is TTX sensitive implying it does involve the mediation by an action potential (214). This is in agreement with the synaptosome work.

GABA also decreases the release of ACh, possibly acting via an indirect effect on DA release (358,572).

As well as ACh affecting DA release, DA can affect ACh release as has been mentioned in the previous section (76,247,248,563), in favour of a FLH. This is also true of GABA (74). The reciprocal nature of these interactions is extensively reviewed by Lloyd (371) and argues against a simple feedback loop. A loop may be involved but the total mechanism cannot be as simple. Alternately, the ACh and GABA

involvement in the striatum may be coincidental to the feedback mchanism and represent other influences on the system. Figure 25 shows the postulated mechanisms that may be involved in an intra-striatal feedback mechanism.

As well as evidence for a local striatal effect there is also evidence for an intra-nigral effect on the control of DA cell activity. This concept was first tentatively suggested by Aghajanian and Bunney in 1973 (6,54). They showed that the local application of DA in the SN caused a slowing of the DA cells. In this experiment, amphetamine had little effect and the, later christened, DA autoreceptors were not thought to have a significant role in the control of cell activity. In 1975 Groves *et al* again put forward the idea. This time a lot more emphasis was placed on a role for the DA autoreceptors in the control of cell activity, as an alternative to the FLH (242). In this paper the hypothesis that the dendrodendritic synapses, that were proposed on the observation of varicosoties in the DA dendrites containing DA (39), could regulate the DA cell activity. Synaptic vesicles had been demonstrated in these varicosities as early as 1973 (252). A small number of "autapses", that is collaterals synapsing with the parent cell have also been seen in the SN (312). To test the hypothesis that dendritically released DA could control DA cells by acting on DA autoreceptors, Groves *et al* (242) infused amphetamine into the SN and striatum and recorded the activity of the DA cells at the same time. These direct injections led to strong depression of the firing rate of the DA cells, and was consistent with a local mechanism of control. Since 1975 much evidence has accumulated in favour of this hypothesis.

It was shown that the inhibitory effect of amphetamine on the SNC cells was blocked by α-methyl-p-tyrosine (243). DA release was shown

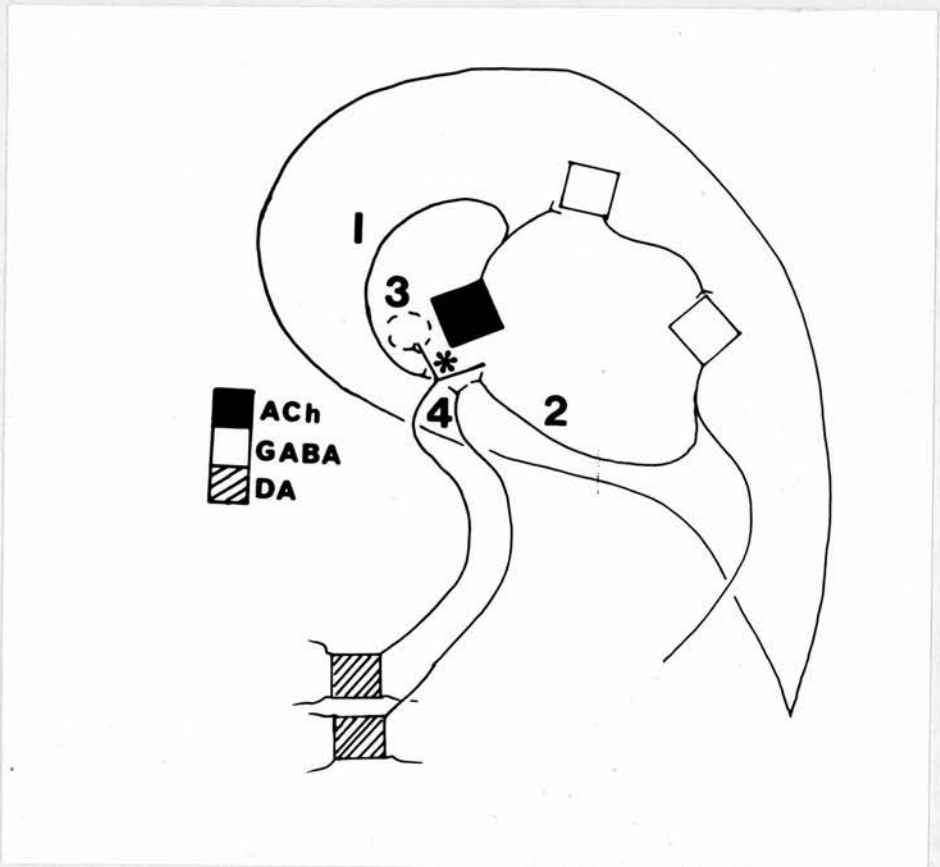


FIGURE 25: Diagrammatic representation of intrastriatal mechanisms for the feedback control of DA turnover.

1. A striatal ACh cell feeds back onto the DA terminal.
2. A striatal ACh cell feeds back onto the DA terminal by way of a GABA interneurone and a collateral from a striato-nigral GABA cell.
3. DA acts directly on its own terminals.
4. A second nigro-striatal DA cell affects the terminals of the first one.

* represents the site of action of all the mechanisms.

to occur in the SN (78) and to be increased by amphetamine and the uptake blocker benztropine (415,451). Nerve ending particles can also be prepared from DA dendrites and these can be shown to release DA (269). DA levels were shown to decrease and the metabolite levels increase after stimulation of the MFB resulting in the antidromic activation of the DA cells (350,351) also K^+ stimulation leads to release (118,211). Uptake of endogenously applied DA can also be shown to occur in SN (118) and release appears to be greater in the SNR, which contains most of the dendrites (118). This is consistent with a role for dendritically released DA.

DA or amphetamine applied locally into the SN does result in decreased release of DA in the striatum as would be expected if nigral DA had a regulatory function (427,428). Similarly, neuroleptics, applied to the SN, will increase DA in the striatum (428). Iontophoretic DA, directly into the SN, will reduce DA cell firing rate (8).

DA can also affect GABA-ergic function in the SN. A large GABA input to the SN comes from the striato-pallidal area (see Section 1.2) and there is also some intrinsic GABA or output GABA cells. Different groups have reported different effects on GABA release. Reubi *et al* (494,495) showed an increase in evoked GABA release in SN slices whereas Arbilla *et al* (14) failed to do this. In fact if large doses of apomorphine were used a slight decrease was observed. In the intact animal, using push-pull cannulae, biphasic results were obtained on GABA release (599). Apomorphine and DA behaving in an opposite manner. This is possibly due to complications from the descending GABA pathway in the whole animal. It can be shown that DA can excite SNR cells which is unusual for this inhibitory transmitter (522). It also seems that DA can affect the release of GABA from the GABA nerve

terminals when these are stimulated by K^+ (566). The exact significance of this is unclear, but it may be involved in the action of the striato-nigral cells on the DA cells. Despite some confusing evidence, it seems that DA does affect GABA in the SN and this suggests a transmitter or modulatory role for DA in the SN.

The existence of DA autoreceptors, both in the striatum and the SN has been demonstrated by autoradiography combined with lesion and binding studies (409). The DA autoreceptors are thought to be of the D2 non-cyclase linked type of receptor, both in the striatum and in the SN. Spiperone binding studies combined with various lesions show this in the SN (483,492) and the striatum (205,492,539).

Biochemistry has also suggested the existence of a DA-AC in the SN (316,463,478), later shown by lesion studies to be on the terminals of the striato-pallido-nigral fibres (203,411,462,478,483,560). Similarly in the striatum the DA-AC receptor has been shown to be on the post-synaptic cells (186,205,539).

Figure 26 shows the postulated feedback mechanisms in the SN.

The current concensus of opinion on the FLH seems to be that it may exist, but that the main function of the striato-nigral pathway is as an output for the striatum and it only secondarily affects DA cell activity, and the feedback control of DA synthesis is in fact controlled by one or more local mechanisms in the SN or the striatum.

A compromise hypothesis was suggested by Bunney and Aghajanian in 1978. They suggest that control of DA cell activity is mediated by both the striato-nigral pathway and the autoreceptors in the SN (58). Skirboll *et al* suggest an interesting point by saying that if the pre-synaptic DA receptors were more sensitive than the post-synaptic ones, low doses of DA agents could possibly result in a net excitatory rather

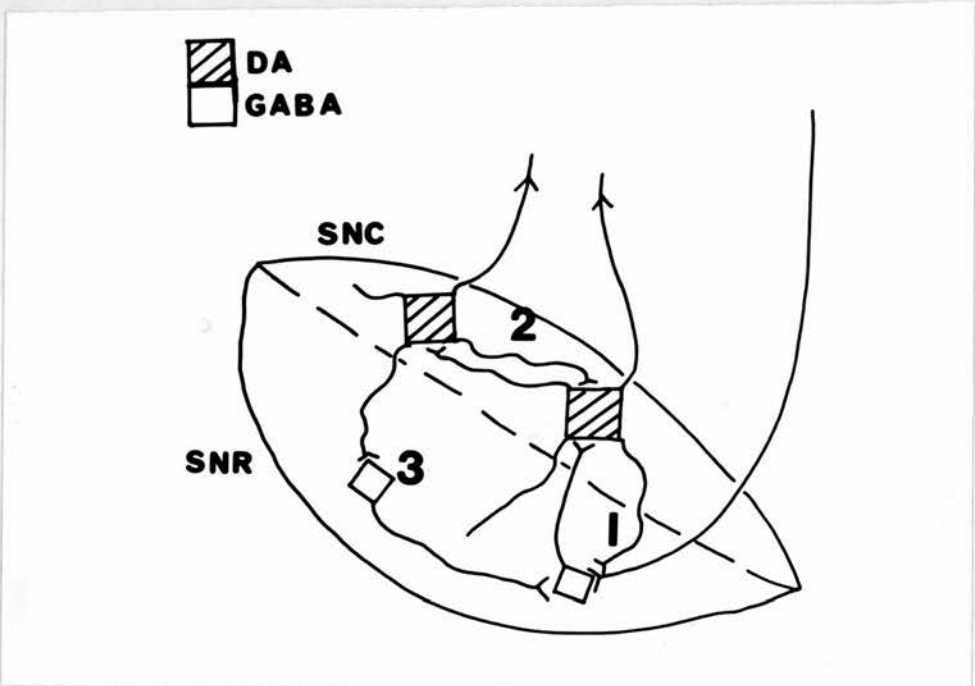


FIGURE 26: Diagrammatic representation of intra-nigral mechanisms for the feedback control of DA turnover.

1. DA from the dendrites of the DA cells, acts on the striato-nigral GABA terminals to affect an interneurone, which then affects the DA cells.
2. Direct lateral (or auto) inhibition between the DA cells.
3. Dendritic DA affects an interneurone, possibly containing GABA, which affects the DA cells via another GABA interneurone.

rather than inhibitory effect (553). This might help to explain some of the conflict in the literature.

An alternative role for the striato-nigral pathway

The unilateral activation of the nigro-striatal DA system leads to contralateral rotation in rats (15) and this is correlated with DA release in the striatum (17). After a unilateral 6-OHDA lesion in the nigro-striatal system rats will turn ipsilaterally when challenged with apomorphine (see Chapter III). Lesions of the striato-nigral pathway will block this behaviour (140,206). Picrotoxin and GABA agonists will also affect the DA dependent turning behaviour of rats, but in a rather complicated way (see review by Pycock, 481). Whether the DA effects are enhanced or blocked appears to depend on where in the SN the drugs are acting (18,489). It seems that the SNR acts as an output station in the processing of DA mediated effects in the striatum, and that the GABA in the striato-nigral pathway mediates this effect (96,140,206,443).

There is still controversy over where the output goes after the SN (see review by Pycock, 481). It may go via the ventromedial thalamus (208,291), the midbrain reticular formation (139,208,408) or the deep layers of the superior colliculus (128,139,208,282), although there is some evidence against the latter (110,335,490) (see also Section 1.4).

Other possibilities for the post-synaptic mediation of the feedback control of DA turnover

Other polysynaptic pathways could conceivably be involved in the feedback control of DA turnover, not involving the striato-nigral path. The crus lesion described (206) does not get the projection from the tail of the striatum to the more lateral parts of the SN (55,588). However, GABA and DA are mostly located in the main body of the

nucleus (277,584) and so the effect is unlikely to be mediated by the lateral fibres. Also the effects of apomorphine and haloperidol on DA cell firing are not affected by lesions getting this part of the striato-nigral pathway (206).

Of the other inputs to the SN (see Section 1.3), the one from the raphe nuclei is the most likely to be involved. Stimulation of the DRN and MRN leads to the inhibition of the nigral DA cells (152,154). It is suggested that 5-HT could cause a tonic inhibitory influence on the DA cells. Lesions of the raphe nuclei also lead to a drop in nigral 5-HT and an increase in striatal dopamine (154). DA agonists have been shown to increase 5-HT levels in the rat brain (491), so the mechanism might conceivably work. The fact that the striatum also receives an input from the median raphe (422,601) suggests the possibility of complex interactions. In further support of a role for 5-HT in the control of DA turnover, 5-HT is also known to be involved in turning behaviour (103,286,287,402,481,613) which may be related to DA dependent rotation, although there is evidence to suggest a non-DA dependent involvement of 5-HT in rotation (440). It is even possible that DA and 5-HT have opposing roles in the control of DA cell activity (115).

However, it is difficult to see how this feedback loop would be completed. It would have to be a very complex circuit.

Another interesting possibility that has not been investigated is that the proposed non-DA nigro-striatal pathway (see Section 1.2) may be involved.

Experimental rational

In the experiments described in this Chapter, an attempt was made to study the control of the activity of SNC DA cells using electrophysiological methods, and paying particular attention to an intra-nigral

mechanism of control. Firstly, it was thought that if DA could be released from the dendrites by antidromically driving the cells from the striatum, it might be possible to see the effect of the released DA on the firing of the cells by constructing peristimulus histograms (PSTHs). In these, the cell firing is time locked to the stimulus so that short-term (several ms) changes in activity can be examined. Such high currents were required to drive the cells antidromically, that what appeared to be interference from the striato-nigral pathway masked any possible effect from antidromically released DA. Complicated sequences of responses resulted from what were presumed to be GABA and SP from this pathway. In order to avoid this interference, lesions of the striatum were made and the effect of driving observed. The earliest lesions used KA injections into the striatum and on examination did not appear to clarify the picture at all. At this stage it was assumed the lesions had not been extensive enough to damage enough of the striatum to prevent the effect and other types of lesion were tried: electrolytic lesions of the striato-nigral fibres where they pass through the crus cerebri, distinct from the ascending DA fibres (206,588). These lesions and other neurotoxic lesions of the striatum (using NMA) also did not affect the pattern. The significance of this is discussed in Section 4.4.

If there is an intra-nigral, DA mediated, lateral inhibition it seemed odd that picrotoxin, a GABA antagonist should block the effects of amphetamine on the DA cell firing, as had been reported (234). In the drug experiments first of all we tried to repeat the experiments with picrotoxin on the effect of amphetamine on DA cell firing. After this, the existence of a mechanism by which dendritically released DA could act via GABA was investigated. DA could act on the striato-nigral terminals to reduce the release of GABA. This would then lead to the

excitation of inhibitory, interneurons themselves possibly GABA containing, which would then cause the inhibition of firing in the DA cells. This is similar to the hypothesis of Grace and Bunney (234) where they also propose a GABA interneurone in the SN which is affected by the release of striato-nigral GABA. In their view, DA was acting in the striatum, while in this view, it is acting in the SN.

To examine this possibility further, we tried to see if the DA feedback obligatorily involved GABA cells or whether the effect on DA cell firing could still be seen after blockade of GABA receptors with picrotoxin. If so, no further effect should be seen with haloperidol after the administration of picrotoxin (which of course will speed up the cells on its own). Haloperidol should not affect the action of picrotoxin. The same rational was used in studying the effects of drugs on the PSTHs. It was hoped that this would show whether any antidromic effect was mediated via GABA receptors or not.

This is further discussed in Section 4.4, after the methods used and the results obtained are given in detail.

4.2 Electrophysiology methods

The spontaneous activity of the dopamine cells in the SNC was recorded extracellularly. These cells were driven antidromically from a stimulating electrode in the striatum. Records of spontaneous activity and PSTHs were made from each cell. The activity of cells from both control and striato-nigral lesioned animals was recorded. The effects of several drugs on the firing of control cells were also studied.

Surgical procedure

All experiments were performed on male albino Wistar rats, ranging in size from 220-300 g. Anaesthesia was induced in a plastic box, with

a 4% mixture of halothane (Fluothane, ICI Ltd) and medical air. A tracheal cannula (outside diameter 2.1 mm, Portex Ltd) was inserted to facilitate breathing. Anaesthesia was then maintained at 1%, with a flow rate of 200-300 ml/min. The tracheal cannula was connected to a Y-piece, along one arm of which the anaesthetic was supplied. The anaesthetic was removed along the other arm by suction.

In drug experiments a cannula (outside diameter 0.63 mm, Portex Ltd) was placed in the femoral vein of one leg, to allow the injection of drugs. If necessary, another cannula (outside diameter 0.75 mm, Portex Ltd) was placed in the adjacent femoral artery to allow the recording of blood pressure. The intravenous cannula was perfused with normal saline at a rate of 150 µl/hr to prevent the cannula blocking. Blood pressure was recorded on a Devices chart recorder, via a transducer (0-75 cm Hg, Type 4-327-L221, Bell and Howell Ltd). The cannula and transducer were filled with normal saline in which was dissolved 50 units/ml heparin sodium (Pularin, Duncan Flockhart Ltd). On flushing the cannula, care was taken to get as little heparin as possible into the circulation of the animal as this could lead to death from bleeding.

After this the rat was laid on a heated blanket (Thermega Ltd) and put in the stereotaxic frame (David Kopf small animal frame) using blunt earbars, tip angle 45°. The tooth bar was set at 2.4 mm below the earbar zero. A midline incision was made to expose the skull and the muscles at the back of the neck. Two burr holes, 2 mm in diameter were drilled with a dental drill to insert the stimulating and recording electrodes. Before inserting the electrodes the dura matter was removed with fine forceps and the brain surface covered with liquid paraffin to prevent the cortex from drying out.

Two approaches were used to position the nigral recording electrode. The first of these was to insert the electrode at 46° to the vertical. The co-ordinates used for this approach were as follows:

4.5 - 5.2 mm posterior to bregma
6.8 - 7.0 mm lateral/oblique to bregma
6.0 - 8.0 mm ventral/oblique from brain surface

Because of the angle of the electrode, the lateral and ventral measurements are also at 45° to the axis of the frame, hence the use of the term "oblique". The more lateral and anterior measurements were used in the bigger animals.

The second approach was to insert the electrode in vertically at the following co-ordinates:

4.5 - 5.2 mm posterior to bregma
1.5 - 2.0 mm lateral to bregma
7.0 - 8.0 mm ventral from brain surface

Again, the more lateral and anterior values were used in the larger animals.

The latter approach allowed more electrode penetrations in each animal before the brain became too damaged to record from. However, in this way, fewer cells could be recorded on each penetration because of the smaller size of the SN when approached from above.

The striatal stimulating electrode was placed in various sites both in the head of the striatum and further back towards the tail. The co-ordinates for two of the sites are given below.

0.5 mm anterior or 1.0 mm posterior to bregma
2.5 mm or 4.0 mm lateral to bregma
4.5 mm ventral from the brain surface.

The position of the electrodes was verified histologically at the end of each experiment, as described in Section 2.1.

In the animals lesioned with neurotoxins, the stimulating electrode was placed as near as possible to the centre of the lesion by inserting the electrode at the same co-ordinates as were used for the injection of the neurotoxin.

Animals which were given picrotoxin were given injections of gallamine triethiodide (Sigma) intravenously. A dose of 16 mg/kg was used and was repeated every 45 min or as necessary. Because of the paralysis induced by the gallamine, these animals were ventilated artificially, using a Bioscience air pump, model No. B15-51190-1. The ventilation rate was set at 60 cycles/min and the volume (2-5 ml) set to maintain the blood pressure as near to control as possible.

Cells which were recorded from animals with an unsteady blood pressure were not used. Slight decreases in blood pressure did occur on the injection of any solution. Also, during surgery on the head, fluctuations in blood pressure occurred which steadied after the operation. Figure 27 shows sample blood pressure traces.

Stimulating procedure

Concentric bipolar stimulating electrodes (Rhodes Medical Instruments Ltd) were used throughout. These had an outside diameter of 0.5 mm. Stimulating pulses were generated by an isolated stimulator (Digitimer Ltd, Type No. 2533), supplying a constant voltage from 0 V to 100 V. Pulse lengths varied from 0.2 ms to 0.5 ms, with a very occasional longer pulse of 1 ms. The frequency of stimulation was either 1/1.1 s or 1/1.3 s. The currents involved were later calculated from a calibration curve, plotted from resistance measurements made at the end of an experiment. The currents used varied from 40 μA to 3000 μA.

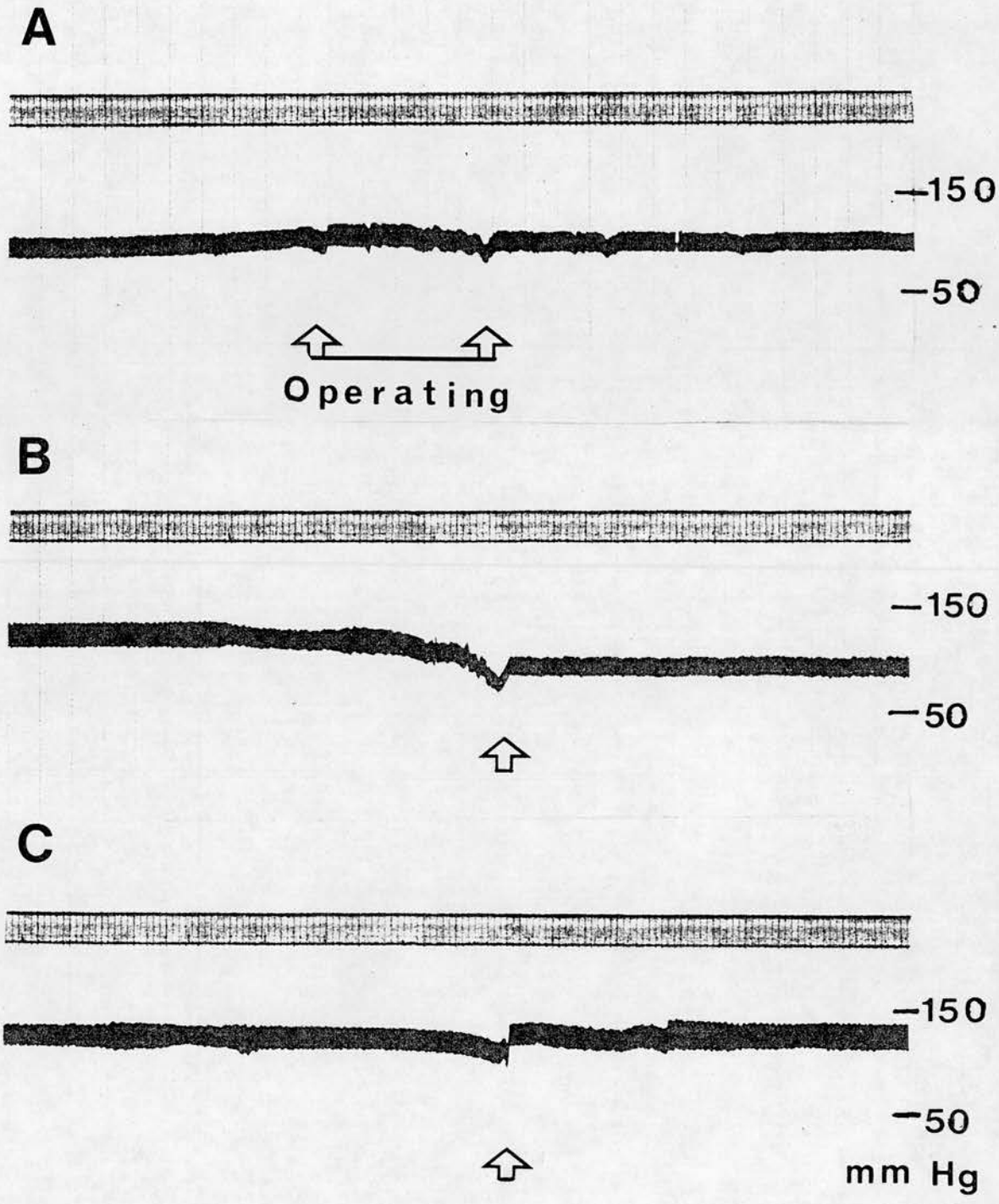


FIGURE 27: Sample blood pressure traces.

A. Control, with variations due to operating on the head.

B. Before and after the start of ventilation.

C. The effect of the injection of 0.3 ml saline.

The timing of the stimulating pulse was controlled from another stimulating unit, capable of generating several pulses to control all the equipment (D 100, Digitimer Ltd). All connections are shown in Figure 28. A typical stimulating site is shown in Figure 29, both in a control and an NMA lesioned striatum. In the lesioned animals, the stimulating electrodes were placed as near as possible to the centre of the lesion. This was done by using the same co-ordinates as for the injection and noting where in the burr hole the needle was positioned. In one experiment, an attempt was made to estimate the extent of stimulus spread. This was done by inserting an array of four electrodes, all of the same type as used in the other experiments, into the striatum of the rat. Measurements were then made on the stimulation strengths, and other parameters needed to drive the same cell antidromically from the different stimulating sites.

Recording procedure

Recording electrodes were made from double barrelled thetaglass (Clark Electromedical Instruments Ltd, TGC 150). These were pulled on a Forth Instruments microelectrode puller, and had a resistance of 45-60 Mohms, as measured on a voltohmeter (W.P. Instruments Ltd, model No. F-29). The electrodes were filled with 2% pontamine sky blue dye (Gurr Ltd) in a 0.5M solution of sodium acetate (BDH), filtered and adjusted to pH 7.7.

The recording electrode was moved through the brain by a microdrive unit (Forth Instruments Ltd) using remote control. While the electrode was being moved through the brain to the correct area, it was moved at 100 µm/s, 2 µm at a time, and while searching for cells in the substantia nigra, the electrode was moved at 4 µm/s. Each electrode could generally be used several times. At the end of the experiment,

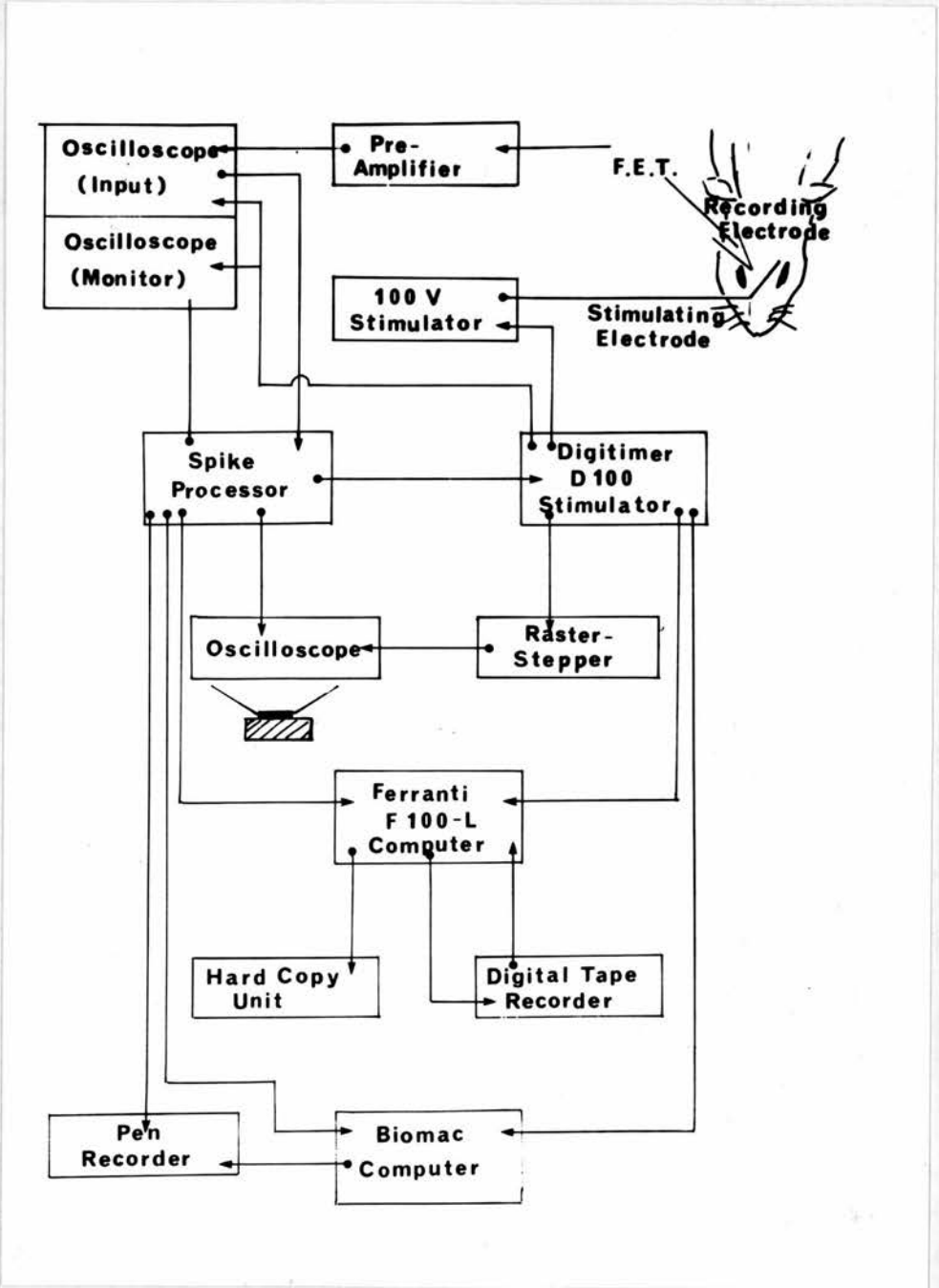
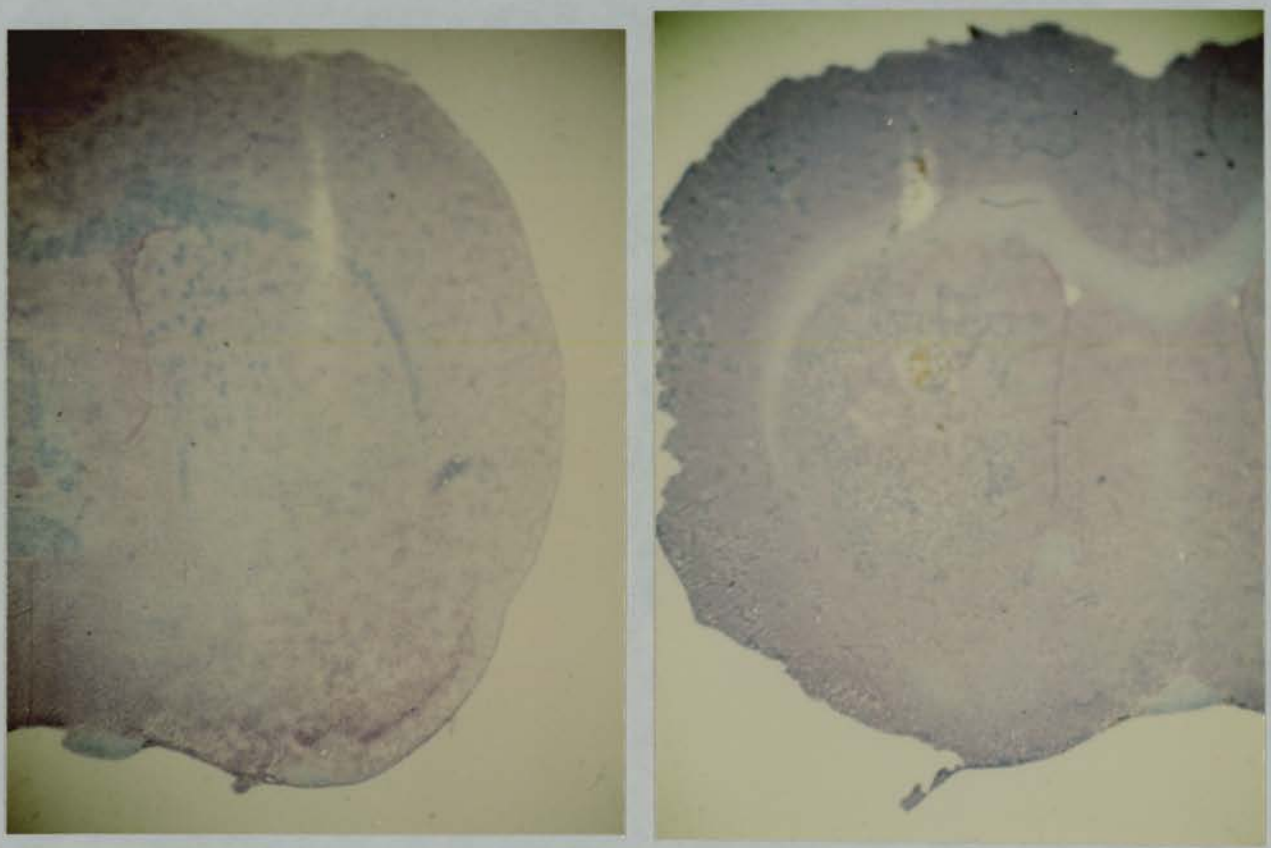


FIGURE 28: Interconnections of the equipment used in the recording experiments. The Biomac computer and the Ferranti computer were not used in the same experiments. Details of the use of the various pieces of equipment are given in the text.



A B

FIGURE 29: Histology of typical stimulating electrode placements stained with luxol fast blue and cresyl violet.

A. In a control animal.

B. In a NMA lesioned animal. The electrode tip can be seen in the centre of the lesion.

or sometimes during the experiment, the position of the electrode tip was marked by ejecting some dye from the tip with negative current (10 μA for 7 min). This left a blue spot in the brain, the position of which could be determined under the microscope. Figure 30 shows a typical electrode placement.

The electrode was connected via a silver wire to the gate of a field effect transistor (F.E.T., Radiospares Components Ltd, No. 2N3819) which was used as a voltage follower and was supplied by two 6 V batteries in series.

The signal was recorded differentially, using a silver wire in the neck muscles as a reference electrode. An earth electrode was attached to the skin at the edge of the incision.

The whole animal, including the F.E.T. was enveloped in aluminium foil to reduce radiofrequency interference to a minimum.

The signal from the preamplifier was fed into a differential amplifier (Tektronix 5A22N) and then into channel 1 of a dual beam storage oscilloscope (Tektronix D.13). In this amplifier the signal is filtered to remove excess noise. The upper limit is 3 kHz and the lower limit is 1 kHz. From the differential amplifier, the signal was fed to a spike processor (Digitimer Ltd, D100), where the nerve action potentials were quantified and the signal digitised. The levels at which the potentials were counted could be set by a window discriminator in the spike processor and these levels could be monitored on channel 2 of the oscilloscope. The digitised signal was then passed on to the data collecting system, described later. The signal could also be used to trigger the D100 for performing a collision test or constructing post-pulse histograms (PPHs). These are also described later in this section. Again, these connections are shown in Figure 28.

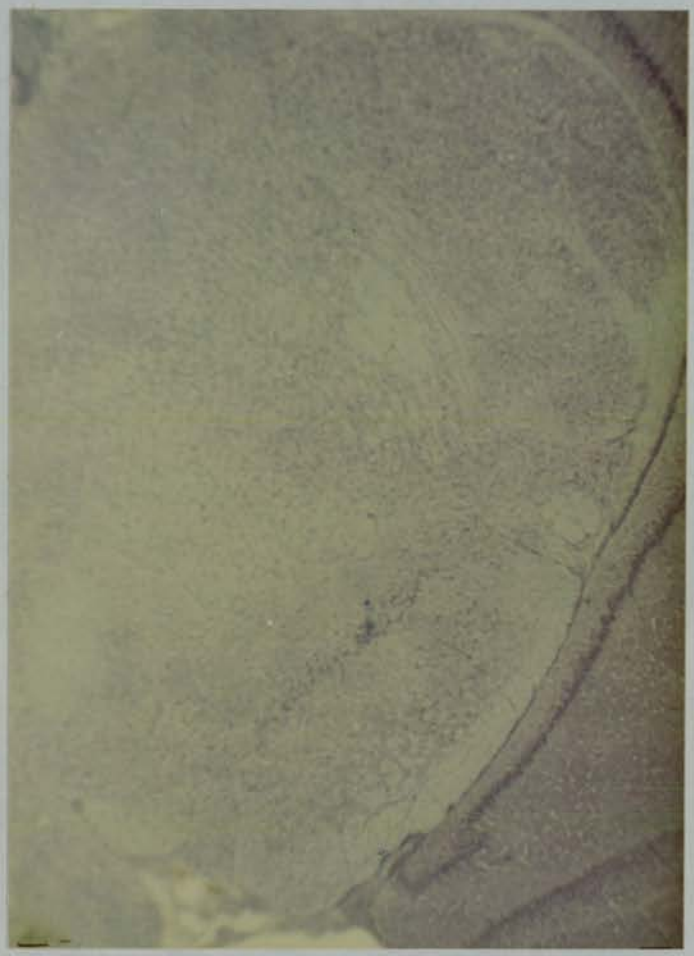


FIGURE 30: Histology of a typical recording electrode position. The blue spot marks the final position of the tip of the electrode.

Antidromic driving

To show that a cell was being antidromically driven, that is being driven backwards towards the cell body and not trans-synaptically, each one was tested for collision and if this did not occur, the cell was rejected. Collision is when a spontaneous spike travelling from the cell body to the terminals meets and cancels out an action potential caused by stimulation and which is travelling from the terminals to the cell body. This is one of the most reliable tests to see if a driven potential is antidromic or not. To perform this test, the D100, set in single cycle mode, was used to trigger the stimulator when it itself was triggered from a spontaneous spike, via the spike processor. The time interval between the spontaneous spike and the stimulating pulse was increased from zero until the driven spike could be seen again. The time interval when the driven spike was just not seen, was taken as the collision time.

Another test used for antidromicity was the following frequency of the response. This was done by applying two stimuli closer and closer together until only one response was seen. The frequency at which two responses could still be seen, was taken as the following frequency. A frequency of greater than 100 was taken as support for antidromicity.

Data collection

- *Spontaneous activity:*

The output from the spike processor was fed into a chart recorder (Servoscribe, or Bryans B600). The number of spikes summated over 10 s intervals was plotted, and changes were noted as an increase or a decrease in this level.

- *Interval histograms:*

In the earlier experiments, the signal from the spike processor was sent to a Biomac 1000 computer (Data Laboratories Ltd) which constructed interval histograms (IHs) and displayed them on a screen. This could then be output to a chart recorder (as above).

The later histograms were constructed by a microprocessor computing system, built in this department and based on the Ferranti F100-L system. The D100 was adapted to send a signal to the microprocessor system which was programmed to display the completed histograms on a graphics display unit (G.D.U.) (Tektronix 4006-1). This information could then be stored on magnetic tape (Tandberg TDC 3000 Digital Cartridge recorder), or a hard copy could be taken (Tektronix Hard Copy Unit, 4613). From either of these the actual values forming the histograms could be determined.

- *Peristimulus histograms:*

Again, the early experiments used the Biomac 1000 to construct the peristimulus histograms, and output them to a chart recorder as above. In constructing these, the D100 was used to co-ordinate the triggering of the oscilloscope, the stimulator and the Biomac. In some cases the pulse was delivered at the start of the histogram, and in others it was delivered 200 m into the histogram in order to provide a control period at the start. The overall lengths of the histograms varied: 40, 80, 640 or 1280 m. The shorter ones were for demonstrating the antidromic response and any early effects, whereas the longer ones showed the later effects, up to about 1 s after the stimulus. The 1280 m ones proved more satisfactory as all effects were not always over by 640 m. However, this meant that the highest frequency of stimulation was one stimulus every 1.3 s (0.77 Hz).

The next set of experiments was done using a Scope Raster-Stepper (W.P. Instruments Ltd, model No. 140A) to construct the PSTHs. Here the histogram was recorded on film as a pattern of dots, built up on a second oscilloscope screen. These were later counted and conventional histograms drawn from these values. In these experiments the D100 was used to trigger the second oscilloscope, via the raster-stepper, as well as the other equipment. In this case, histograms were either 50 or 1000 ms in length. Again the shorter ones are to show antidromic and early effects and the longer ones to show effects up to 800 ms after the stimulus. In the longer histograms the stimulus was applied at 200 ms. Here the stimulation frequency used was one every 1.1 s (0.9 Hz).

The last experiments again used the microprocessor system. The D100 was also modified to trigger the computer at a set time in the stimulating cycle. It also triggered the oscilloscope, the stimulator and the raster-stepper which was used to monitor the histograms as they were building. Again the histograms could be displayed on the GDU and could be stored on tape or as a hard copy.

All data on tape could later be reread or calculations performed on it, by another microprocessor computer, the Ferranti F100-L Development system.

Experiments on spontaneous activity

The spontaneous activity of all cells was routinely recorded. The effects of several drugs on the spontaneous activity was observed. The effect on the interval histograms was also investigated. All drugs were applied intravenously through a cannula in the femoral vein. The exact doses of the drugs varied and details are given in the results

section, but the drugs used are listed here, with the range of doses used: haloperidol, 0.05-0.2 mg/kg (Serenace, Searle, 5 mg/ml ampoules diluted in saline); picrotoxin, 1.5-4 mg/kg (Sigma); apomorphine, 0.1-0.4 mg/kg (Macfarlane Smith Ltd); amphetamine, 1-2 mg/kg (Koch Light Laboratories). For a single stage injection the volume of injection was 1 ml/kg.

Experiments on peristimulus histograms

Measurements made on the antidromic activity were as follows: antidromic threshold, antidromic latency, collision time and following frequency.

For each cell a series of histograms were made at different stimulation strengths, to observe the full range of effects of stimulation on that cell and to obtain thresholds for all effects. For each cell one or two control PSTH's were constructed, using the same procedure, but with the stimulator switched off. This was used in the analysis of antidromic effects. Another type of control histogram was the PPH. Here the stimulator is also switched off and the computer is triggered by a spontaneous spike. The D100 was left on single cycle so each histogram was 1 s long plus an interval while the system waited for the next spontaneous spike.

This was done both on control cells and on cells from animals with a lesion in the striato-nigral pathway, either in the striatum or in the fibres as they travel to the substantia nigra.

The effects of picrotoxin and haloperidol on the PSTH's were also studied by constructing the histograms before and after intravenous application of the drugs. This was only done on control cells.

PSTHs taken from the biomac or the raster-stepper were taken over 64 sweeps and those on the Ferranti computer over 50 sweeps.

Analysis

- *Analysis of post stimulus histograms:*

A non-parametric approach, using Wilcoxon Rank Sum tests, was used to estimate the effects of lesions on the antidromic threshold and the various other thresholds measured, the antidromic latencies and the firing rates. The mode and range of the antidromic latency were also plotted as these were more meaningful than the mean.

The estimation of any excitations or inhibitions in the PSTHs was done by eye in the preliminary experiments, but it was felt that numerical values and a consistant quantitative analysis of the data was needed. In order to do this a control period was used to calculate a mean value for the number of spikes in a 20 ms period in each cell. This control period was either a complete 1 s histogram constructed as the PSTHs were, or the control 200 ms at the start of one or more of the PSTHs.

The mean number of spikes in 20 ms was then used to calculate limits above and below which the number in a test period could be said to differ significantly from the control one. To find these limits, the following equation, based on a Poissonian distribution for the spontaneous activity was used. The distribution of firing rates in the control histograms did not differ signficiantly from Poissonian distribution, so this approach seemed valid.

$$\lambda_2 = \lambda_1 + \frac{Z^2}{2t_2}\left[1 \pm \sqrt{1 + \frac{4\lambda_1}{Z^2} \times \frac{t_2}{t_1}(t_1 + t_2)}\right]$$

λ_1 = mean control firing in 20 ms, summed over 50 sweeps.

λ_2 = limits between which the mean firing in a test period of 20 ms was considered normal

t_1 = the number of 20 ms periods used to obtain λ_1

t_2 = the number of 20 ms periods in the test period, used to find the test mean firing value

Z = a constant, 1.96, giving 95% confidence limits.

An example is given below to show how this operates in practice, and what problems can arise in the application of the method.

Mean value of spikes in 20 ms in control period = 4.9
Number of 20 ms periods used to obtain the above = 50

Values of 20 ms bins in the histogram to be tested:

13	14	9	8	55	31	7	2	4	4	0	0	0	0
7	19	40	8	19	32	63	0	0	2	0	13	7	20
11	17	12	12	12	9	8	2	5	11	8	10	14	12

The limits calculated from the above equation are:

for t_2 = 3, 2.9 - 8.12
for t_2 = 1, 2.04 - 11.54

The values above were summated over 60 ms periods and the average for 20 ms found. This was done forward and backward from the point of stimulation, and this leads to the omission of the first and last numbers. Below are the results of this averaging and how they compare with the calculated limits, an excitation indicated by a +, an inhibition by a -, and no difference by a ∘.

10.3	=	∘	22.3	=	+
11.7	=	∘	13.7	=	+
9.7	=	∘	0.7	=	-
55*	=	+	1.3	=	-
31*	=	+	12.7	=	∘
7*	=	-	13.7	=	+
3.3	=	-	9.7	=	∘
0	=	-	6.0	=	-
8.7	=	∘	10.7	=	∘

(* see later - these three are still uncombined 20 ms bins.)

This histogram has a short strong excitation, followed by an inhibition and then a sequence of effects lasting up to 1 s.

The choice of sample time is important. 20 ms was convenient for several reasons. Firstly, it was the minimum period of resolution

that could be obtained from the raster-stepper patterns on the oscilloscope; secondly it is a long enough time to have a reasonable number of spikes in each bin, even in the slowly firing cells; and thirdly, and almost incidentally, in the PSTHs constructed on the computer using 50 sweeps this number corresponds to the firing rate of the cell in Hz. The number in 20 ms and firing rate, of course, did not correspond in the histograms taken from the raster-stepper oscilloscope pictures, where 64 stimuli were used.

The length of bin used to calculate the mean firing must be the same in both the control and test cases, although this need not have been 20 ms as was used throughout in these analyses. In the example quoted above 20 ms spells were used in the control period, and as 60 ms test periods were used, t_2 had to equal 3.

It was decided to use 60 ms test periods as this seemed the best compromise between losing information by having too long a sample time (that is aliasing), or to obtain many spurious effects from having too short a sample time. Also a shorter test period would have resulted in unrealistic limits and any effect would have been difficult to show. It was also thought best to use 95% confidence limits as this ensured there was a significant effect, while not expecting too much of the data. It is unlikely that the histograms themselves are more than 95% consistent.

Although it was decided to use 60 ms periods for the analyses, the first 60 ms after the stimulus required a different treatment. Very often this was complicated by the presence of a large stimulus artefact, leading to very high count in that bin. Also, above antidromic threshold, the antidromic spike was often included in this bin. This meant that the analysis of the first 20 ms, and even occasionally the

second 20 ms, was difficult. Therefore, in order to keep the other bins in line in the analysis of a series of PSTHs, lower values of t_2 were used in the 60 m after the stimulus. Sometimes a very short early excitation, lasting only about 20 ms would be seen in this bin. If this happened, it was treated with a value of t_2 equalling 1.

The PSTHs constructed from the oscilloscope raster-patterns were already in 20 ms bins and the numbers from these were grouped and averaged by hand. These values were then compared with limits calculated on a programmable calculator (Hewlett Packard 97). Early data from the F100 computer was collected in 4 ms bins and then reduced to 20 ms bins. The summated values were obtained on line. These values were recorded on the hard copy unit and processed as above. The later data from this system were stored on magnetic tape and processed later entirely by the F100 development system. This gave comparisons with the limits on a printout similar to the example above, leaving the values around the stimulus as 20 ms bins, allowing later examination by hand.

The data obtained in this way were drawn as single lines, a space indicating an inhibition, a thin line a period of normal firing and a thick line an excitation (see Figure 46). This allowed easy comparison between cells and between different histograms from the same cell.

- *Analysis of the action of drugs on peri-stimulus histograms:*

The PSTHs taken after the intravenous administration of drugs were analysed as above. Any changes in the response to stimulation caused by the drug were then described qualitatively.

- *Analysis of the action of drugs on the spontaneous activity:*

In the cases of single drug administration, a paired t-test was done on the mean firing rate before and after the drug application. However, due to low numbers in the other groups where more than one drug was administered each cell was described individually and any overall effect was discussed qualitatively, with no further attempt at analysis.

4.3 Electrophysiology results

Recordings have been made from a total of 281 DA neurones in the SNC. An outline of all the data recorded is given in Tables 9 and 10. Not included in these tables are cells that were recorded from but that no experimental procedures were performed on. Most of these however, are included in Table 13, showing the distribution of types of antidromic response (see later in this Section). Information from many of these neurones is used more than once in the rest of the Chapter.

Tables 9 and 10 summarise all the data that has been described and discussed in the rest of the Chapter. Also discussed are the basic properties of the cells in each group, for example the antidromic latency and firing rates of the units recorded.

All stimulus strengths are quoted in coulombs, to avoid the problem arising from different pulse lengths (see Section 4.2).

Activity and antidromic driving of the dopamine cells

All subsequent sections will deal with data collected from action potentials (APs) both firing spontaneously and affected by orthodromic and antidromic activation from the striatum. The basic properties of the recording of these APs is described here and will be assumed in the rest of the Chapter.

	Animal group:		
	control	lesioned	partially lesioned
Preliminary data	35	16	-
Single[1] PSTHs	14	5	-
Series of PSTHs	131	27	29

[1] These are from cells which were lost before more than one, or possibly two, PSTHs could be recorded.

TABLE 9: Summary of data described in Chapter IV - non-drug experiments.

	Drugs									
	H	P	H then P	P. then H	Apo + H[5]	Apo + P[5]	Amph + H[5]	Amph + P[5]	Amph	Apo
On[1] PSTHs	16	7	3	4[3]	-	-	-	-	-	-
On[2] spontaneous activity	17	20	4	4[4]	5	7	2	5	16	1

H = Haloperidol; P = Picrotoxin; Apo = Apomorphine; Amph = dl-amphetamine

[1]some cells are included in more than 1 group - total cell number = 23
[2] " " " " " - total cell number = 55

[3]one cell was given Apo between H + P

[4]as 3, but another one was also given P after H

[5]drugs given in any order

TABLE 10: Summary of drug experiments.

All APs studied in this Chapter were shown histologically to be recorded from the SNC. An example of a typical recording site is shown in Figure 30. These units are thought to be the DA neurones that project to the striatum as they were all antidromically driven from the striatum and had shapes and firing rates corresponding to those previously described (250). All cells resembled the Type I neurone of Guynet and Agahjanian.

The firing rate was usually constant for long periods of time, varying from unit to unit, from 0.9 to 9.8 Hz. The shape of the AP, however, varied during the course of an experiment, and bore no relation to the antidromic latency of the action potential. The shape change was probably due to the electrode edging closer to or further away from the cell, or possibly due to some extent of damage to the cell caused by the electrode. The most noticeable change in shape involved separation of the initial segment (IS) spike from the full somato-dendritic one (SD). Figure 31 shows examples of the shapes of extracellularly recorded DA cell APs.

All the units used were thought to be DA cells on the strength of the above characterisation. It was unlikely that any were axons, but the possibility does exist. All units are referred to as cells from here on.

The measurements quoted below include information from the preliminary data and the later studies. The antidromic latencies from each group of animals is given in Table 11, as is the firing rate. The mode and the range are given as well as the mean in the case of the antidromic latency, as the distribution was skewed, as shown in Figure 32.

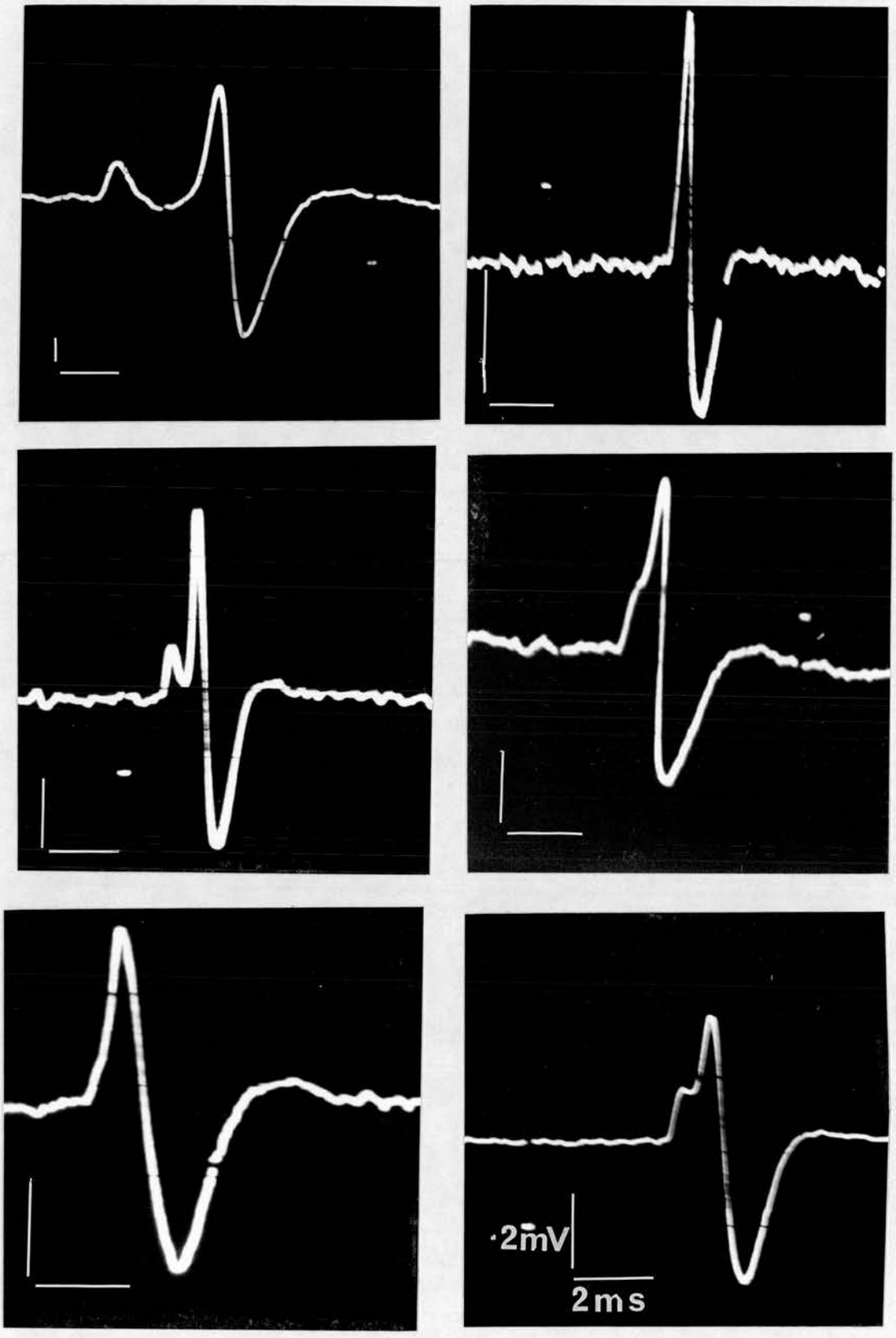


FIGURE 31: Examples of various shapes of DA action potential.

Animal group	n	Antidromic latency: Mean (ms)	Mode (ms)	Range (ms)	Mean firing rate (Hz)
Control	177 (169)	12.4 (0.3)	10	6 - 23	(4.6 (0.1))
Lesioned	48	11.8 (0.6)	9	6 - 23	5.6 (0.3)*
Partially lesioned	29	*10.7 (0.6)	9	6 - 20	4.9 (0.4)

TABLE 11: Antidromic latency and spontaneous firing rate.

* indicates groups that are significantly different from control.
Figures in brackets are standard errors.

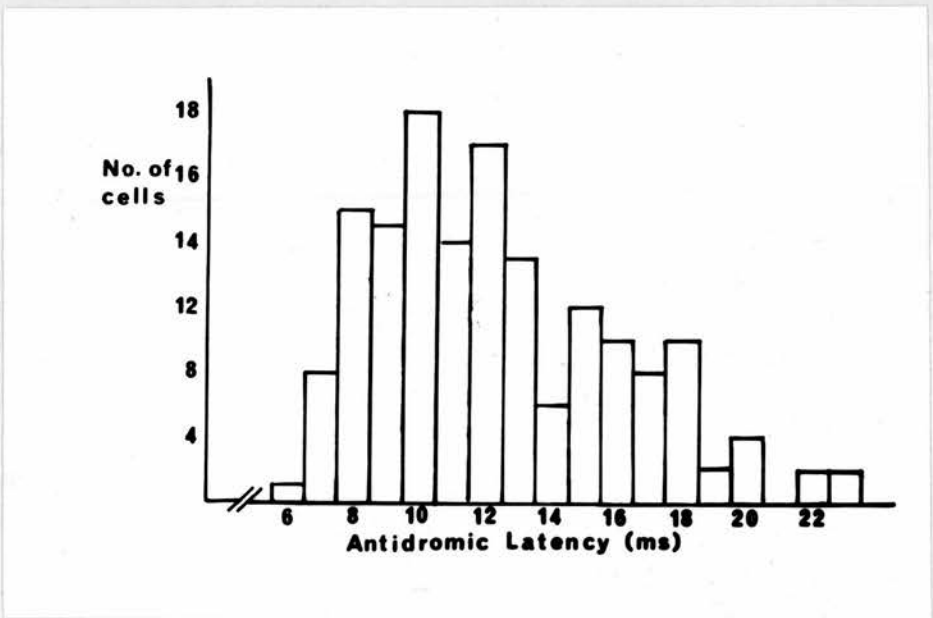


FIGURE 32: Histogram showing the skewed distribution of antidromic latency in SNC DA cells on striatal stimulation.

A Wilcoxon Rank Sum test showed that there was no significant difference in the antidromic latencies of cells from the control and lesioned groups of animals ($P > 0.1$), but that there was a difference between the control group and the partially lesioned group ($P < 0.01$). Where there was a latency variation (see later) the value occuring most at antidromic threshold was used. This was generally the longest value.

Using both a Students t-test and a Wilcoxon Rank Sum test, the firing rate of the control and partially lesioned groups did not differ significantly ($P > 0.1$). Those from control and lesioned groups of animals did differ ($P < 0.002$).

The collision test method was used to verify the antidromic nature of the driving in all cells used. The collision time was taken as the longest time interval between a spontaneous spike and the stimulus that still led to collision, that is the cancelling out of the spontaneous and driven action potentials. A longer interval than this allows the spontaneous spike to pass the stimulation point before the stimulus is applied and the antidromic pulse can be initiated. Figure 33 shows collision: three stimuli are applied, but only the two later ones result in an antidromic response, the one with the shortest interval from the triggering spontaneous spike does not give an antidromic response, i.e. it has collided.

Theoretically the collision time should be equal to the antidromic latency plus the refractory period of the cell. However, in many of these recordings, the collision time was equal to or even less than the antidromic latency (the time from the stimulus to the onset of the antidromic response). This was due to the triggering system used in these experiments. The stimulus was not triggered until the falling

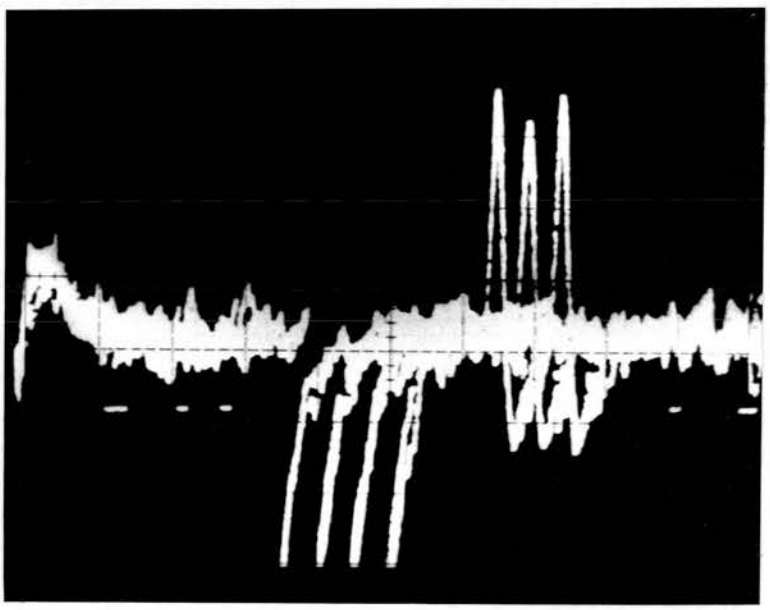


FIGURE 33: Demonstration of collision on the oscilloscope trace. Four stimuli are given and the resulting traces superimposed. The time interval between the spontaneous spike triggering the stimulus and the stimulus was increased by 1 ms each time. Only the stimuli with the longer intervals are effective antidromically driving the cell.

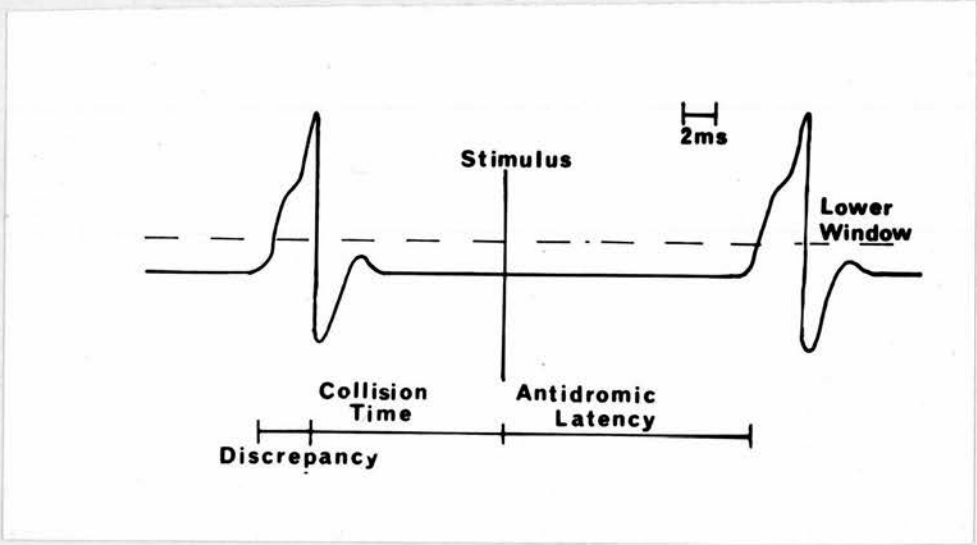


FIGURE 34: Diagrammatic representation of the relation between collision time and antidromic latency. The collision time (12 ms) was taken from the time that the falling phase of the triggering action potential crosses the lower window, until the stimulus. The antidromic latency (15 ms) is measured from the stimulus to the start of the initial segment component of the antidromic spike. This leads to a discrepancy (3 ms) approximately the length of the AP.

phase of the action potential crossed the lower level set by the window discriminator. Depending on action potential shape and the level the windows were set at, this could be as much as 4 ms after the start of the action potential. However it is the start of the action potential that is taken for the antidromic latency measurement. This leads to an apparent reduction of the collision time in relation to the antidromic latency. Figure 34 shows this diagrammatically, using a rather extreme example.

The following frequencies of most of the cells were also tested. This was done by applying two stimuli closer and closer together until only one response was seen. The frequency at which two responses were just seen was taken as the following frequency. If the stimulus artefact was large it could be difficult to see the first response. It was possible to tell whether the cell was following at this frequency by noting the latency of the visible response. This should be longer than the latency in response to a single stimulus by the amount between the two stimuli. Figure 35 shows an antidromic response following at 500 Hz.

A following frequency of greater than 100 Hz is taken as strong evidence for an antidromic response.

The antidromic response to a series of stimuli either consisted of the IS component, the whole SD spike or a mixture of the two. Sometimes the SD spike is evoked, but it is split partially or fully into the IS and SD components. Figures 36 and 37 show examples of single and several superimposed responses. Table 13 outlines the frequencies of the various types of antidromic response in all the groups of cells described. The significance of this is covered in the discussion.

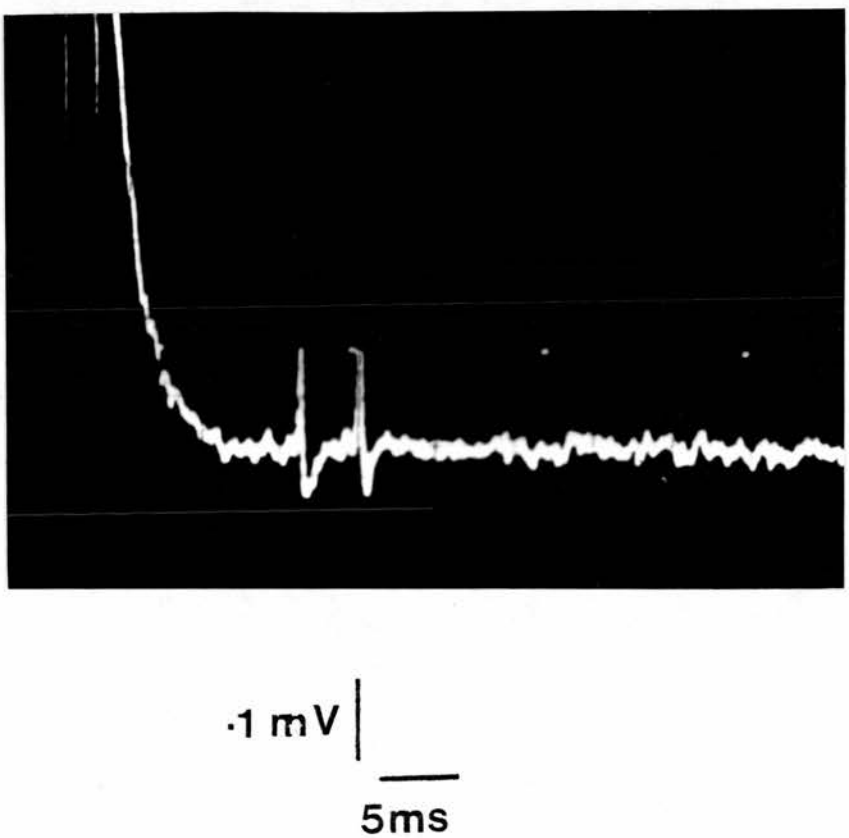


FIGURE 35: Example of a cell following a stimulation frequency of 500 Hz. The two stimuli are applied 2 ms apart and a response to both is still seen.

FIGURE 36: Examples of the responses to single stimuli in SNC DA cells on stimulation of the striatum.

A. IS component only.

B. SD component only.

C. A split response showing both IS and SD components.

The insert in each case shows the shape of the spontaneous AP.

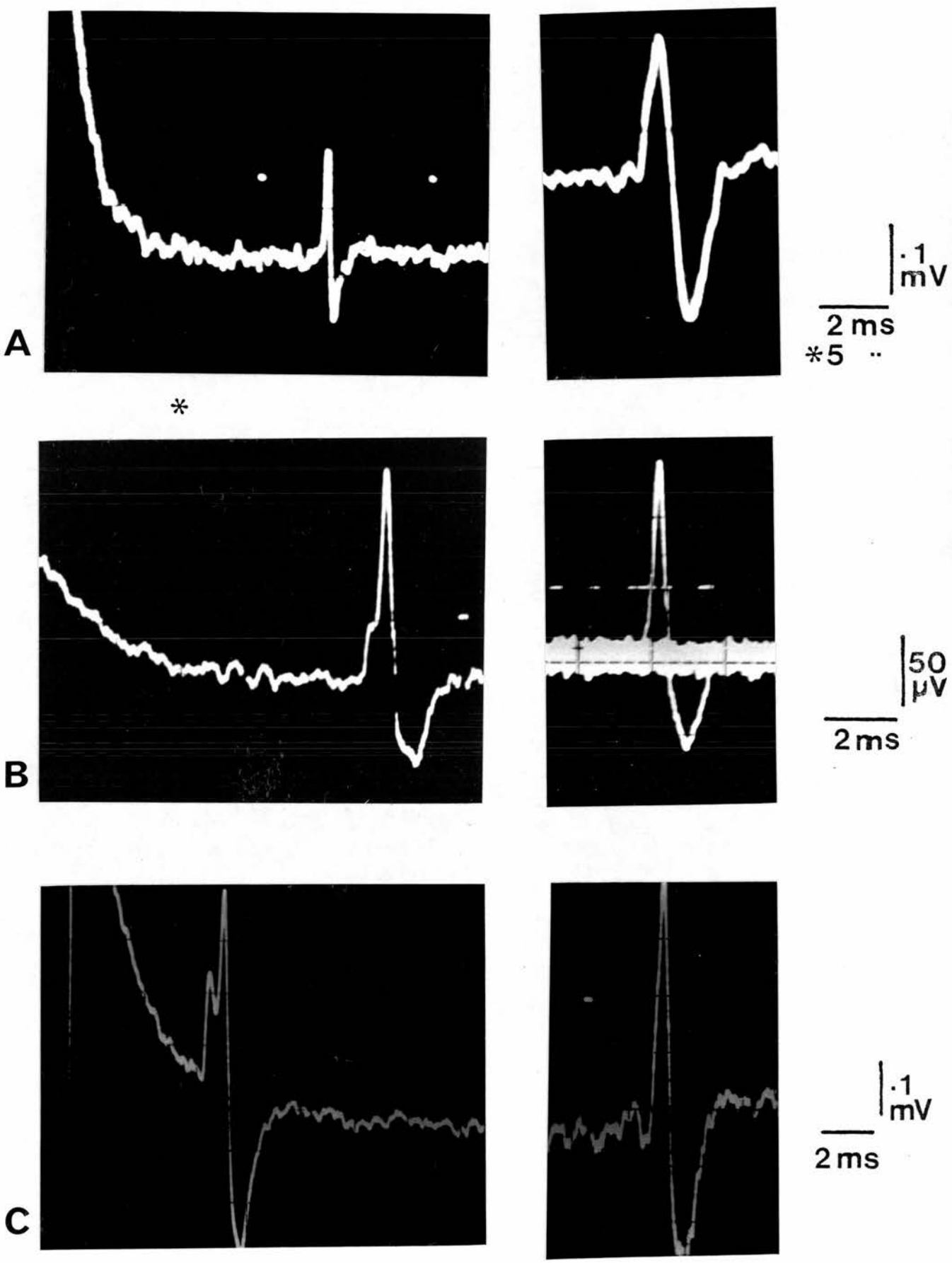

A
*
B
C
.1 mV
2 ms
*5
50 µV
2 ms
.1 mV
2 ms

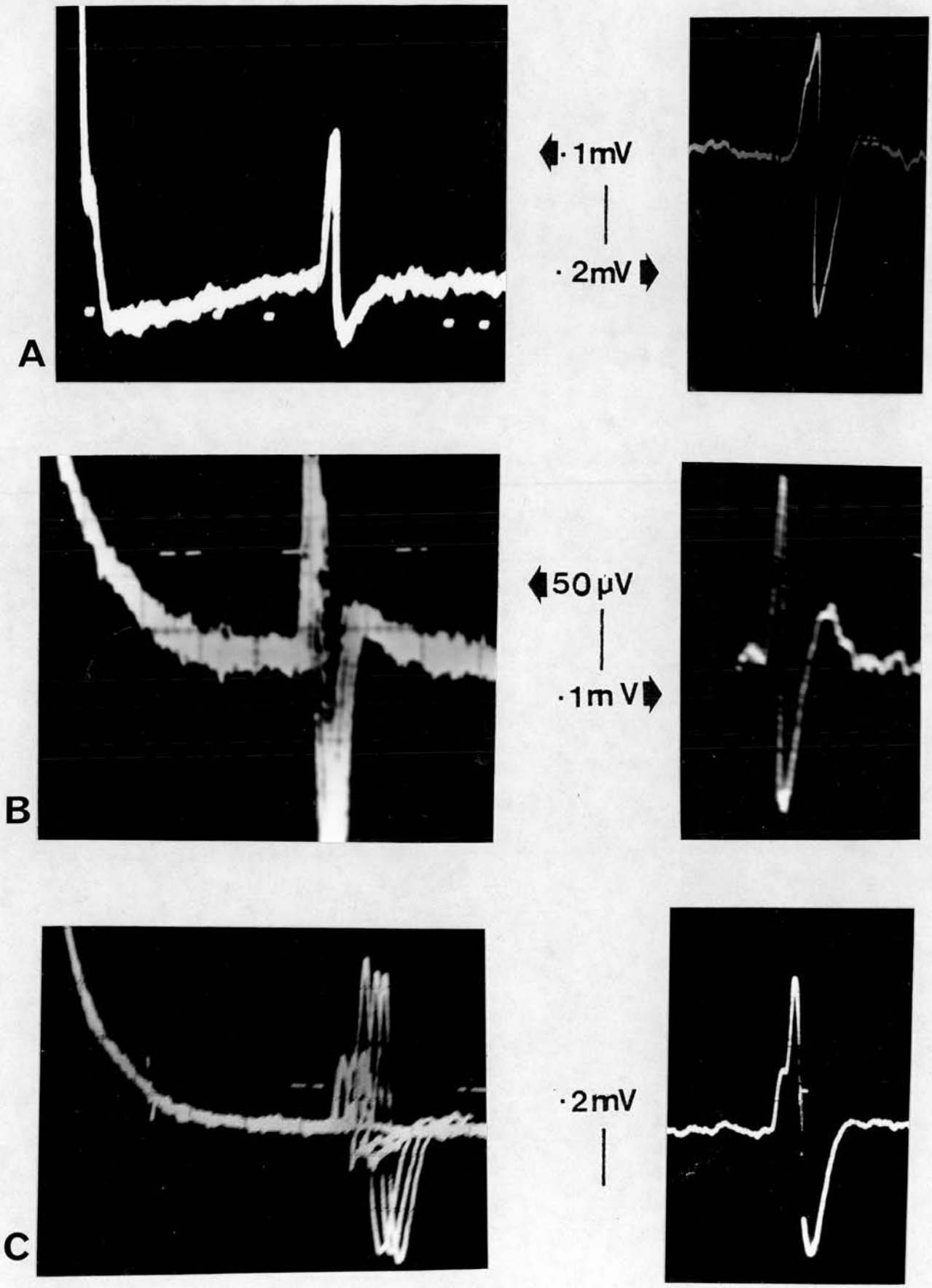
FIGURE 37: Examples of several superimposed responses in SNC DA cells in response to striatal stimulation.

A. All IS components.

B. Mostly SD component.

C. Mixed IS and SD responses.

The insert shows the shape of the spontaneous AP in each case.


A
·1mV
·2mV
B
50 µV
·1m V
C
·2mV
—— 2ms in each case

Animal group	IS only	IS >SD	IS = SD	SD > IS	SD only	Total
Control	65	66	22	18	5	176
Lesioned	13	19	5	4	6	47
Partially lesioned	12	13	3	0	1	29
Total	92	104	33	25	12	267

TABLE 13: Division of driven APs into IS, SD and both components of the spike. Data is from all three animal groups.

In some cells the antidromic latency is not constant despite the fact that collision occurs. Some cells show a distinct double or triple latency whereas others show continuously varying latencies over several ms. Sometimes the latency reduces as the stimulation strength increases. Figure 38 shows examples of this "latency jump".

Stimulus spread

Four electrodes were placed in the striatum of one rat. The array crossed the nucleus from the antero-medial portion to the posterior and lateral portion, not quite reaching the edge. Each cell was driven from one electrode and then the current necessary to drive it from the other electrodes was measured. This was attempted on 7 cells. In 4 of these, electrode 1 could drive the cell but electrode 2 could not. The other three were driven by more than one electrode. The first was driven by electrode 1 at a threshold of 112 nC, by electrode 2 with a threshold of 1500 nC. It was not driven by the other 2 electrodes. The second of these was driven by electrode 1 with a threshold of 40 nC, by electrode 2 at 60 nC and by electrode 3 at 3000 nC. It was not driven by electrode 4. The third cell was driven by all 4 electrodes. Electrode 1 drove the cell with a threshold of 60 nC, electrode 2 at 240 nC, electrode 3 at 400 nC and electrode 4 at 3000 nC.

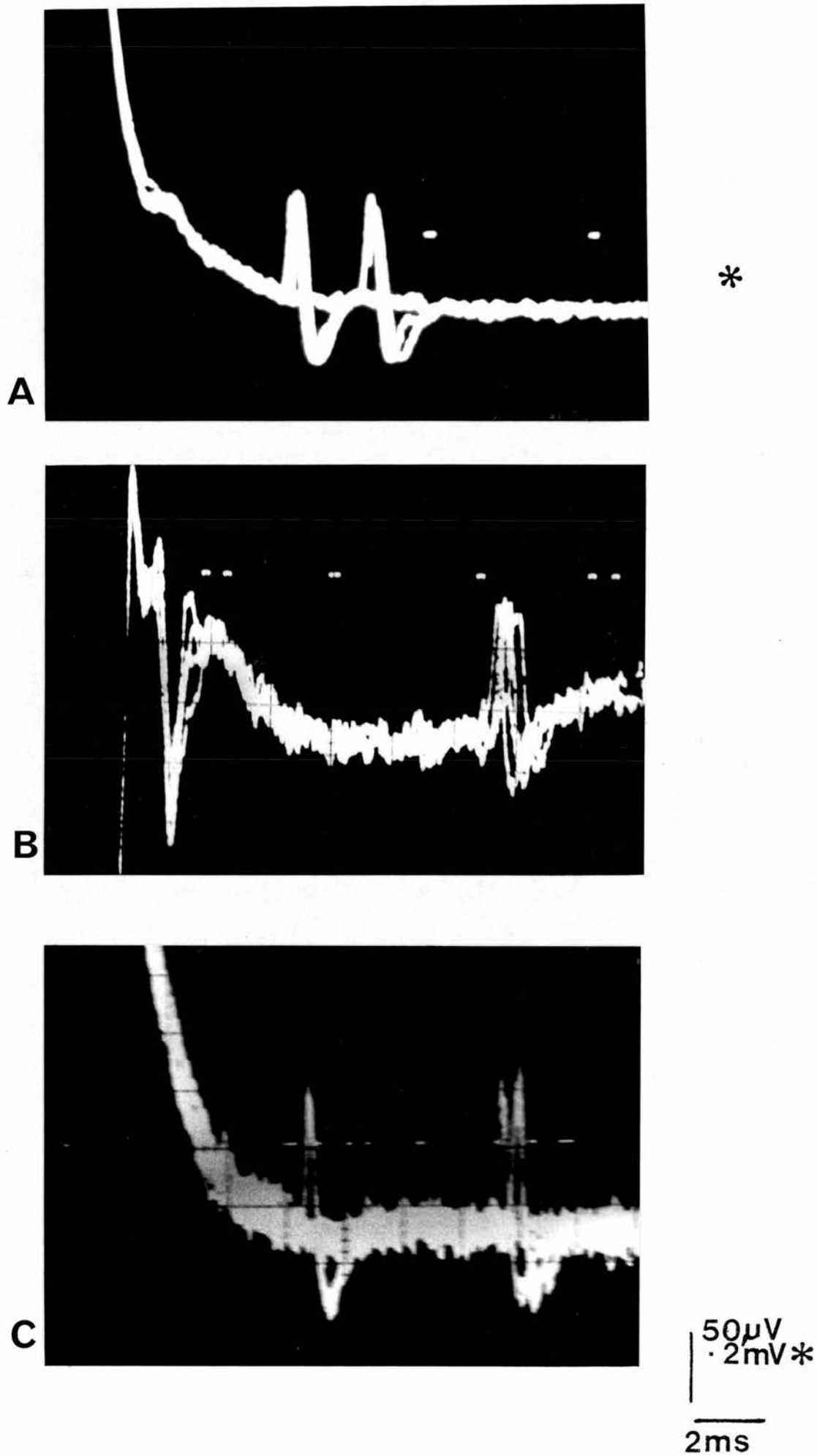
From these crude experiments it can be estimated that even at the highest stimulus strengths used, current spread was not much more than 6 mm and likely to be less. At the lower strengths the area of spread is less, between 2 and 4 mm.

FIGURE 38: Examples of latency variations. Several superimposed responses to stimulation of the same cell are shown in each trace.

A. Two distinct latencies at the same stimulus strength.

B. Continuously variable latency at one stimulus strength.

C. Distinct latency jumps, the earlier response being elicited at a higher stimulus intensity than the later.


A
B
C
*
50µV
·2mV*
2ms

Preliminary studies

- *Preliminary stimulation studies:*

In all these preliminary experiments only one PSTH was recorded for each cell, either at or above the threshold for antidromic activation. In some cases a note was made of the threshold for any other effects of stimulation. These other effects were often complex and consisted of a series of excitations and inhibitions of the normal firing in the period after stimulation, often lasting up to 1 s after the stimulation. The antidromic threshold had a mean value of 311 nC and this varied from 54 nC to 1500 nC. The mean "effect" threshold was 69 nC and this varied from 40 nC to 200 nC. Plotting antidromic threshold and effect threshold and using a Spearman Rank Correlation test, it was seen that the two are only just correlated at the 95% level. The scatter diagram is shown in Figure 39a, and Figure 40 shows examples of the PSTHs that were obtained from striatal stimulation. These PSTHs were collected on the Biomac 1000 and were printed out on a Servoscribe or a Bryans chart recorder. It proved impossible to get accurate numerical values from these histograms as the resolution was not good enough to separate the individual columns. (Each PSTH is made up of 1000 columns.) When printed at a faster paper rate, the PSTHs were unwieldy and it became apparent that the response time of the recorder was not fast enough to keep up with the Biomac output. If the output speed was reduced, the time taken to print the PSTH became unreasonable while on line.

Despite the lack of accuracy in estimating the effects of stimulation, due to the non-numerical nature of the data, it was clearly seen that a complex response resulted from striatal stimulation. This complex response was attributed to orthodromic activation of the striato-nigral pathway, which would be expected to have a lower threshold due to the different

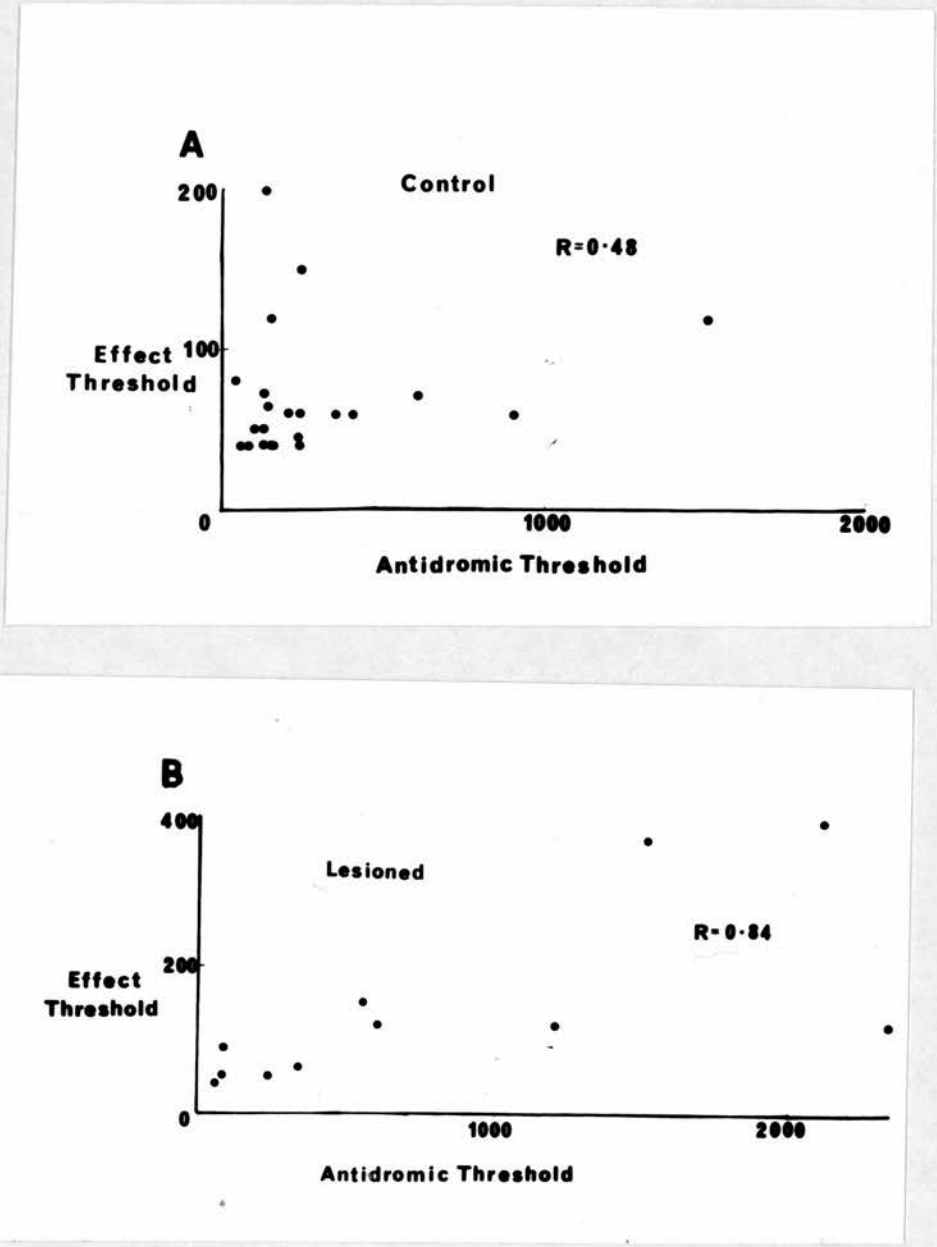


FIGURE 39: Scatter diagram of the correlations between antidromic threshold and effect threshold in the preliminary experiments.

A. Control animals. There is a significant correlation at $P < 0.05$, but not at 0.02.

B. KA lesioned animals. Here there is a correlation between the two at $P < 0.01$.

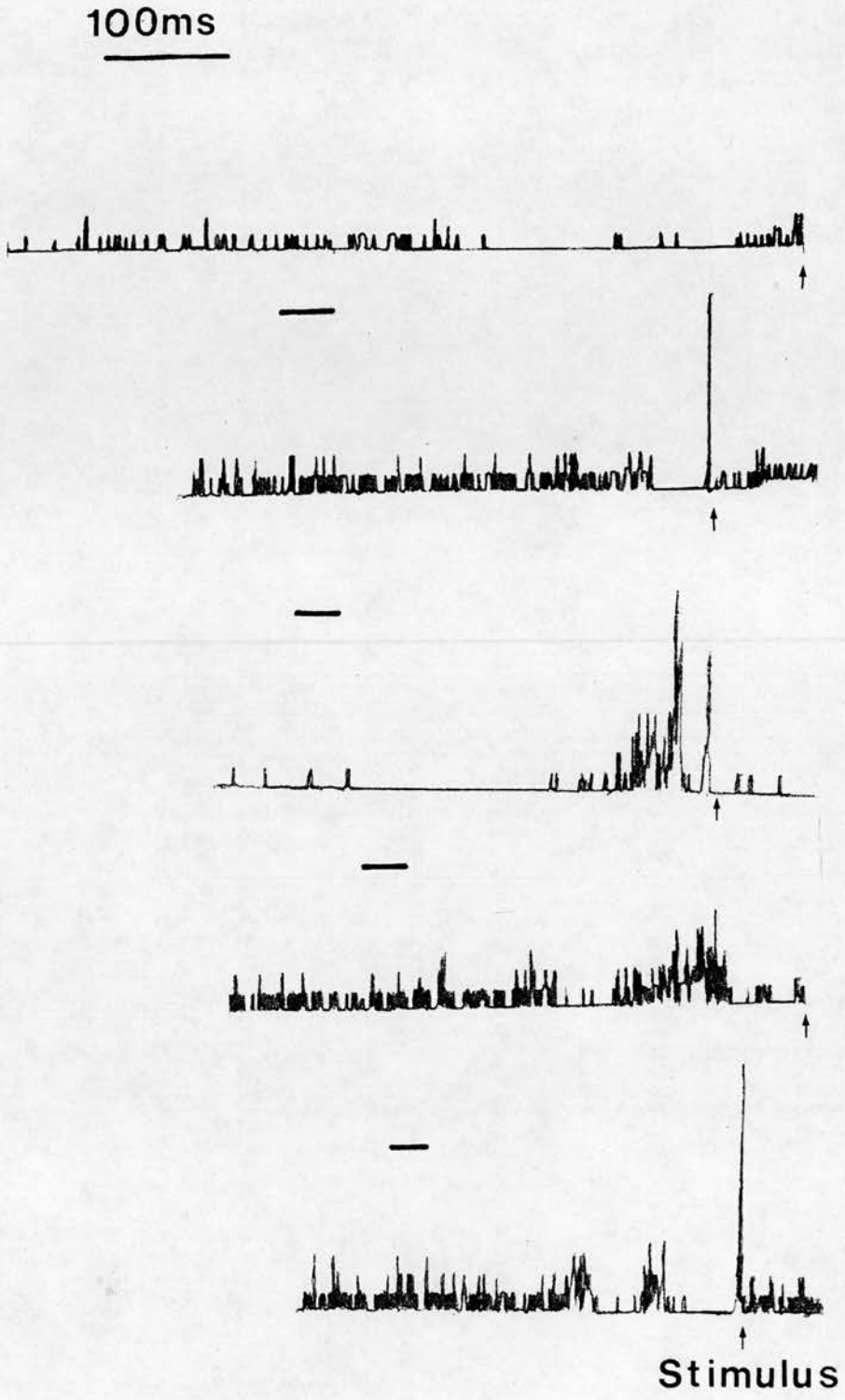


FIGURE 40: Examples of PSTHs recorded on striatal stimulation from SNC DA cells in control animals. These were collected on the Biomac computer and printed by the chart recorder.

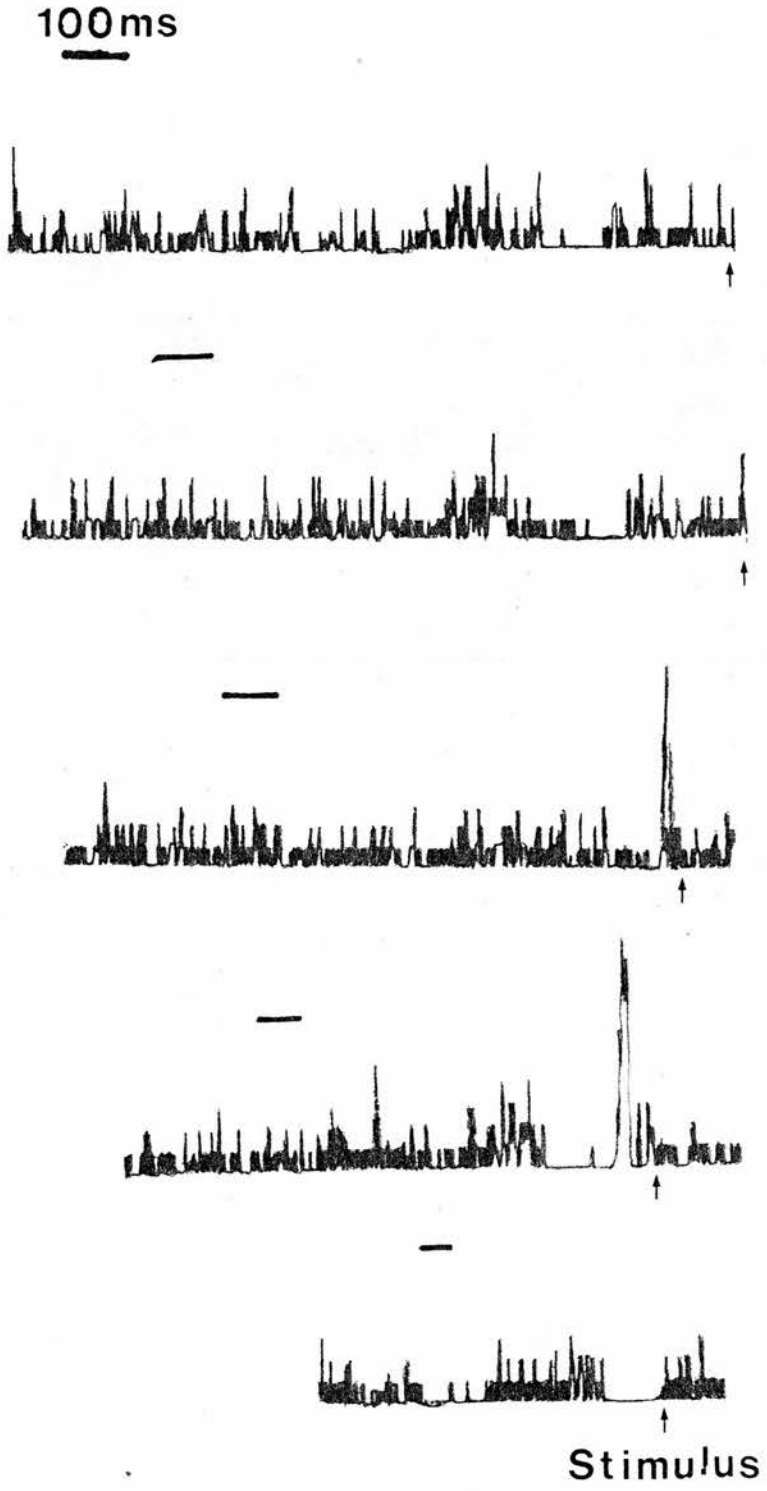


FIGURE 41: Examples of PSTHs recorded on striatal stimulation from SNC DA cells in KA lesioned animals. The data was collected on the Biomac computer and printed by the chart recorder.

nature of the fibres. It was thought that if the striato-nigral pathway were lesioned by removing the striatal cell bodies with kainic acid, the response patterns would be simplified, and an analysis of any purely antidromic effect could be attempted.

Initial experiments with KA lesions, however, did not appear to simplify the pattern very much if at all. However, using a Wilcoxon Rank Sum test, a significant increase in the threshold for the other effects was recorded ($P < 0.05$). The mean was 143 nC with a range of 40 nC to 400 nC. This time a plot of this against the antidromic threshold (mean 828 nC, ranging from 60 nC to 2400 nC) showed a stronger correlation on the Spearman Rank test ($P < 0.01$). This is shown in Figure 39b and examples of the PSTHs obtained from these lesioned animals is shown in Figure 41.

In all, 11 lesioned animals were used and an assessment of the extent of each lesion is given in Table 12. All animals were considered to have large striatal lesions, either on the grounds of a reduction of striatal enzymes or on histological evidence. The biochemistry was given slightly less weight in deciding on the extent of the lesion as only the portion of the striatum anterior to the injection site was assayed and this may have included normal tissue, whereas the histology showed the tissue immediately at the injection site. An example of a KA lesioned striatum, showing cell loss and gliosis is illustrated in Figure 11 in Section 3.2.

- *Preliminary experiments with picrotoxin:*

From the results from six early cells it became apparent that 1.5 mg/kg was a threshold dose, sometimes being effective and sometimes not, for both an effect on DA cell firing and for causing convulsions in the animal. This made it difficult, in some cases impossible, to record

Rat No.	Turns/30 min I	Turns/30 min C	GAD: control	GAD: lesioned	CAT: control	CAT: lesioned	Histology
1	66	7	375	225	146	106	-
2	101	0	-	-	-	-	Wide area of damage
3	153	1	-	-	-	-	As 2 + enlarged ventricle
4	130	7	-	-	-	-	As 2
5	140	12	47	0	228	18	-
6	257	0	29	0	202	73	-
7	169	4	-	-	-	-	Large, slightly posterior lesion
8	271	22	177	132	369	177	As 2 + enlarged ventricle
9	160	0	154	26	222	14	As 2
10	144	44	95	59	262	26	-
11	105	11	118	15	39	27	As 2

Biochemistry results are expressed as nmol/mg protein/hr. Control and lesioned striata from each animal were analysed separately. Positive identification of a lesion was made on a combination of criteria. I - ipsilateral turns, C - contralateral turns.

TABLE 12: Profile of KA lesioned animals used in preliminary lesion experiments.

after the injection of the drug, so for later experiments, gallamine paralysis and artificial respiration was used. Despite these problems the experiments with amphetamine and picrotoxin described in a later section of the results were successfully carried out.

To summarise, from these early experiments it became clear that high currents were needed to drive the nigral DA cells antidromically, and that these currents caused interference from other neurons acting on the DA cells orthodromically. From the results from the KA lesioned animals it seemed that the simplest possibility - that the striato-nigral fibres may not account wholly for the effects of stimulation - might be the case, although the threshold for these effects did seem to be higher in the lesioned animals. More experiments were needed with carefully assessed lesions. It was also obvious that some form of quantification was needed as it was difficult to estimate the extent of effects consistently and accurately by eye. The later experiments attempt to do this. Also in all future picrotoxin experiments, gallamine paralysis was used.

Description of the lesions

The lesioned animals described above and in the rest of the Chapter consist of several animals with electrolytic lesions in the crus cerebri and several with KA or NMA lesions in the striatum. The KA lesions have been already described. All the rats turned in response to apomorphine, and had lesions identified histologically (see Section 3.3). Table 14 shows the turning values for all the animals and the levels of GAD in both nigrae of the NMA lesioned rats.

The partially lesioned animals also turned in response to apomorphine, but histologically these lesions were not as extensive as those considered as lesioned animals. The one partially lesioned NMA animal only differed in that there was no GAD drop in the ipsilateral SN. This was taken to

a) NMA lesions

	GAD in SN nmol/hr/mg protein		Turns in response to 2 mg/kg apomorphine in 30 min ipsi/contralateral
lesioned	79	44	153/0
	240	118	132/0
	92	35	181/3
	212	92	178/2
	194	61	191/0
Partially lesioned	231	237	924/1

b) electrolytic lesions

Turns in response to 2 mg/kg apomorphine in 30 min ipsi/contralateral	
278/29	
78/6	
95/3	
66/9	
160/8	
130/4	106/18
75/6	130/4
115/5	150/3
117/0	71/0

TABLE 14: Characterisation of lesions used in electrophysiology experiments: a) NMA lesions in the striatum; b) electrolytic lesions of the striatonigral pathway in the crus cerebri.

mean that the lesion might not be extensive enough for the purpose of the experiment, i.e. to ensure that current does not activate the striato-nigral pathway.

Antidromic and effect thresholds

In order to follow up the difference in correlations between the antidromic thresholds and effect thresholds (the lowest strength at which an effect is seen) in the lesioned and control groups, hinted at in the preliminary data, the same correlations that were done in the preliminary section were done on the later data where more careful measures of the effect thresholds were available. The Spearman Rank Correlation Test showed strong correlations between antidromic threshold and effect threshold in the control group ($P < 0.001$), and the lesioned group ($P < 0.01$) but the partially lesioned group did not show this correlation ($P > 0.1$). Table 15 gives the mean values and the range for these thresholds. The preliminary data was not used here as it was less accurate. Figure 42 shows the scatter diagram of the correlation of the two thresholds in the control animals.

The Wilcoxon Rank Sum test was used to see if there was any significant difference in the antidromic or effect thresholds between the lesioned group of animals and the controls and the partially lesioned animals and controls. No significant differences were seen between the antidromic thresholds of the control and either lesioned group, or between the effect thresholds of the control and either lesioned group.

Post-pulse histograms

In order to control for the possibility that each time a SN DA cell fires, there follows a set pattern of firing, PPHs were constructed. If this set pattern exists, any effect would not be seen in histograms

Cells from	Control animals	Lesioned animals	Partially lesioned animals
Antidromic threshold	451.1 (n = 142)	362.6 (n = 32)	514.8 (n = 28)
Effect threshold	77.8 (n = 125)	58.2 (n = 26)	71.1 (n = 27)

All values are in nC.

TABLE 15: Mean values of the antidromic thresholds and effect thresholds in each group of animals. There is no difference between the control group and lesioned groups. There is a correlation between antidromic threshold and effect threshold in each case (see text for details).

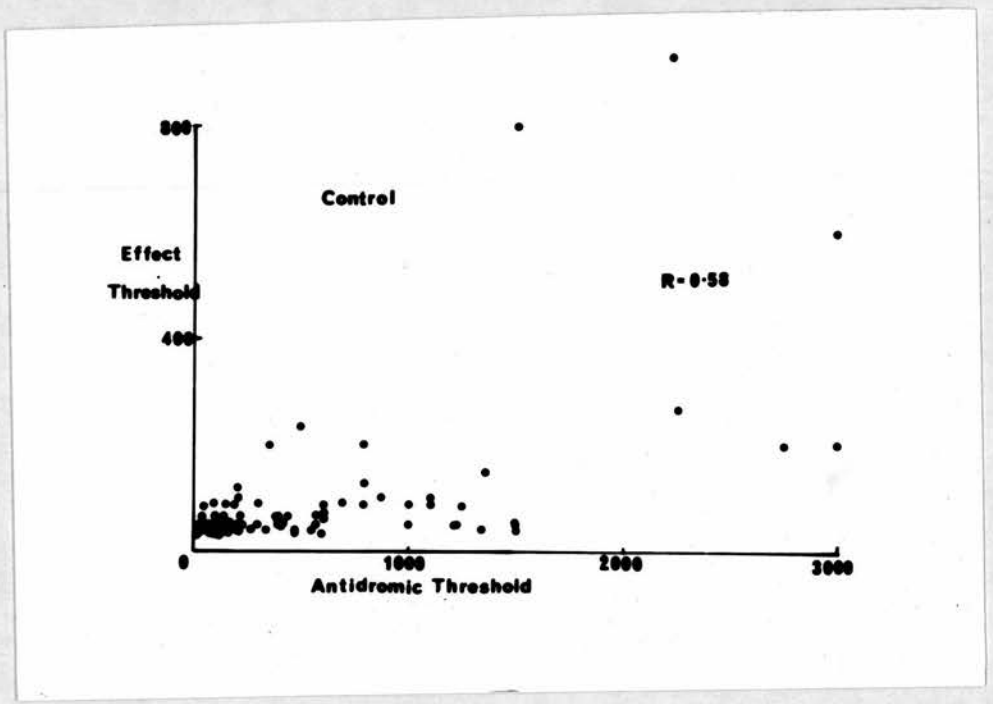


FIGURE 42: Scatter diagram of the correlation between antidromic threshold and effect threshold in the later control data (see text for details). Here there is a significant correlation between the two thresholds ($P < 0.001$).

made up of randomly selected 1 s periods, as were the control histograms used. The PPHs were constructed by starting each sweep in the control histogram at a spike, triggering the system, set in single cycle mode, with a pulse from the spike processor. This would show any inherent fluctuations in the post AP period. A typical PPH is shown in Figure 43. In all, these PPHs were obtained from 17 cells. These were analysed in the same manner as the PSTHs, and in very few was there any trace of any periods of firing above or below normal. There was usually a short inhibition after the triggering pulse. The mean value of this was 46 ms (s.e. 12.4). The range of the inhibition was from 8 ms to 220 ms. A single value of 220 ms and one of 104 ms caused an increase in the mean which otherwise would have been much lower - without these two values the mean inhibition was 30.9 (s.e. 4.6). The fact that the only inherent pattern of firing linked to the firing of the cell is a short post-spike inhibition, means that the large deviations from normal in the PSTHs are indeed caused by the stimulation of the striatum and are not an artefact caused by time locking the spikes. This result would have been expected as the patterns were also seen, below antidromic threshold. Because of this short inhibition, care should be taken in interpreting short initial inhibitions, immediately after the antidromic spike as these could reflect this functional "refractory period" of the cell.

Initial effects of striatal stimulation

The only immediately obvious classification of the PSTHs from the 206 cells recorded from all the groups of animals, was a division into a group which had a short latency (<60 ms) excitation, which was short lasting and relatively strong, and those which had not. Figure 44a shows an example of an early excitation. An example of a cell with no early excitation is shown in Figure 46. The decision to use 60 ms as the set

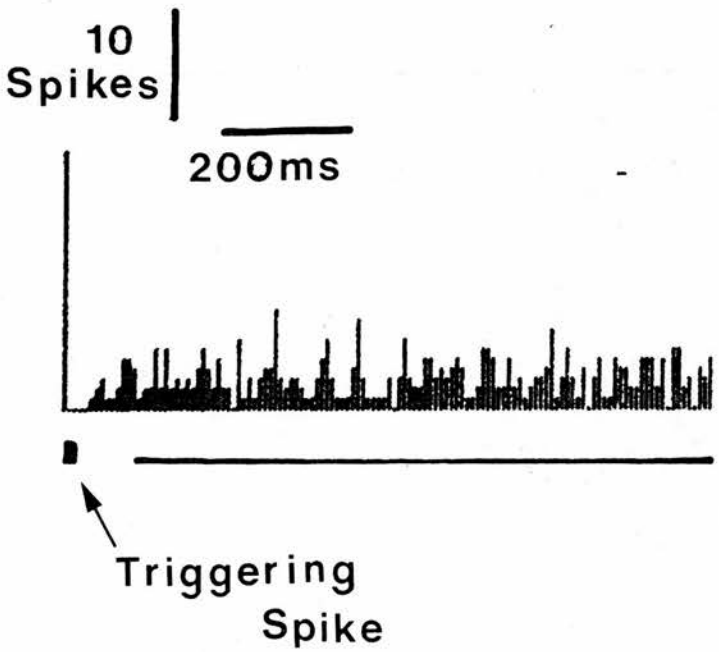
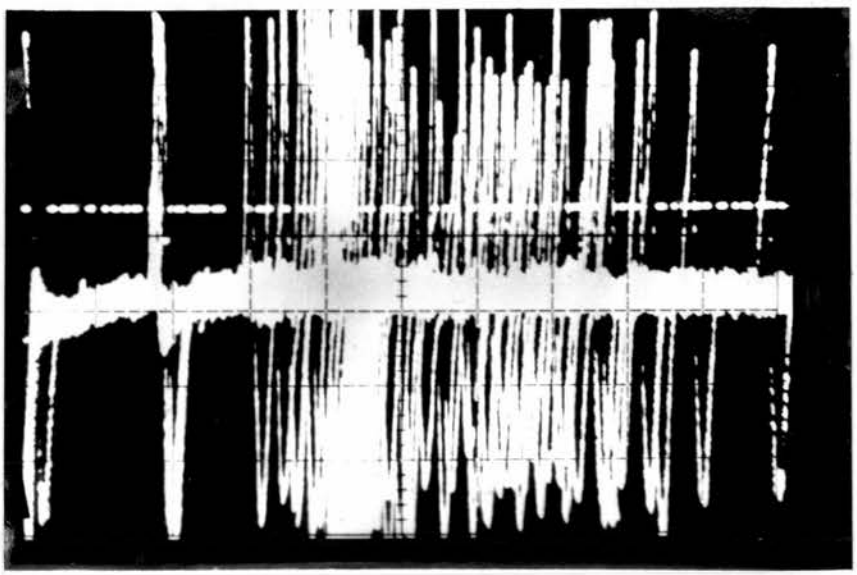


FIGURE 43: Example of a PPH, showing the short post-pulse inhibition in DA cell firing.



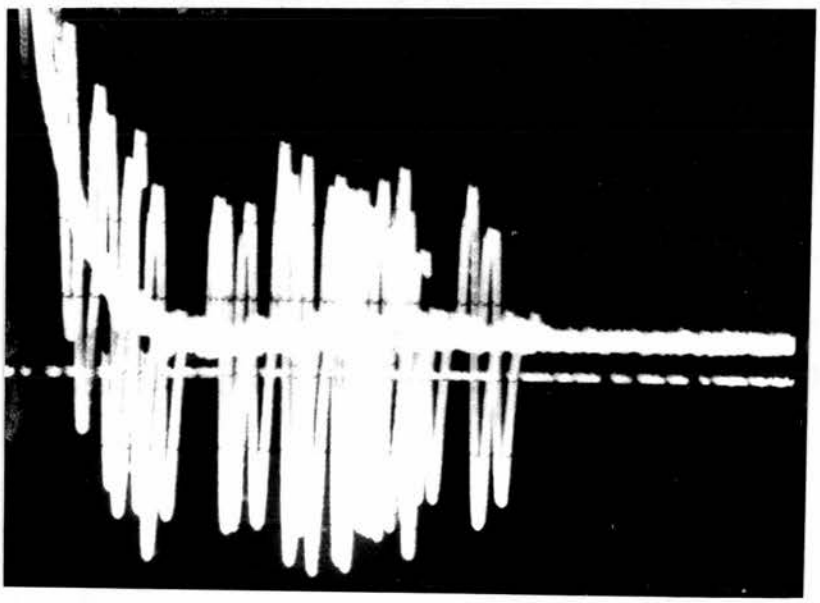


FIGURE 44: Oscilloscope traces showing the early excitation resulting from striatal stimulation.

A. Latency of excitation is greater than that of the antidromic spikes.

B. Latency of excitation is less than that of the antidromic spike and shows the two effects superimposed.

Both traces show 5 superimposed sweeps.

time interval within which excitation had to occur included was arbitrary, but 60 ms seemed to be functionally relevant as this short lasting excitation never started after 60 ms, although a few lasted longer than the 60 ms. A total of 8 out of 67 cells (6 control, 1 lesioned and 1 partial lesioned) with an early excitation had an excitation carrying on after the 60 ms period, some up to a few hundred ms after.

Excitations starting before 20 ms after the stimulus were difficult to measure as they tended to become tangled with the stimulus artefact and the antidromic spikes. When this occurred, collision was also more difficult to show, as spontaneous collision with the excitation occurred before the antidromic spike could reach the electrode. This only occurred to a significant extent in 3 cells one of which is illustrated in Figure 44b.

- *Peri-stimulus histograms with an early excitation:*

The frequency of occurrence of the early excitation was determined and a 2 x 2 frequency table was analysed with a chi-squared goodness of fit test, comparing each of the two lesioned groups with the control. The control group was used to generate expected values and the test values were shown not to be significantly different from control in the frequency of occurrence of the early excitation ($P > 0.1$).

The threshold stimulus strength for the appearance of the early excitation in each of the two test groups was compared with the control group, using a Wilcoxon Rank Sum test and were shown not to differ either ($P > 0.1$).

As the effect was short lasting in most cases, it was decided not to measure the length of the effect but only its extent, counting the number of spikes above the mean predicted by the equation given in the analysis section. Only the firing during the first 60 ms was included. The 3 cells

whose early excitation tangled with the antidromic spike and stimulus artefact were completely excluded from the analysis as an accurate estimate of the extent of the excitation was impossible to obtain.

A stimulus intensity that was as close to twice the threshold for the effect as possible was chosen for the analyses. Due to the limited number of histograms from some of the cells this could not always be done. Strengths varying from threshold to four times threshold were used with only 4 cells out of 189 above this. Twenty-one cells had no information on thresholds. These were included in the analyses of the effect extent, but not of the effect threshold. Omitting these PSTHs from the threshold effects leads to the differing n values in Table 16. This table summarises all the above information and gives mean values with standard errors for occurrence, threshold and extent of the early excitation.

- *Peri-stimulus histograms with no early excitation*

The other group of PSTHs either showed an initial inhibition of firing when compared with control or showed a delayed effect, which in most cases was an inhibition. In three cases, however, the delayed effect was a late excitation. Two came from control animals and one from a lesioned animal. Sometimes there would be an initial or delayed inhibition occurring at a stimulus strength above that at which a late excitation appeared. In a case like this the threshold for the appearance of the inhibition was used in this section, although in the previous section where the antidromic and effect thresholds were compared, it was the lowest threshold that was used, that is the threshold for any effect at all.

As the inhibitions were much more varied than the excitations, the latency and the length of the effect was measured as well as the extent,

Animal Sp	"Early excitation" cells: Threshold (nC)	Extent*	Occurrence	No "early excitation" cells: Threshold (nC)	Latency (ms)	Length (ms)	Extent+
Control ± s.e.	455 ± 104	18.6 ± 1.6	46	98 ± 19	33.0 ± 0.6	164 ± 9	31.4 ± 2.0
(n)	(38)	(43)	143	(80)	(90)	(90)	(90)
Lesion ± s.e.	284 ± 57	17.6 ± 4.6	10	58 ± 8	15.0 ± 7.3	153 ± 16	31.9 ± 5.4
(n)	(8)	(10)	32	(17)	(20)	(20)	(20)
Partially + s.e.	225 ± 70	20.3 ± 2.2	12	89 ± 24	34.3 ± 12	159 ± 21	28.3 ± 3.8
lesioned (n)	(12)	(12)	29	(13)	(14)	(14)	(14)

* Extent - No. of spikes above mean for period of excitation
\+ Extent - " " below " " " inhibition

TABLE 16: Characterisation of the initial effects in PSTHs recorded from SN DA cells, resulting from striatal stimulation. No significant differences occur, using Wilcoxon Rank Sum test or χ^2 test. The latter test was used for the occurrence of the early excitation.

which this time was summed over the whole length of the inhibition. The extent of the inhibition was taken as the number of spikes below the expected number for that period, calculated from the control firing.

The same rules for choosing which stimulus intensities were chosen for the analyses as in the previous section were used.

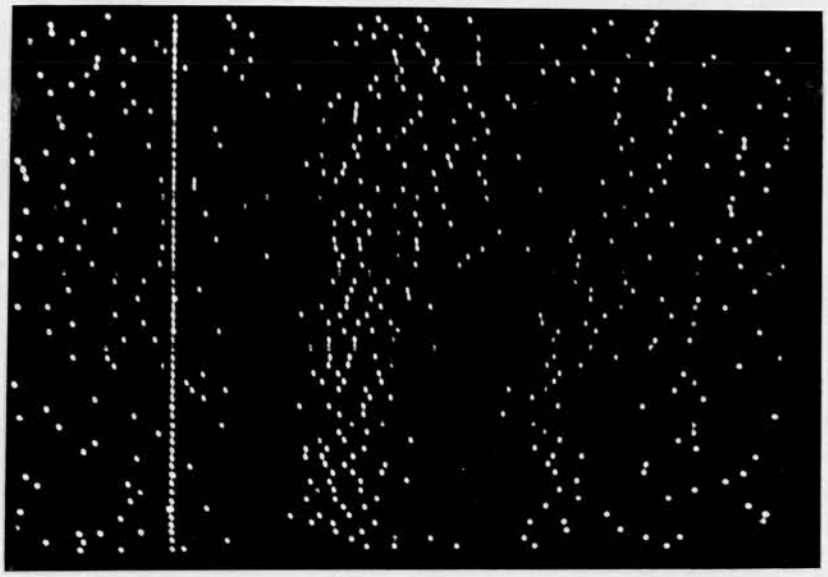
The three PSTHs which had delayed excitations as their only initial effect, over all intensities of stimulation were not used in the analyses, nor were another four cells which did not show a clear effect over the stimulation intensities used. Hence only 90 of the 97 cells in this group have been included in the analyses.

The mean values for the effects of this group of cells has also been included in Table 16.

Later effects of striatal stimulation

The initial effects seen in the histograms were often followed by a complex sequence of changes in firing, the most common effect being a late excitation. Later inhibitions and even third excitations or inhibitions were also seen. These later effects could be short lasting, or they could last up to the end of a PSTH. Only one cell showed only an initial effect at the stimulus intensities used and no cells did not respond in some way to stimulation. Figure 45 shows the oscilloscope pattern constructed with the raster-stepper and figure 45b shows the histogram drawn from it. Figure 46 is an example of a PSTH constructed by the F100 computer system.

Of the later effects, the most common one was a late, second excitation illustrated in Figure 46. This was generally longer lasting than the initial excitation and although occasionally could be, were not usually as great in extent. It seemed to occur more strongly in cells which also had an initial excitation, but this observation was not investigated further due to comparative and quantification difficulties.



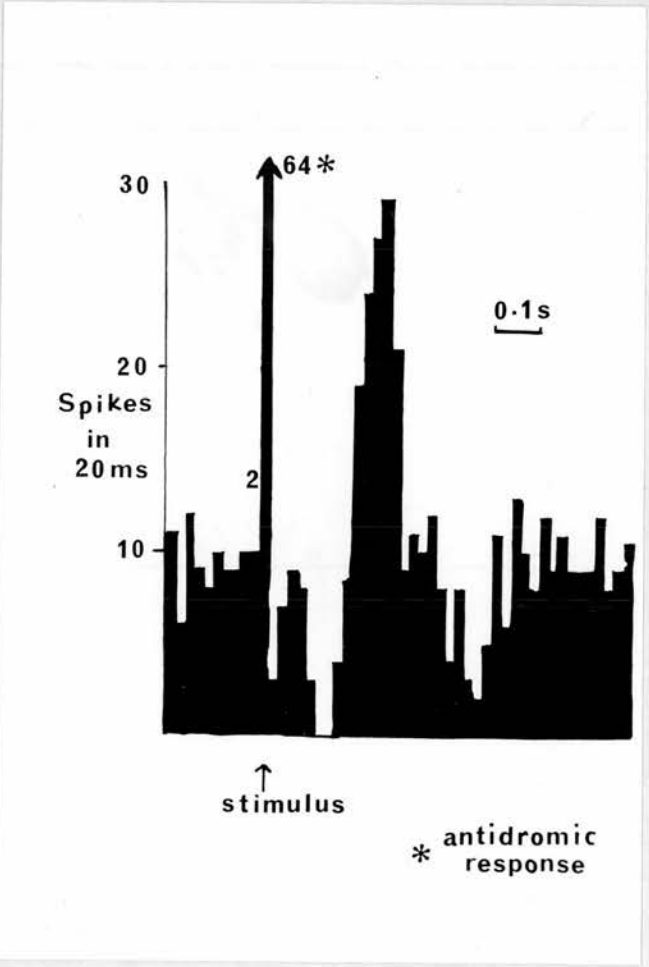


FIGURE 45: Example of the oscilloscope pattern resulting from the use of the raster-stepper to construct histograms. The graph plotted from this scatter is also shown.

A total of 64 sweeps was made.

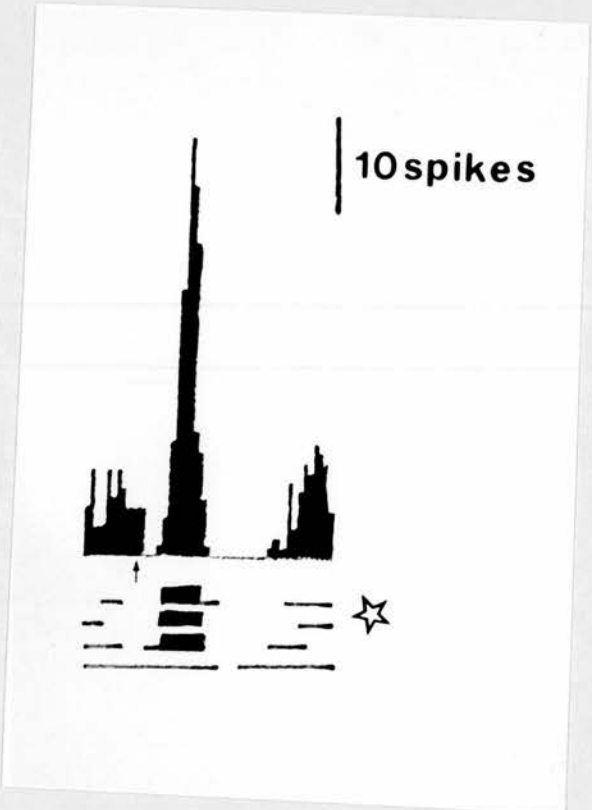


FIGURE 46: PSTH constructed by the Ferranti computer, showing a late excitation. The bars drawn under the PSTH indicate whether or not the particular period of firing is above control (▬), the same as control (—) or below control ( ). (See text for details of the analysis resulting in these levels.) The PSTH is 1 s long. The star indicates the position of the antidromic threshold.

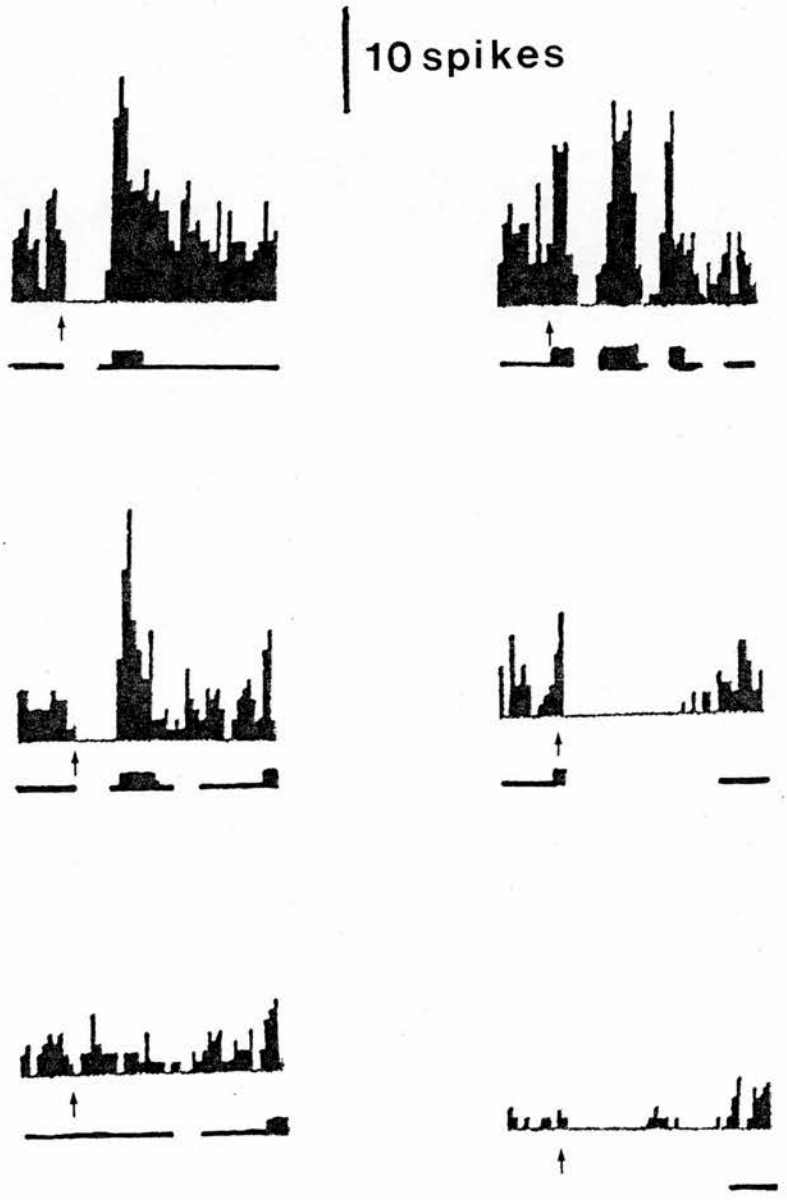


FIGURE 47: Sample PSTHs from control cells. The scale bar is the same in each case and each PSTH is 1 s long. All are taken from the Ferranti computer, but these are also typical of those obtained from using the raster-stepper.

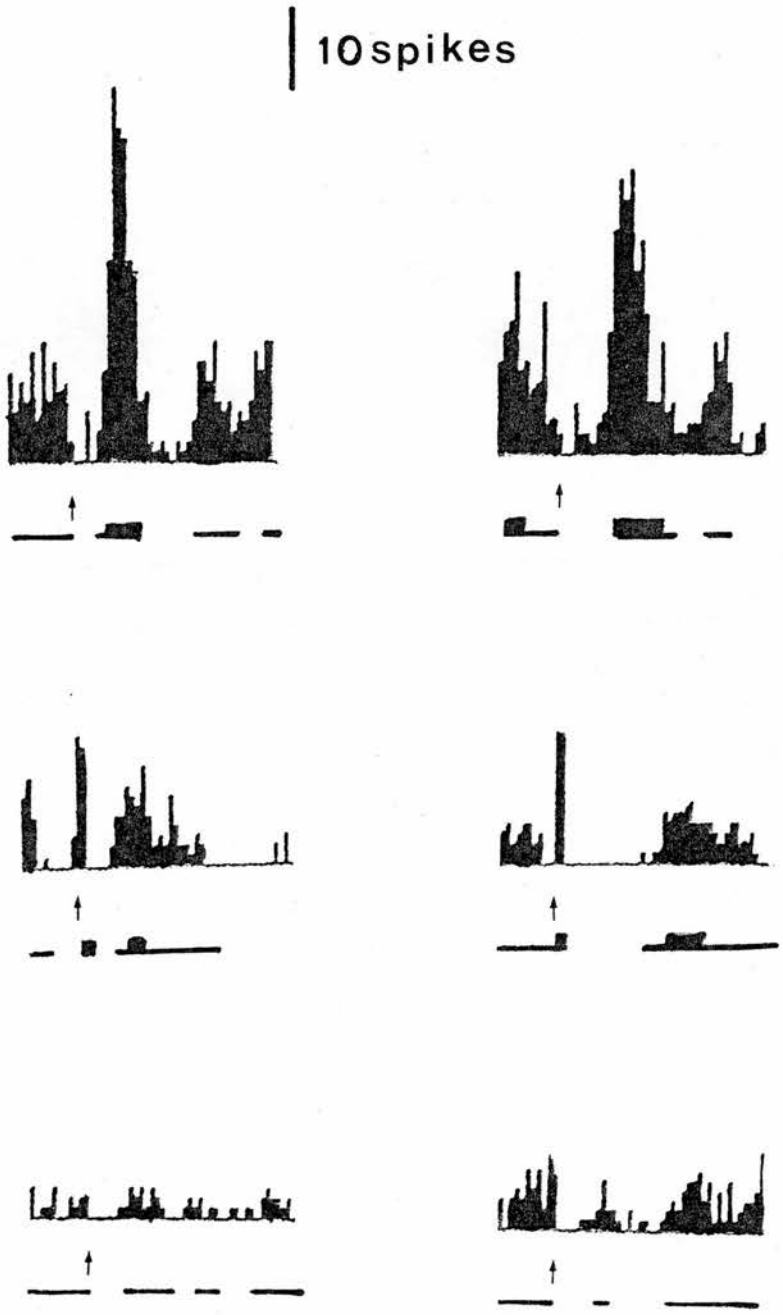


FIGURE 48: Sample PSTHs from cells from lesioned animals. See Figures 46 and 47 for description of the histograms.

	Description of change in pattern as stimulus strength increased	Initial effect	Control animals	Lesioned animals	Partially lesioned animals
1	Effects generally increased	Early excitation	28	7	10[1]
		No early excitation	76[2]	13	8
2	Later effects decreased as initial effects increased	Early excitation	4	-	1[3]
		No early excitation	3[4]	1	1
3	Initial effects decreased as later effects increased	Early excitation	7	-	-
		No early excitation	6	2	3
4	Initial effect decreased as a later effect appeared	Early excitation	0	-	-
		No early excitation	1[5]	1[5]	-
5	Little change occurred	Early excitation	2[6]	-	1
		No early excitation	6	3	5
6	Not enough information to classify	Early excitation	3	2	-
		No early excitation	7	3	-

TABLE 17: Division of later effects of striatal stimulation on nigral cell firing.

[1] In 4 cells a secondary excitation decreased as stimulus strength increased, in others any secondary excitation increased with stimulus strength.
[2] In 1 cell, an initial inhibition disappeared at the top stimulus strength.
[3] A delayed inhibition decreased as a secondary excitation increased, to join with the early excitation.
[4] In 1 cell a late excitation appeared and then disappeared as stimulus strength increased.
[5] These cells may have had early excitations if the stimulus strength could have been increased. The initial inhibition had gone at the top strength.
[6] In 1 cell an early excitation appeared and disappeared as the stimulus strength increased.

It was decided not to attempt to quantify the later effects at all as they varied so much in latency and extent, also it would be very difficult to choose which stimulus intensities to use and to justify the choice, especially if a comparison with a different group of cells was to be made. An attempt at a qualitative description is made below and a few histograms with diagrammatic representation of the changes caused by a change in stimulus strength are shown in Figures 47 and 48. A larger collection of data is also demonstrated in Appendix B, as it was felt it was useful and important to have more information than could be conveniently coped with in the text.

On a qualitative basis, the series of histograms can be divided into six groups:

1. effects generally get larger as stimulus strength increases;
2. the second effect decreases as the initial effect gets larger, as the stimulation strength increases;
3. the initial effect decreases as the second effect gets larger, as the stimulation strength increases;
4. the initial effect is split by the appearance of a new effect as the stimulation strength increases;
5. very little change in pattern results from increasing stimulus strength;
6. not enough information to classify.

Table 17 shows the numbers of cells that fall into each category. It can be seen from the table that the most common effect is for the patterns to increase as the stimulus intensity increases. This is true for both classification groups and for both control and operated animals.

The other four effects also have a similar distribution amongst the various groups.

The above classification, or rather description, shows no correlation with the relative positions of the recording and stimulating electrodes in the brain. Most combinations of effect grouping and histology positions were seen.

The action of drugs on the effects of striatal stimulation

It was hoped to be able to compare PSTHs before and after drug treatment by using a chi-squared test on each cell to see if the response pattern was different after the drug and if so, to then examine the PSTHs more closely to describe the effect, on a cell by cell basis. However, if PSTHs taken at the same stimulation strength in the same cell or two "off" periods are compared before and after a drug (allowing for the change in spontaneous rate), they show as being significantly different by this test ($P < 0.05$). This was seen even when the two patterns were identical when described by the equation given in Section 4.2. Due to this inherent variation in the PSTHs from one cell, it was clear that the test was not suitable and it was decided, as in the previous section, only to describe the effects from observation.

- *Haloperidol:*

Haloperidol at 0.05 or 0.1 mg/kg, caused little change in the pattern of response to striatal stimulation, either above antidromic threshold or at a lower strength, nearer the effect threshold. However, there did appear to be a slight, but consistant reduction in the amount of inhibition in the PSTHs. 0.05 mg/kg seemed to be a maximal dose as there was little difference in the effect of this dose and the higher dose. Sixteen cells were used to construct the PSTHs above antidromic threshold.

In 10 of these the reduced inhibition was in the form of a shortening of the latency of a delayed excitation or the shortening of an initial inhibition. In 1, there was a decrease in a delayed inhibition, secondary to an early excitation. In 2, there was generally less inhibition, and although in 1 the initial inhibitory component seemed to be larger there was much less inhibition later in the PSTH. In the final 3, there was very little change at all.

Only 13 cells were used to construct PSTHs near the effect threshold. The same general pattern was observed as mentioned above, but the effects, both before and after the drug are less than at the higher stimulus strength.

Looking at the numbers in the bins in the PSTHs, rather than just the pattern, did not alter the conclusions about the effects of the drug. In some cells the delayed excitation was stronger or less delayed, whereas in others it is weaker and prolonged. Figure 49 shows some examples of histograms at antidromic threshold before and after haloperidol. The extent of significant effects is also indicated below each PSTH (see legend to Figure 46).

It seems, therefore, that haloperidol may reduce the amount of inhibition produced by striatal stimulation, although it proved impossible to provide statistical support for this.

- *Picrotoxin:*

Picrotoxin at 2 mg/kg had a much more pronounced effect than the haloperidol on the response to striatal stimulation. In 3 out of 7 cells recorded, picrotoxin caused the appearance of an early excitation, not present before the drug. In 1 cell the threshold for an existing early excitation was very much reduced. In another 2 cells the existing early excitation is, if anything reduced, and later excitations are

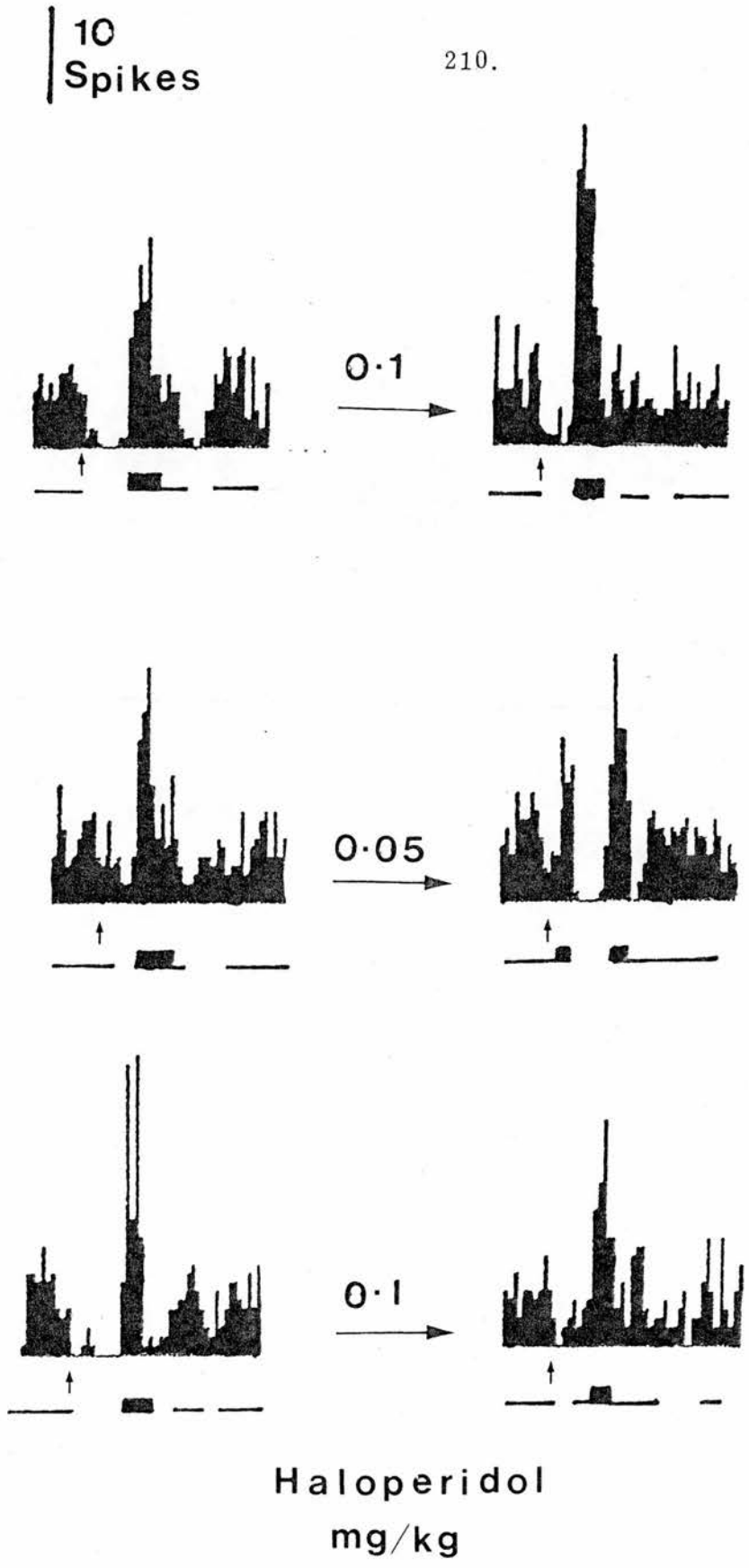


FIGURE 49: PSTHs taken before and after haloperidol. All are taken at just above antidromic threshold. See Figure 46 for a description of the histograms.

increased substantially. In one cell there was little change in firing rate or the shape of the IHs, and correspondingly there was no change in the response to striatal stimulation. This may have been because 2 mg/kg was below the threshold dose for an effect in this animal. This dose was chosen because it was known to be submaximal.

In the cells where the drug introduced a strong early excitation, the later inhibition tended to be increased although this was not always the case. Also where the early excitation was introduced by the drug, a later secondary excitation was reduced in size, corresponding to an increased inhibition, but contrasting to the increase in a secondary excitation in the case where the early excitation was already present, and reduced by the drug. Figure 50 shows examples of PSTHs taken before and after picrotoxin. Figure 51 shows a strong excitatory response to striatal stimulation not present before the administration of picrotoxin.

It seems therefore that picrotoxin causes an increase in the amount of excitation resulting from striatal stimulation, although again it was not possible to give statistical support for this.

- *Haloperidol and picrotoxin combined:*

Haloperidol, 0.1 mg/kg, given after 2 mg/kg of picrotoxin does not seem to cause the same effects after picrotoxin as it does before. This combination of drugs was tried on 4 cells. If anything, the amount of inhibition is increased, going both by the patterns and by the numbers in the PSTHs. The excitatory component of the response did not seem to be affected. In one case, due to a high spontaneous firing rate, after 4 mg/kg picrotoxin apomorphine was added before the haloperidol (0.05 mg/kg). The apomorphine prolonged the inhibition and this was reversed partly by the haloperidol, but not quite to the picrotoxin level. This is more in line with the action of haloperidol on cells with no pretreatment.

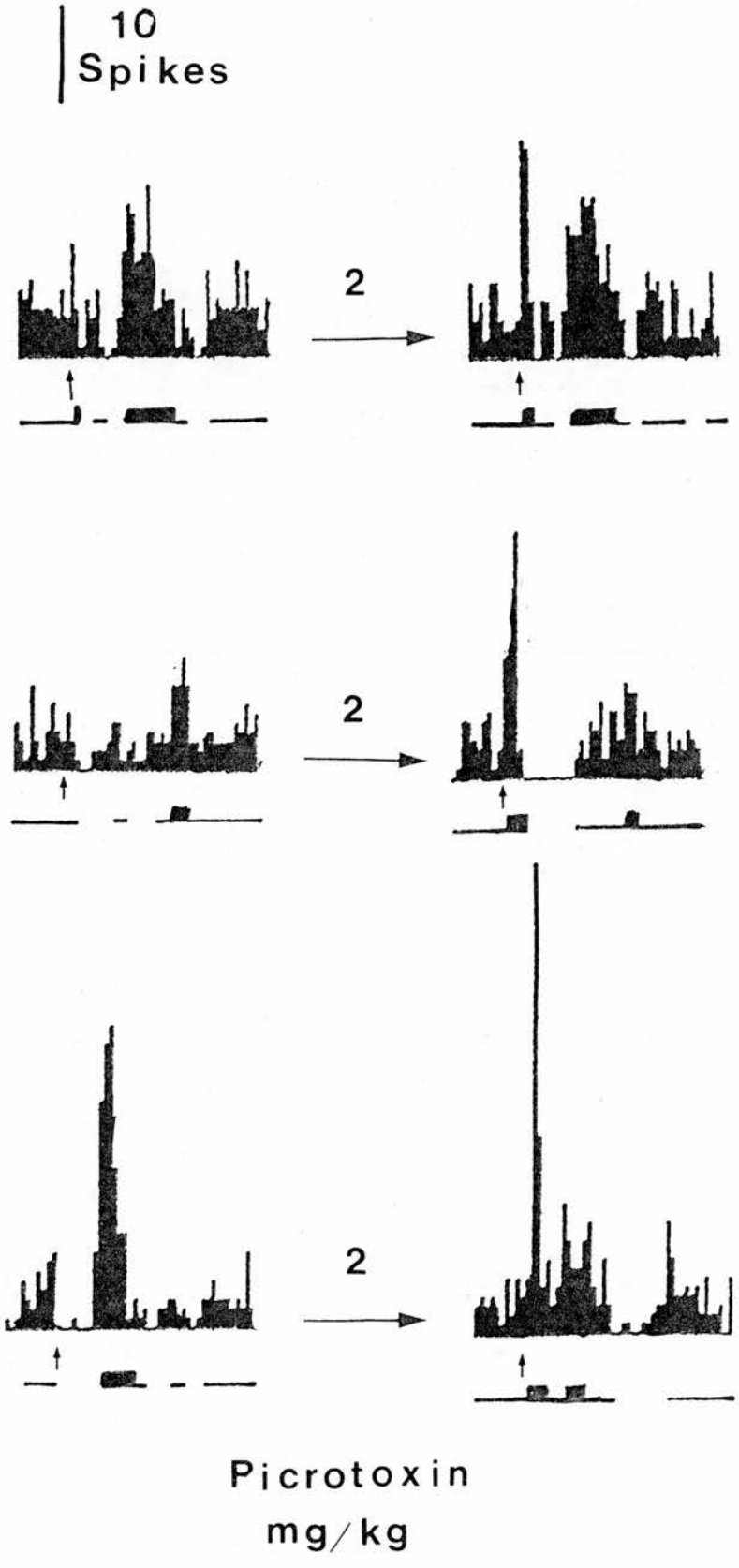


FIGURE 50: PSTHs taken before and after picrotoxin. For description of the histograms, see Figure 46.

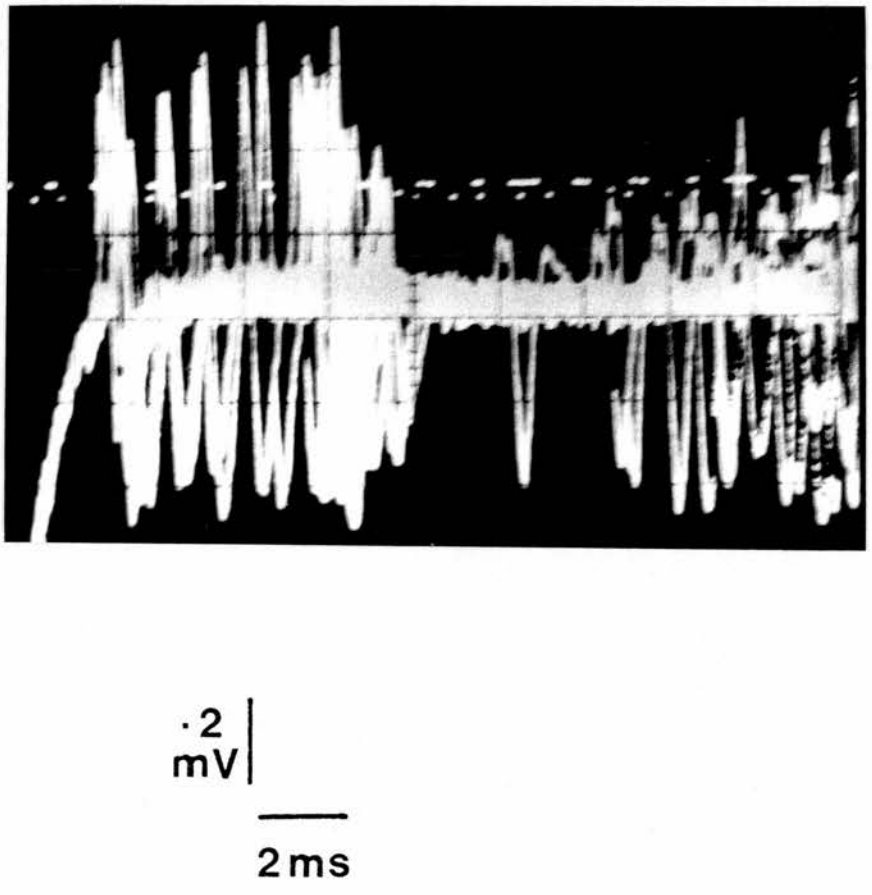


FIGURE 51: Example of a strong excitation in response to striatal stimulation after 2 mg/kg picrotoxin. No excitation was present before the picrotoxin was given.

It is possible that apomorphine is somehow competing with the picrotoxin to reduce its effect. This is further considered in the Discussion. PSTHs from the 4 discussed cells are shown in Figure 52.

If picrotoxin, 2 mg/kg, was given after 0.05 mg/kg haloperidol, it seemed to cause the same changes as it did in the control case. In 2 out of 3 cells an early excitation was introduced where there was not one before, and in the third an initial one was increased. The latter effect is different from controls where early excitations already present were reduced. Where the initial excitations were introduced, this may have been due to the shortening of the latency of a later excitation. In one case this would also mean the disappearance of an initial inhibition. In the case where the early excitation was increased a secondary inhibition was also reduced. In this particular PSTH there were no later excitations. All these are shown in Figure 53.

It seems therefore that haloperidol does not affect the action of picrotoxin on the effects of striatal stimulation.

The effects of drugs on the spontaneous firing of SN DA cells

- *Amphetamine and picrotoxin:*

In order to determine the effects of picrotoxin on the response of the SN DA cells to amphetamine, the response of 16 cells to amphetamine was first determined. A dose of 1 mg/kg was shown to reduce significantly the firing rate of the DA cells ($P < 0.001$, Student's paired t-test). In a further 3 cells in which a higher dose was used (2 or 4 mg/kg) inhibitions no greater than those observed above were seen. To test whether this effect was reduced after picrotoxin, the drop in firing rate resulting from a 1 mg/kg dose of amphetamine before and after a 1.5 mg/kg dose of picrotoxin were compared using a paired t-test. In order to

FIGURE 52: PSTHs resulting from striatal stimulation taken initially and then after picrotoxin followed by haloperidol (see Figure 46 for description of histograms).

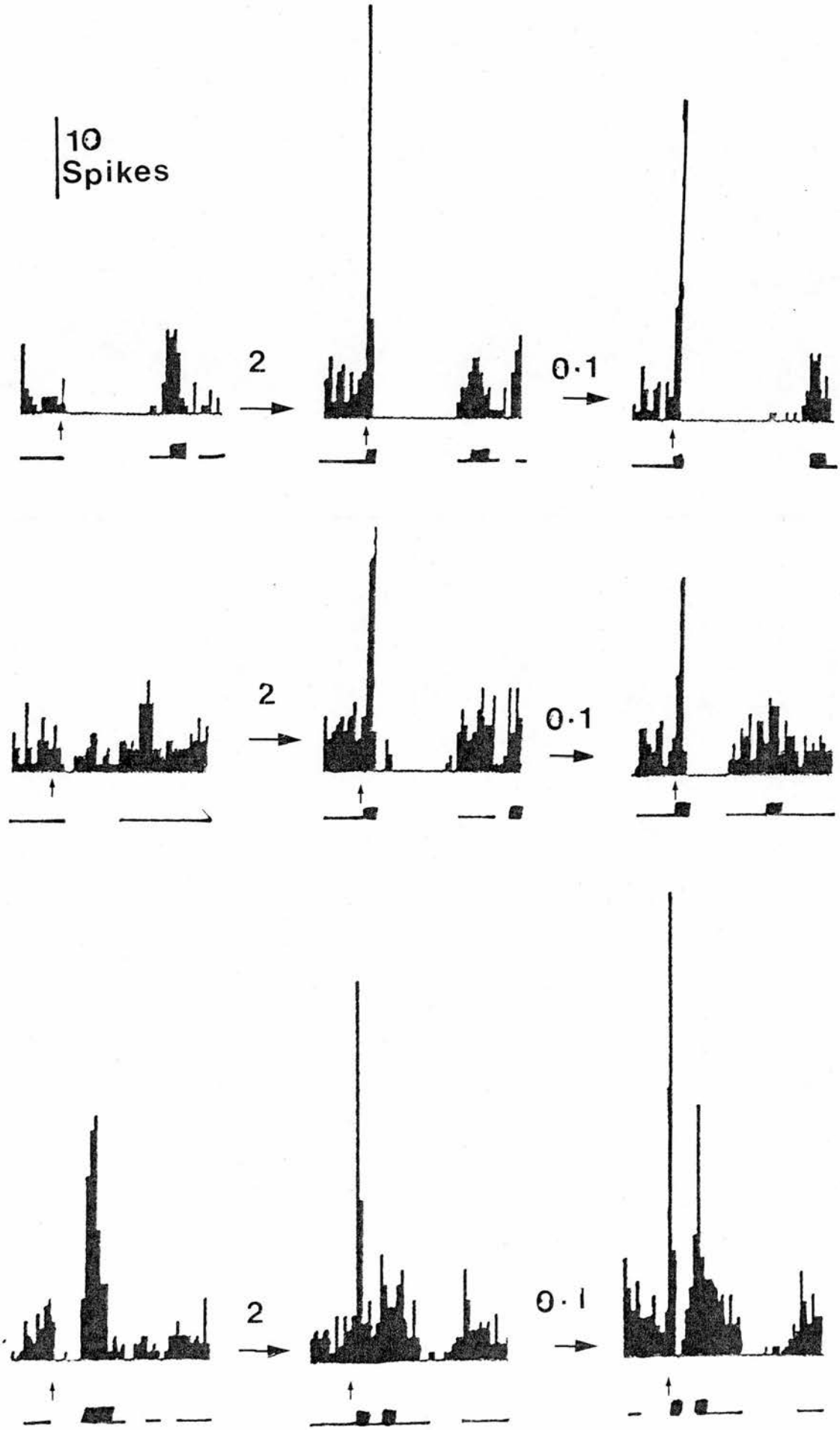
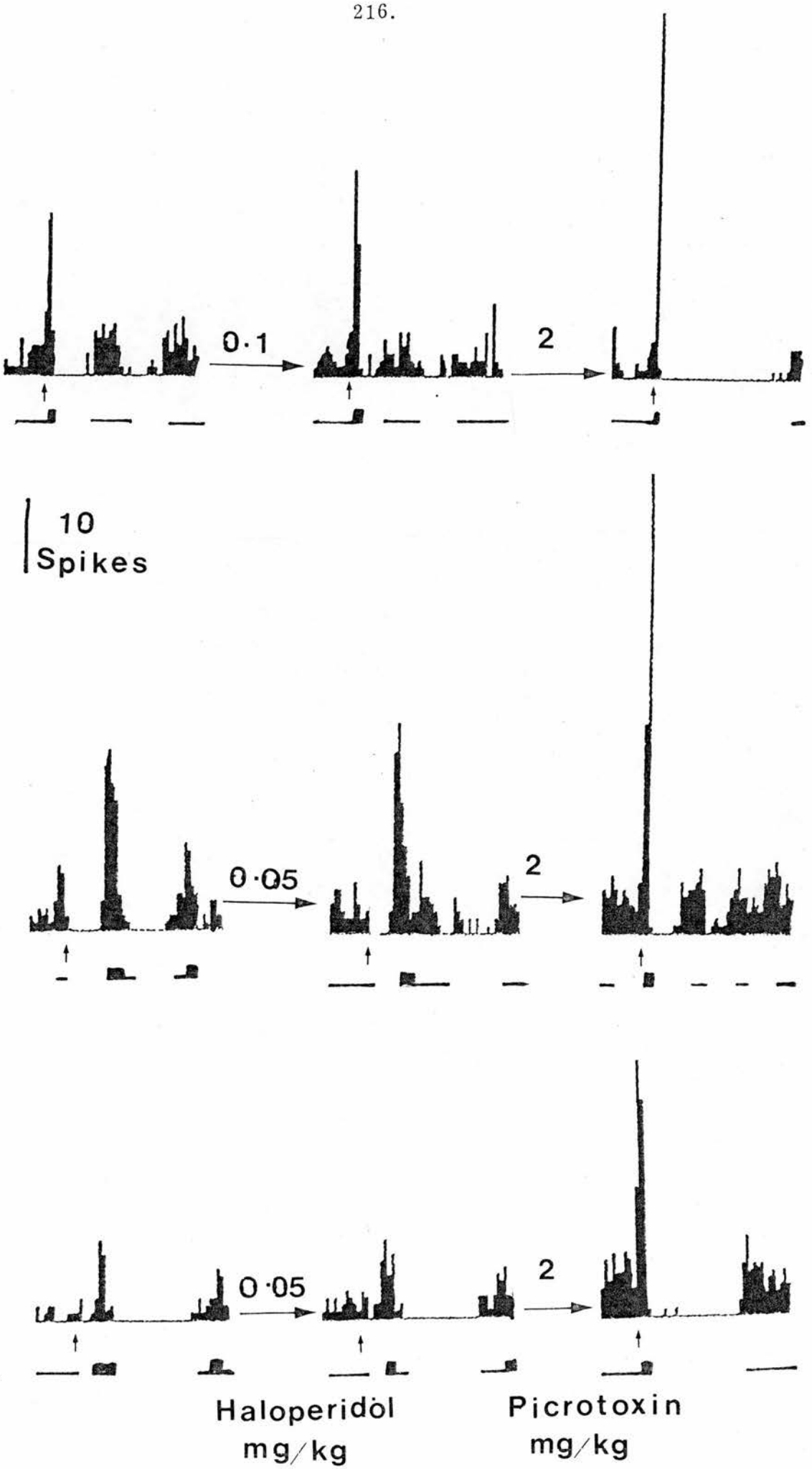

10
Spikes
2
0·1
2
0·1
2
0·1
Picrotoxin
mg/kg
Haloperidol
mg/kg

FIGURE 53: PSTHs in response to striatal stimulation, taken initially and after haloperidol followed by picrotoxin (see Figure 46 for description of histograms).


0·1
2
10
Spikes
0·05
2
0·05
2
Haloperidol
mg/kg
Picrotoxin
mg/kg

control for a reduced effect of a second injection of amphetamine, the same was done on the drops caused by two consecutive injections of amphetamine, 45-60 min apart, a time comparable to that between the injections when picrotoxin was also used. There was a significant reduction in the drop in firing rate caused by amphetamine after the picrotoxin ($P < 0.001$). Such a reduction in effect was not seen after the second injection of amphetamine, when no picrotoxin was given in between ($P > 0.1$). The reduction of the amphetamine effect by picrotoxin is similar to that caused by haloperidol and confirms the observations of Bunney and others (see Section 4.1) in support of the feedback lopp hypothesis, that picrotoxin blocks the action of amphetamine on SN DA cells.

It was decided that due to the variability in the control firing rates of the cells used, the numbers in the different groups were too low to attempt a quantitative analysis of the effect, but the traces shown in Figure 54 are typical of all. Also included in this figure is an example of the blocking action of haloperidol on the effect of amphetamine on the DA cells.

- *Picrotoxin and haloperidol:*

In order to further investigate the interaction of DA and GABA, the combined effects of picrotoxin and haloperidol were studied. Before this was done, however, the effects of these drugs on their own were investigated. Both picrotoxin and haloperidol were shown to significantly increase the firing rate of the DA cells, again using a paired t-test ($P < 0.001$ in both cases).

It was decided not to attempt the quantitative analysis of the size of the effects due to the high initial variability. Plots of the concentration against firing rate, including the standard error, show how variable the

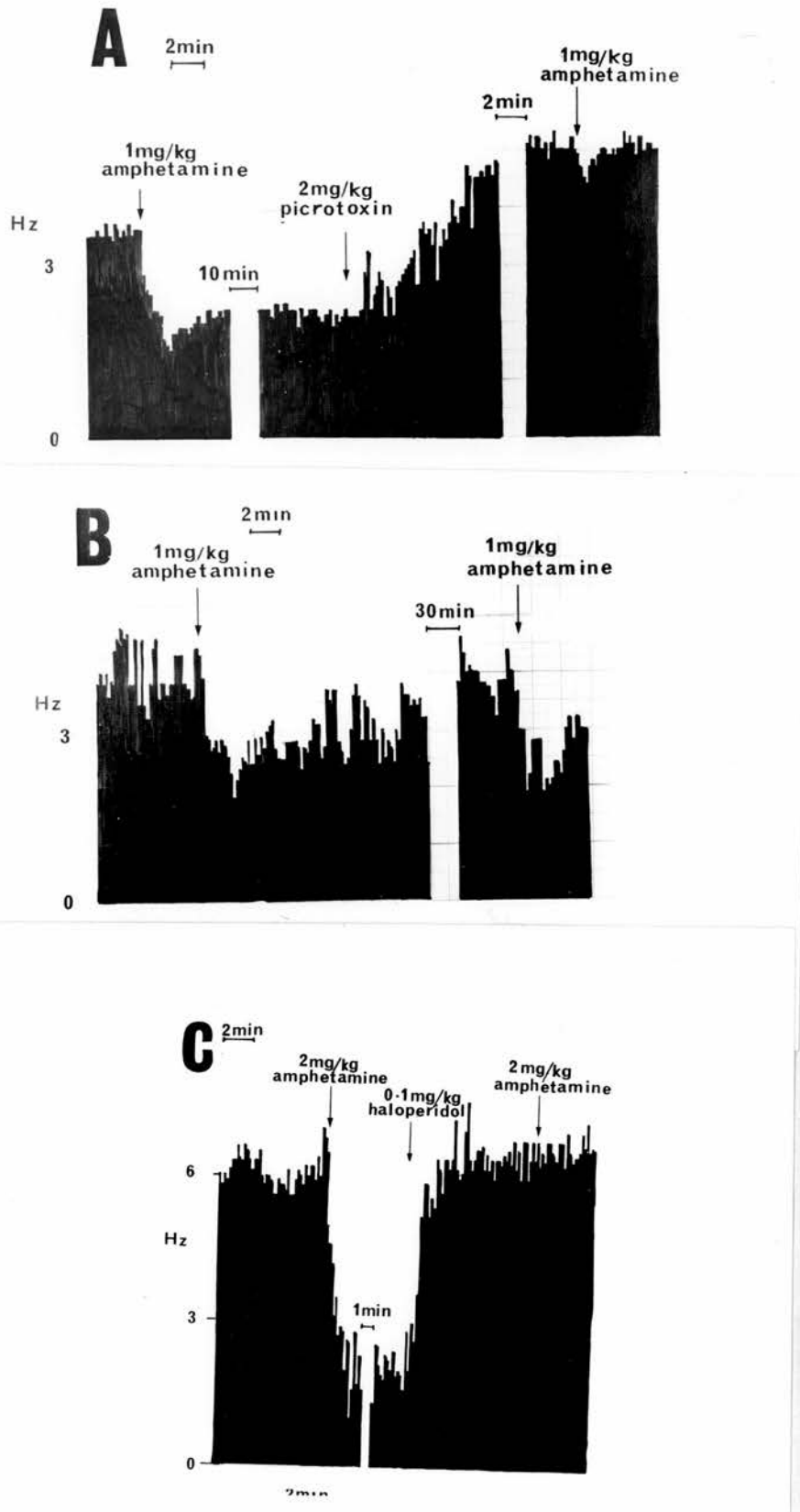


FIGURE 54: Effects on the spontaneous firing of SNC DA cells of:

A. Amphetamine followed by picrotoxin and more amphetamine.

B. Amphetamine followed by a second dose of amphetamine.

C. Amphetamine followed by haloperidol and then more amphetamine.

firing rates are and how difficult it would be to quantify the dose-response effect realistically. These are shown in Figure 55, a and b.

It was decided to use 2 mg/kg of picrotoxin and 0.05 mg/kg of haloperidol when used as the initial drug in the pair, and 2 mg/kg picrotoxin and 0.1 mg/kg haloperidol when it was the second of the pair. The initial doses were chosen as they seemed to be mostly submaximal, although occasionally 0.05 mg/kg haloperidol did produce an effect, not increased by the further addition of haloperidol. Also occasionally 0.05 mg/kg of haloperidol did not affect the firing rate and so another 0.05 mg/kg was added.

It can be seen from Table 18 that these two drugs, haloperidol and picrotoxin, are additive, irrespective of the order they are applied in. An attempt was made to compare quantitatively the effect of haloperidol and picrotoxin on their own to the effect of each after the other. It was shown that there was no significant difference between the control effect of haloperidol and the effect after picrotoxin ($P > 0.1$), nor between the control effect of picrotoxin and the effect after haloperidol ($P > 0.05$).

- *Depolarisation block and the effects of apomorphine:*

One of the biggest problems with the drugs increasing the firing rate of the DA cells is that the cells do not appear to be able to fire much faster than 10 Hz. A few may fire a little faster, and a few may not reach this level, but it is an approximation of the level when firing patterns change dramatically. This may mean that the sample of cells used in the haloperidol and picrotoxin experiments above may not be representative of the whole population, as in my experience the experiment could not be done on a cell firing at 6 Hz or more to start with.

Examination of the interval histograms from these cells show that the pattern of firing may have changed, while the mean firing rate has

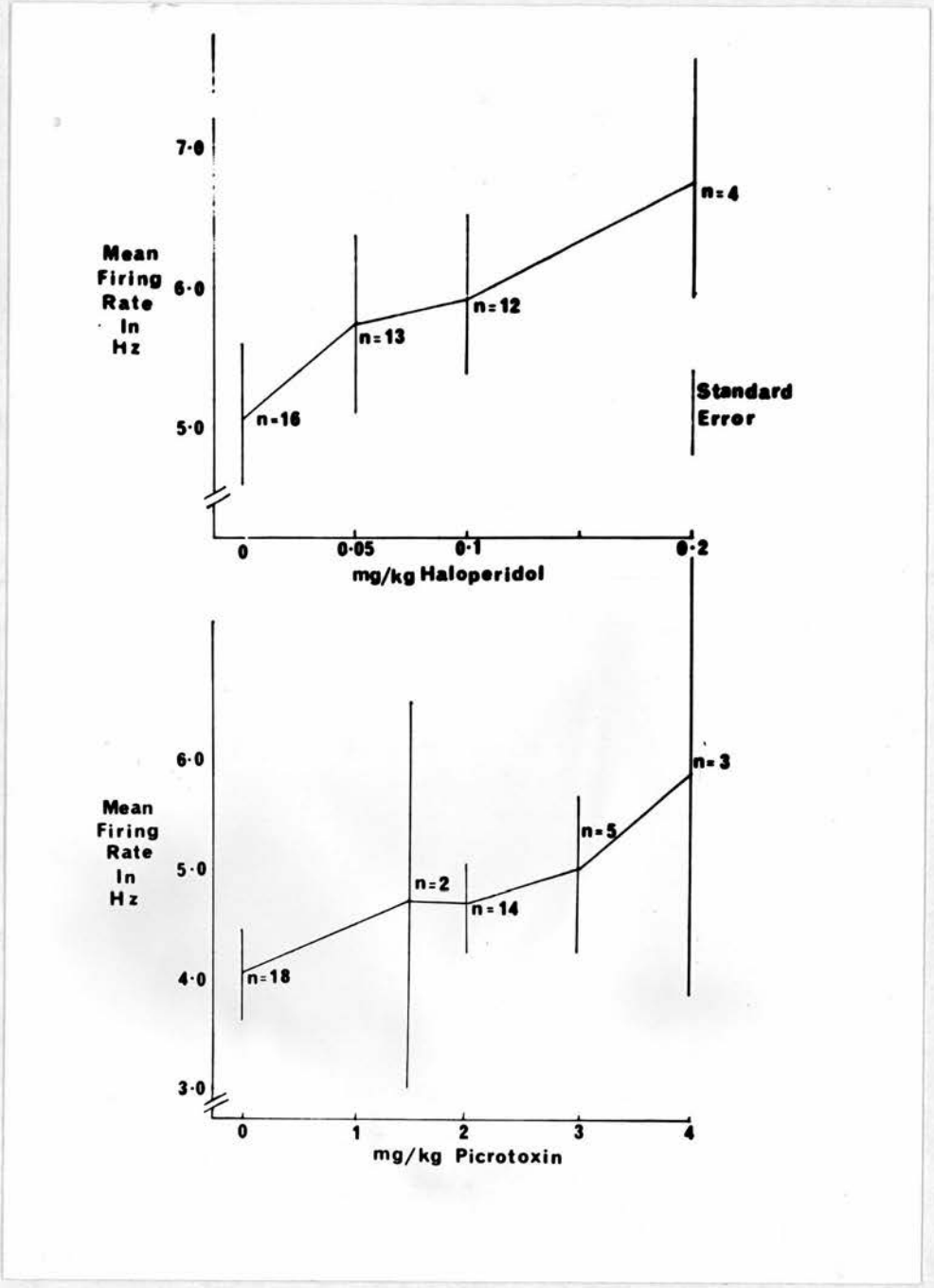


FIGURE 55: Dose response relation of haloperidol and picrotoxin, showing the great variability in the effects of the drugs.

No pretreatment			After 0.05 mg/kg haloperidol
0.89	0.54	0.80	0.97
0.95	0.04	1.42	1.05
0.62	1.63	0.45	2.09
-0.27	1.05	0.55	0.91
0.69	0.46		

TABLE 18a: Changes in firing rate (Hz) caused by 2 mg/kg picrotoxin, with and without 0.05 mg/kg haloperidol pretreatment. There is no significant difference between the two cases (Wilcoxon Rank Sum test).

No pretreatment			After 2.0 mg/kg picrotoxin
0.78	-0.08	1.16	-0.78
0.46	0.51	1.30	0.85
0.89	0.56	0.55	1.79
0.30	1.27		2.40

TABLE 18b: Changes in firing rate (Hz) caused by 0.1 mg/kg haloperidol, with and without 2 mg/kg picrotoxin pretreatment. There is no significant difference between the two cases (Wilcoxon Rank Sum test).

remained much the same, indicating that firing rate alone is not a good indication of whether a cell has been affected or not. The usual pattern is for the cell to start firing in short, fast bursts, leading to a biphasic interval histogram, or even one with just a very early peak, depending on the frequency of the bursts. Figures 56 and 57 show IHs from some cells treated with haloperidol or picrotoxin. The main significance of this for these experiments is that when the cells start bursting the size of the action potential reduces towards the end of the burst, possibly even below the level of the noise, meaning that not all the action potentials will be counted, or that as the IS and SD components often become more separated, each action potential is counted twice. Figure 58 shows an oscilloscope trace of a "bursting" cell. This bursting effect was only seen extensively after picrotoxin, but a change in the IHs of haloperidol treated cells did sometimes show similar changes.

An extension of this observation is that sometimes, after picrotoxin, the cell will increase its firing rate greatly, increase the frequency of overall firing of bursts and then reduce in size gradually or abruptly until it cannot be detected over the background noise. It was supposed that the cells were firing so fast that they had gone into depolarisation block, and that a drug known to reduce the firing rate after a picrotoxin induced increase (apomorphine) might restore the firing of the cell. This effect of apomorphine in reducing the firing rate of cells after picrotoxin is marked and was observed in 3 cells one of which is illustrated in Figure 59a. A complete cessation of firing was seen in 7 cells given picrotoxin at 3 or 4 mg/kg. Apomorphine was tried on 6 of these and some firing was restored. This was difficult to quantify and a description of the behaviour of each cell is given in Table 19. Traces from two of these cells are illustrated in Figure 59b.

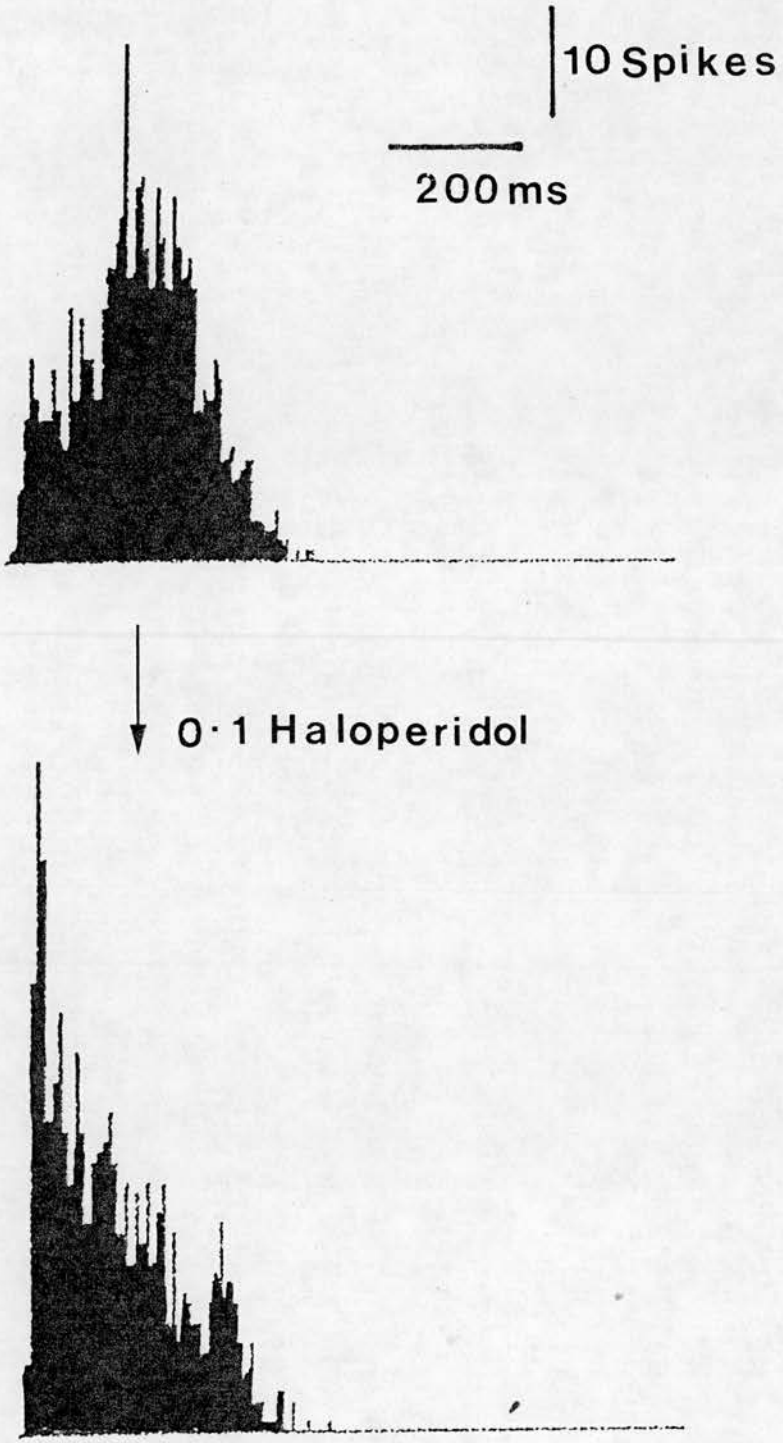


FIGURE 56: IHs before and after haloperidol 0.05 mg/kg, showing a decreased mode and a change of shape.

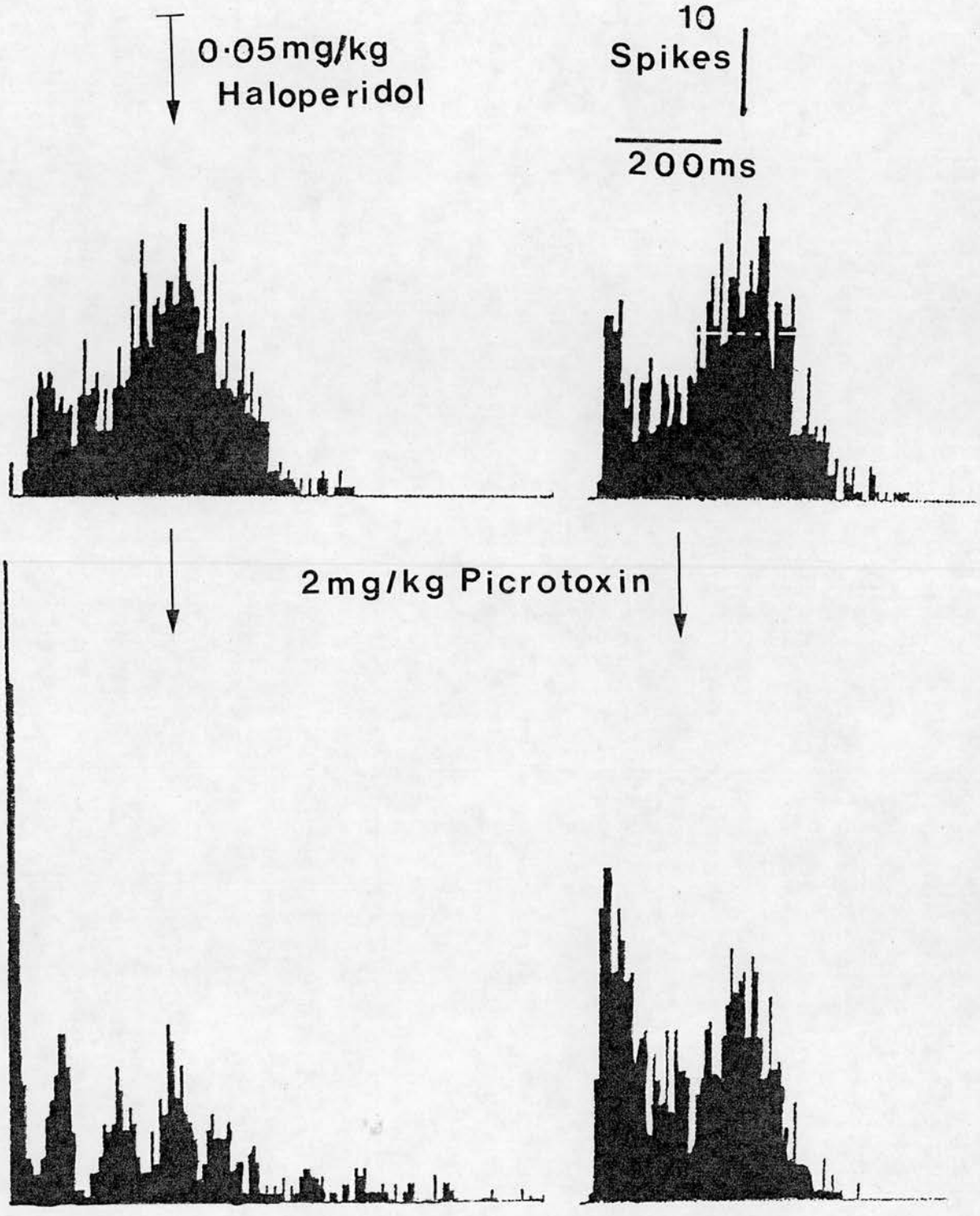


FIGURE 57: IHs before and after 2 mg/kg picrotoxin, showing a decreased mode and a shape change.

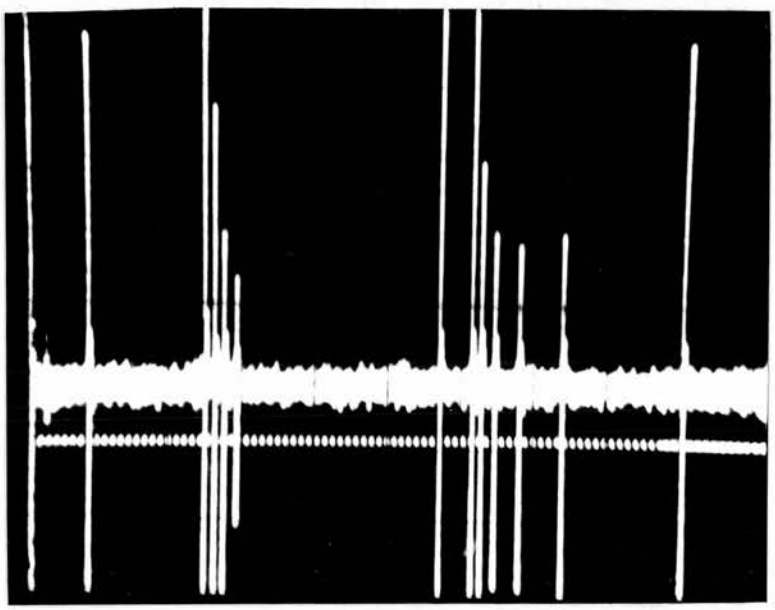


FIGURE 58: Oscilloscope trace of a burst in cell firing after 2 mg/kg picrotoxin, showing the steady decrease in AP size during the burst.

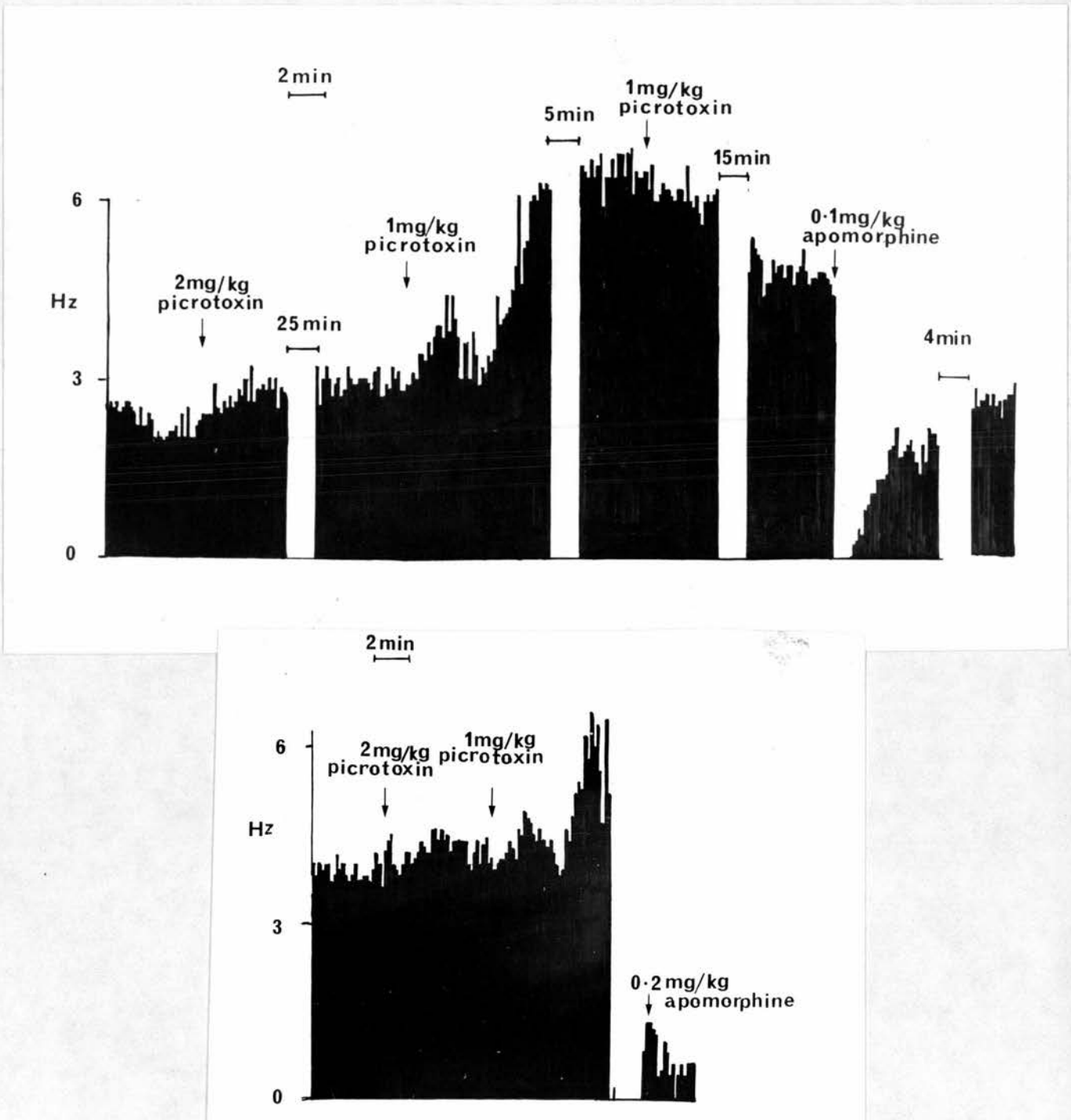


FIGURE 59: Changes in the spontaneous activity of a cell caused by:

A. Administration of picrotoxin followed by apomorphine.

B. Administration of apomorphine after picrotoxin had caused the cell to enter depolarisation block.

	Initial firing rate (Hz)	First dose of picrotoxin (mg/kg)	New firing rate (Hz)	Second dose of picrotoxin (mg/kg)	Effect of second dose of picrotoxin on firing	Dose of apomorphine (mg/kg)	Effect of apomorphine on cell firing
1	3.75	3	——————→		"depolarisation"	1.0	Bursts of fast firing occurred, especially in relation to stimulation.
2	4.65	2	5.60	2	"depolarisation"	1.0	Variable firing rate, 2-6 Hz.
3	5.68	2	6.73	2	"depolarisation"	0.2	Some firing, very "bursty" and uncountable due to entanglement with the noise.
4	3.93	2	4.39	1	Rate increased to 5.74 Hz and then depolarised after several minutes.	0.1	Restored firing at about 0.8 Hz.
5	*c*5.00	2	*c*7.00	1	Rate increased to about 8 Hz then "depolarised".	0.2	Cell fired for a spell at about 2 Hz and was then lost.
6	6.49	2	7.38	2	Rate increased to 9.61 Hz then suddenly "depolarised".	1.0	No effect.

TABLE 19: Effects of apomorphine on the firing of cells caused to go into "depolarisation" block by picrotoxin.

The fact that apomorphine can restore some form of firing would support the concept that picrotoxin can cause a state of depolarisation block in the DA cells.

As no haloperidol treated cells entered this apparent state of depolarisation block, it was not possible to see if apomorphine would do the same to them. However, it does seem that some effect of apomorphine on the DA cells remains after 0.05 mg/kg haloperidol (see Figure 60). As only 2 cells were tested with apomorphine itself it was not possible to say quantitatively whether the reduction in firing rate was reduced by haloperidol, as has been reported.

One cell appeared to be inhibited by amphetamine after a dose of picrotoxin sufficient to cause bursting in the cell, although the total rate was not very high (4 Hz). However, as the cell was bursting very fast, with a mode in the IH of 10 ms, the amphetamine application could have coincided with the onset of depolarisation block. If this was the case, the amphetamine unlike apomorphine, did not restore firing. This might have been expected from the results, mentioned earlier, that picrotoxin blocks the effects of amphetamine.

In summary, therefore, it was seen in these experiments that picrotoxin does block the effects of amphetamine, but not that of apomorphine and that the latter may even restore firing after onset of a depolarisation block caused by picrotoxin. It was also seen that the effects of picrotoxin and haloperidol are additive, regardless of the order in which they are applied.

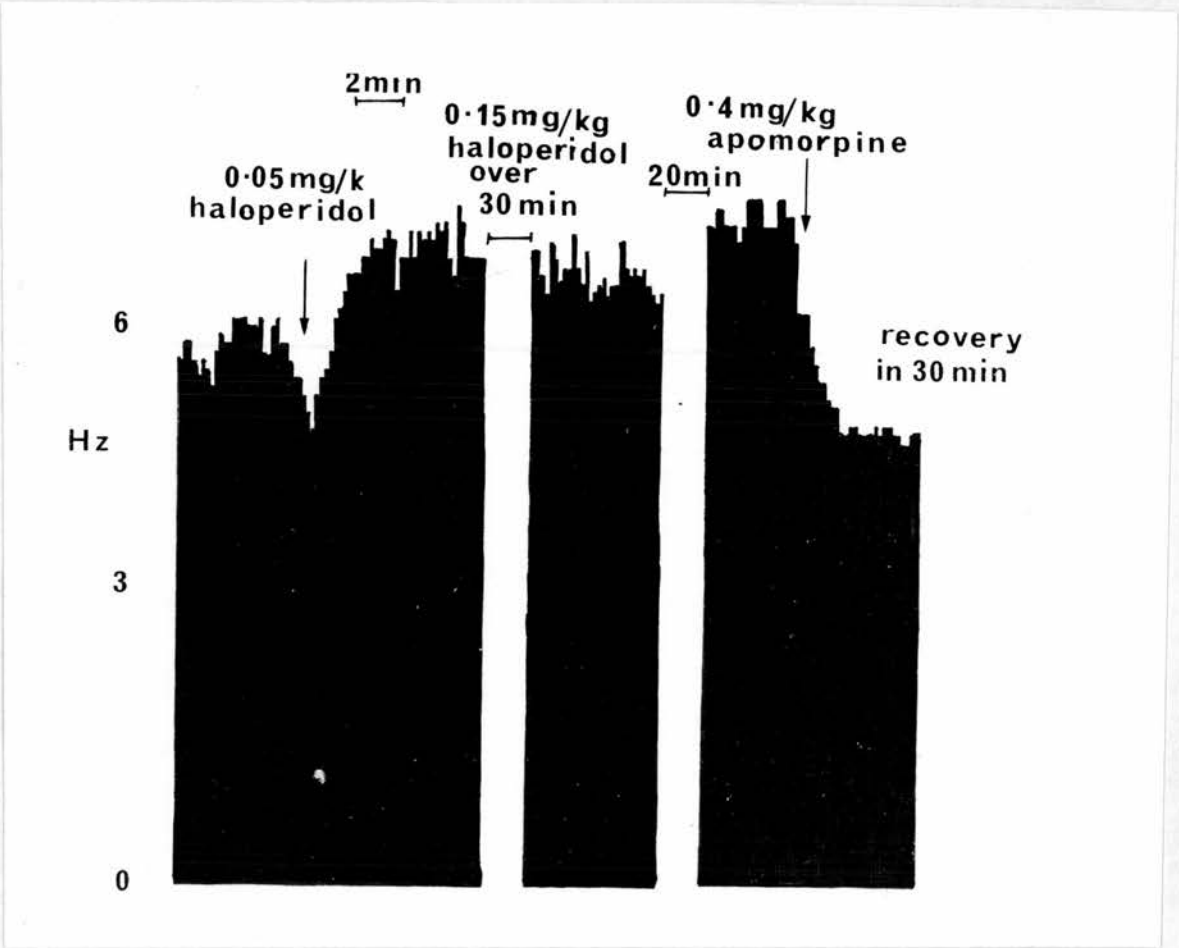


FIGURE 60: Changes in spontaneous firing rate of SNC DA cells caused by haloperidol followed by apomorphine.

4.4 Discussion

Review of results

All DA cells recorded from were characterised by antidromic driving from the striatum. The threshold for antidromic driving was correlated with that for any effect in both control and lesioned animals. There was no difference in the antidromic thresholds, nor in the effect thresholds nor in the extent of any of the effects measured, in any of the groups of animals. The only effect of the lesions was to increase the spontaneous firing rate slightly in the lesioned animals, and to decrease the antidromic latency in partially lesioned animals.

The effects of picrotoxin and haloperidol in increasing the spontaneous firing rate were additive, regardless of the order in which they were administered. The effect of amphetamine was blocked by picrotoxin, but that of apomorphine did not appear to be. Apomorphine could even cause firing in cells that had entered depolarisation block after picrotoxin. Haloperidol may have reduced the amount of inhibition in PSTHs resulting from striatal stimulation. The effect of picrotoxin was to increase excitations and to cause some excitations that were not there before the administration of the drug.

Dopamine action potentials

Other workers have found that the APs from cells shown to be DA ones are very homogeneous in nature (6,62,136,235,250). Cells identified as DA ones by their position in the SNC, intense fluorescence after iontophoresis of l-DOPA (6,62) and/or by antidromic driving from the striatum (136,235,250) all had similar characteristics. These characteristics consist of a relatively slow firing rate with wide action potentials. The APs have been described in some studies as typically biphasic, and greater than 2 ms long in some studies (6,62), but in others

they have been reported to be much longer, 4-6 ms, typically triphasic with a late positive wave and a marked initial segment component (250). The firing rate and pattern seems to depend on the anaesthetic used. In animals that were anaesthetic free and gallamine paralysed, the firing rate has been reported to be between 2 and 6 Hz and to be steady (6,62). If chloral hydrate was used the rate seemed faster, 3-9 Hz showing bursting patterns with a decrease in AP width and size during the burst (6,62). However, a later study has shown that firing in chloral hydrate anaesthetised animals was more variable between animals and steady (0.5-8.0 Hz) (250). On the other hand, ketamine anaesthetised animals have been reported to have DA cell firing rates of 3-6 Hz (136).

Injection of 6-OHDA into the nigro-striatal path several days before the experiments resulted in an almost complete loss of this type of cell and this has been used as evidence that the type of AP described above belongs to a DA cell (6,250). Experiments combining extracellular recording with the intracellular injection of l-DOPA show intense fluorescence in this type of recorded neurone. This is not seen in non-DA cells (235).

The APs described in the experiments in Section 4.3 all resemble those mentioned above. In the halothane anaesthetised rat, there appear to be a combination of the characteristics from the others. Cell firing rate varies from 1-9 Hz, and shows bursting patterns in quite a few cases. Many of the APs are wide, up to 6 ms, and have an initial segment component. However, narrower ones are seen, although none are less than 2 ms wide and not all have the initial segment component. The shape and size of the cell often varied between the alternatives during the course of an experiment and this has been attributed to the

movement of the electrode towards or away from the cell, changing its orientation to the soma.

From the above it was assumed that all the cells used in this study are, in fact, all DA cells.

An increase in firing rate of SNC DA cells after striatal KA lesions has been previously reported (531), although not all groups of workers have seen this increase after the lesions (413).

In this study, although the range in firing rates was not different in control and striato-nigral lesioned rats, the mean rate was more in the lesioned group than the control group or the partially lesioned group. The other characteristics were unaltered by the lesions. The change in firing rate is probably due to the removal of a tonic inhibitory input from the striato-nigral GABA projection (see Section 1.2).

Antidromic driving of substantia nigra dopamine cells

As has already been mentioned, all cells used in this study were antidromically driven from the striatum. Guyenet and Aghajanian (250) have also identified SNC DA cells by means of antidromic driving from the striatum. The DA cells, called by these authors Type I cells, were only antidromically activated from the striatum and not from any of the other areas tested and all fitted the description given above. Other groups of workers have also antidromically activated DA cells from the striatum (136,87,89,413). One of these groups of workers (136) also found some SNR cells that were antidromically activated from the striatum and some of these were also driven from the thalamus. The shape of the APs of these cells was more like the non-DA, Type II cells of Guyenet and Aghajanian, that is much narrower (less than 2 ms) and they always responded with the full spike.

From the antidromic latency the authors mentioned above calculate conduction velocities of from 0.3-1.0 m/s, mostly averaging at about 0.5 m/s (87,189,136,413). This value corresponds well with the values obtained in this study. Assuming a distance of approximately 4.5 mm, the conduction velocities in this study ranged from 0.2-0.75 m/s. These values are necessarily approximate as the exact distance and point of activation cannot be ascertained. This value is consistant with that expected from fine non-myelinated fibres that the nigro-striatal pathway is reported to consist of (see Section 1.2).

The mean conduction velocity of the SNR fibres driven from the striatum was 4.1 m/s, corresponding to a latency in the case of the experiments described in Section 4.3 of just less than 1 ms. No antidromic latencies this short were observed in this study. The shortest was 6 ms. From this it was concluded that none of the cells used in this thesis were of the fast conducting type.

The antidromic latency was shorter in the partially lesioned group when compared with that of the controls. However, the lesioned group showed no differences. The latency in the lesioned group, however, was shorter than in the control cases, but the difference was not significant. Intracellular studies have suggested that an inhibitory input may lengthen the time between the stimulus and the IS component of the antidromic spike (249), so the removal of a tonic inhibitory input to a system may result in the shortening of the stimulus-IS interval. This may be the reason for the shortening of the latency in this case. Removal of a tonic inhibitory input would also be consistent with the increase seen in the spontaneous firing rate in the lesioned group. The reason that the lesioned group did not show a drop in latency and the partially lesioned group did not show a drop in firing rate may have been because

the operated groups are relatively small and the changes themselves are small. Larger samples may have resulted in significant effects for both measures in both operated groups.

Collision, the cancelling out of a spontaneous or orthodromically driven spike by an antidromically driven one is thought to be the best means of proving antidromicity. Other methods are a high frequency response to repeated stimulation, a constant latency of response, stability of the antidromic threshold and the shape of the driven AP (367). AP shape is not a good indicator for antidromicity in SNC DA cells. In many areas of brain, the antidromic spike will be split into the IS and SD components, whereas the spontaneous or orthodromic spikes are not. In the case of the SNC DA cells, the APs are split under normal circumstances and so this test is not suitable. Stability of threshold is also not very useful in this case. Normally in antidromic driving, there is a stimulus strength that results in 100% invasion but any lower strength will result in no invasion, i.e. it is all or none. However, if the stimulating electrode is in a terminal area where one of several points may be activated, not necessarily by the same stimulus strength, this may result in not quite 1 : 1 responding at some stimulation strengths. Also if the cells are spontaneously active, a stimulus arriving during the relative refractory period needs to be higher to drive the cells than otherwise. This will also lead to less than 100% response. Both of these conditions apply to SNC DA cells. Variations in latency result from several factors including the activation of different branches in a terminal area (leading to distance latency jumps) and also it can take a longer time for the AP to invade the cell in the relative refractory period and so there are variations in the IS-SD time. Both these types of latency variations have been reported for SNC cells driven from the striatum (89).

These latency variations were also seen in the experiments described in this Section 4.3, although only the stimulus-IS interval was measured.

The other two criteria, collision and following frequency, have both been measured in these experiments, and collision is taken as the definitive test, as various factors can affect following frequency, usually in some way related to an increased membrane potential.

The principal of collision is that the cell can be antidromically invaded after a minimal delay between a spontaneous or orthodromically driven spike and the stimulus, the collision time. A delay any less than this will cause the orthodromic and antidromic spikes to cancel out. The minimum delay should be equal to the antidromic latency of the cell plus the refractory period. In the experiment described in Section 4.3 the collision time was often equal to or less than the antidromic latency. This would not be expected according to the above. Explanations for this observation have been suggested (200). These reasons are based on changes in the electrical properties of the axons but the reason in this case is due to the method of triggering used - the stimulator was triggered by the falling phase of the spontaneous spike, but the antidromic latency was measured to the first appearance of the IS spike (see Figure 34).

Cells that did not collide were excluded from the results in this Chapter. However, in some cases collision with a spontaneous spike was difficult to demonstrate due to an early strong orthodromic excitation. If any orthodromically driven spikes occurred after the stimulus, but before the antidromic response, it collided with the antidromic pulse so it was not observed. However, as the shape of the antidromic and orthodromic spikes usually differed it was possible to estimate the collision time if the test was repeated often enough.

A very high stimulus strength has been reported to be necessary for the antidromic activation of the SNC DA cells. Values of 100-250 μA, 50-2000 μA and 40-3000 μA with pulse widths of 0.05-0.5 ms have been quoted (87,89,136,250). These values compare well with those used in the experiments described earlier (Section 4.3). These vary from 35-3000 μA with pulse widths from 0.2-0.5 ms, with occasionally longer pulses of 1 ms.

The antidromic thresholds were the same in all the groups of animals. It might have been expected that the threshold would be lower in the lesioned animals as an inhibitory input had been removed. That the input had been removed was suggested by the increase in spontaneous firing rate seen in the lesioned animals. This was not reflected in the antidromic threshold. It is worth noting, however, that the thresholds were of the same order in the NMA lesioned animals as in the control animals. This implies that the lesion has not affected the axons in the nucleus and supports the specific action of the neurotoxin on cell bodies and not axons of passage.

Other studies have reported that the full SD spike is frequently not generated by stimulation of the striatum and only the IS component appears (87,89,250). Guyenet and Aghajanian (87) comment that the few cells giving 100% SD responses also have thresholds for activation lower than most. It is not clear why this should be so. It might be thought that invasion of the soma leading to the full spike might be easier if there was less inhibitory input, but the antidromic threshold depends on the ease of activation of the terminal and not the cell body so they would not be expected to be related. However, if there is a low threshold for antidromic activation, less of the inhibitory striato-nigral fibres will be activated and so the cell body will be less inhibited.

The relation between the proportion of SD invasion and antidromic threshold could be due to the coincidental existence of the reciprocal pathways between the nuclei.

The relation between proportion of full SD spikes and low threshold was not seen in the cells described here, however it was seen that few cells exhibited the full SD spikes all the time. The usual response was a combination of the two components.

In most studies, antidromic driving has been used primarily for the purpose of identifying the cells as SNC cells, so the split into IS and SD components is not important. In the experiments in this study, the main purpose of antidromic driving, as well as for identification, was to cause the release of dendritic DA in the SN. If only the IS of the cell is driven, it is unlikely that much DA will be released from the dendrites. However, as the desired effects are likely to be caused by a lateral inhibition in the SN as well as, or instead of, auto-inhibition, it is likely that sufficient DA cells will be induced to release DA on striatal stimulation. However, failure of the full SD spike could lead to a dilution of any effect of antidromically released DA and may explain the lack of effects described later in the discussion. Stimulation of the MFB does result in the release of DA in the SN, therefore it is possible to get the dendrites to release DA by this method (350,351). Problems and variation arise when the effect on specific areas are studied. In this case no relation was seen between the proportion of full SD spikes and the pattern in the longer PSTHs (Table 17) so it is possibly not important in view of the possible complications in the PSTHs from other sources (see later).

Later effects of striatal stimulation

The effects of striatal stimulation on SN cell firing have been widely reported, but fewer studies have been done on identified DA cells.

Complicated response patterns to striatal stimulation have been reported in SNC DA cells (87,153,250,413). The effects can be seen in PSTHs constructed during the 1 s period after striatal stimulation. Series of excitations and inhibitions can be seen lasting as long as 1 s. The most common response is an inhibition lasting several hundred ms, but facilitations of firing do occur as the first effect of stimulation. What appear to be rebound excitations also occur as well as distinct late excitations and inhibitions. Occasionally, oscillatory sequences of responses occur, returning to normal anything up to 1 s after the stimulus. All these responses to striatal stimulation are also seen in this study.

In two of the studies mentioned above, not all the cells are driven antidromically from the striatum: in one, 68 out of 116 are (87) and in the other, 14 out of 65 are (413). In the third, all are antidromically activated, but only 12 cells are used to constructed PSTHs 1 s long (250) and in the fourth no cells are antidromically activated (153).

Collingridge and Davies (87) explain the complex sequences as being caused by inhibitory GABA and excitatory SP inputs from the striatum. Even very late effects could be explained by the striato-nigral fibres terminating on very distal dendrites, and they suggested that several hundred ms might be needed for the effects of activation of these synapses to be seen in the soma. Dray *et al* (153) suggest that the inhibition could be caused by the GABA component of the striato-nigral pathway, but do not suggest what the excitatory transmitter might be.

Guyenet and Aghajanian (250) describe a long lasting suppression of firing after the appearance of an antidromic spike, and use this as further support in differentiating SNC and SNR cells. The SNR cells show a much shorter inhibition after antidromic driving. Guyenet and Aghajanian do not attempt to explain this observation.

Nakamura *et al* (413) observe excitations and inhibitions in non-antidromically driven cells but do not try to explain these. The assumption is that the inhibition is due to the GABA-ergic input from the striatum as the inhibition in non-antidromically driven cells is reduced after KA lesions of the striatum. They explain the inhibition seen in the antidromically driven cells as being due to dendritically released DA. The evidence in support of this is that this inhibition is not shortened by striatal lesions (16 out of 97 cells from lesioned animals were antidromically driven) but that it is shortened by intravenous haloperidol and iontophoretic trifluoperazine. Nakamura *et al* (413) use the suppression of firing after the antidromic spike reported by Guyenet and Aghajanian (250) to support their observations on this "antidromic inhibition", although Guyenet and Aghajanian themselves do not suggest this as an explanation.

The results presented in this study do not agree with the existence of a prolonged inhibition after every antidromic invasion. Antidromic activation is followed by a number of types of response: inhibition (short and long); facilitation (generally short) or a delayed effect, either facilitatory or inhibitory. The numbers in this study are much higher than those in these two studies (180 antidromically activated control cells as opposed to 12 (250) or 14 (413). As a large number of antidromic spikes were followed by the type of inhibition described by these two groups it may only have been because of low numbers that this was

the only effect seen. Also, in these two studies, a range of stimulation intensities was not tried, so the variety of responses might be expected to be less.

The lack of change in the "antidromic inhibition" seen by Nakamura *et al* (413) after KA lesions of the striatum is interesting. However, in this study, no change was seen in any of the effects on lesioning the striatum (see Section 4.3). Complicated excitations and inhibitions still appear after the lesion at the same stimulus strengths as without a lesion. That these other effects still exist after the lesion does agree with Nakamura *et al* (413) who report that all effects seen without a lesion are seen with one, except that the initial inhibition is shorter. These authors chose not to discuss the origin of these effects, and concentrated only on the effect seen after antidromic activation (16 out of 97 lesioned cells and 14 out of 65 control cells).

There are several explanations of why the complex response patterns are still there after striato-nigral lesions. Firstly, there exists the possibility that the lesion is not large enough and some striato-nigral fibres escape damage and the effects are caused by substance P and GABA as suggested by Collingridge and Davies (87). Secondly, the current spread might be sufficient to spread to other areas, espcially if the electrode is near the edge of the striatum. It could also activate other SN afferents as they pass through the striatum. Thirdly, it is possible that the effects are indeed all mediated by antidromically released DA from the SNC dendrites.

The possibility that the lesion is not large enough is not likely to be the whole explanation. Both the results presented here and those of Nakamura *et al* (413) come from animals with extensive lesions as shown by histology. Also in the NMA and KA lesioned animals used in this study

extensive damage is suggested by biochemistry. All animals also have marked asymmetry in the response to apomorphine indicating a significant loss of the striato-nigral pathway. In the NMA lesioned animals, the stimulating electrode was placed in the centre of the lesion and the complicated patterns were seen even when the lowest currents were used. In these cases it is unlikely that the effects seen in the PSTHs are due to spread beyond the lesion.

If the stimulating electrode was near the edge of the striatum, current spread could activate other areas projecting to the SN. The most likely areas for current to spread to are the globus pallidus and the nucleus accumbens. Both of these areas are known to project to the SN (see Section 1.2). The input from the accumbens is thought to be GABA-ergic and inhibitory on the SN cells. It is not clear which portion of the SN it goes to, but as the SNC dendrites course through the SNR, this does not matter. SNC cells could be affected by an input to either part of the SN. The globus pallidus also has an inhibitory GABA-ergic input to the SN and it has been suggested that there is an excitatory component as well (see Section 1.2). Excitations could also result from antidromic activation from the globus pallidus of subthalamic cells. The subthalamic nucleus sends collaterals to both the globus pallidus and the SN, and the projection is supposed to be excitatory (137), and to go to the SNR. Again the input could affect the dendrites of the SNC cells in this area. An action on the distal dendrites has already been used to explain long latency effects in the SNC cells.

Stimuls spread to other brain areas was a problem in these experiments as the currents necessary for antidromic activation were so high. From the control experiment on stimulus spread, it was seen that current spread at the highest strengths used may have been as much as 6 mm,

although it was considerably less at the lower, more usual currents. This experiment can, at best, only give a very rough estimate of the stimulus spread, as it is impossible to tell at which point in the striatum the fibre is being activated. It could be at the terminal or further back along the axon. As the fibres pass through a large part of the striatum before synapsing with their target cells a large proportion of the axon is available for activation. This means that the values obtained for the current spread may, in fact, be misleading.

The more posterior the stimulating electrode was, the greater the problem of the current spread. The crus cerebri lesioned animals had more posterior placements of the stimulating electrode, and so stimulus spread may have accounted for some effects in these cases. In these animals the position of the stimulating electrode is not necessarily related to the lesion position, whereas in the NMA lesioned animals, the electrode was in the centre of the lesion and current would therefore be less likely to spread beyond the area of the lesion. As complex effects were still seen in cells from these animals, it seems that the effects are less likely to be caused by stimulus spread than would appear from the crus lesioned animals.

Activation of fibres passing through the striatum in the internal capsule might possibly lead to excitation in the SNC cells. The pathway for the proposed cortico-nigral pathway is not clear, but it is likely to pass through the striatum with other corticofugal fibres. If this is the case, stimulation of the striatum could lead to effects in the DA cells. These effects are likely to be excitatory since the proposed transmitter for the cortico-nigral pathway is glutamate (see Section 1.3). Excitation could also result from activation of the cortico-substhalamic pathway, by a polysynaptic mechanism. The subthalamic nucleus has been reported to be excitatory in the SN (137).

The final possibility is that the effects are indeed caused by antidromic release of DA from the dendrites. That none of the effects of striatal stimulation is altered by a striato-nigral lesion would suggest that this could be the case. It has been reported that the initial inhibition after an antidromic spike was not reduced by a striato-nigral lesion, however when the initial inhibition did not follow an antidromic spike, it was reduced (413). This supports the suggestion that the inhibition after the antidromic spike is mediated by dendritically released DA. The lesion studies described in Section 4.3 did not show this effect. However, the fact that antidromic and effect thresholds are equally correlated in lesioned and control animals does support the suggestion of an inhibition by dendritically released DA. If the two thresholds were not correlated in control animals but were in lesioned ones, it would be a clear indication that the effect seen after that lesion was due to antidromically released DA. Rather than explaining the fact that the two thresholds are correlated in both lesioned and control animals by saying that all the effects are mediated by dendritic DA, it seems more likely that the high degree of reciprocal topography in the two systems linking the SN and the striatum is reflected in the correlation in the control animals. In the lesioned group it may be because the effects are partly mediated by dendritic DA release.

The fact that there is no difference in the effect thresholds in the lesioned and control animals would also point to effects being mediated by the dendritic release of DA. However, it seems extremely unlikely that the early, large, initial excitations are due to the dendritic release of DA. These are more likely to be caused by the mechanisms described above. However, later excitatory effects might be a rebound excitation after the inhibitory effect of dendritically released DA.

It seems most likely that the effects of striatal stimulation observed after lesions of the striato-nigral pathway are due to the effects of dendritically released DA and to the activation of cortico-fugal fibres in the internal capsule as they pass through the striatum, either by a mono- or polysynaptic pathway.

Drugs on the effect of striatal stimulation

Very few studies have looked at the effects of drugs on PSTHs of SNC cell activity after striatal stimulation. Most have studied the effects on spontaneous activity. Dray *et al* (153) and Collingridge and Davies (87) have looked at the effects of bicuculline and strychnine on PSTHs similar to those used in this study. Dray *et al* (153) do not make it clear whether they are looking at SNC or SNR cells, although they do separate the cells and say there is less inhibition in the SNC ones than the SNR ones. Division into cell type is only done on position in the SN. Nakamura *et al* (413) look at the effect of intravenous haloperidol and iontophoretic trifluoperazine on the inhibition seen immediately after an antidromic spike.

Collingridge and Davies (87) and Dray *et al* (153) show that the amount of initial inhibition is reduced by bicuculline. Collingridge and Davies also report that the amount of excitation in the PSTHs is increased and some excitations appear that were not there before the drug. The increase in excitation reported by these workers on the application of iontophoretic bicuculline is also seen in this study on the administration of intravenous picrotoxin. In the results described in Section 4.3 the effect of picrotoxin in causing an increase in excitations was so great that any effect on the inhibitions was masked and, if anything, the inhibitions were increased. The cells seemed to fire so fast when stimulated from the striatum after the drug that they briefly enter a

state of depolarisation block (see later in Discussion) apparently leading to a paradoxical enhancement of the inhibition. The greater effect seen in this study as compared to these other workers is possibly due to the fact that the picrotoxin is administered intravenously in this case. This means that the drug will get to more of the SN than if it were applied iontophoretically and so will lead to a greater reduction of inhibitory GABA input.

Nakamura *et al* (413) have shown that intravenous haloperidol and iontophoretic trifluoperazine reduce an inhibition seen after the antidromic invasion of the cell. This effect is also seen in these experiments using intravenous haloperidol. The effect is small and not very consistant. The haloperidol is most likely to be causing this effect by acting in SN as the cells are being stimulated after the point at which haloperidol could act in the striatum. This is evidence that the effects that are reduced by haloperidol, are mediated by dendritically released DA. The fact that the effect of haloperidol is small may be because of the fact that the full SD spike is not always generated and that only part of the observed inhibition is due to dendritic DA. A much greater effect of haloperidol may be seen if enough information could be gathered from cells responding with 100% full spikes. However, as the effect is liable to be the result from lateral as well as auto-inhibition this may not, in fact, help as it is impossible to tell whether or not neighbouring cells are responding with a full spike or not. Haloperidol given after picrotoxin might help as the picrotoxin would remove any inhibitory input to the cells and so a greater degree of SD invasion would occur. However, it seems that systemic picrotoxin caused such a degree of early excitation that spontaneous collision with the orthodromic excitation generally prevents antidromic invasion of the cell body after the drug. A reduced

effect of haloperidol after picrotoxin could be explained by the fact that no DA is being released dendritically due to spontaneous collision from the early excitation resulting from striatal stimulation after picrotoxin. As no DA is released, haloperidol will not be able to block its effects.

Analysis of the post stimulus histograms

Other studies using PSTHs very rarely attempt to analyse them, and the extent of effects are generally estimated by eye. On this basis it is difficult to be consistent in deciding what is and is not an excitation or an inhibition. A recent paper by Dorrscheidt (149) discusses this problem and describes several models that could be used for the statistical analysis of this type of PSTH. He shows that the assumption that the data are Poissonian results in the widest confidence limits for a statistical test. This means that statistical tests based on this distribution will give fewest false positive results. In Section 4.3 "control" PSTHs were taken and used to generate a mean and calculate limits above and below which a test bin would be considered to be significantly different from control. These control PSTHs were constructed as the others, but with the stimulation unit switched off.

A Poisson process is one in which the occurrence of one event does not affect the time of occurrence of the next. If the distribution is Poissonian a histogram built up by triggering the stimulator off a spontaneous AP (a post-pulse histogram, PPH) should be completely flat. This is not in fact the case and a slight "trough" is seen immediately after the triggering spike. This effect is seen in the autocorrelation histograms (ACHs) described by Wilson *et al* (628,629). In fact the trough seen in these is longer than the one seen in this study, but numbers here are not high. Also the ACH is constructed by superimposing

the time period after each spike on top of that of the previous one. In this study the time after each spike is superimposed on that of the first spike after the previous time interval is over. This might lead to a difference in the resulting histogram. In the results described in Section 4.3, after the initial short trough, the PPHs are flat implying a random firing pattern. Wilson *et al* (628) only see this in slower firing cells and in faster firing cells oscillatory patterns are seen. Again this may be due to the difference in construction of the histograms. However, the PPHs resemble the PSTHs in their construction and so provide a better control than ACHs. That the PPH is flat even in relatively "bursty" cells means that the effects seen in the PSTHs are not merely linked to the time locked firing of the DA cells. This in itself does not argue against the possibility of the effects of striatal stimulation being caused by dendritically released DA as the firing is random when no stimulus is applied, but large areas of the SN will be induced to fire synchronously on the striatal stimulation. This will also synchronise the effects of the released DA.

Wilson *et al* (628) showed that the initial trough in the ACh was reduced by haloperidol. They suggest that this could be simply due to the increase in firing rate. If the cell rate increases, the interval between spikes obviously decreases and so will the initial trough of an ACH. However, as the trough sometimes reduced in length even when the cell did not actually increase its firing rate was taken to show that the haloperidol was blocking the effect of dendritically released DA. Haloperidol was added to too few cells in which PPHs were obtained to say whether this study supports this view or not. However, the results from interval histograms would suggest that the pattern of firing can be changed while leaving the overall rate very much the same. This may be

related to the phenomenon of depolarisation block discussed later in this Section.

Effect of drugs on the spontaneous activity of DA cells

In view of the accumulating evidence against the FLH (see Section 4.1), it was surprising that GABA antagonists blocked the effects of amphetamine on the spontaneous firing of these cells (57,58), and speeded the DA cells up on their own (87,619). What was even more confusing was that GABA agonists had been reported to cause excitations of DA cells as well as inhibitions (374,616,619). That picrotoxin increases the firing rate of DA cells and blocks the inhibitory action of amphetamine has been taken as evidence for the FLH (57) and attempts have been made to explain the paradoxical effects of GABA agonists by proposing a GABA interneurone in the striatum (234 and Section 4.1).

The experimental results described in Section 4.3 confirm the finding that picrotoxin does speed up the DA cells and that it does block the effects of amphetamine and so would support a role for the GABA-ergic striato-nigral pathway in the feedback control of the activity of SNC DA cells.

Studies of the effects of drugs on the spontaneous activity of DA cells after lesions of the striato-nigral pathway are to some extent conflicting. Striatal KA lesions have been reported to reduce the effect of haloperidol on DA cell turnover (347) whereas apomorphine has been reported still to be effective after the lesion (24). Electrolytic lesions in the striato-nigral pathway have also been reported not to affect the effect of amphetamine on cell firing rate (206). On the other hand, Bunney and Aghajanian (58) have reported that it needs five times the dose of amphetamine to produce inhibition of cell firing after a KA lesion

of the striatum and that it is not possible to produce complete inhibition of firing in the DA cells with non-fatal doses of amphetamine after the lesion. In the same paper (58) the authors also report that picrotoxin reversed the inhibition of firing caused by amphetamine in control animals but had no effect in the lesioned animals. The reason given by these authors for this effect was that the feedback control of the DA cells is mediated partly by the striato-nigral pathway and partly by DA released from the dendrites in the SN. The experiments described in this thesis do in principal agree with this conclusion.

First of all there is the observation, already mentioned that picrotoxin speeds up the DA cells and blocks the action of amphetamine. This suggests that GABA-ergic processes do have a role in controlling the rate of the DA cells. However, this could be an intra-nigral process rather than a striato-nigral one. That haloperidol and picrotoxin are additive, on the firing rate of the DA cells regardless of which one is administered first suggests that there is both a DA-dependent mechanism and a GABA-dependent mechanism for controlling DA cell firing rate, and that the two are independent. This is supported by the fact that apomorphine is still effective in reducing the cell firing rate after a dose of picrotoxin large enough to increase the firing rate maximally. The large increase in rate suggests that the GABA input is effectively blocked and so apomorphine must be acting on DA receptors that do not exert their effect via GABA cells, most probably in the SN. This is also supported by the fact that haloperidol can still affect the firing rate after picrotoxin by as big an amount as it did without it. Haloperidol need not act via GABA receptors.

There appears to be a difference in the actions of apomorphine and amphetamine. As amphetamine releases DA from terminals and

dendrites and apomorphine acts on DA receptors, it might be expected that the two drugs would have the same effect on cell firing. However, the effect of amphetamine is blocked by picrotoxin, and the effect of apomorphine is not, or at least not to such an extent (Section 4.3). There are reports that suggest that the release process is different in terminals and dendrites, but as amphetamine does release DA from nerve ending particles prepared from the SN, and K^+ will not (269), it does not explain the difference observed. It may be that DA agonists have a different affinity for the different types of receptor: post synaptic cells in the striatum are less sensitive to iontophoretic DA and intravenous apomorphine than the DA cells in the SN are (553). However, as amphetamine releases DA this would still not explain a difference in the action of DA and apomorphine. It may be that DA and apomorphine do not have the same affinities for the different DA receptor types and that the distribution of the two types is sufficiently different in the two areas to result in a difference in action: apomorphine acting in the SN and/or striatum and the DA released by the amphetamine acting primarily in the striatum. Alternately, it may be a matter of the amount and location of DA released by the amphetamine. It is difficult to decide on a dose of apomorphine that is equivalent to the amount of DA released by the amphetamine. Also, release of DA will occur only at release sites, whereas the apomorphine may be able to act at possible extrasynaptic sites as well. The experiments comparing the effect of apomorphine before and after picrotoxin need to be done as has been for amphetamine.

Depolarisation block

The possibility that DA cells are capable of entering a state of "depolarisation block" has been discussed by Bunney and Grace (60,61). These experiments investigate the difference between the effect of acute

and chronic haloperidol on the firing of DA cells. Acute administration leads to an increase in firing rate, but in chronically treated animals there is a lack of spontaneous activity in the DA cells. Firing could be induced in these cells by the application of the normally inhibitory agents DA and GABA. The silent cells were also unresponsive to glutamate applied iontophoretically, a procedure normally causing excitation. Picrotoxin also had no effect in the cells from chronically treated animals. KA lesions of the caudate nucleus before the chronic administration of the haloperidol prevented the loss of spontaneous firing in these cells. The effect was explained by the overactivation of excitatory striato-nigral input to the cells caused by DA blockade in the striatum and leading to state of tonic depolarisation block in the SNC DA cells. Any inhibitory input to these cells will reduce the depolarisation and so allow the cells to resume firing. Picrotoxin and glutamate will have no effect as they tend to increase the depolarisation.

The experiments described in Section 4.3 also suggest that picrotoxin itself can cause the cells to enter depolarisation block as it can speed the cells up to the point where continuous depolarisation occurs. As the rate increases the cells begin bursting, and decrease in size with the cell disappearing into the noise at the end of the bursts. The firing can to some extent be restored by the application of apomorphine, an inhibitory agent.

The picrotoxin experiments do not help clarify whether chronic haloperidol acts in the striatum, as Bunney and Grace's results with KA lesions would suggest, or in the SN via autoreceptors.

The shape changes in the IHs and the reduction of the initial trough in the ACHs of Wilson *et al* (628) with no increase in firing rate, could be due to some extent of depolarisation block occurring, the

reduction in rate by the depolarisation offsetting the increase due to the blockade of inhibitory receptors. The problem of depolarisation block should always be considered in studying the effects of drugs on the firing rate of SNC DA cells as it is possible to change the pattern of firing without altering the rate. IHs or ACHs should always be constructed as well as noting the firing rate. An apparent decrease in rate might well be due to increased depolarisation. The conclusion by Wilson *et al* (628) that the reduction in initial inhibition after firing of DA cells by haloperidol, even when rate is unaffected, is evidence for dendritic DA release may not be valid, although it is the most likely explanation. The possible involvement of depolarisation block ought to be considered and excluded, possibly by observing action potential shape.

Conclusions and relation to the mechanism of control of dopamine cell activity

Lesions of the striato-nigral pathway cause very few changes to the effects of striatal stimulation on DA cell firing. There does, however, appear to be an increase in the firing rate of DA cells in the lesioned animals and possibly a reduction in the antidromic latency when the cells are driven from the striatum. These effects may be due to the decreased inhibitory input to the striatum after the striato-nigral lesion.

The lack of effect of the lesions on PSTHs taken over the 1 s period after the stimulus, was taken to mean that a significant proportion of the effects seen in normal animals is not due to activation of the striato-nigral pathway, but to other causes. The other possible causes include stimulus spread to other areas (minor except at the highest stimulation strengths), activation of other afferent fibres, mono- or polysynaptically, in the internal capsule as it passes through the striatum

and finally to the effects of antidromically released DA from the dendrites in the SN. The final possibility is supported by the effects of haloperidol on the PSTHs.

Because of the lack of a clear effect of antidromic driving on the DA cells it was not possible to say much about an intra-nigral mechanism of feedback control of DA cell activity from the stimulation studies. All that can be said is that because haloperidol causes a slight decrease in the inhibition, the inhibition is possibly mediated to some extent by dendritically released DA.

The action of drugs on spontaneous activity was more helpful in further elucidating the mechanism of the control of the DA cell firing rate. As picrotoxin blocked the effects of amphetamine on the cell firing rate, it is evident that GABA-ergic mechanisms are involved in controlling the firing rate of these cells to some extent. The fact that the action of apomorphine is not blocked by picrotoxin implies that there could also be an independent DA mechanism. This is further supported by the fact that haloperidol and picrotoxin cause additive effects regardless of which one is added first. This suggests that the action of DA agents on DA cell firing need not be mediated by GABA-ergic processes, but that GABA-ergic processes can affect the DA system in their own right.

The GABA-ergic mechanism is likely to involve a GABA-ergic interneurone in order to explain the paradoxical effects of GABA agonists on the DA cell firing and as such it is not possible to say whether the picrotoxin sensitive component of the action of DA takes place in the striatum or in the SN. From lesion work it seems likely that it could be in the striatum. The crucial experiment would be to repeat the combined haloperidol and picrotoxin experiment in striato-nigral

lesioned animals. If the two drugs were still additive, it would then strongly suggest that the DA-GABA interaction took place in the SN. However, if they were not, it would imply the striatum was involved.

The GABA independent mechanism of control of DA cells activity may be the result of a direct lateral or auto-inhibition in the SN with released DA acting directly to inhibit the DA cells. Alternately, inhibitory nigral interneurones or afferent terminals other than the striato-nigral ones could be involved.

There is evidence for a role of glycine in controlling the activity of the DA cells. Glycine has been shown to reduce the firing rate of DA cells (188), and it can be released from SN slices by K^+ and this is a Ca^{++} dependent process (329,330,331). However, it seems that this process is affected by GABA rather than by DA (330,331) and so this may not be involved in a DA inhibition of the DA cells in the SN. The glycine interneurones may be involved in the GABA-dependent mechanism. It has also been shown that dendritically released DA can release 5-HT from afferent terminals in the SN (273). Also 5-HT is known to inhibit the firing of DA cells when activated by glutamate (7) so this mechanism might also affect the turnover of DA and DA cell activity.

CHAPTER V

General Discussion

In this thesis the functioning of the nigro-striatal pathway has been investigated from two points of view. Firstly, an attempt was made to further elucidate the role of DA in the nigro-striatal pathway. The results presented suggest that DA in the nigro-striatal pathway is involved in the expression of motor behaviour. There may also be another component to the role of DA here. This may be involved with the ability to generalise knowledge already acquired and to apply it in new situations, or it may be an involvement with learning. There also appears to be a role for DA in motivational behaviour, possibly in the initiation of movement. Although this may be striatal in origin, it is more likely to result from mesolimbic DA. The interaction of the mesolimbic DA system and the extrapyramidal system is discussed by Mogenson *et al* (403). Here the relations between the VTA and its projection to the nucleus accumbens is discussed in light view of its projection to the globus pallidus. It is suggested in this review that the drives that result in the initiation of movement originate in the limbic or cortical areas and the information is passed via the nucleus accumbens to the globus pallidus. From there the behaviour of the animals can be affected, the nature of the response being determined by the striatum.

The second approach for studying the functioning of the nigro-striatal system was to study the firing of the individual DA cells and to look at how this is controlled. It appears that the DA cells are under the control of a local intranigral feedback mechanism, as well as a striato-nigral feedback loop. From the introduction there would also appear to be an intra-striatal mechanism affecting DA release in the striatum.

The reasons for the existence of two mechanisms controlling DA cell firing rate is unclear. It is possible that the striato-nigral pathway,

which also mediates the output of the striatum to the executing systems of the animal feeds back onto the DA cells as a means of monitoring whether the signal that had been processed in the striatum had been passed or not. If the striato-nigral input to the SNC was a monitoring system it would be a means of ensuring that the action was terminated once the need for it was over. An intra-nigral mechanism might be a feedback mechanism in the more literal sense, that is it tries to offset any change to the system, however caused. It might also be a means of lateral inhibition, sharpening the signal to aid the integration in the striatum. The intra-striatal mechanism for the feedback may also be involved in lateral inhibition for this purpose. It may simply be a means of preventing an excess of DA occurring in the striatum.

Insight into both aspects of the functioning of the nigro-striatal DA system will be of help in understanding the various diseases related to this pathway (150,151). The most studied of these is Parkinson's Disease. If the mechanisms controlling the turnover of DA were understood better, it might be possible to treat the disease more effectively. DA has also been implicated in aetiology of schizophrenia (571) as DA antagonists greatly improve the symptoms of this disorder. However, patients treated with DA antagonists frequently develop side effects, that resemble the symptoms of Parkinson's Disease. A better understanding of the control mechanisms might lead to a better treatment for the disease. Also an understanding of the function of DA in the behaviour of an animal is important in deciding how to treat these diseases. One worrying experiment shows that in a passive avoidance test, rats will learn the task under the influence of a neuroleptic, but do not show that they have learned until the effect of the drug has worn off (185). This means that the aversive stimuli must have been felt by

the animals and must have had enough effect on them for the animals learn to escape. However, the learned response could not be shown while the animals were under the influence of the drug. This may mean that psychotic patients treated with these drugs are still feeling the effects of their abnormality, but cannot express their reaction to it. If the role of DA were better understood, the effect that neuroleptics have on these patients might also be better understood, and the cause of the disease rather than the symptoms could be treated.

Conclusions

Evidence has been presented in this thesis to support both a motor effect and another effect of DA in the nigro-striatal pathway. It is possible, however, that the other effect is mediated by the mesolimbic DA system as the 6-OHDA lesions used in the experiments also affect this. The second component could be involved in learning or in the generalisation of behaviour. More likely it is involved with the motivation for or initiation of movements. Evidence is also presented to support the existence of two mechanisms of control for the DA cells in the SN. One of these involves the striato-nigral pathway and the other involves the local release of DA in the SN. The effects of striatal stimulation on the SNC DA cells has also been investigated and it seems that all the effects that have been attributed to this pathway may not necessarily be mediated by this pathway, but by other means. The evidence for this comes from the fact that lesions of the pathway do not change the pattern of response to striatal stimulation.

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APPENDICES

APPENDIX A: Raw Data from Paw Pressing Experiments

In this Appendix, all the raw data is given for the results presented in Chapter III. The mean and standard errors (s.e.) given in Tables B to J are those used to plot the graphs in Figures 15 to 23. Table A gives the information used to calculate the correlations between lever and paw preference.

The following tables show the total combined number of presses in four sessions except where the number is specifically stated. The relevance of the data is discussed in Section 3.3 in relation to the correlation between lever and paw preference.

TABLE A(i): Control animals.

Left lever:			Right lever:			Left lever:			Right lever:		
left paw	right paw	other presses	left paw	right paw	other presses	left paw	right paw	other presses	left paw	right paw	other presses
163	6	19	363	45	260	2	235	3	5	347	8
4	2	2	729	5	96	19	13	9	40	540	34
154	404	277	38	5	14	643	63	7	1	0	0
826	61	56	5	1	1	1	653	11	6	18	0
699	7	13	5	2	1	379	163	32	20	4	6
1	789	21	1	7	5	3	6	0	0	622	0
12	186	13	5	547	3	677	0	0	7	0	0
332	363	182	97	7	7	37	10	3	521	3	12
49	505	35	73	15	36	430	1	0	327	0	1
48	677	20	193	36	100	0	0	0	0	604	0
13	2	2	277	409	155	3	46	3	3	515	4
0	31	0	0	611	0	0	67	0	5	632	3
373	170	88	31	5	5	6	1	4	377	0	2
27	405	8	6	33	42	370	4	10	299	0	2
16	301	21	27	98	2	409	2	2	102	3	3
1	402	7	2	112	1	53	21	11	453	23	16
10	405	14	17	99	17	49	95	12	27	437	12
570	11	32	117	5	54	50	27	7	486	5	6
33	594	31	7	24	9	21	42	14	421	92	123
606	0	5	11	0	6	4	542	4	29	11	6
500	0	4	21	0	4	0	481	1	3	246	1
3	462	4	4	2	1	352	0	0	217	2	4
349	2	6	160	2	12	160	579	77	28	14	4
642	1	3	17	1	2	3	1	0	647	2	0
209	257	62	7	6	23	10	5	6	730	15	33
20	84	12	159	372	47	1	7	0	556	56	232
546	87	24	27	16	7	1	5	6	4	685	7
8	57	26	469	261	52	4	3	0	582	18	164
677	7	15	5	1	4	11	15	1	660	12	22
6	3	1	650	3	11	394	34	33	240	6	17
719	40	60	13	8	6	0	7	1	624	0	2
663	3	4	29	1	2	1	1	0	479	143	129
2	4	0	13	568	4	0	2	0	17	525	64
17	9	8	551	61	35	13	40	163	624	5	9
371	124	41	43	9	14	540	0	3	11	2	2
4	23	2	55	623	8	2	184	1	0	28	1
148	424	49	9	343	20	2	184	3	19	294	15
66	777	12	0	2	0	6	48	3	19	294	15
901	0	0	4	0	1	10	0	1	546	0	1
213	325	43	63	8	15	0	0	0	113	695	46
760	14	20	111	1	2	26	647	2	7	2	3
33	0	2	686	0	2	240	0	5	271	0	1
348	31	26	104	69	72	8	29	5	11	565	26
859	10	5	67	0	0	1	105	1	16	502	114
28	523	64	3	56	4	729	2	2	2	0	2
32	4	1	580	4	74	420	34	8	25	35	26
627	86	44	14	9	4	5	220	10	3	15	3
386	243	25	255	10	2	115	94	81	67	14	17
15	625	242	9	10	9	413	68	22	21	29	8
730	15	36	127	8	18	172	386	83	9	7	5
35	0	3	768	4	65	315	4	22	272	5	3
825	1	12	120	1	3	21	672	6	16	41	2
841	5	5	121	5	3	4	732	1	3	25	1
795	9	1	14	0	4	63	605	30	4	8	1
595	198	33	21	31	9	490	80	72	21	0	0
603	10	26	54	15	17	46	97	21	69	472	19
590	1	16	39	8	8	14	34	6	547	3	52
18	344	9	10	458	13	30	19	15	788	6	4
327	20	6	536	20	7	602	2	2	136	3	8
0	38	0	1	727	3	1	39	0	16	559	10
118	15	37	4	476	2	0	386	1	7	449	11
72	612	59	7	19	3	431	20	15	3	10	2
2	13	0	0	734	4	64	8	9	105	7	18
4	3	0	110	245	34	3	2	5	740	1	7
31	6	1	63	321	138	806	0	3	20	1	5
						707	0	2	3	1	1

TABLE A(ii): a) 6-OHDA lesioned animals

	Left lever:			Right lever:			
	left paw	right paw	other presses	left paw	right paw	other presses	
Control	603	10	26	44	16	17	
	490	1	16	39	8	8	
	18	344	9	9	458	13	
	0	38	0	1	727	3	
	48	7	23	0	625	2	
	72	612	59	7	19	3	
4 weeks after 6-OHDA injected contralaterally to the initially preferred paw	3	510	1	3	103	4	*
	0	556	1	0	24	0	*
	115	15	23	378	17	17	*
	12	0	2	544	23	17	*
	50	0	2	450	6	1	*
	183	2	7	335	3	6	*
8 weeks after 6-OHDA injected contralaterally to the initially preferred paw	0	1142	3	6	50	9	+
	0	1115	2	0	21	0	+
	62	0	16	1023	12	26	+
	5	0	0	1074	0	5	+
	26	0	1	941	0	4	+
	98	0	10	1083	4	5	+

*3 sessions; +5 sessions

TABLE A(ii): b) 6-OHDA lesions, no pretraining

Left lever:			Right lever:		
left paw	right paw	other presses	left paw	right paw	other presses
71	0	0	443	1	2
115	0	2	344	0	3
40	2	1	526	0	0
34	3	6	829	1	4
35	2	5	559	1	1
37	0	0	504	0	0
7	2	0	907	0	0

TABLE A(iii): 1 or 2 weeks after an injection of NMA into the striatum contralateral to the initially preferred paw

Left lever:			Right lever:		
left paw	right paw	other presses	left paw	right paw	other presses
3	657	5	4	4	1
552	4	5	0	8	0
586	12	3	61	1	0
21	672	6	16	50	3
413	38	28	150	0	3
900	8	9	5	0	0
1	928	5	32	34	13
1	2	0	15	894	16
1	11	1	20	808	4
1	881	21	1	20	6

TABLE A(iv): During local anaesthesia of initially preferred paw. All numbers here are for 3 sessions

Left lever:			Right lever:		
left paw	right paw	other presses	left paw	right paw	other presses
1	5	3	8	579	8
472	2	0	75	0	1
606	0	1	22	0	1
18	87	20	5	72	621
567	0	0	159	0	0
2	0	0	531	9	11
1	481	8	0	28	5
0	5	664	0	5	17
1	2	1	21	522	5
4	663	43	0	11	3
16	565	84	0	1	1
0	3	23	14	37	481
0	8	2	7	549	13
8	784	3	2	22	0

TABLE A(v): 8 weeks after local anaesthesia of initially preferred paw (no residual effect). Here the totals are for 5 sessions except * which is 4 sessions.

Left lever:			Right lever:			
left paw	right paw	other presses	left paw	right paw	other presses	
93	10	9	1119	181	23	
197	1140	0	2	5	8	
1020	40	120	0	6	26	*
9	14	2	256	951	9	
1240	97	18	7	24	3	
1240	4	1	1	1	0	
0	8	0	0	1300	0	
15	3	1	956	366	118	
646	623	15	9	29	0	

* 4 sessions

TABLE B: Pressing behaviour before and after an injection of 6-OHDA into the MFB contralateral to the initially preferred paw.

	Pre-operation				Operation + 1 week			Operation + 4 weeks			Operation + 8 weeks				
Session:	1	2	3	4	1	2	3	1	2	3	1	2	3	4	5
Total presses															
Rat 1	135	193	179	199	89	102	123	191	208	227	222	255	263	265	214
2	117	157	206	182	75	114	116	179	198	206	213	220	226	241	239
3	168	220	218	245	76	51	118	194	193	166	234	218	238	238	211
4	169	181	210	210	72	87	115	194	196	203	237	248	235	235	230
5	150	169	173	207	64	63	58	161	168	180	171	215	185	207	202
6	188	188	187	212	87	82	100	168	184	182	203	255	248	259	232
mean	154.5	184.7	195.5	209.2	76.2	83.2	105.0	181.2	191.2	194.0	213.3	235.2	232.5	238.3	221.3
s.e.	10.5	8.9	7.5	8.4	3.4	9.6	1.8	5.8	5.6	9.0	9.9	7.9	10,8	8.7	5.9
Preferred presses															
Rat 1	125	156	176	190	18	7	4	5	1	0	3	2	1	0	0
2	98	150	205	176	25	5	0	0	0	0	0	0	0	0	0
3	159	187	214	242	13	1	11	18	12	2	2	2	3	2	3
4	169	179	209	209	56	20	15	9	2	0	0	0	0	0	0
5	87	161	171	207	9	5	6	1	9	1	0	0	0	0	0
6	150	148	151	181	20	38	4	7	0	1	2	1	1	0	4
mean	131.3	163.5	187.7	200.8	23.5	12.7	6.7	6.7	3.2	0.7	1.2	0.8	0.8	0.3	1.2
s.e.	13.7	6.5	10.3	9.9	6.9	5.7	2.2	2.7	1.9	0.3	0.5	0.4	0.5	0.3	0.7
Other presses															
Rat 1	10	47	3	9	71	95	119	186	207	227	219	253	262	265	214
2	19	7	1	6	50	109	116	179	198	206	213	220	226	241	239
3	9	33	4	3	63	50	107	176	182	164	232	216	235	236	208
4	0	2	1	1	16	67	100	185	194	203	237	248	235	235	230
5	53	8	2	0	55	58	52	160	164	179	171	215	185	207	202
6	38	40	36	31	61	44	96	161	184	181	201	254	247	259	228
mean	21.5	22.8	7.8	8.3	52.7	70.5	98.3	174.5	188.0	193.3	212.2	234.3	231.7	240.5	220.2
s.e.	8.2	7.9	5.7	4.7	7.9	10.6	9.9	4.7	6.2	9.3	9.8	7.8	10.6	8.4	5.9

TABLE C: Pressing behaviour before and after a break of 1 week in the training sessions

	Initial				+ 1 week			
Session:	1	2	3	4	1	2	3	4
Total presses								
Rat 1	103	189	215	259	242	246	253	297
2	194	189	310	285	291	282	297	327
3	123	165	230	217	224	228	254	162
4	190	258	358	264	313	269	216	242
5	155	148	257	196	173	276	251	233
6	163	209	196	173	241	291	235	244
7	195	200	270	227	249	287	275	241
8	229	240	155	232	262	329	271	294
9	146	193	230	216	265	279	198	174
10	144	239	251	274	290	295	253	214
11	129	192	179	223	265	216	220	206
12	113	159	184	184	224	169	215	209
mean	157.00	198.42	236.25	229.17	253.25	272.25	244.80	236.92
s.e.	11.08	9.73	16.70	10.26	10.71	8.83	8.35	14.28
Preferred presses								
Rat 1	102	177	213	241	222	218	237	280
2	86	107	135	91	179	276	292	233
3	53	107	187	196	196	205	237	156
4	34	113	312	254	209	257	210	231
5	12	20	102	98	112	217	182	213
6	161	209	158	243	217	291	235	244
7	181	181	126	128	204	253	197	187
8	226	219	128	206	253	300	255	294
9	145	193	229	216	265	279	198	174
10	128	224	245	274	285	295	253	214
11	127	192	177	223	264	203	220	206
12	60	138	113	145	135	98	111	46
mean	109.58	156.67	177.08	184.58	219.42	241.00	218.92	200.00
s.e.	18.40	17.54	18.20	17.79	17.04	16.57	13.12	17.36

Other presses/

TABLE C (cont):

	Initially				+ 1 week			
Session:	1	2	3	4	1	2	3	4
Other presses								
Rat 1	1	12	2	18	20	28	16	17
2	108	82	175	194	120	6	5	4
3	71	58	43	21	28	23	17	6
4	156	145	49	20	28	12	6	11
5	143	154	155	98	61	59	69	20
6	2	0	38	30	24	0	0	0
7	14	19	94	99	45	34	78	57
8	4	21	27	26	9	29	16	0
9	1	0	1	0	0	0	0	0
11	16	15	7	0	5	0	0	0
12	2	0	2	0	1	13	0	0
13	53	21	71	39	89	71	104	163
mean	47.58	43.92	55.33	45.42	35.83	22.92	25.92	14.87
s.e.	16.88	15.88	17.02	16.64	10.77	6.67	10.45	6.42

TABLE D: Pressing behaviour before, during and after injection of local anaesthesia into the initially preferred paw

						Local anaesthesia				8 weeks after local anaesthesia				
	Session:	1	2	3	4	1	2	3	4	1	2	3	4	5
Total presses	Rat 1	119	132	132	152	207	193	204	181					
	2	82	192	161	136	170	210	176	197					
	3	41	144	161	182	187	204	237	248					
	4	156	232	178	223	201	249	249	334					
	5	157	192	152	197	215	271	240	286					
	6	153	199	186	164	101	177	195	-	258	284	278	297	324
	7	140	149	206	211	138	188	197	-	281	293	291	261	227
	8	117	185	202	204	206	248	258	-	245	270	316	294	332
	9	152	182	204	215	224	191	137	-	162	234	258	285	299
	10	225	230	231	268	248	245	231	-	301	310	293	278	273
	11	218	210	178	226	228	231	217	-	221	275	225	252	277
	12	168	126	169	229	180	213	174	-	232	281	250	246	300
	13	170	166	190	254	173	204	201	-	225	280	274	279	294
	14	179	184	118	240	270	293	274	-	315	281	258	313	290
	mean	148.4	173.1	176.3	207.2	209.1	222.6	212.1		248.9	278.7	271.4	278.3	290.7
	s.e.	13.0	12.8	8.1	10.2	10.5	9.2	9.6		15.5	6.8	9.0	7.3	10.2
Preferred presses	Rat 1	106	126	82	116	2	4	5	6					
	2	52	181	144	127	0	2	0	0					
	3	32	136	158	182	0	0	0	0					
	4	111	180	175	221	23	0	0	0					
	5	147	173	122	176	0	0	0	0					
	6	46	123	140	148	9	0	0	-	3	25	86	25	52
	7	94	115	151	210	0	0	1	-	2	0	21	65	111
	8	106	172	200	205	0	0	0	-	0	90	308	291	328
	9	144	181	203	213	20	2	0	-	38	121	65	34	7
	10	190	208	225	254	4	0	0	-	221	290	281	262	259
	11	218	209	179	226	2	10	2	-	220	272	223	252	277
	12	166	126	169	229	8	12	18	-	231	281	250	246	300
	13	145	139	182	242	7	0	0	-	170	202	228	231	240
	14	119	111	85	165	10	0	0	-	132	105	199	161	157
	mean	118.3	155.2	158.2	193.7	6.1	2.1	1.9		113.0	154.0	184.5	174.1	192.9
	s.e.	17.6	9.1	11.2	11.4	2.0	1.1	1.3		34.0	37.0	34.0	35.3	38.3
Other presses	Rat 1	13	6	51	36	205	186	199	175					
	2	12	17	3	9	170	208	176	197					
	3	9	8	3	0	187	204	257	248					
	4	45	52	3	2	178	249	249	334					
	5	10	19	30	21	275	276	240	286					
	6	107	76	46	16	172	177	195	-	255	259	192	272	272
	7	46	34	55	1	138	188	196	-	279	293	270	196	116
	8	11	13	2	1	206	248	238	-	245	180	8	2	4
	9	8	1	1	2	204	189	137	-	124	113	193	251	292
	10	35	22	6	14	224	245	231	-	80	20	12	16	14
	11	0	1	1	0	226	221	215	-	1	3	2	0	0
	12	0	0	0	0	172	201	156	-	1	0	0	0	0
	13	25	27	8	12	166	204	205	-	55	78	48	48	54
	14	60	73	33	75	260	293	274	-	183	176	59	152	133
	mean	27.2	24.9	17.3	13.5	194.5	220.3	212.0		135.9	124.7	87.1	104.1	98.3
	s.e.	7.9	6.8	5.6	5.5	8.4	9.4	10.5		36.3	36.5	34.3	37.9	38.5

TABLE E: Pressing behaviour before and after injection of KA into the striatum contralateral to the initially preferred paw

Session:	1	2	3	4	1	2	3	4	5
Total presses									
Rat 1	185	162	128	157	199	151	125	147	251
	153	171	183	214	147	182	179	200	227
	202	219	204	229	173	214	212	198	164
	166	180	197	226	118	143	131	118	139
	149	146	135	172	150	167	153	167	237
mean	171.0	175.0	169.4	199.6	157.4	171.4	160.0	166.0	203.6
s.e.	9.97	12.22	15.59	14.74	13.58	12.59	16.09	15.57	21.97
Preferred presses									
Rat 1	173	139	105	115	128	100	58	30	97
2	153	167	182	213	94	26	5	1	0
3	122	135	145	155	44	41	30	23	3
4	134	135	154	163	59	73	43	30	60
5	129	139	122	169	11	0	3	0	2
mean	142.2	143.0	141.6	163.0	67.2	48.0	27.4	16.8	32.0
s.e.	9.26	6.07	13.27	15.66	20.22	17.56	11.06	6.78	19.87
Other presses									
Rat 1	12	23	23	42	71	51	67	116	152
2	0	4	1	1	53	156	187	199	227
3	80	84	59	74	139	173	182	174	101
4	34	45	43	63	59	70	88	88	79
5	20	7	13	0	139	166	151	167	235
mean	29.2	32.6	27.8	36.0	92.2	123.2	135.4	148.8	168.8
s.e.	13.85	14.77	10.40	15.38	19.32	25.91	24.56	20.30	23.49

TABLE F: Pressing behaviour before and after injection of NMA into the striatum contralateral to the initially preferred paw

(i) Immediate swappers

Session	1	2	3	4	1	2	3	4
Total presses								
Rat 1	147	205	207	203	198	216	212	219
2	150	217	239	229	210	236	223	261
3	114	157	156	197	133	211	207	208
4	109	187	184	163	145	155	171	159
5	171	163	173	194	286	242	240	217
6	138	187	195	168	170	149	153	183
7	127	152	183	195	110	127	154	167
8	82	145	156	180	179	152	178	162
9	116	165	186	177	230	229	242	257
10	196	219	224	223	254	256	259	257
mean	135.0	179.7	190.3	192.9	191.5	197.3	203.9	207.0
s.e.	10.4	8.6	8.5	6.9	17.5	14.8	12.0	12.0
Preferred presses								
Rat 1	134	205	207	201	16	3	2	1
2	145	213	239	229	1	1	0	0
3	92	148	155	192	24	1	2	10
4	107	182	184	163	37	0	1	0
5	141	148	135	189	6	2	0	0
6	133	172	179	165	1	1	7	4
7	57	97	99	140	4	0	0	0
8	55	109	142	160	6	0	1	0
9	96	129	168	168	10	4	2	0
10	172	202	221	223	29	4	0	0
mean	113.2	160.2	156.3	183.0	14.2	1.6	1.5	1.5
s.e.	12.2	12.9	20.4	9.1	4.1	0.5	0.7	1.0
Other presses								
1	13	0	0	2	182	213	210	218
2	5	4	0	0	209	235	223	261
3	22	9	1	5	109	210	205	198
4	2	1	0	0	108	155	170	159
5	30	15	28	5	280	240	240	217
6	5	15	16	3	169	148	146	179
7	70	55	84	55	106	127	154	167
8	27	35	14	20	173	152	178	162
9	10	6	6	3	196	224	239	237
10	15	8	2	0	210	250	259	257
mean	18.9	14.8	15.1	9.3	174.2	195.4	202.4	205.5
s.e.	6.5	5.5	8.2	5.4	17.5	14.3	12.3	12.2

TABLE F: Pressing behaviour before and after injection of NMA into the striatum contralateral to the initially preferred paw

(ii) Gradual swappers

Session:	1	2	3	4	1	2	3	4
Total presses								
Rat 1	125	141	154	139	193	186	182	203
2	93	49	38	36	123	135	165	173
3	145	155	166	178	178	194	188	232
4	161	176	223	206	207	171	201	167
5	150	179	200	195	167	195	186	189
mean	134.8	140.0	156.2	150.8	173.6	176.2	184.4	192.8
s.e.	12.0	23.8	32.0	30.9	14.3	11.2	5.8	11.6
Preferred presses								
Rat 1	115	136	153	135	178	161	108	50
2	80	42	36	35	90	28	4	3
3	123	142	162	167	159	111	68	54
4	157	175	219	206	191	137	99	7
5	95	148	169	157	108	7	33	15
mean	114.0	128.0	147.8	140.0	145.2	88.8	62.4	25.8
s.e.	13.1	22.7	30.2	28.7	19.7	30.3	19.7	10.9
Other presses								
1	10	5	1	4	15	25	74	153
2	7	7	2	1	33	107	161	170
3	22	13	4	11	19	83	120	178
4	4	0	3	0	12	25	99	158
5	36	21	23	35	57	184	139	174
mean	9.6	9.2	6.6	10.2	26.0	84.8	118.6	166.6
s.e.	3.3	3.6	4.1	6.5	7.2	29.6	15.2	4.8

TABLE G: Pressing behaviour before and after electrolytic lesions of the striato-nigral pathway in the crus cerebri contralateral to the initially preferred paw

Session:	1	2	3	1	2	3
Total presses						
Rat 1	97	106	54	138	149	132
2	90	136	132	169	112	120
3	50	127	106	146	151	157
4	63	80	74	85	96	112
5	47	61	91	97	122	124
mean	69.4	102.0	91.4	127.0	126.0	129.0
s.e.	10.2	14.0	13.3	15.6	10.6	7.7
Preferred presses						
Rat 1	67	76	34	7	7	2
2	50	90	123	9	28	41
3	21	36	50	15	25	18
4	53	76	68	4	5	5
5	23	55	82	32	36	35
mean	42.8	66.6	71.4	13.4	20.2	20.2
s.e.	9.0	9.5	15.2	5.0	6.1	7.8
Other presses						
Rat 1	30	30	20	131	142	130
2	40	46	9	160	84	79
3	29	91	56	131	126	129
4	10	4	6	81	91	107
5	24	6	9	65	86	89
mean	26.6	35.4	20.0	113.6	105.8	106.8
s.e.	4.9	15.9	9.3	17.6	11.8	10.3

TABLE H: Pressing behaviour before, during and after training the rats to press with the other paw

	Initially				During retraining					8 weeks after retraining			
Session:	1	2	3	4	1	2	3	4	5	1	2	3	4
Total presses													
Rat 1	135	200	232	232	172	192	226	231	219				
2	102	190	296	250	241	319	267	244	186				
3	186	192	275	241	239	209	280	284	214				
4	226	229	254	222	123	229	236	217	232				
5	146	172	149	261	241	220	240	217	228				
6	122	216	251	235	250	230	256	270	275				
7	242	286	256	297	86	158	210	223	146	168	249	200	225
8	291	282	297	327	296	297	228	268	236	173	247	235	252
9	224	228	254	160	150	153	188	174	161	269	218	229	207
10	313	269	216	242	218	206	233	229	241	220	301	249	272
11	173	276	251	233	291	239	255	257	230	195	253	228	242
12	148	161	161	172	214	128	184	224	205	208	239	330	247
13	130	164	194	185	286	249	223	206	203	241	275	260	276
mean	187.50	217.31	237.38	235.15	215.92	217.62	232.77	232.62	213.54	210.60	254.60	247.30	245.90
s.e.	18.50	11.64	12.83	12.80	18.21	14.97	7.83	8.61	9.52	13.70	10.10	15.50	9.20
Preferred presses													
Rat 1	81	70	190	185	118	36	27	18	1				
2	80	181	253	221	193	151	37	6	4				
3	148	106	115	70	98	12	8	11	(3)				
4	171	204	235	221	64	68	14	2	0				
5	132	168	143	261	*88	58	2	1	1				
6	97	213	251	235	151	27	80	6	4				
7	222	218	257	280	79	150	118	52	11	65	139	41	60
8	171	276	292	323	161	123	44	34	15	12	8	49	147
9	196	205	237	156	22	15	8	14	3	11	52	133	122
10	289	257	210	231	51	11	2	9	3	5	1	28	160
11	112	217	182	213	124	19	4	1	3	85	196	199	217
12	148	161	161	172	190	63	58	41	40	110	221	301	241
13	90	90	103	122	167	68	23	2	0	0	1	3	161
mean	149.00	182.00	200.69	206.92	115.85	61.62	32.69	15.15	6.77	41.10	88.30	107.90	151.90
s.e.	17.05	17.24	15.96	18.57	15.01	13.99	9.71	4.64	3.02	16.90	33.30	41.20	23.30
Other presses													
Rat 1	54	130	42	47	54	166	199	213	218				
2	22	9	43	29	48	168	230	240	240				
3	68	86	160	171	141	197	272	273	(211)				
4	55	12	19	1	59	161	222	215	232				
5	12	4	6	0	153	162	238	216	227				
6	25	3	0	0	99	203	176	143	266 (276)				
7	20	28	16	17	7	8	92	171	135	103	110	158	165
8	120	6	5	4	135	174	184	234	221	161	239	186	105
9	28	23	17	6	128	138	186	160	185	258	166	96	75
10	24	12	6	12	167	195	231	220	238	215	300	221	112
11	61	59	69	20	157	220	251	256	227	106	51	29	25
12	0	0	0	0	24	65	146	162	165	89	15	29	5
13	40	74	91	63	119	181	200	204	203	241	274	257	114
mean	33.00	34.31	36.46	28.46	99.30	153.77	201.62	208.23	212.92	167.60	165.00	139.40	85.90
s.e.	5.74	11.24	12.90	1.13	15.09	16.26	13.24	10.90	9.87	26.70	42.00	34.20	21.00

TABLE I: Pressing behaviour before and after an injection of KA in the striatum ipsilateral to the initially preferred paw

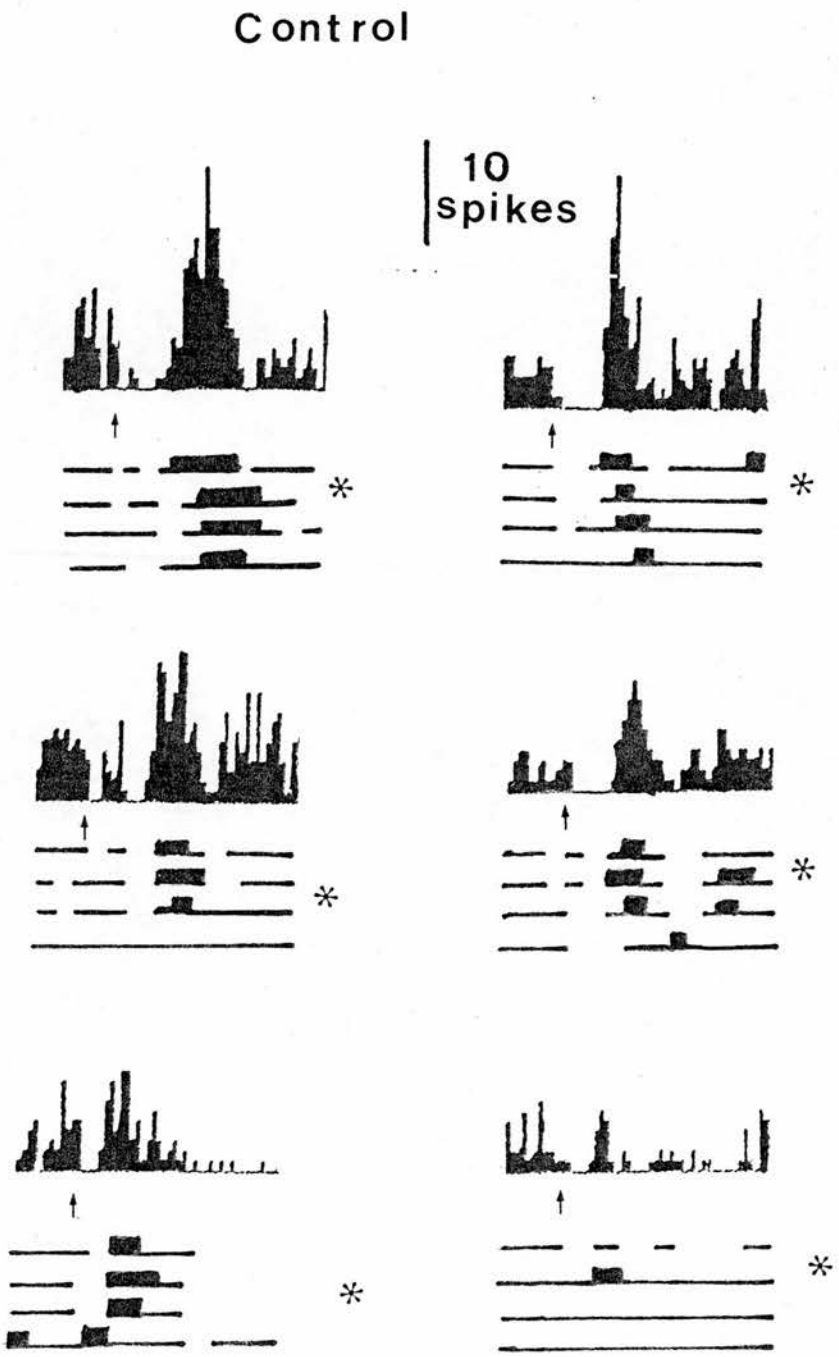
Session:	1	2	3	4	1	2	3	4	5
Total presses									
Rat 1	96	146	133	144	258	148	165	172	223
2	139	144	134	155	136	150	150	139	172
3	155	161	176	229	28	90	119	115	129
4	146	277	175	212	97	104	100	107	107
5	151	138	129	163	150	150	123	139	235
mean	137.4	173.2	149.4	100.6	132.2	128.4	129.9	134.4	173.2
s.e.	10.69	26.22	10.69	16.80	36.26	13.02	11.64	11.36	25.14
Preferred presses									
Rat 1	96	142	130	140	257	147	164	172	221
2	130	136	118	126	113	124	142	133	170
3	134	144	168	225	28	90	119	109	127
4	118	185	158	179	86	102	89	96	98
5	147	121	114	154	139	145	118	137	230
mean	125.0	145.6	137.6	164.8	124.6	121.6	126.4	129.4	169.2
s.e.	8.60	10.64	10.81	17.41	37.87	11.36	12.61	13.07	25.72
Other presses									
Rat 1	0	4	3	4	1	1	1	0	2
2	9	8	16	29	23	26	8	6	2
3	21	17	8	4	0	0	0	6	2
4	28	92	17	33	11	0	11	11	7
5	4	17	15	9	11	5	5	2	5
mean	12.4	27.6	11.8	15.8	9.2	6.4	5.0	5.0	3.6
s.e.	5.25	16.30	2.71	6.30	4.12	4.98	2.07	1.90	1.03

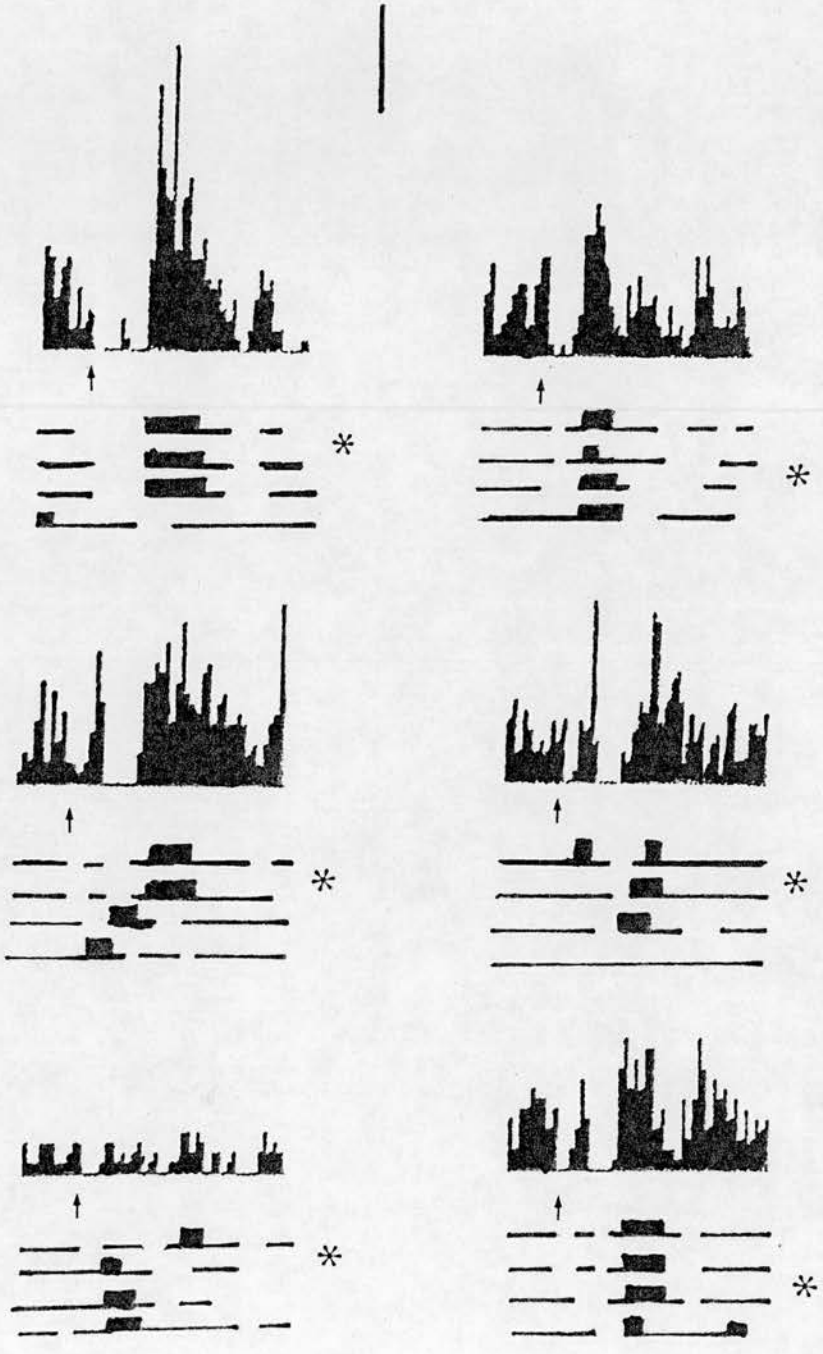
TABLE J: Pressing behaviour before and after an electrolytic lesion in the striato-nigral pathway in the crus cerebri ipsilateral to the initially preferred paw

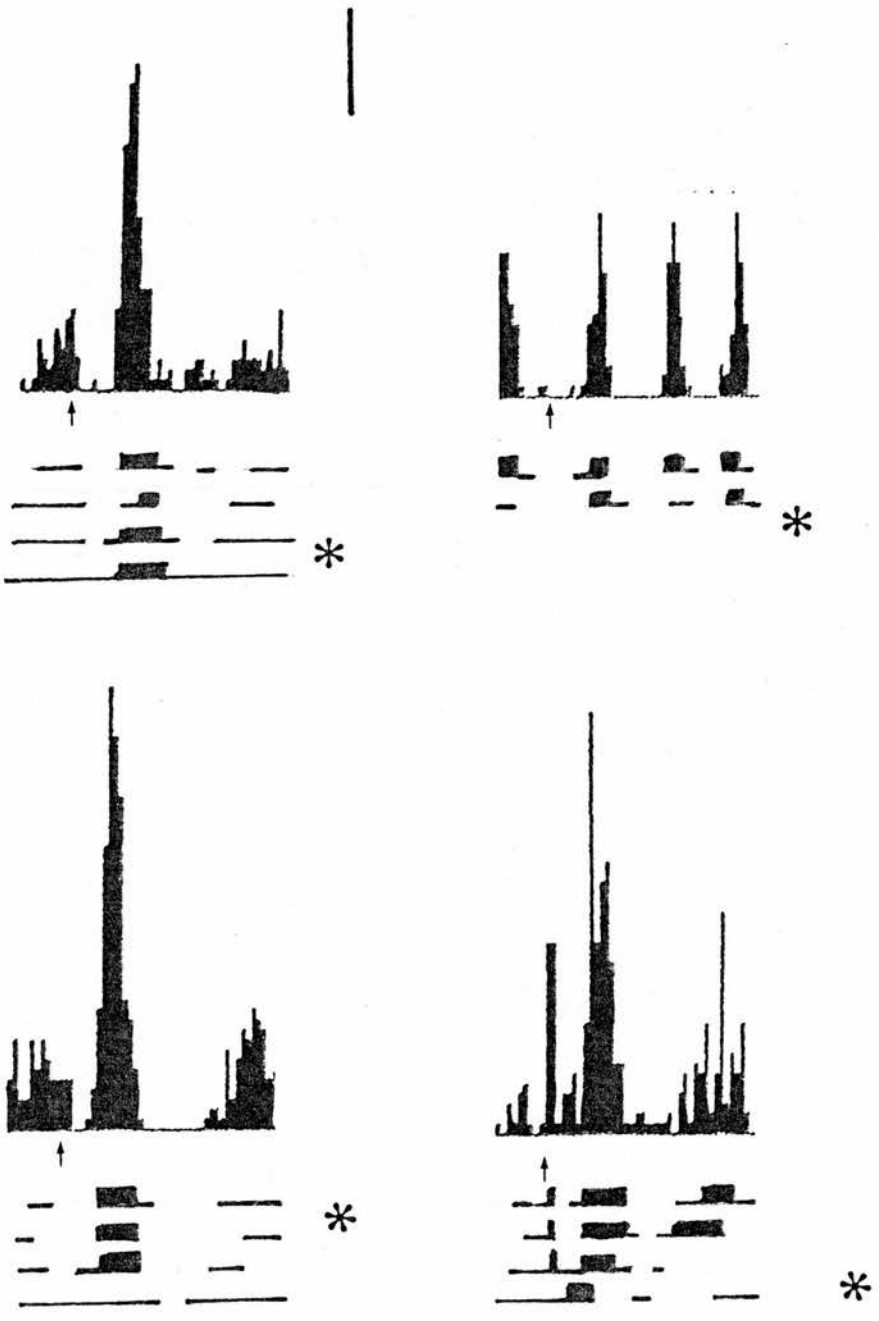
Session:	1	2	3	4	1	2	3	4
Total presses								
Rat 1	159	118	180	143	136	143	137	162
2	154	166	171	187	174	161	161	180
mean	156.5	142.0	175.5	165.0	155.0	152.0	149.0	171.0
s.e.	2.5	24.0	4.5	22.0	19.0	9.0	12.0	9.0
Preferred presses								
Rat 1	72	89	118	118	132	142	157	162
2	154	166	171	187	174	161	161	180
mean	113.0	127.5	144.5	152.5	153.0	151.5	149.0	171.0
s.e.	41.0	38.5	26.5	34.5	21.0	9.5	12.0	9.0
Other presses								
Rat 1	87	29	62	25	4	1	0	0
2	0	0	0	0	0	0	0	0
mean	43.5	14.5	31.0	12.5	2	0.5	0	0
s.e.	43.5	14.5	31.0	12.5	2	0.5	0	0

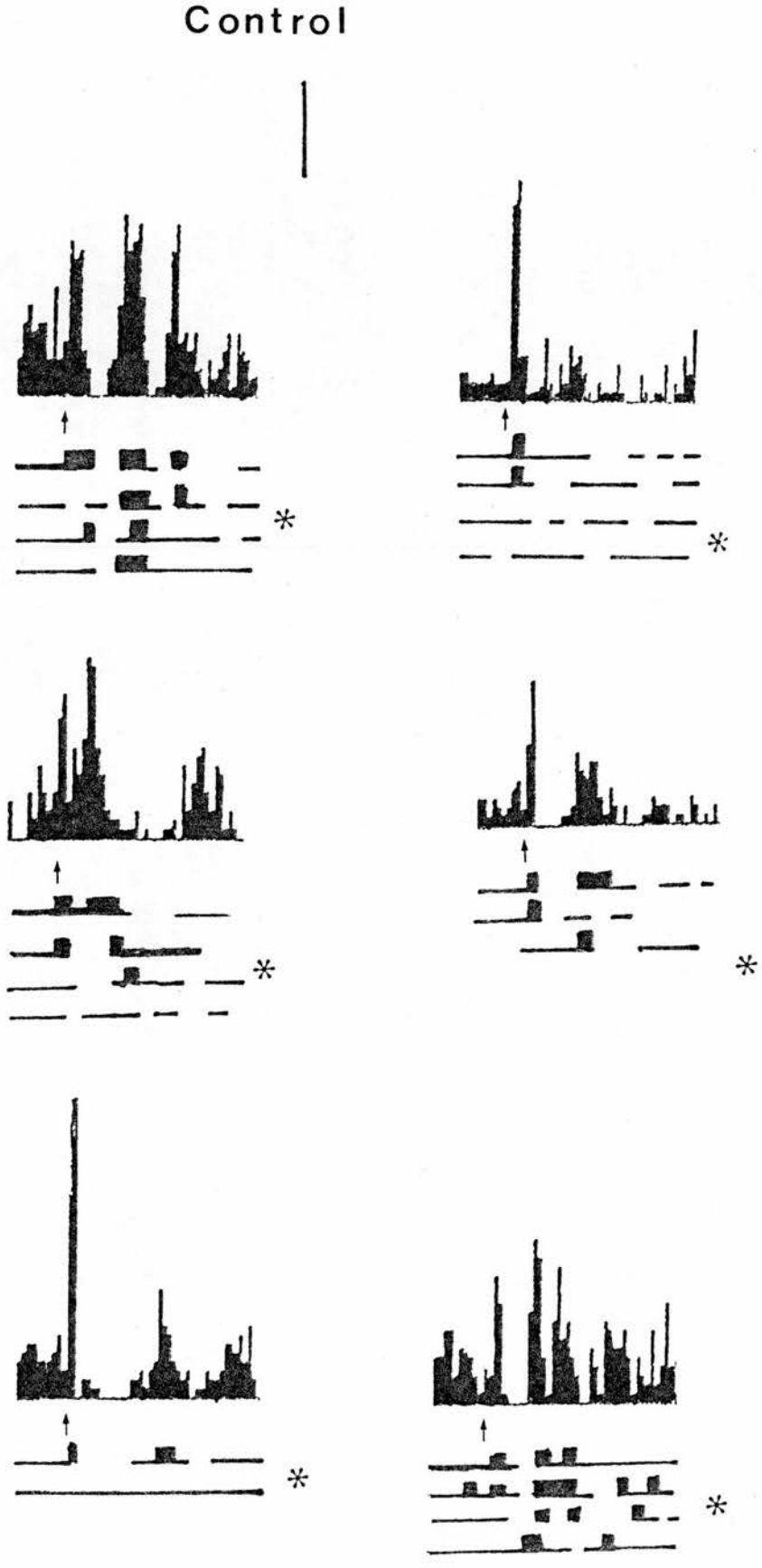
APPENDIX B: Examples of post-stimulus histograms resulting from striatal stimulation in control and lesioned animals

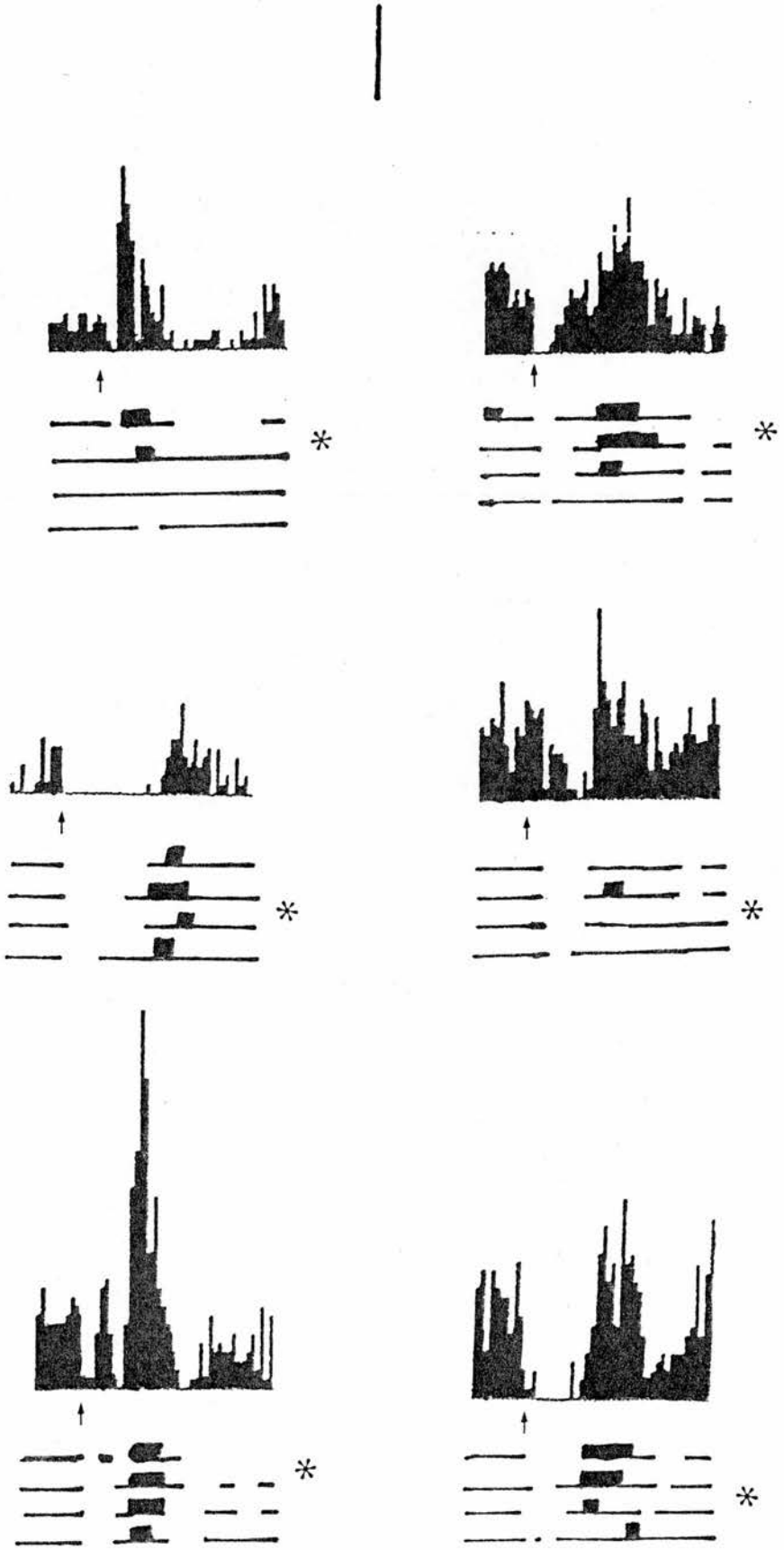
The PSTHs on the following pages are all 1 s long and the verticle scale bar is for 10 spikes in each case. They are grouped together, as far as possible into similar groups, and give a reflection of the variation in the whole population of cells. The differing thickness of lines underneath the graphs indicates how the pattern of response varies with stimulus strength. The top line is the highest strength (illustrated). A blank indicates an inhibition of firing, a thin line normal firing and a thick line an increase in firing rate. The arrow indicates the point of stimulation in each case and the asterisk marks the antidromic threshold. Artifacts and spikes due to the antidromic spike have been removed to allow clear visualisation of the effects.

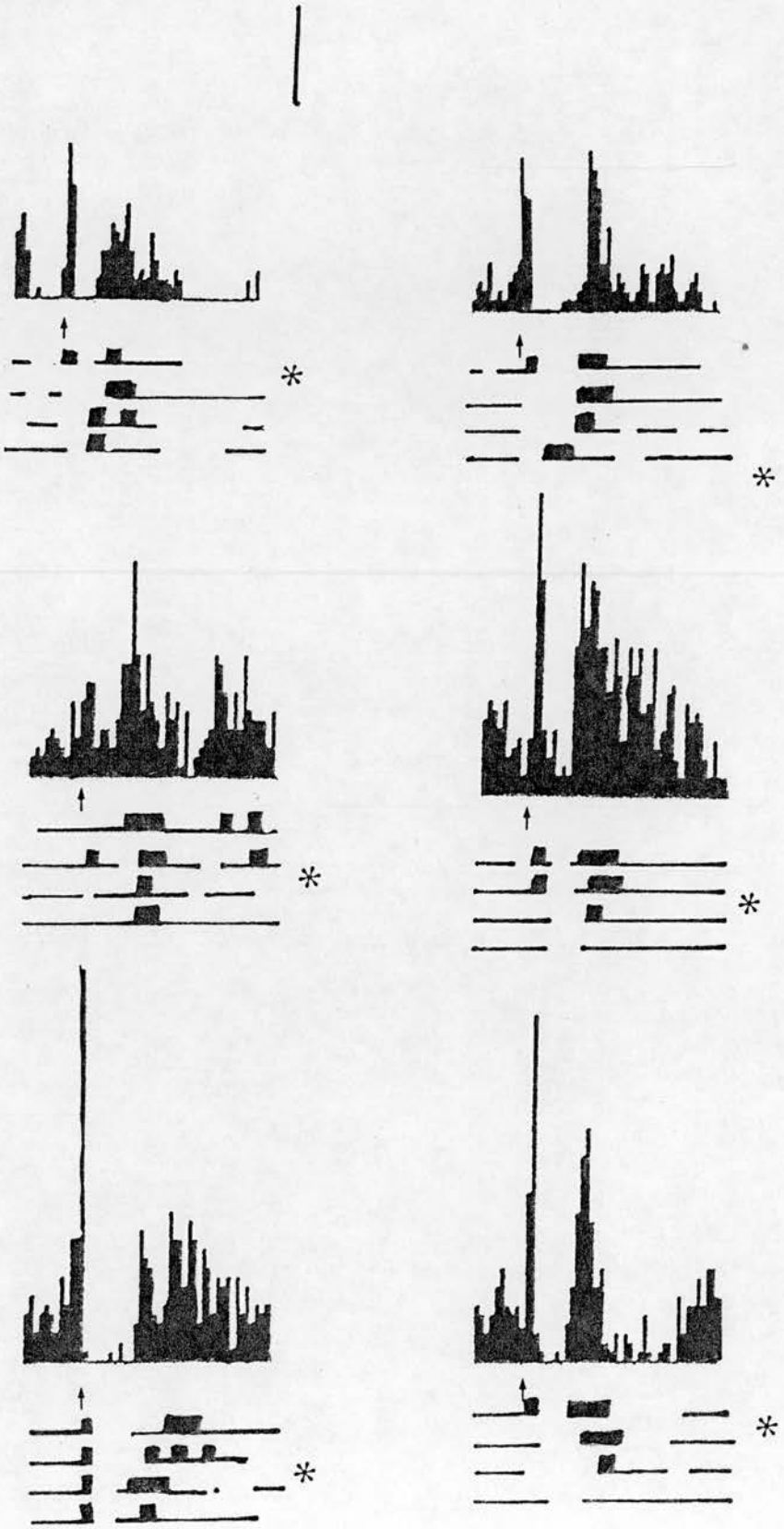

Control
10
spikes

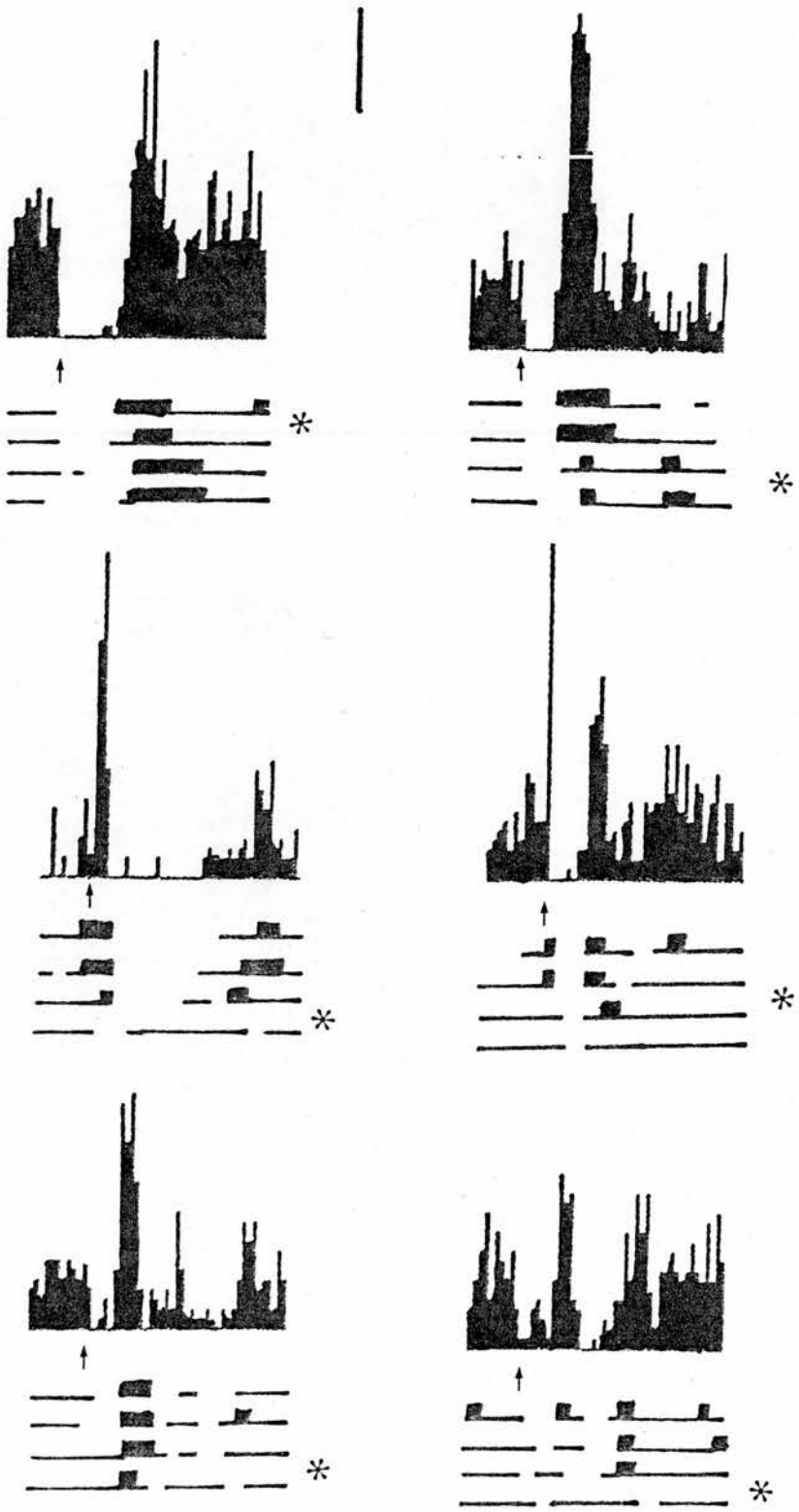

Control


Control
*
*
*
*


Control


Lesioned


Lesioned


Partially
Lesioned