NEUROPHARMACOLOGICAL STUDIES ON THE INTERACTIONS OF SOME PUTATIVE PEPTIDE TRANSMITTERS WITH THE NIGROSTRIATAL AND MESOLIMBIC DOPAMINE SYSTEMS

by

Robert Denham Pinnock B.Sc.

A thesis submitted in support of the Degree of Doctor of Philosophy of the University of Southampton.

September, 1980.

INDEX

CHAPTER TWO. METHODS

CHAPTER THREE. RESULTS

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

PHYSIOLOGY AND PHARMACOLOGY

Doctor of Phil osophy

NEUROPHARMACOLOGICAL STUDIES OF THE INTERACTION OF SOME PUTATIVE PEPTIDE TRANSMITTERS WITH THE NIGROSTRIATAL AND MESOLIMBIC DOPAMINE SYSTEMS.

by Robert Denham Pinnock

The experiments described in this thesis are an investigation of the interactions of some peptides with dopamine in areas of the rat brain known to be components of the ascending dopamine pathways. Most attention was paid to drug interactions at the cellular level using conventional iontophoretic techniques in three brain regions, the substantia nigra, the caudate nucleus and nucleus accumbens. Behavioural studies of the locomotor activity produced by some of these peptides after injection into the nucleus accumbens or ventral tegmentum of chronically cannulated rats were also carried out. By using a series of substance P fragments it was found that the 1-9 N-terminal of the molecule previously found inactive has similar iontophoretic effects in the substantia nigra and similar behavioural actions in the ventral tegmentum as substance P. Two hypothalamic releasing hormones, TRH and MIF and some analogues of them were found to be inactive after iontophoretic application into any of the three brain areas. These compounds also failed to potentiate hyperactivity produced by amphetamine. The peptide BW180C thought to be an opiate agonist produced inhibitions of cell activity in the nucleus accumbens. Behaviourally it caused a long term hyperactivity after injections into either the nucleus accumbens or ventral tegmentum. None of the effects could be reversed by classical antagonists such as nalaxone or putative peptide antagonists.

By using both atypical neuroleptics and semirigid dopamine analogues the study was extended to find if there was any evidence from iontophoretic studies to support a multiple dopamine receptor hypothesis. ADTN was found to produce a more powerful inhibition of firing on nigral compacta neurones than has been reported for other dopamine agonists. The atypical neuroleptic sulpiride blocked the effects of dopamine both on these neurones and on those in the caudate nuclues. Our results with this and another neuroleptic fluphenazine imply that both are acting at a common site. These results are discussed with regard to the multiple dopamine receptor theories.

ACKNOWLEDGEMENTS

I am very grateful to Dr. G.N. Woodruff for his supervision and suggestions over the past three years. I would also like to thank Dr. M.J. Turnbull for his interest in the peptide studies, Professor K.A. Munday in whose department the electrophysiology experiments were performed and ICI Ltd., for allowing the use of their laboratories for the behavioural studies.

I am also grateful to Dr. J.A. Poat for measurement of tritiated dopamine and noradenaline levels in the testing of release from micropipettes, N.N. Petter for measuring MIF levels in similar experiments, J.W. Grocott for the data from peripheral studies with substance P analogues and J. Shaw for the data from peripheral studies with opiate agonists and antagonists.

My thanks are extended to Mr. N. Dale for photography of figures, the electronics department for maintenance of equipment, to the staff of the animal house for consistently providing animals in good condition, and to Mrs. M.P. Reilly for her patient and very efficient typing of this thesis. I am also indebted to Dr. J.A. Poat for correcting the first draft.

Finally my thanks go to my parents for their encouragement and financial support and most of all to my wife without whose co-operation the project would have been incomplete.

ABBREVIATIONS

Ach	Acetylcholine
ADTN	2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene
C	Centigrade
CNS	Central Nervous System
cAMP	Cyclic adenosine 3',5'-monophosphate
cGMP	Cyclic guanosine 3',5'-monophosphate
DA	Dopamine
1-Dopa	1-3,4,-Dihydroxyphenylalanine
DLH	DL Homocysteic acid
Flu	Fluphenazine
Fig	Figure
g	gram
GABA	Gammaamino butyric acid
5-HT	5-hydroxy tryptamine
Hz	Hertz
I.P.	Intraperitoneal
I.V.	Intravenous
Kg	Kilogram
µg	Microgram
µl	Microlitre
µmol	Micromole
M	Molar
ml	Millilitre
mM	Millimolar
mg	Milligram
mV	Millivolt
µV	Microvolt
MΩ	Megohm
nA	Nanoamp
NA	Noradrenaline
nmol	Nanomole
3,4-diOHNom	3,4,-dihydroxynomifensine
nM	Nanomolar
NaCl	Sodium Chloride
6-OHDA	6-hydroxydopamine
S.C.	Subcutaneous
S.E.M.	Standard error of the mean

ABBREVIATIONS (contd.)

SNC	Substantia nigra compacta
SNCDcell	Substantia nigra compacta dopamine cell
SNR	Substantia nigra reticulata
μ	micron

CHAPTER 1. INTRODUCTION

1.1 SUBSTANCE P

1.1.1. Isolation and structure

Substance P is an undecapeptide with the following structure :

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

Although an impure form which contracts the isolated guinea pig ileum (GPI) was first extracted from the horse hypothalamus in 1931 by Gaddum & Von Euler, its amino acid sequence was only determined in 1971 by Chang et al. Since then its physical properties have been examined. Interactions between different substance P molecules in solution do not occur readily and and the peptide does not possess a secondary structure in aqueous or organic solvents until high (10^{-3}M) concentrations are reached (Mehlis et al 1975, Mehlis et al 1980).

1.1.2. Distribution of substance P in the brain

Substance P is unevenly distributed in the CNS. It was first extracted from the hypothalamus. Its distribution has been studied using bioassay, radioimmunoassay and immunohistochemistry. The highest concentrations of substance P are found in the substantia nigra (11 pmol/mg protein and the lowest concentration in the cerebellum (0.02 pmol/mg protein) (Brownstein et al, 1976). Substance P positive cell bodies, fibres and nerve terminals have been identified by immunohistochemistry (Cuello & Kanazawa 1978). The results from these techniques correlates with the distribution by bioassay (Lembeck & Zetler 1971). Recently attempts to map substance P containing nerve tracts have been made. In these studies specific knife cuts of the nerve tracts caused a loss of immunoreactivity or fluorescence in the area innervated and a build up of fluorescence on the cell body side of the knife cut. Using these techniques several pathways have been proposed (Fig. 1.1). A review of these pathways has been published (Emson, 1979). A binding study agreed with the distribution of substance P by these methods (Nakata et al, 1978).

1.1.3 Structure activity studies in peripheral preparations

Peptides which are hypotensive, contract intestinal preparations and have sialogogic activity are known as "tachykinins". Structure activity studies on tachykinins were begun before the purification of substance P in 1971. The tachykinin eledoisin was isolated in 1962 by Erspamer & Anasti and structure activity studies were performed on it and another tachykinin, physalaemin, using sialogogic activity in the rat as a bioassay (De Caro

(1966). When the structure of substance P was determined it was realized that all tachykinins are structural analogues of substance P. Relative potencies of tachykinins using parallel bioassay in eighteen different preparations permits an easy discrimination between eledoisin and the amphibian tachykinins and even a clear cut distinction between substance P and the other tachykinins. Substance P being the least active tachykinin in most preparations. This is illustrated in Table 1.1. However, it has been observed that variations in experimental design may be responsible for different activity ratios to those previously found (Szeli et al, 1977) (see Table 1.1). Variations in the relative activity of portions of the substance P molecule on identical preparations have been reported. For example, (Table 1.2) the C-terminal 4-11 octapeptide is less potent (Yanaihara, et al. 1977) or more potent than the parent molecule (Bury & Mashford, 1974, Bergmann, 1974, Oehme, et al. 1977, Chipkin, et al. 1979). There is evidence that the N-terminal of the molecule is important in determining the potency of fragments while the C-terminal hexapeptide is thought to be important for binding to the receptor (Oehme, et al. 1977).

From the peripheral studies mentioned and others it was deduced that the C-terminal hexapeptide was necessary to elicit activity comparable to substance P on peripheral preparations (see Table 1.2) and that generally speaking the tachykinins all possess the C-terminal sequence :- Phe-X-Gly-Leu-Met-NH_2, where X = Ile, Phe, Tyr or Val, and replacements of these amino acids leads either to a reduction or loss of activity (Mroz & Leeman, 1978). Finally on the basis of differences of activity in different assay systems Mroz & Leeman in their review (1978) conclude that "actual differences in receptors seem likely".

It is of interest that while removal of the methiomine residue causes a complete loss of activity (Yanaihara, et al. 1977), substance P sulfoxide, which is readily formed in dilute solutions, has 36% of the sialogogic and 40% of the hypotensive activity of substance P in the rat, as well as 80% and 40% of the immunoreactivity of substance P directed against two different antisera, (Floor & Leeman, 1980) which may affect both tissue estimates and structure activity studies using these methods.

1.1.4. Structure activity relationships in the CNS

The invitro hemisected baby rat spinal cord (Otsuka & Konishi, 1974) was

first used for systematic structure activity studies of substance P in the CNS. It was found that removal of the 1-5 N-terminal amino acids did not cause any serious loss of motoneurone depolarizing activity (Otsuka & Konishi, 1977). The potencies of the fragments being roughly in parallel with their hypotensive and spasmogenic actions (Table 1.2). Differences are apparent between these and earlier studies where the same authors found the pyrrolidone carboxylic acid 6-11 fragment to be twelve times less potent than substance P in the frog spinal cord (Otsuka, Konishi & Takahashi, 1975) and twelve to fifteen times more potent that substance P in the rat spinal cord. An invivo iontophoretic study in the cat spinal cord (Piercey & Einspahr, 1980) agrees with the invitro rat spinal cord studies. However, the iontophoretic study found the 1-10 N-terminal fragment had some activity although it is without effect on the GPI. In a substance P binding study, substance P displacement by the C-terminal fragments only partly correlated with that expected from both peripheral and central studies (illustrated in table 1.2). The 6-11 fragment was potent while the 5-11 and 4-11 fragments were unexpectedly weak. As would be expected from a different species, displacement of substance P by either eledoisin or physallaemin was very poor (Nakata, et al. 1978). Similarly in frog spinal cord preparations the amphibian peptides were more potent in depolarizing neurones than substance P (Otsuka, Konishi & Takahashi, 1975). However, iontophoretic experiments in the locus coeruleus found that physalaemin, ERP (an analogue of substance P 6-11) and substance P were equipotent in exciting neurones (Guyenet & Aghajanian, 1977). Differences between substance P and ERP are apparent, for example a study in the amygdala found desensitization after repeated application of substance P or ERP, but if ERP was applied after substance P no cross desensitization occurred (Ben Ari, et al. 1978). It must be stressed that this type of study is uncommon because of the technical problems with iontophoreses which make quantitative interpretations difficult. In addition there may be problems of peptides degrading inside electrodes (Gozlan, et al. 1977).

1.1.5. Metabolism of substance P

Axons and terminals do not contain ribosomes and hence have little capacity to synthesise proteins. Substance P is thought to be synthesised in the soma. The incorporation of (^{35}S)-methionine into substance P in isolated rat dorsal root ganglia is blocked by the protein synthesis inhibitor cycloheximide, suggesting a conventional ribosomal synthetic process

(Harmar, Schofield & Keen, 1980). The experiments in which substance P immunofluorescence accumulates on the soma side of knife cuts of substance P tracts (see 1.1.2.) suggest that the peptide is transported down the axons and concentrated in vesicular subcellular fractions. Evidence for this comes from radioimmunoassay and E.M. cytochemistry studies in rat brain (Cuello, et al 1977).

A calcium-dependent, potassium-stimulated, release of substance P like immunoreactivity has been shown to occur from hypothalamic slices and synaptosomes (Iversen, Jessell& Kanazawa, 1976, Schenker, Mroz & Leeman, 1976). Electrical stimulation of the dorsal root or high potassium also causes a calcium dependent release in the isolated rat spinal cord (Otsuka & Konishi, 1976). This can be abolished by opiates, an effect which is naloxone reversible and does not occur in nigral slices (Jessell& Iversen, 1977).

Once released rapid removal of the peptide occurs. This is believed to be by enzymatic degradation rather than reuptake, since using I^{125}-Tyr_8-Substance P Iversen, Jesselland Kanazawa (1976) were unable to demonstrate uptake of the molecule. There is now general agreement that degradation does occur although the exact nature of the enzymes responsible is controversial. Blumberg & Teichberg(1980) suggested a metallo endopeptidase involvement while Lee, Arregui & Iversen (1979) disagree.

1.1.6. Mechanism of action

When substance P is applied to neurones in the brain by iontophoresis the majority of reports found a slow onset exitatory effect which is of long duration (Davies & Dray, 1976, Guyenet & Aghajanian, 1977, Sastry, 1978 and Piercey & Einspahr, 1980). The exact ionic mechanism of the response is unclear. Otsuka & Konishi (1977) using the isolated spinal cord preparation found the reversal potential for the excitation to be near zero suggesting a sodium event. In cuneate neurones an increase in membrane resistance to potassium orchloride occurred (Krnjevic, 1977). This latter suggestion is supported by the dorsal root potential studies by Krivoy, et al. (1979) which indicate a modulatory role in synaptic transmission. However, using a similar intracellular recording technique to Krnjevic (1977), Zeiglegansberger & Tulloch (1979) could detect no changes in membrane conductance. Using the frog neuromuscular junction (NMJ) Steinacker (1976) found substance P had a potent inhibitory effect on synaptic

transmission which is calcium dependent, which is of interest since substance P has been shown to decrease calcium uptake in rat brain synaptosomes and mitochondria. The high concentration of substance P necessary to reduce transmission at the frog NMJ may reflect a different potency in a different species. In the GPI* substance P may also act by interfering with the transport of calcium ions (Szeli, et al. 1977). Neurochemical studies have found that while substance P stimulates adenylate cyclase in rat brain homogenates this ability does not correlate well with those areas which contain the highest concentration of peptide (Duffy, Wong & Powell, 1975). Studies on identified neurones in the locus coeruleus suggest that it has a separate site of action from cholinergics and opiates (Guyenet & Aghajanian, 1979) but give no clue to its cellular action. So while it is clear that substance P is not acting directly on another known transmitter receptor, it is unclear whether or not it has a common mechanism of action at the cellular level.

1.1.7. Possible role of substance P in the CNS

There is evidence that substance P plays an important role in the transmission of painful sensations. Thus spinal nociceptive units are consistently sensitive to substance P (Henry, 1976). A possible mechanism for the pain gate proposed by Melzack & Wall (1965) has been suggested by Jessel & Iversen (1977). Their experiments show that the release of substance P could be blocked by opiates in spinal cord slices but not in nigral slices. Taken in conjunction with the presence of enkephalin interneurones and substance P terminals in the superficial laminae of the dorsal horn (Hökfelt et al. 1977) Jessel & Iversen proposed that enkephalin interneurones prevent the release of substance P by acting presynaptically, (Fig. 1.2). This is supported by experiments in which enkephalin was shown to decrease action potential duration and substance P release from sensory neurones grown in culture (Mudge, Leeman & Fishbach, 1980). Furthermore, capsaicin (whose chronic administration renders animals insensitive to painful stimuli) is capable of producing a calcium dependent release of substance P in the spinal cord (Theriault, Otsuka & Jessel, 1979). However, other studies using iontophoretic techniques could find no effects of substance P at sites in the substantia gelatinosa where met-enkephalin reduces the transmission of nociceptive impulses (Duggan, et al. 1979).

In the brain the evidence for a function of substance P is again controversial. There appears to be a link between substance P and certain neurologi-

* guinea pig ileum

al and psychiatric disturbances. A reduction in substance P is found in the substantia nigra of post mortem Huntingtons Chorea victims (Kanazawa, et al. 1977). This is also seen in the substantia nigra of rats chronically treated with haloperidol (Hong, Yang & Costa, 1978). It is of interest that substance P degradation was inhibited by the angiotensin converting enzyme inhibitor SQ20881 (Lee, Arregui & Iversen, 1979). Furthermore a reduction in nigral angiotensin converting enzyme was found in the substantia nigra of early onset schizophrenics (Arregui et al. 1977) and a reduction in converting enzyme in both the striatum and substantia nigra of Huntington's Chorea victims and rats given intrastriatal kainic acid lesions (Arregui, Emson & Spokes, 1978).

Intraventricular injections of substance P stimulated the turnover of dopamine, noradrenaline and 5HT (Carlsson, et al. 1977) and increased locomotor activity (Rondeau, et al. 1978). This stimulation of locomotor activity was produced by the C-terminal hexapeptide (Kubicki, 1977). Consistent with a role in the substantia nigra, iontophoretic application of substance P causes typical slow onset long duration excitations (Davies & Dray, 1976). Unilateral nigral injection of a wide range of doses results in contralateral rotation (James & Starr, 1977, Olpe & Koella, 1977, Diamond, et al. 1979). Bilateral injections cause stereotypy which can be blocked by 6OHDA lesions of the nigro striatal tracts (Kelley & Iversen, 1979). These findings suggest that substance P activates the ascending dopamine systems. Consistent with this suggestion is the observation that infusion of substance P into the nigra causes an ipsilateral increase in ^{3}Hdopamine release in the caudate nucleus (Cheramy, et al. 1977, Cheramy, et al. 1978). Substance P increased both uptake and release of dopamine by nigral synaptosomes (Silbergeld & Walters, 1979). Bilateral substance P injections into the VTA increase locomotor activity, an effect which can be blocked by 6OHDA lesions, or infusions of neuroleptics into the nucleus accumbens where the A10 dopamine neurones terminate (Kelley, Stinus & Iversen, 1979).

Cells in the VTA which receive a substance P input from the habenula (Cuello, et al. 1978) are excited by both substance P, acetylcholine and electrical stimulation of the habenula (Sastry, 1978, Ogata, 1979). Consistent with the small diameter of substance P fibres the latency of excitation is some 25msec.

Two techniques were used in the studies with substance P. The first was

electrophysiological similar to the iontophoretic study of Davies & Dray (1976). The second was behavioural and relies on the locomotor response produced by Substance P (Kelley, Stinus & Iversen, 1979).

The use of a limited number of N and C-terminal fragments of substance P enables the structural requirements for substance P in the substantia nigra and VTA to be compared with those in the spinal cord and peripheral preparations. With some of the fragments it may be possible to show if the slow onset and long duration of action during substance P iontophoresis is due to a breakdown product of substance P. One of the greatest problems in substance P research is the lack of a suitable antagonist. Iontophoresis of some putative antagonists would show if they are active at the neuronal level and would be of use in confirming substance P as the transmitter in a striatonigral pathway.

Assay system	Physalaemin	Eledoisin	Substance P	Author(s)
Rabbit B.P.	100	25-30	120-250	
Guinea Pig Ileum	100	30-120	50-60	Erspamer, Erspamer and Linari, 1977.
Rat Salivation	100	30-40	20-25	
Dog Urinary Bladder	100	1000-5000	50	
Frog Spinal Cord	100	75	10	Otsuka, Konishi and Takahashi, 1975.
Substance P binding (IC50 3.17×10^{-9})	100	59	317×10^{4}	Nakata et al., 1978
Iontophoresis (locus coruleus)	100	100 (ERP)	100	Guyenet and Aghajanian, 1977.
GPI max response on longditudinal muscles	100	<SP	<Phys	Szeli, et al. 1977.
Drinking in Pigeons	-	10 x <SP	100	Evered, Fitzimons and DeCaro, 1977.

Table 1.1. Comparison of the effects of three different tachykinins on various test preparations to show the differences in potency. Data is modified from the original papers so that Physalaemin produces a response of 100%. (ERP) is eledoisin related peptide not the true eledoisin. In the case of drinking in pigeons physalaemin was not tested.

	Substance P fragment											
Assay system	1-11	2-11	3-1	4-11	5-11	6-11	7-11	8-11	9-11	1-7	1-10	Author(s)
Dog femoral artery blood flow	100	68	128	164	104	65	4.2	0.5	0.4	-	-	
Guinea pig ileum	100	60	157	201	126	108	2.2	-	-	-	-	Bury & Mashford, 1974.
Rabbit ear vein	100	94	107	128	98	48	0.9	-	-	-	-	
Guinea pig ileum	100	70	172	264	127	109	3	-	-	-	-	Bergman et al. 1974.
Guinea pig ileum	100	-	-	130	-	-	3	-	-	0.3	-	Chipkin et al. 1979.
Guinea pig ileum	100	100	100	40	170*	200*	1	1	1	-	0.1	Yanaihara et al. 1977.
Frog spinal cord	100	-	-	7.5	-	2.5*	0.6	0.05	0.02	-	-	Otsuka, Konishi & Takahashi, 1975.
Rat spinal cord	100	60-90	40-100	80-100	200-1200*	500-1200*	2	0.02	.008	-	0.05	Otsuka & Konishi, 1977.
Iontophoresis in rat spinal cord	100	100	100	100	100	100	<<100	<<100	<<100	-	<100	Piercey & Einspahr, 1980.
Displacement of substance P binding. (IC50 3.17×10^{-9})	100	-	0.11	0.8	5700	250	0.82	-	-	-	-	Nakata et al. 1978.

Table (1.2). Comparison of the effects of fragments of substance P molecule on different assay systems. Data has been modified from the original papers so that substance P produces a 100% response on all assays. * indicates that the fragment of substance P has a cyclized glutamine residue at the n-terminus which stabilizes the molecule.

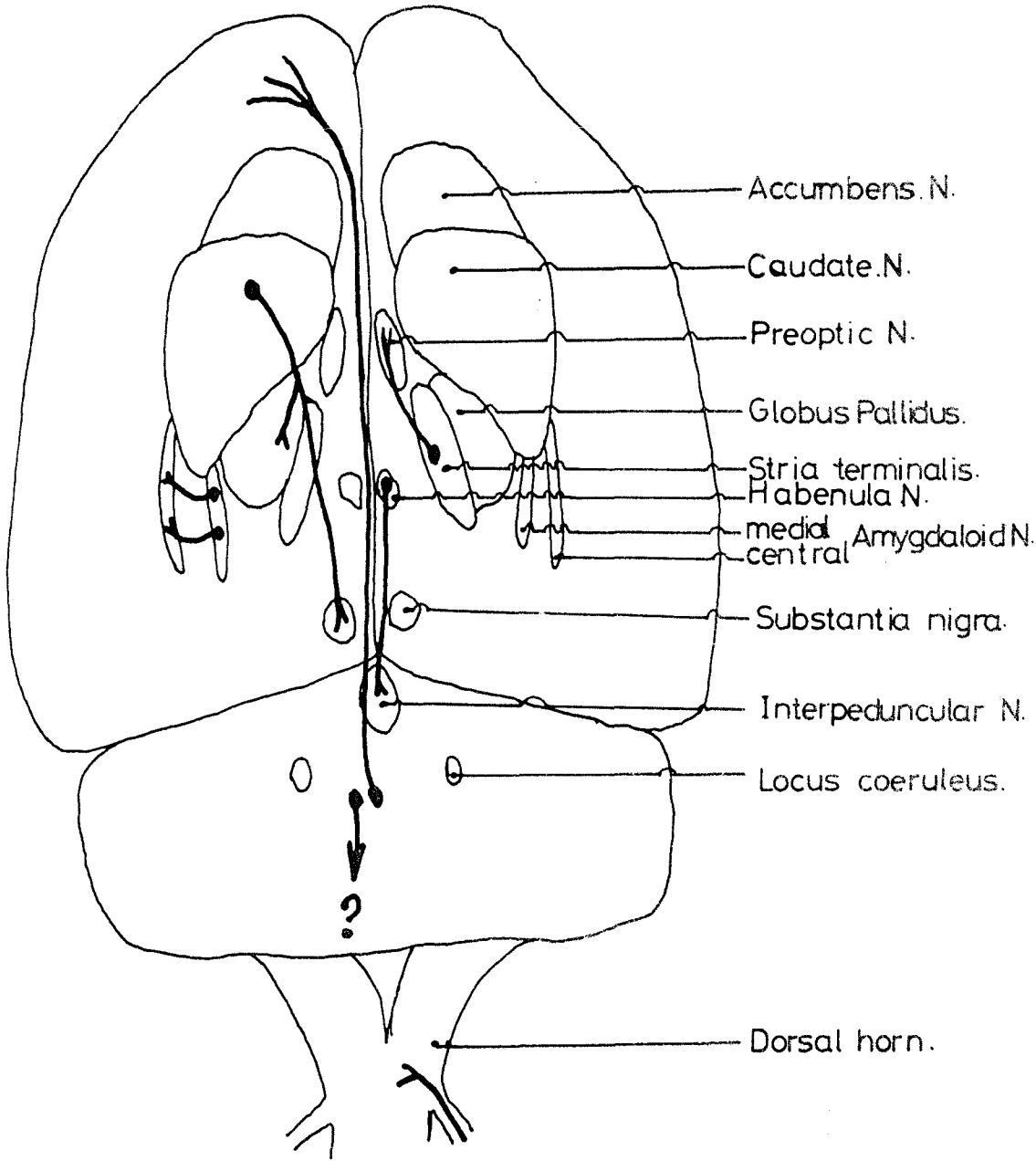
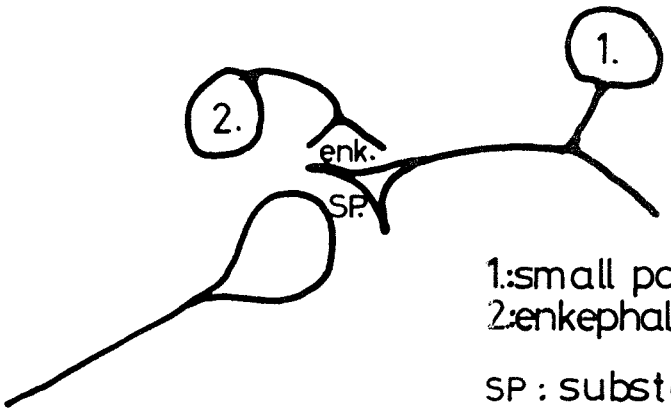


Figure (1.1). Horizontal section through the rat brain to show some of the proposed substance P pathways (data from a review by Emson, 1979). (Substance P pathway :- ●———).



1:small pain primary afferent.
2:enkephalin interneurone.

SP : substance P.
enk: enkephalin.

Figure (1.2). proposed transmitters at the spinal pain gate (Jessel and Iversen 1977.).

1.2. MSH release inhibiting factor (MIF)

1.2.1. General Pharmacology of MIF

A tripeptide which inhibited the release of Melanocyte Stimulating Hormone (MSH) from the pituitary was first isolated from bovine hypothalamus in 1971 (Nair, Kastin and Schally). MSH release from the pituitary is thoughtto be controlled by tripeptides. The most potent of these contains the C-terminal fragment of oxytocin; L-prolyl-L-leucyl-glycinamide (Celis, Taleisnik and Walters, 1971), while the C-terminal fragments of lysine and arginine vasopressin (Fig. 4.3) also have this property to a lesser degree (Celis, Hase and Walters, 1972). In this thesis the tripeptide L-prolyl-L-leucyl-glycine amide will be referred to as MIF. An extrahypothalamic role has been proposed for MIF. For example in the 1 - 10 mg/Kg range MIF will attenuate puromycin amnesia in mice (Van Ree and DeWied, 1977), others find low (5mg/Kg) doses potentiate this effect (Muchu and Kalant, 1979). However most of the literature deals with an interaction of MIF with dopamine systems in the brain. However the topic is contentious as there has been relatively little work on this peptide. By using electrophysiological techniques we hoped to clarify some of the aspects of the proposed interactions between MIF and dopamine.

1.2.2. Interactions of MSH and MIF with dopamine

The MSH activity of rat pituitary glands was decreased by treatment with neuroleptics (Kastin and Schally, 1966). Phenothiazines caused pigmentation to occur in both lizards and man (Carter and Shuster, 1978). One of the side effects of neuroleptics during the treatment of psychosis is the appearance of extrapyramidal disturbances. Thus, there appears to be a relationship between MSH and drugs, such as the neuroleptics which are thought to affect dopamine systems in the brain.

Using a histofluorescence technique to detect changes in activity, it was found that MSH will increase the firing rate of dopamine containing cells (Lichtensteiger and Lienhart, 1977). More support for a central action of MSH comes from studies in which intravenous MSH caused abnormal EEG patterns and changes in a variety of behavioural and psychological tests in man (Kastin, et al. 1966, Kastin, et al. 1975).

Parkinsons disease is thought to be caused by a loss of dopaminergic function of the ascending nigrostriatal tracts, in which in conjunction with a

reduction in nigrostriatal dopamine levels, a loss of pigmentation in the substantia nigra occurs (section 1.5.1.).

Dopamine and melanin share the first stages in their synthetic pathways, additionally, the tissues containing them are derived from the same embryological site (Fitzpatrick, Seigi and McGugan, 1961). This prompted Cotzias, et al. (1967) to use B-MSH and L-dopa as anti-Parkinsonian agents. However, they found while L-dopa was effective, B-MSH excacerbates the condition. Since then B-MSH levels have been found to be raised in Parkinsonian patients (Shuster, et al. 1973) and these authors suggested that both endogenous and exogenously administered MSH caused L-dopa to be converted to melanin thereby worsening the condition. It seemed reasonable to Shuster, et al. (1973) that an MSH inhibiting factor could be of potential therapeutic value in Parkinsonism.

Early clinical studies with acute intravenous injections of MIF in conjunction with L-dopa showed promising results (Barbeau, 1975) which were repeated (Gonce and Barbeau, 1978, Schnieder, et al. 1978). Behavioural studies have supported a synergistic effect on dopamine systems, with MIF (0.1 - 30mg/Kg) greatly potentiating dopamine effects. This effect was present in hypophysectomized as well as intact animals, which have normal MSH secretion (Plotnikoff, et al. 1971) thus negating a direct MSH effect (Plotnikoff, Minard and Kastin, 1974, Voith, 1977). MIF will reverse reserpine sedation (Plotnikoff, et al. 1973) and antagonize fluphenazine induced catalepsy (Voith, 1977). Oxotremorine induced tremor appears to be abolished with low but not high doses of MIF (Björkman and Sievertsson, 1977). In animals with unilateral 6OHDA lesions of the nigrostriatal tracts, ADH, oxytocin and MIF all cause ipsilateral rotation when injected ICV but not when injected directly into the substantia nigra, suggesting an interaction with presynaptic dopamine nerve terminals (Schulz, Kovacs and Telegdy, 1979). In striatal slices from intact, but not hypophysectomised animals, pretreated with MIF, a dose dependant increase in dopamine synthesis occurred (Friedman, Freidman and Gershon, 1973). Increased brain dopamine levels after MIF treatment have been reported by other authors (Pugsley and Lippman, 1977). Spirtes, et al. (1976) however, found the response variable. Animals which showed a potential response to L-dopa sometimes failing to show increased dopamine levels. Furthermore, Plotnikoff, Minard and

Kastin, (1974) failed to find any significant changes in brain dopamine levels following chronic MIF administration in spite of the animals responding behaviourally to MIF and L-dopa. This was confirmed in the study of Kostrzewa, Kastin and Spirtes (1975) who found no changes in dopamine levels or synthesis. One of the possible reasons for these discrepancies is the large dose range employed. For example 0.1 mg/Kg potentiated L-dopa while 30 mg/Kg is the optimum dose for reversing oxotremorine induced tremor. Doses of 0.1 mg/Kg in conjunction with L-dopa have been reported to be effective against harmine tremors (Huidoborotoro, Carolis and Longo, 1975). Haloperidol catalepsy was reversed by a high (100 mg/Kg) dose of MIF given 30 minutes before but not after the haloperidol (H. Wheeler personal communication). It thus appears that the timing of drug administration is significant. Chronic but not acute MIF treatment antagonized fluphenazine catalepsy (Voith, 1977) and morphine catalepsy (Chiu and Mishra, 1979). Thus it is unclear at which point MIF has this anticataleptic action.

The cellular effects of MIF are unclear. While results measuring changes in the cAMP levels are unclear (Christensen, et al. 1976) an increase in cGMP was reported in the thalamus after chronic MIF treatment (Spirtes, et al. 1978) but no changes were seen in nuclei containing high densities of dopamine terminals.

There have been few reports on the distribution of MIF in the brain. From autoradiographic studies after intravenous ^{3}H -MIF injections, high levels of radioactivity were seen in the pineal gland and anterior, posterior and intermediate lobes of the pituitary. Although MIF has a plasma half life of nine minutes (Redding, et al. 1973) it is possible that low CNS levels occurred because only limited amounts of MIF would reach the brain after passage through the systemic circulation (Dupont, et al. 1975). In support of this suggestion, intra carotid rather than intravenous injection resulted in significant amounts of radioactivity in the cerebral ventricles. After ICV injections large amounts were taken up into the septum, putamen, globus pallidus and hippocampus (Pelletier, et al. 1975). More recently a binding study found a high affinity saturable binding to calf caudate membranes (Chium Wong and Mishra, 1980). Since neither technique discriminates between the intact peptide and its metabolites and since it is not established that the tripeptide or a metabolite are responsible for the behavioural effects, it is unclear whether or not MIF actually has a receptor. In

spite of this a direct effect on the post synaptic membrane of caudate neurones, rather than a modulation of dopamine transmission was favoured by those who found no change in amine levels, turnover or uptake (Spirtes, et al. 1976, Kastin, Coy and Bymaster, 1979).

Preliminary clinical studies found low not not high doses of MIF were effective in some types of depression (Ehrensing and Kastin, 1978). This correlates with the bifasic effects seen in reversing oxotremorine tremor (Björkman and Sievertsson, 1977).

1.3. Thyrotropin Releasing Hormone

1.3.1. General pharmacology of TRH

Another tripeptide which has been isolated from the hypothalamus is Thyrotropin releasing hormone or TRH, which has the structure: L-pyroglutamyl-L-histidyl-L-prolinamide. It is thought to be involved in the regulation of thyroid hormones. Intravenous TRH causes an increase in plasma TSH which can be blocked by simultaneous thyroxine administration. (TRH has a plasma $\frac{1}{2}$ life of $\sim$ 4 min). Its actions have been reviewed by Schally, Arimura & Kastin (1973).

1.3.2. TRH in the CNS

The distribution of ^{3}H-TRH has been studied after intravenous injection followed by autoradiography. Most of the label accumulated in the pituitary, and smaller amounts were found in the kidney and liver. However, none of the label was located in the brain (Dupont, et al. 1972). Apart from its function as the regulator of TSH secretion there is evidence for an extrahypothalamic role. Indirect immunofluorescence techniques showed high levels of TRH-like immunoreactivity located in nerve terminals particularly in the dorsal part of the nucleus accumbens, lateral septal nuclei and several motor nuclei of the brain stem and spinal cord (Hökfelt, et al. 1975). This peptide appears to be present in large granular vesicles approximately 1000 Å in diameter, (Johansson, et al. 1978). A binding study by Burt and Snyder (1975) showed a saturable high affinity component present in brain tissue with the exception of the cerebellum. TRH with D substituted amino acids was less active than TRH indicating the binding site had some specificity for the native peptide. It has also been found that intracisternal 6OHDA treatment causes a substantial decrease in immunoreactive TRH material in the posterior cortex and forebrain, while other treatments such as thyriodectomy or hypophysectomy are without effect (Winokur, et al. 1978).

Reversal of hibernation in animals can be achieved within three hours of injecting TRH into the hippocampus (Stanton, Winokur and Beckman, 1980). Artificially induced sedation by either barbiturate or ethanol can be reversed by TRH given peripherally or centrally (Prange, et al. 1974, Tache, et al. 1977, Carino, et al. 1976). Although no specific mechanism has been proposed for this there does appear to be a link between TRH and catecholamines in temperature regulation from the following observations:-

If ICV injections are given to animals there is a drop in core temperature similar to that produced by imipramine, calcium and noradrenaline (Metcalf, 1974). It has also been found that TSH potentiates the effects of imipramine in depressed patients (Prange, et al. 1970) and it was this that prompted psychiatrists to use TRH to treat depression. Early clinical trials indicated a brief ameliorating effect (Prange, et al. 1972) in this and also in some forms of schizophrenia (Wilson and Lara, 1973). Changes in blood pressure and EEG after intracisternal injections of 20ng-20µg TRH (Beale, White and Huang, 1977) and intraperitoneal administration caused a longer latency of onset of REM sleep and changes in spike intervals in the MRF and hippocampus (Korany, Whitmoyer and Sawyer, 1977). More significantly after four days TRH treatment (1mg/kg I.P.) multiple unit activity in the brain stem and hypothalamus (recorded with chronic electrodes) becomes like that of animals treated with imipramine (Korany, et al. 1976). Intracisternal or I.V. injections of TRH or an analogue MK177 cause changes in EMG activity in anaesthetized cats (Yarbrough, McGuffin and Clineschmidt, 1979) a similar effect occuring in spinalized animals accompanied by an increase in the number of spontaneous motoneurone potentials (Cooper and Boyer, 1978). This is confirmed by the intracellular studies on frog motoneurones which show an increase in conductance, possibly to sodium, the depolarization rarely reaches threshold suggesting TRH also has a modulatory role (Nicoll, 1977). Thus it is possible TRH has a function in the spinal cord as well as in the brain.

Electrophysiological studies using iontophoresis show a variety of effects. Predominantly inhibitory effects are seen in the cerebral cortex and hypothalamus (Moss, Dudley and Kelly, 1978, Dyer and Dyball, 1974), ventromedial hypothalamus, cerebral cortex and cerebellar cortex (Renaud, Martin and Brazeau, 1975). The acetylcholine response in the cortex is potentiated by both TRH and MK177 (Yarbrough, 1977). Winokur and Beckman, 1978, however, failed to observe this effect in cortex, septum or hypothalamus. They also found that the peptide did not alter responses to dopamine although TRH did have inhibitory action of its own in all three areas (Winokur and Beckman, 1978). Additionally, in the cortex some investigators found the response to glutamate is selectively depressed while the response to acetylcholine is not, the peptide also inhibits spontaneous activity (Renaud, et al. 1979, Renaud, Martin and Brazeau, 1976, Renaud and Martin, 1975).

The turnover of acetylcholine was increased while the content was decreased

in the parietal cortex after intracisternal TRH (Malthe Sørenssen, et al. 1978). However, other authors can find no change in acetylcholinesterase activity or choline uptake and release from cerebral synaptosomes (Renaud, et al. 1979). This latter study leads to the suggestion of an interaction between TRH and the glutamate receptor. Chronic administration of TRH for ten days produced a dose and time dependent increase in cerebrocortical 5HT, dopamine and tyrosine hydroxylase activity but no change in noradrenaline levels (Argarwal, Rastogi and Singhal, 1977) while others found an enhancement of cerebrocortical noradrenaline turnover and suggested this is responsible for its antidepressant activity (Constantinidis et al. 1974). A calcium dependent potassium stimulated release of TRH-like-immunoreactivity has been demonstrated from hypothalamic and septal synaptosomes. In the hypothalamus but not the septum, dopamine or apomorphine stimulated the release of TRH-like material, the response being inhibited by phenothiazines (Schaeffer, Axelrod and Brownstein, 1977). Slices of nucleus accumbens will release dopamine in a calcium-dependent fashion when stimulated with high potassium, TRH will enhance this release while having no effect on ^{3}H-dopamine accumulation, dopamine binding or dopamine stimulated adenylate cyclase, indicating that it does not act directly on a dopamine receptor. This effect is specific for accumbens tissue since it does not occur in caudate slices (Kerwin and Pycock, 1979). In this case TRH appears to modulate dopamine release in the accumbens while Schaeffer, Axelrod and Brownstein (1977) found the opposite is true.

Locomotor activity caused by both l-Dopa and pargyline is potentiated by peripheral administration of TRH in normal, thyroidectomized and hypophysectomised rats and mice, implying that the phenomenom is independent of the release of thyroid hormones (Plotnikoff, et al. 1972, Plotnikoff, et al. 1974). TRH given over ten days produces a dose and time dependent increase in spontaneous locomotor activity in young rats which is accompanied by an increase in cerebrocortical 5HT and dopamine levels (Argawal, Rastogi and Singhal, 1977). Acute injections of 10-20mg/kg TRH have been found to cause a short term increase in locomotor activity, similar to the effect seen if TRH is injected bilaterally into the accumbens but not the caudate of chronically cannulated animals. This effect can be blocked by peripheral or centrally administered neuroleptics (Miyamoto and Nagawa, 1977) or 6OHDA lesions (Green and Heal, 1978). TRH does not produce rotation in animals with unilateral nigrostriatal lesions, nor does it enhance

amphetamine turning (Heal and Green, 1979) while both amphetamine and TRH increase the release of tritiated dopamine from accumbens slices (Miyamoto, et al. 1979) suggesting that in this area it acts by releasing dopamine rather than by direct stimulation of the post synaptic dopamine receptors.

Thus there is evidence that both MIF and TRH interact with nigrostriatal and mesolimbic dopamine pathways. While dopamine receptors are located on the postsynaptic membrane of the target neurones in the caudate nucleus and nucleus accumbens, presynaptic receptors occur on the nerve terminals and also on the cell body, it is assumed that the receptors on the cell body are the same as those on the terminals. We therefore decided to undertake iontophoretic experiments in the substantia nigra, caudate nucleus and nucleus accumbens with TRH and MIF to see if dopamine responsive cells were also affected by these compounds and the MIF analogues which were available to us. Behavioural experiments were also carried out to see whether or not peripheral administration of either TRH or MIF affected the response to the catecholamine releasing agent amphetamine administered either directly into the nucleus accumbens or peripherally. The effect of MIF pretreatment on the loss of spontaneous exploratory behaviour caused by haloperidol was also observed.

1.4. Opioid peptides

1.4.1. Opioid peptides in the CNS

Opioid peptides are those which, in one or more invitro assay systems, share actions with opiate alkaloids. The actions of both opioid peptides and alkaloids are reversed by antagonists such as naloxone. The opioid peptides include B-endorphin and the pentapeptides methionine and leucine enkephalin. There is good evidence for these peptides as putative transmittersin the brain. Synthesis of these peptides is by a conventional ribosomal process. The enkephalins are present in nerve terminals, are unevenly distributed in the brain with high concentrations in the globus pallidus and substantia gelatinosa and low concentrations in the cerebellum. Pathways from striatum to the substantia nigra and also from amygdala to the striaterminals have been proposed. Enkephalin containing interneurones are thought to occur elsewhere in the CNS. Calcium dependent release of enkephalins followed by hydrolysis occurs from enkephalin nerve terminals. This evidence is adequately reviewed by Emson (1979). Iontophoresis of enkephalins in most brain regions causes inhibition of neuronal firing. The iontophoretic data has been reviewed recently by North (1979). Opiates are stereo specific, for example the (-) isomer of Naloxone reverses the iontophoretic effects of enkephalins while the (+) isomer is inactive (Gayton, Lambert and Bradley, 1978). Behavioural responses to enkephalins include analgesia akinesia and wetdog shakes. These have been reviewed by Emson (1979) and Lord, et al. (1977).

While there is a great deal of literature on the various opioid peptides and their fragments the work in this study was concerned with interactions of opiates with dopamine systems.

1.4.2. Interactions between dopamine and opiates

The highest concentrations of opiate receptors are found in the limbic and extrapyramidal systems and there appears to be a close overlap between dopamine and enkephalins, (Kelley, Stinus and Iversen, 1980, review by Emson, 1979). Following 6OHDA pretreatment there is a decrease in caudate opiate binding suggesting that the opiate receptors may be located presynaptically on the dopamine nerve terminals (Pollard, Llorens-Cortes and Schwartz, 1977, Pollard, et al. 1978). This is supported by experiments which show that while morphine has no effect on dopamine sensitive adenylate cyclase in vitro, it does produce an increased cyclase activity in rats chronically pretreated with morphine. This suggested that morphine blocks

dopamine transmission, which leads to dopamine supersensitivity (Iwatsubo, 1977). Single cell recordingsfrom the locus coeruleus show that intravenous morphine inhibited cell firing (Korf, Bunney and Aghajanian, 1974), while in the substantia nigra intravenous morphine caused excitation of dopamine compacta cells (Iwatsubo and Cluoet, 1977) and inhibits reticulata cells (Finnerty and Chan, 1979). All of these effects are reversed by (-)-naloxone. Peripheral administration of narcotic analgesics has been found to increase locomotor activity while decreasing brain catecholamine content (Rethy Smith and Villarreal, 1971). The VTA and the nucleus accumbens are the two mesolimbic areas thoughtto be involved in the hyperactivity response to opiates. If morphine or d-Ala^2 metenkephalin (DALA) are infused into the accumbens a naloxone reversible hyperactivity occurs (Pert and Sivit, 1977), sometimes preceded by a period of hypoactivity (Costall, Fortune and Naylor, 1978). Self stimulation was increased after VTA morphine injections (Broekkamp, Van Dongen and Van Rossum, 1977) and an increase in locomotor activity was seen after bilateral VTA injections of morphine or enkephalins (Iversen, et al. 1978, Broekkamp, Phillips and Cools, 1979). These effects could be reversed by naloxone or 6OHDA pretreatment (Kelley, Stinus and Iversen, 1980). α, and B endorphins have similar locomotor effects to enkephalins when injected into the VTA, naloxone or 6OHDA pretreatment abolished these responses (Stinus, et al. 1980). These facts are taken as evidence than an intact dopamine system is required for the locomotor response.

While there have been studies on the conformation of opiod peptides (Smith and Griffin, 1978) details of the conformation of enkephalins at the receptor site are complicated by the possibility that there may be three types of receptors. These respond to three different drug types and have been classified accordingly, μ (morphine),k (ethylketocyclazocine), δ (N-Allylnorphenazocine). This classification was suggested by the differences in pharmacological responses to different types of narcotic analgesics and their inability to substitute for each other in the suppression of withdrawal symptoms in the addicted chronic spinal dog preparation (Martin, et al. 1976). The possibility of different receptor types is of importance in explaining the differences observed between enkephalins and endorphins (Lord, et al. 1977) and differences between various synthetic morphine drugs. With the synthetic morphine drugs for example, EEG and behavioural parameters showed a nearly linear relationship between increasing central excitatory activity and diminishing analgesia (Labella, Pinsky and Havlicek, 1979).

It is assumed from studies on peripheral preparations that the guinea pig ileum (GPI) has mostly μ while the mouse vas deferens (MVD) contains more δ than μ enkephalin receptors, since in the latter preparation enkephalin effects are less easily reversed than morphine effects by opiate antagonists. While the GPI contains more k receptors than the MVD it is unclear what role k receptors have (Lord, et al. 1977, Waterfield, et al. 1978).

While both μ and δ sites have been detected in the dorsal root and spinal cord by the use of (^{3}H)-nalorphine and (^{3}H)-d-Ala^2-d-Leu^5 enkephalin (Fields, et al. 1980), there was no evidence for k sites. However, the binding of (^{3}H)-ethylketocyclazocine, a specific k agonist, follows the distribution of other opiates in the brain (Miller and Simon, 1979).

1.5. DOPAMINE

1.5.1. Dopamine - Distribution, pathways in the CNS

Dopamine was first discovered as an intermediate in the synthetic pathway from phenylalanine to noradrenaline. Subsequently there has been considerable evidence to show that dopamine is a neurotransmitter with a physiological role different to that of noradrenaline. Dopamine appears to have specific actions in both the periphery and central nervous system (review by Ungerstedt, 1978). In the brain there is good evidence for several dopamine pathways, however the only ones considered in detail here are the nigrostriatal and mesolimbic projections.

In the early studies on catecholamine containing cell bodies it was found that there were high densities of fluorescence in cells in areas A9 and A10 in the rat brain, (Dahlstrom and Fuxe, 1965). Although there was no clear borderline between the two groups of cells it was proposed that the nigrostriatal pathway arose from the A9 area whereas the mesolimbic and mesocortical projections arose from the A10 area. A more recent study using a combination of histochemical techniques suggest that the A9 and A10 cells form a single nuclear group. In contract to the noradrenergic innervation which is relatively homogenous and distributed in a fairly even plexiform manner in most areas, the dopamine neurones have a highly topographical organized projection but there is no clear distinction between VTA (A10) and substantia nigra (A9) dopamine cells (Fallon and Moore, 1978, Simon, le Moal and Calas, 1979, Beckstead, Domesick and Nauta, 1979, Guyenet and Aghajanian, 1978). The actual soma of the dopamine neurones are non spherical, having a very large dendritic tree extending into the reticulata of the nigra (Juraska, et al. 1977, see figure 1.3).

1.5.2. Biochemistry of dopamine release, receptor binding and postsynaptic actions

A potassium stimulated release of dopamine has been shown to occur from striatal but not nigral synaptosomes (Hardy, et al. 1980). A similar report has found that while caudate dopamine terminal preparations will release dopamine after potassium or amphetamine stimulation, nigral dendrite preparations will only release dopamine if amphetamine is also present (Hefti and Lichtensteiger, 1978). This supports the hypothesis that nigral dendrites release dopamine when the cells depolarize. Thus when the cells fire dopamine may act on both somatic and pre and post synaptic receptors.

Dopamine receptors have been studied using radioligand binding assays. Specific saturable binding of tritiated dopamine was reported by Seeman, Chau-Wong and Lee in 1974. More recently ^{3}H-ADTN has been used as a dopamine agonist ligand (Davis, Woodruff and Poat, 1980, Roberts, Woodruff and Poat, 1977). Antagonists such as (+)-butaclamol, (^{3}H)-spiroperidol and (^{3}H)-sulpiride will also bind to dopamine receptors (Burt, et al. 1979, Therodorou,et al. 1979). Dopamine receptor binding has proved a useful method of screening potential agonists and antagonists. A review of dopamine receptor binding has recently been published (Titeler and Seeman, 1979).

After interaction with a receptor a change in biological parameters occurs which leads to a change in membrane permeability. How this actually happens is a controversial topic which will not be dealt with in detail here, but it has been suggested that adenosine -3, 5-monophosphate (cAMP) functions as the second messenger in the action of dopamine. An adenylate cyclase which was specifically stimulated by dopamine was first shown to be present in homogenates of rat striatum by Kebabian, Petzhold and Greengard (1972) and subsequently in other areas in the CNS. The dopamine sensitive adenylate cyclase has proved to be a useful model of post-synaptic dopamine receptors (Iversen, 1975). While most studies have used homogenates the dopamine sensitive adenylate cyclase has also been demonstrated in slices from the rat striatum (Munday, Poat and Woodruff, 1974). The activity of the cyclase remains after surgical or chemical lesions (6OHDA) of the ascending dopamine neurones, suggesting the enzyme is located post-synaptically (Krueger, et al. 1976).

1.5.3. Iontophoretic dopamine pharmacology in the caudate nucleus and nucleus accumbens

Dopamine was first found to inhibit neuronal firing in the cat caudate nucleus by Bloom, Costa and Salmoiraghi (1965). In a detailed study of the nigrostriatal pathway it was found that cells which respond to nigral stimulation with a slow latency inhibition were also inhibited by dopamine (Feltz, 1969). The response to dopamine was increased in cats with 6OHDA lesions (Feltz and De Champlain, 1972). A monosynaptic excitatory response to nigral stimulation was also found on cells which were not affected by dopamine (Feltz, 1969, Feltz and McKenzie, 1969). This excitation was not affected by 6OHDA lesions (Feltz, 1972), but was abolished by perfusions of haloperidol over the caudate nucleus (Feltz, 1971) and was absent in animals

anaesthetized with barbiturate (Feltz and Albe-Fessard, 1972). This is of interest since Bloom, Costa and Salmoraighi (1965) found that intravenous or iontophoretic barbiturates abolished the excitatory responses to acetyl choline. Thus the evidence suggests that there is more than one nigrostriatal pathway. Extracellular studies such as these, generally found that dopamine inhibited either spontaneous or glutamate induced firing, and was reversed by neuroleptics (Bunney and Aghajanian, 1976, Woodruff, McCarthy and Walker, 1976, Bevan, et al. 1978, Skirboll, Grace and Bunney, 1979). In contrast to this, intracellular studies usually found dopamine produced a depolarization (Koscis and Kitai, 1978, Bernardi, et al. 1978, Herrling and Hull, 1980). This depolarization is accompanied by a reduction in the number of action potentials, thus the extracellular studies do actually correlate with the intracellular studies. However, the mechanism by which a depolarization leads to a decrease in firing rate is unclear, and it is uncertain which ions are involved. Recently a comprehensive review of nigrostriatal neuropharmacology has been published (Dray, 1979) in which some explanations have been offered.

1.5.4. Effect of drugs on the cell bodies of the nigro-striatal tract

It was logical to assume that drugs which block postsynaptic dopamine receptors might cause an increase in the firing rate of dopamine at the post synaptic receptors. Conversely drugs which are dopamine agonists or stimulate dopamine release, such as apomorphine or amphetamine would be expected to inhibit the neurones by a feedback mechanism. These changes in firing rate occur as expected when single cell recordings were made from dopamine containing cells in the substantia nigra or VTA. The changes in firing rate correlated with variations in dopamine metabolism measured by altered DOPAC levels (Bunney, et al. 1973). The inhibitory response to amphetamine is blocked by the tyrosine hydroxylase inhibitors an effect which is reversed by l-dopa (Bunney, Aghajanian and Roth, 1973).

It is possible that the inhibitory responses to intravenous drugs could be mediated by presynaptic dopamine receptors. The possible presence of these presynaptic receptors was demonstrated in the following way. If the impulse flow in dopamine neurones was stopped by several acute procedures, such as cutting the tract or administration of δ-hydroxy butyrate, there was an increase in striatal dopamine synthesis in dopamine nerve terminals. This synthesis could be reversed by administration of apomorphine which is thought to act particularly potently on dopamine pre-synaptic receptors

(Walters and Roth, 1976). These presynaptic receptors are thought to be present on the soma of the dopamine containing neurones where they have been called "autoreceptors" (Aghajanian and Bunney, 1977). They are thought to be dopamine receptors rather than other receptors from iontophoretic studies in both the A9 and A10 areas where the response to dopamine was blocked by trifluoperazine or fluphenazine, but not by the α-blocker piperoxane or the β-blocker solatol. Noradrenaline was equipotent with dopamine in producing inhibition of the neurones, while the β-agonist isoprenaline the α agonist clonidine and 5HT were inactive and amphetamine was only weakly active. Both GABA and glycine inhibitions were unaffected by dopamine antagonists (Aghajanian and Bunney, 1977, Collinbridge and Davies, 1979, Ruffieux and Schultz, 1980). There is some electrophysiological evidence that the soma-dentritic autoreceptor is more sensitive to both intravenous apomorphine and iontophoretic dopamine than the postsynaptic receptors in the striatum (Skirboll, Grace and Bunney, 1978). A close relationship between reticulata and compacta cells has been demonstrated. Iontophoretic dopamine was found to excite reticulata cells, possibly indirectly via compacta cells (Ruffieux and Schultz, 1980). Additionally using simultaneous recordings from bothareas it has been shown that when GABA inhibits a reticulata cell there is a corresponding increase in compacta activity. Glutamate causes the opposite effect, suggesting the compacta receives an inhibitory GABA input from the reticulata (Grace and Bunney, 1979).

1.5.5. Electrophysiology of the mesolimbic and nigrostriatal pathways

As would be expected from the histological studies, which show the ascending dopamine pathways are morphologically similar, electrophysiological characterization gives a similar result. After electrical stimulation of the caudate nucleus antidromic spikes are seen in two different types of cell in the ipsilateral substantia nigra compacta. These can be easily distinguished. The first type are dopamine cells of the type classed by Bunney, et al. (1973) with a long extracellular AP. They can be antidromically identified from various locations along the nigrostriatal tract, the conduction velocity is very slow (0.5m/s), typical of unmyelinated fibres and frequently only the axonal component of the antidromic spike occurs. These cells are inhibited by iontophoretic dopamine, intravenous apomorphine and are absent after 6OHDA lesions of the nigrostriatal tract (Guyenet and Aghajanian, 1978). A second type of antidromically activated cell have also been found, these have a much faster conduction velocity. These cells which appear to be nondopaminergic were still present after 6OHDA treatment

(Guyenet and Aghajanian, 1978). In the VTA similar antidromic spikes to those found in the compacta dopamine neurones are found. While there appears to be a branching of neurones in the caudate and accumbens there are few cases of a neurone projecting to both the caudate and the accumbens from a single cell in either A9 or A10. Similar to the nigrostriatal system there appears to be a faster conducting nondopaminergic projection from the VTA to the cortex, accumbens and rostral striatum (Deniau, Thierry and Feger, 1980, German, Dalsass and Kiser, 1980, Yim and Mogenson, 1980). Intrastriatal injections of Kainic acid which cause a loss of cell bodies but do not destroy axons or terminals, do not cause a loss of the antidromic spikes (Nakamura, et al. 1979, Kondo and Iwatsubo, 1980) although there appears to be less post stimulus inhibition of cell firing than is normally seen. An inhibition of firing in nondopamine cells is seen after striatal stimulation, this can be blocked by picrotoxin or bicuculline (Crossman, Woodruff and Walker, 1974a, Feltz & b, 1971, Davies, Dray and Tongroach, 1978) suggesting a striato nigral feedback inhibition.

Electrical stimulation of the substantia nigra produces a variety of effects in the ipsilateral striatum including a slow latency inhibition on cells inhibited by dopamine (Feltz, 1969) and a slow latency excitation which is absent in barbiturate anaesthetized animals (Feltz and Albe-Fessard, 1972).

Thus there is still uncertainty over how many striato nigral and nigro striatal pathways there are, it has not been made easier by the use of different species of animals in many of these studies.

1.5.6. Behavioural effects of dopamine

Pijnenberg, Woodruff and Van Rossum (1973) first discovered a possible involvement of the nucleus accumbens in the control of locomotor activity. These authors found that bilateral injections of ergometrine into the nucleus accumbens of conscious rats produced a strong and longlasting locomotor stimulation. This was abolished by haloperidol or pimozide but not by a tyrosine hydroxylase inhibitor (α-methyl-p-tyrosine). Shortly afterwards a similar effect was observed with dopamine in MAOI treated rats (Pijnenberg and Van Rossum, 1973). Very similar but more potent, longer lasting stimulations were seen with the conformationally restricted analogue of dopamine ADTN (Elkhawad and Woodruff, 1975). The locomotor

component of hyperactivity manifested by the nucleus accumbens is important in another behaviour pattern. It was found that after unilateral striatal lesions of dopamine terminals, an intact mesolimbic system was required for the contralateral rotation produced by amphetamine. Additionally, the ipsilateral rotation caused by apomorphine was enhanced after bilateral destruction of mesolimbic dopamine terminals. Thus the nigro striatal lesion produced an assymetry while the mesolimbic system dictated the degree of rotational response (Kelly and Moore, 1976, Kelly and Moore, 1977, Pycock and Marsden, 1978).

Certain neuropathalogical disturbances appear to be related to dopamine dysfunction. Amongst these are Parkinsonism and Schizophrenia. In Parkinsonism there is thought to be a loss of nigrostriatal dopamine neurons (Hornykiewicz, 1979) which results in postsynaptic supersensitivity (Lee, et al. 1978). L-dopa treatment produced therapeutic results in this condition (Cotzias, et al. 1967). In schizophrenia overactivity of the mesolimbic dopamine systems is thought to occur. The evidence for this is mostly pharmacological, as the one property shared by neuroleptics is their ability to block dopamine receptors (reviews by Snyder, et al., 1974 and Carlsson, 1978).

The antipsychotic drugs are derived from several different parent molecules (see table 1.3). A new class of antipsychotic drug, the substituted benzamides, have been introduced recently. An example of these is sulpiride. Sulpiride has only a partial spectrum of activity of the classical neuroleptics. In particular it does not block striatal dopamine stimulated adenylate cyclase which has been widely used as a model for post-synaptic dopamine receptors (Elliot, et al. 1977, Trabucchi, et al., 1975). While it was thought that the substituted benzamides produced less catalepsy than classical neuroleptics (review by Jenner and Marsden, 1979) this effect may be due to the impermeability of the blood brain barrier. Since the earlier work it has been shown that sulpiride administered intra-cerebroventricularly was almost as potent a catalaptogen as haloperidol (Honda, et al. 1977) and will block ADTN hyperactivity when injected into the nucleus accumbens as potently as fluphenazine (Woodruff and Andrews, 1979).

Partly on the basis of the lack of effect of sulpiride on adenylate cyclase it has been suggested that there is more than one type of receptor for dopamine. These have been classed as DA1 which are post synaptic linked to the

cyclase, and DA2 which are pre and not linked to the cyclase (review by Kebabian and Calne, 1979). Other invitro studies support a two receptor hypothesis. In 1978 Titeler et al. suggested from experiments which showed that binding of tritriated dopamine agonists and antagonists was bi phasic, that they were possibly binding to two high affinity sites. Later studies in which kainate lesions were shown to decrease striatal cAMP values to a greater degree than tritiated neuroleptic binding sites, suggested that butyrophenones are preferentially labelling presynaptic (DA2) receptors (Schwarczet al. 1978). These haloperidol binding sites were located mainly on the terminals of afferents from the cerebral cortex. A diagram of these different DA sites is given in fig. 1.4.

The structure of dopamine at its receptor site has been the subject of much work. The principle of free rotation about single carbon-carbon bonds means that dopamine is a flexible molecule. In solution it can exist in extended, or anti, and folded, or gauche forms (Bustard and Egan, 1971, Rekker, Engel and Nys, 1972). One approach to the problem of the conformation of dopamine at its receptor site has been the use of rigid dopamine analogues. The drug norsalsolinol contains the folded form of dopamine. As a result of studies on invertebrate dopamine receptors, using norsalsolinol and a variety of substituted phenylethylamine derivatives, it was suggested (Woodruff, 1971) that the active conformation of dopamine was contained in the molecule 2-amino-6,7-dihydroxy 1,2,3,4 tetrahydronaphthalene (ADTN). In the extended form, dopamine can exist in the α or β rotomeric extremes corresponding to 2 amino, 5,6-dihydroxy (56 ATN) and 2-amino-6, 7-dihydroxy 1,2,3,4 tetrahydronaphthalene respectively. Since the original proposition that ADTN was the active molecule it has been shown to be a potent dopamine agonist, whereas 56 ATN and norsalsolinol have been found to be less active or inactive in behavioural, biochemical and electrophysiological assays (Woodruff, et al. 1977, Woodruff, et al. 1979, Woodruff, McCarthey and Walker, 1976). ADTN can itself exist in two enantiomeric forms as it has an ayssymetric carbon atom next to the nitrogen atom. It has been shown that the R-(+)-enantiomer is the more potent of the two in behavioural, biochemical (Andrews, et al. 1978) and invertebrate preparations (Batta, Woodruff and Walker, 1979).

The experiments described in this thesis were intended to show whether sulpiride would affect dopamine responses mediated via auto receptors or post synaptic receptors in the striatum. In addition by using both

substituted and rigid analogues of dopamine such as ADTN, the structural requirements for the somatodendritic autoreceptors could be determined.

Drug class and drug	Relative affinity for muscarinic receptors $^{1}/_{IC50}$	Frequency of extrapyramidal side effects
DIBENZODIAZEPINE		
Clozapine	385.0	1 (v. low)
PIPERIDINE PHENOTHIAZINE		
Thioridazine	66.7	2 (low)
ALKYLAMINO PHENOTHIAZINE		
Promazine	5.2	3 (medium)
Chlorpromazine	10.0	
Trifluopromazine	10.0	
PIPERAZINE PHENOTHIAZINE		
Acetophenazine	0.91	4 (high)
Perphenazine	0.93	
Trifluoperazine	0.91	
Fluphenazine	0.91	
BUTYROPHENONE		
Haloperidol	0.21	5 (v. high)
SUBSTITUTED BENZAMIDE		
Sulpiride	no data	2 (low)

Table 1.3. Different classes of antipsychotic drugs, data modified from Snyder et al. 1974.

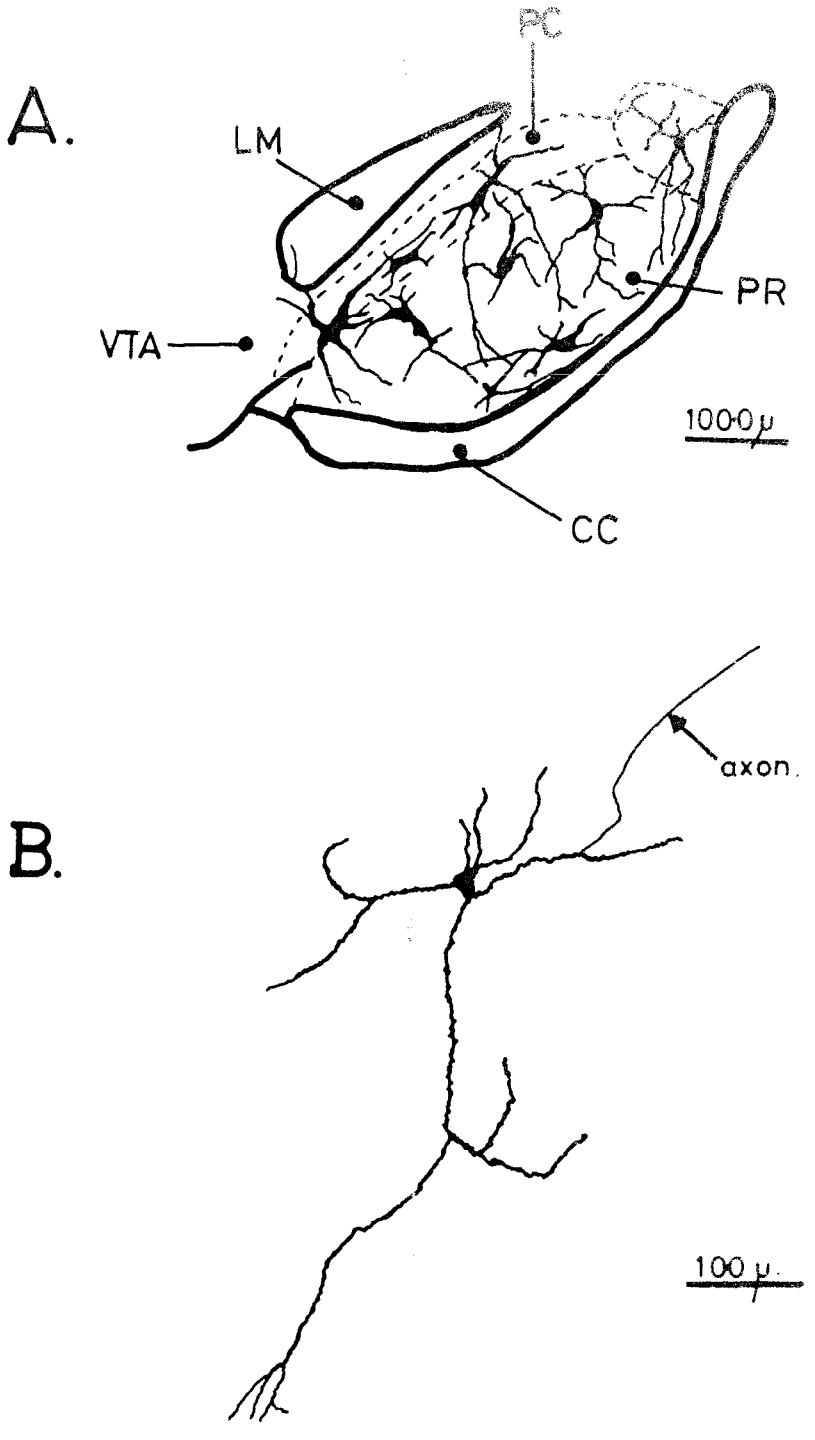
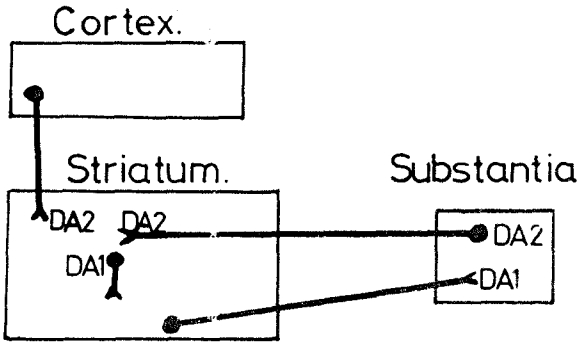


Figure (1·3)· A: coronal section through the rat substantia nigra to show the structure of both compacta & retiaulata neurones. (from Juraska, et al. 1977). LM; lemniscus medialis, VTA; ventral tegmentum, PC; pars compacta, PR; pars reticulata, CC; crus cerebri·

B: coronal section of a compacta neurone·



DA1 = cyclase linked dopamine receptors.

DA2 = non cyclase " " " .

Figure (1.4) Distribution of dopamine receptors in the nigrostriatal system. (from Kebabian and Calne 1969.)

CHAPTER 2. METHODS

2.1. Electrophysiology

2.1.1. Preparation of animals

Male albino wistar rats weighing between 150 and 200 g were anaesthetized with urethane 1.5-2g/kg, or chloral hydrate 350mg/kg. In the latter case the animals were cannulated via the left or right femoral vein for the administration of more anaesthetic or other drugs. In many cases the chloral hydrate anaesthetized animals were maintained on 1-2% halothane in 100% O_2. This allowed a lighter level of anaesthesia to be used during the experimental period. To enable the electrodes to be accurately positioned in their respective brain areas, the rat's head was secured in a Narishigè stereotaxic frame and the micromanipulators zeroed on the skull landmark Lambda. It was found that the animals maintained their body temperature if lightly anaesthetized. The skin on the animal's head was parted and the skull overlying the area to be studied was removed with a dental drill. The dura was carefully removed and the exposed cortex was covered in 0.9% saline to prevent drying of exposed tissue. Co-ordinates used for the brain areas studied were taken from the stereotaxic atlas of the rat brain by Konig and Klippel (1963) and corrected for the weight of the rat. Co-ordinates for the following areas in a 150gm rat were: substantia nigra A 1.8-2.2, L 1.6-2.2, D 6.4-8.0, ventral tegmental area A 1.6, L 0-0.6, D 6.8-7.8, nucleus accumbens A 8.9-9.8, L 0.8-2.0, D 5.0-6.2, caudate nucleus A 6.5-9.6, L 1.0-3.8, D 2.0-6.2, the depth D being measured from the surface of the brain.

2.1.2. Recording and iontophoresis

Extracellular recordings and iontophoresis were achieved by the use of specially constructed six or eight barrel microelectrodes. The construction of these has been previously described (Crossman, Woodruff and Walker, 1974) but in this study several modifications were employed. Prefibred pyrex glass tubing (Clarke Electromedical GC150-15, 1.5mm o.d., 0.86mm i.d.) was cut into 7cm lengths. Seven lengths of electrode glass were then held together with heat shrink tubing (R.S. 4.8mm) in a manner shown in the figure 2.1 (A) where the tubes were held together with wire springs. The bundle of glass tubing was mounted in a Narishigè vertical electrode puller. The central section of the glass was heated to softness, the heating coil was left on while the bottom chuck of the electrode puller was rotated through 180 degrees to fuse the individual barrels together. The glass was allowed to cool and then the electrode was pulled in the usual manner to

the appropriate dimensions by adjusting the heating current and magnetic pull. The seven barrels were then held together permanently by placing some super epoxy adhesive round the base of the shaft (fig. 2.1, B). The electrode tip was then broken back under microscopic control with a clean glass rod to produce the desired tip diameter and shape, approximately 7μ diameter (fig. 2.1, D). The shafts of six of the seven barrels were then bent outwards over a fine flame to accomodate the iontophoretic leads (fig. 2.1, C). A single barrel electrode was pulled from the same type of glass and two right angle bends were made near the shaft by using a small flame, so that its shape roughly corresponds to the outline of the multibarrel electrode. The two microelectrodes were held in micromanipulators and under microscopic control were brought alongside one another with the tip of the single barrel electrode protruding some 10-20μ beyond the multibarrel assembly tip. The tips of the electrodes were secured together by applying super epoxy adhesive along their length. When this was set after approximately ten minutes, super epoxy adhesive was applied around the base of the two shafts, firmly holding them together, the finished electrode tip is shown in fig. 2.2.

Figure 2.3 illustrates the equipment used for recording and displaying action potentials. Extracellular recordings were made through the protruding barrel of the microelectrode, which was filled with 2% pontamine sky blue dye in 0.5M NaCl. A silver wire was inserted into this recording barrel and connected to a differential field effect transistor which acts as a voltage follower. The signal was then increased by a Tektronix 122 low-level preamplifier, passed through a 50Hz filter and displayed on a Tektronix 502A oscilloscope. The signal was then passed through variable high and low pass filters to remove as much high and low frequency background noise as possible before passing through a spike processor (Digitimer D 130). The digital output from this was displayed on a servoscribe penrecorder as a continuous ratemeter recording. An output was fed into a loudspeaker to assist in finding neurones and also into a polygraph (Grass 79 D) or camera (Nikon Komden Kogyo Co. Ltd., Continuous Recorder PC 2A) for continuous spike records. When required permanent recordings of the extracellular potentials were made on a Racal Thermionic Store 4 tape recorder, such data could then be processed at leisure.

Of the iontophoretic barrels one always contained one of the following, 1.5M NaCl, 0.02M Na Acetate, 0.02M Na Tartrate. These were used to balance the

net current at the electrode tip to zero, or to expel ions in order to check for the effects of iontophoretic currents. The remaining barrels each contained one of the drugs which are listed in table 2.1 with the concentration and pH used in the experiments. D, L-Homocysteic acid and L-Glutamate were ejected as anions. All other drugs were ejected as cations, as it was assumed from their structures that at low pH they would be positively charged. Catecholamines were dissolved in 8mM tartaric acid to prevent degradation during iontophoresis. MIF was dissolved in either tartaric acid (3mM), acetic acid (20mM) or distilled water. TRH, enkephalin analogues, substance P and analogues were dissolved in 20mM acetic acid. All other drugs used for iontophoresis were dissolved in distilled water. The pH of drug solution was adjusted to the appropriate value by the addition of small quantities of 0.1M HCl or NaOH. Drugs given intravenously were dissolved in 0.9% saline and the pH adjusted to 7.4 if necessary.

The resistance of the electrodes varied between 10 and 100MΩ and were sometimes greater than this. In spite of this it was often found that current balancing was unnecessary in the substantia nigra although in the nucleus accumbens and caudate nucleus it was required. Retaining currents of 5-10nA were used to keep drugs in the electrodes. Ejecting currents were usually in the 30-90nA range. When comparing the potencies of drugs regular pulses were used to overcome the "warm up" effects due to the removal of drug from the tip of the electrode by the retaining currents (Bradshaw, Roberts and Szabadi, 1973). The iontophoretic currents were monitored on ammeters and controlled by a custom built 5 channel iontophoresis unit.

2.1.3. Release of drugs from microelectrodes

While it is known that peptides such as substance P are released from iontophoretic electrodes (Krnjevic and Morris, 1974), it is uncertain whether the tripeptide MIF is. In view of this uncertainty it was necessary to test for release. Four multibarrel electrodes were constructed as previously described. Five barrels of each electrode were filled with MIF (50mM) the pH having been adjusted with HCl prior to filling the electrode. The recording barrel of the electrode remained empty. This enabled a maximum drug current of 500nA to be passed from each electrode, 100nA through each barrel. In order to eject nMole quantities of drug an ejection time of 4-5 hours was thought to be sufficient. As there is no sensitive bio or immunoassay the peptide was measured chemically by amino acid analysis and thin layer chromatography.

Four different combinations of drug solution and current polarity were used:

Electrode 1, MIF pH 4.5 500nA eject 5 hours
" 2 " 4.5 " retain "
" 3 " 7.0 " eject "
" 4 " 7.0 " retain "

The MIF was iontophoresed into 1ml of distilled water in a Quickfit MF 24/0 tube, a silver earthing wire was immersed into the distilled water while the iontophoretic leads were inserted into the electrode barrels. The samples were freeze dried in their individual tubes and analysed for peptides (N. Petter, ICI Ltd.). All glass was cleaned with chromic acid before use.

The release of noradrenaline and dopamine from iontophoretic electrodes was also measured. These experiments were performed in conjunction with Dr J.A. Poat of the Dept. of Physiology and Pharmacology, Southampton University. A mixture of ^{3}H-dopamine (6.2 Ci/mmole)and cold dopamine (0.2M) was made up and injected into one barrel of a multibarrel electrode. The electrode was lowered into a 2ml volume of tartaric acid (0.5mg/ml, 3mM) while a constant 10nA retaining current was being applied to the filled barrel. Three different ejection currents were then applied for the following times with a sixty second retaining period between each ejection current, a one minute period was allowed for changing the sample tubes for different current intensities.

60nA : 4 x 1 min
30nA : 4 x 1 min
15nA : 10 x 1 min

Between each ejection a retaining current of 10nA was applied to stop spontaneous leakage of drug. The quantity of dopamine ejected into the 2ml of tartaric acid was then found with a scintillation counter and the average quantity released per nA of ejecting current worked out. This value was compared by the students t-test with the value for noradrenaline obtained by an identical method.

2.1.4. Stimulation and identification of neurones

Bipolar stainless steel electrodes (Rhodes Medical Instruments, SNEX 100) with a tip separation of 0.25mm and a coaxial configuration were stereo-

taxically positioned into the required areas using the stereotaxic atlas of the rat brain (Konig and Klippel, 1963). For the caudate nucleus of a 150 gm rat the electrode was inserted at an angle of 15^{o} in the coronal plane and the following co-ordinates were used, D being measured from the surface of the brain: L 4.6, A 6.0-8.5, D 2.8-5.5. For the substantia nigra the electrodes were positioned vertically at the following co-ordinates in a 150 gm rat: A 1.8-2.2, L 1.6-2.2, D 6.4-8.0.

The stimulation pulses were generated by a Devices gated pulse generator 2521, timed by a Devices digitimer 3290 and delivered to the stimulating electrode by a Devices isolated stimulator 2533. By using the digitimer single pulses or trains of pulses could be given at regular intervals. The effects of stimulation were monitored on a storage scope DM35A triggered by the digitimer. The stimulus current was indirectly measured by recording the voltage of the stimulus and dividing it by the resistance of the stimulating electrode. The parameters of stimulation were single square wave pulses of 0.1-1mA, 0.1-0.5msec duration delivered at between 0.1 and 100Hz. A block diagram of the apparatus is given figure 2.3.

Identification of neurones was achieved by monitoring the size, shape and latency of extracellularly recorded action potentials on a DM35A Tektronix storage oscilloscope. Antidromic potentials and orthodromic excitatory potentials could be easily detected with this system, however orthodromic inhibitions were less easily seen.

2.1.5. Analysis of Data

Electrophysiological data was presented in three different ways. Post-stimulus evoked responses were photographed from the storage oscilloscope. Spike records were made either on continuously moving film or on a polygraph. Rate meter recordings were made in three different forms, number of spikes per one, four or ten seconds. An example of the three different types of ratemeter recording are shown in figure 2.5.

2.1.6. Verification of electrode positions

In order to verify that the recordings were made from the correct brain areas pontamine sky blue was ejected, as an anion, from the recording barrel by passing a 5 μA current for ten minutes (Hellon, 1971). The brain was fixed at the end of the experiment by perfusing the rat through the aorta with 10% formol saline. The position of the dye spots was verified

histologically by examining serial slides of the brain which had been cut on a freezing microtome.

For verification of the position of the stimulating electrode, electrolytic lesions were made at their tips by passing a current of 2mA for 10 seconds.

Table (2.1.). List of drugs used and their source. The concentration and pH of solutions used for iontophoresis is given. Details of drugs given peripherally or centrally are given in the text. The structures of drugs are given in the appendix unless otherwise stated.

Drug	Concentration	pH	Source
Acetylcholine chloride	200mM	4-5	B.D.H.
ADTN:2-amino-6,7-dihydroxy-1, 2,3,4-tetrahydronaphthalene HBr.	40mM	4-5	Dr.J.D. McDermed
d-Amphetamine sulphate			S.K.F.
Angiotensin II	27mM	4-5	CIBA Geigi
Apomorphine HC1			MacFarlane Smith Ltd.
BW180C;d-leu^{2},met^{5}-enkephalin	100mM	4-5	Burroughs Wellcome
Chlorpromazine			May and Baker
CRM221;methylated TRH	50mM	4-5	Reckitt and Coleman Ltd.
Chloral Hydrate			B.D.H.
Dopamine HC1	200mM	4-5	Koch-Light
3,4-dihydroxy nomifensine	20mM	3-4	Hoechst
139462	50mM	3-4	Dr. J.S. Morely
Fluphenazine HC1	100mM	3-4	Squibb
GABA;gamma-amino-butyric acid	200mM	4-5	B.D.H.
L-glutamate	200mM	8-9	B.D.H.
Haloperidol			Janssen-Pharmaceutical
Halothane (Fluothane)			I.C.I.
His-Pro;histidyl-proline	60mM	4-5	Dr. B. Morgan
D L H;D,L-homocysteic acid	200mM	8-9	Koch -Light
MIF;MSH releasing factor	50mM	3-5	Sigma
96955	50mM	3-5	J.S. Morley
96956	50mM	3-5	J.S. Morley
96957	50mM	3-5	J.S. Morley
Nomifensine maleate	100mM	2-3	Hoechst
L-Noradrenaline bitartrate	200mM	4-5	Koch -Light
Sagatal			May and Baker
Substance P (SP)	0.08mM	3-4	Beckman
SP1-2	0.7mM	3-4	J.S. Morley, I.C.I.
SP1-9	0.1mM	3-4	structures in figure
SP4-9	0.2mM	3-4	" " "
SP5-9	0.2mM	3-4	(4.3) " " "
SP6-11	saturated soln.	3	" " "
SP6-11pyroglutamyl analogue	" "	3	" " "
spant			" " "
erp			" " "
aa			" " "
aa			" " "

Table (2.1) continued.

Drug	Concentration	pH	Source
(±),(+),(-)-sulpiride	100mM	5.5	Ravizza s.p.A.
TRH-thyrotropin releasing hormone	50mM	4-5	Sigma
p-tyramine HCL	200mM	4-5	Koche Light

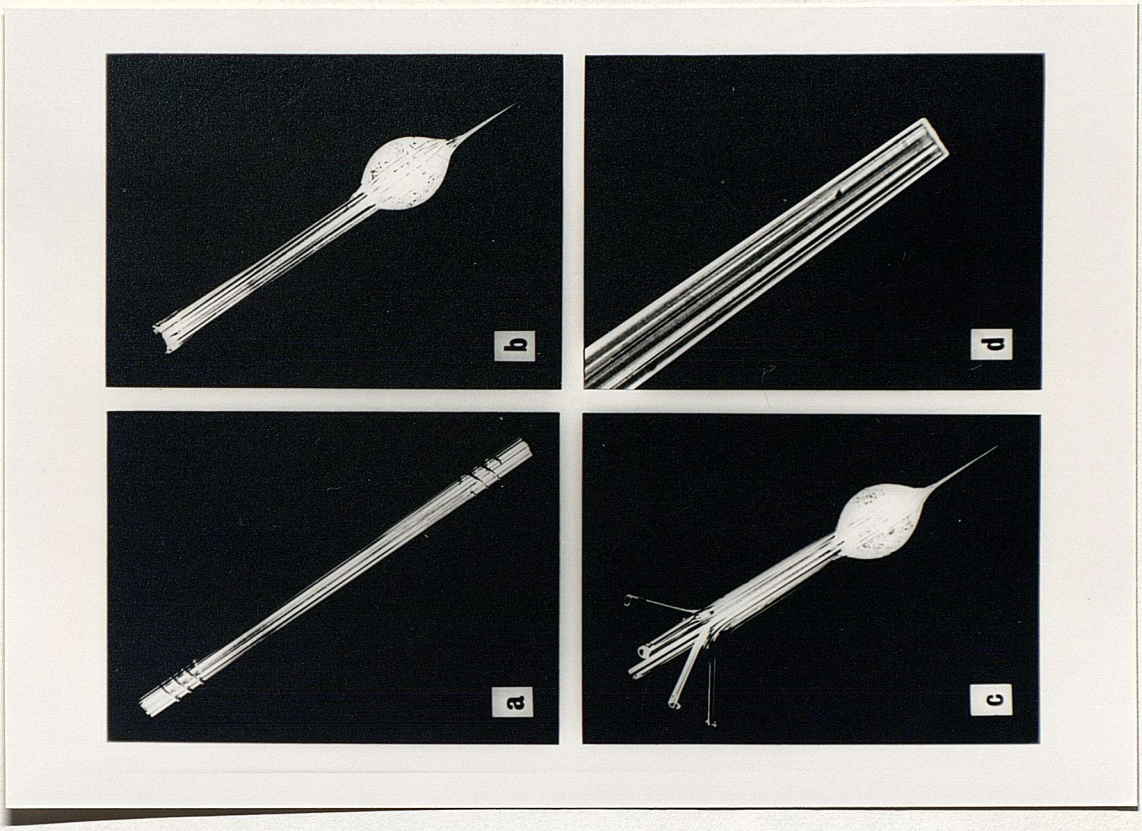


Figure 2.1. Stages in the construction of a multibarrel electrode, see text for details, from S. Batta, Ph D thesis.

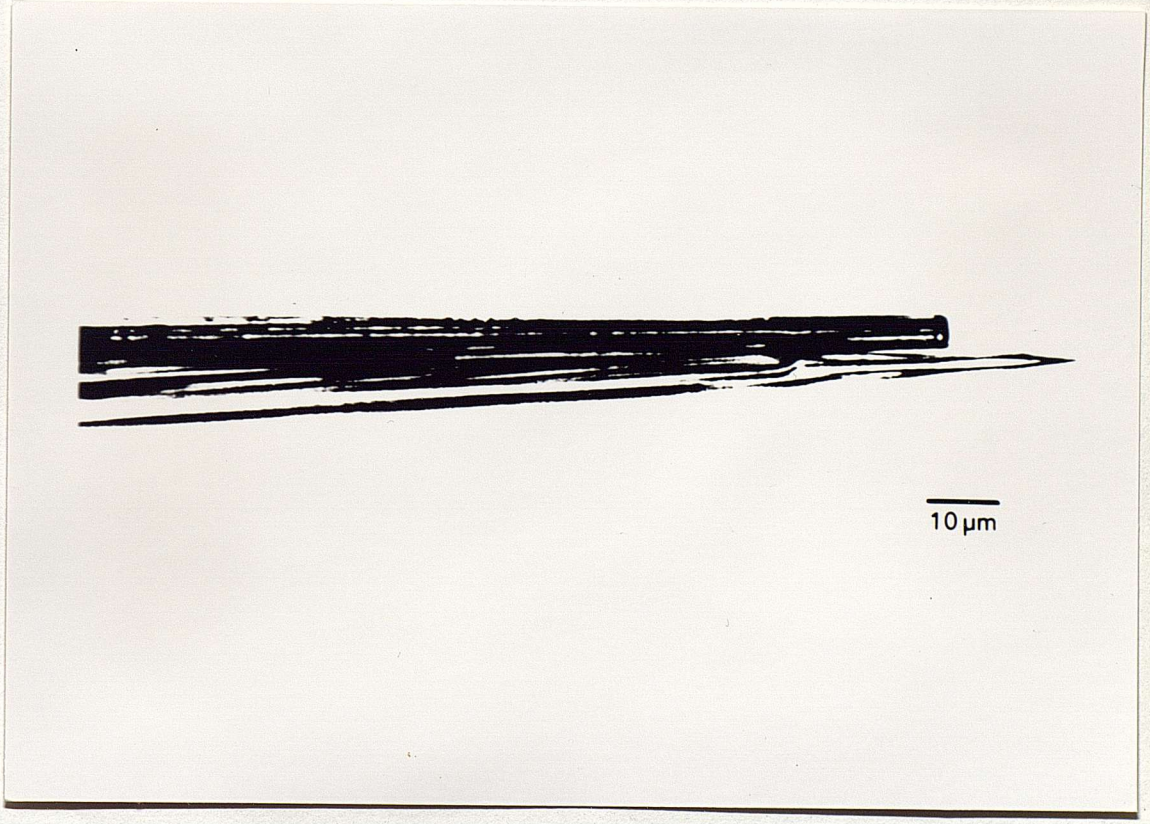


Figure 2.2. Magnified photograph of the tip of a recording and iontophoresis electrode. The recording barrel protrudes some 20u, from Crossman, Woodruff and Walker, 1974.b

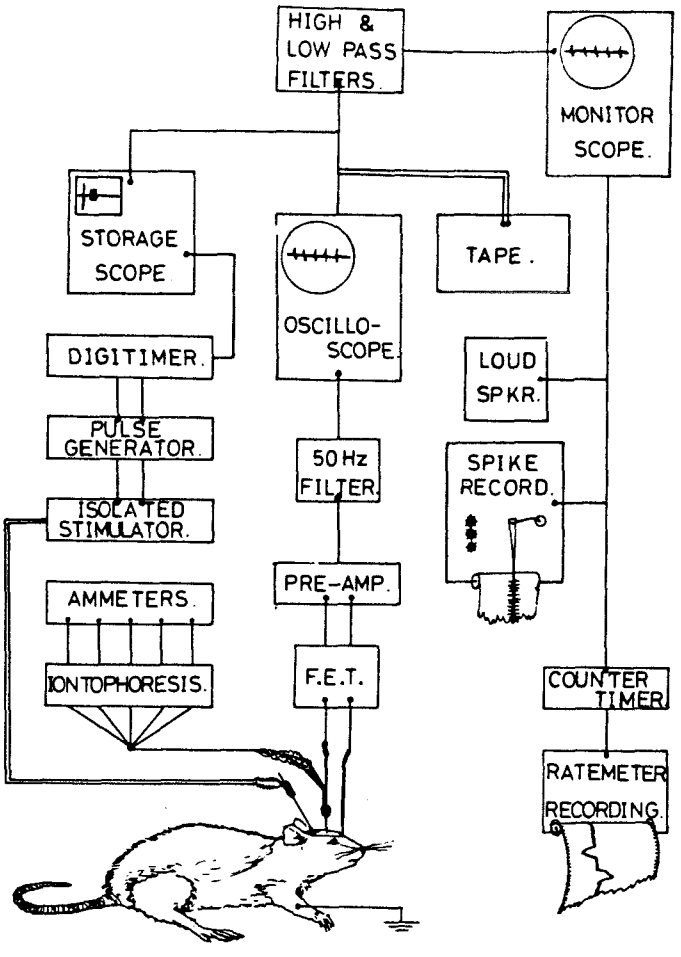


Figure 2.3. Block diagram of the recording, stimulating and iontophoresis equipment.

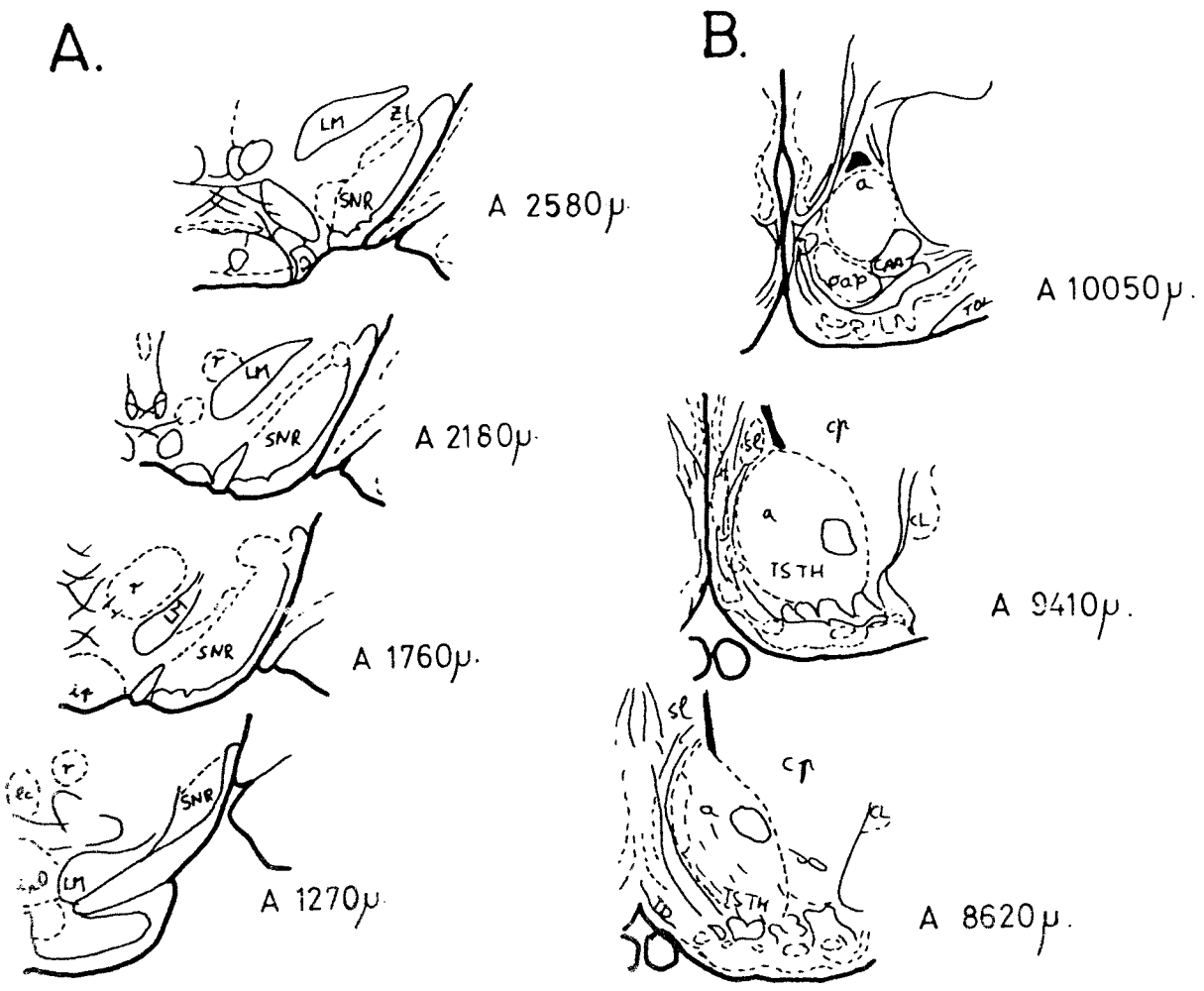


Figure (2-4). Serial coronal sections through the rat brain at the level of A: the substantia nigra, and B: the nucleus accumbens.
(From Konig & Klippel)

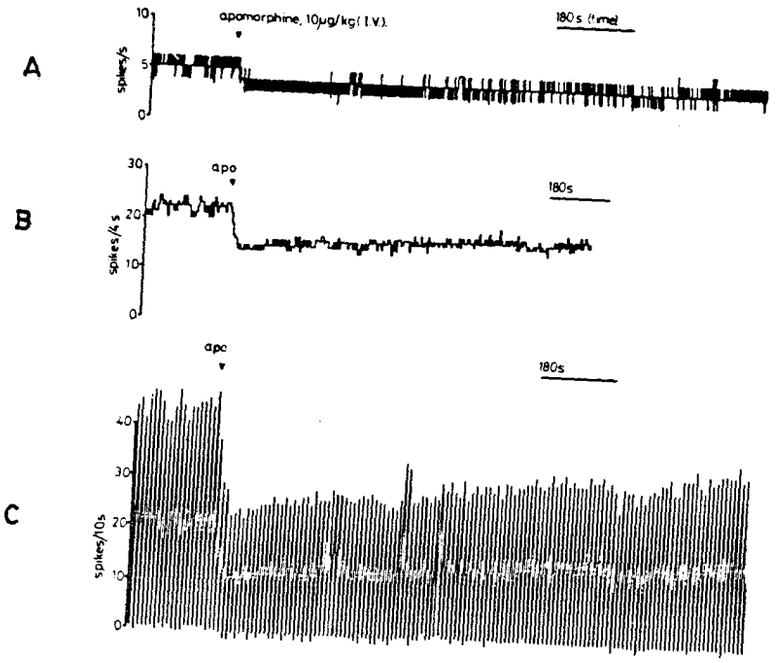


Figure 2.5. Three ratemeter recordings made simultaneously from a neurone showing the three different forms in which an identical drug response have been illustrated in other figures.

2.2. Behaviour

2.2.1. Preparation of animals for chronic cannulae

Intracerebral injections into the nucleus accumbens and ventral tegmental area of conscious rats were achieved via permanently-implanted cannulae. The cannulae were made to the design shown in figure 2.6. Male wistar rats weighing between 180 and 200 gm were anaesthetized with 4% Halothane in 100% O_2 and their heads secured in a stereotaxic frame (David Kopf 900). A saggital incision was made in the scalp and the skin retracted to expose the skull. The surface of the skull was thoroughly cleaned to remove connective tissue and allowed to dry. The features on the skull surface were then easily visible. The co-ordinates for the cannulae to be aimed at were worked out using the stereotaxic atlas of the rat brain by Konig and Klippel (1963) and corrected for the weight of the rat. Co-ordinates for the following areas in a 150 gm rat were: nucleus accumbens A 9.4, L 1.2, D 6.0, ventral tegmental area A 1.6, L 0.6, D 6.8, the depth D being measured from the surface of the brain. In order to avoid puncturing major blood vessels and to permit easier placement of cannulae, the cannulae were implanted at an angle of 12^{o} to the vertical plane. Thus the co-ordinates on the skull surface for the nucleus accumbens cannulae were: 9.4mm anterior from the lambda and 2.4mm from the saggital suture, for the VTA cannulae 1.7mm from the lambda and 2.3mm from the saggital suture. In both accumbens or VTA cannulated animals a similar procedure was adopted for implantation of cannulae. Using a 1.0mm dental steel burr, two holes were drilled in the skull approximately 4mm anterior from lambda and 1mm either side of the saggital suture. A 1/8 inch stainless steel screw was placed into each of these. The purpose of these two screws was to secure the subsequently applied dental cement to the skull. After placing the screws in these holes two further holes were drilled through the skull at the appropriate co-ordinates for either accumbens or VTA cannulae. The dura beneath these holes through which the cannulae were to be inserted was punctured with a sterile hypodermic needle. A cannula, sterilized in absolute alcohol and dried, was attached to the holder on the frame and slowly lowered at an angle of 12^{o} into one of the holes. The cannulae was then fixed in position using acrylic dental cement (DeTrey) and after allowing the cement to set for 15 minutes, the cannulae holder was carefully unscrewed and withdrawn. The procedure was repeated to implant the cannulae on the other side and cement was applied liberally around both cannulae and the screws so that there were no sharp points to cause irritation to the animal. The cement was allowed to harden for 20 minutes, the cannulae holder was removed and the stylets were inserted into the cannulae. A small smount of

antibiotic powder (Cicatrin) and local anaesthetic (Lignocaine) was then applied, and the incision closed with cotton sutures. This prevented infection and reduced irritation in the immediate post operative period. The rats were allowed to recover in a warm room. Over the next few days they were accustomed to handling and were ready for use within one week of surgery.

2.2.2. Intracerebral and peripheral injections

Drugs were injected into the nucleus accumbens or VTA (ventral tegmentum) of the conscious, manually restrained animal via the previously implanted cannulae. A 5 µl glass microsyringe (Hamilton) fitted with a length of polythene tubing (Portex PP10) and a 30 gauge needle (90° flat cut tip) was used for this. It was necessary for the tip of the injection needle to protrude from the end of cannulae by a certain distance depending on the brain area. As the cannulae were of standard dimensions, this was achieved by fitting a plastic sleeve to the needle of a length such that the exposed portion extended below the cannulae tube by a distance placing the needle tip at the correct injection site. The drug was delivered in a 0.5 µl volume of CSF over a period of 10 seconds. The cannulae stylets being replaced immediately after injections. A period of 48 hours between injections was allowed.

Peripheral injections were given either Intraperitoneally (I.P.) or subcutaneously (S.C.). Drugs were dissolved in 0.9% saline. A list of the drugs used and their source is contained in figure 2.4.

2.2.3. Experimental procedure

With the exception of one group of experiments, animals were placed in their activity cages at least half an hour before drug administration, so that they could become acclimatized to a new environment. The activity cages work on the same principle as animex cages. Eight animals were placed in eight activity cages, four were injected with drugs, the remaining four with control vehicle. Activity was counted every five minutes in both horizontal and if necessary vertical planes and printed both numerically and as a punched tape. The length for which activity was counted depended on the drug being used. Short acting drugs such as amphetamine were monitored for 1½ hours whereas longer lasting drug effects such as those produced by BW180C were monitored for 4 hours. Further drug administrations could be carried out during the period of recording, the counts during the

injection period being discarded.

2.2.4. Analysis of data

The data stored on punched tape could be run through a computer terminal and processed by computer, to give either a table or a graph of the mean, standard error and significance of the treatment verses control data (students unpaired t-test). Due to the inflexibility of the program this could not be used for all data. When this was the case data was sorted manually and then the statistical analysis was calculated using a programmable Hewlett-Packard calculator. Results were presented as histograms or graphs of horizontal movements against time.

2.2.5. Histological procedures

The rats were anaesthetized with a lethal dose of barbiturate.
Once anaesthetized the histological procedure was identical to that used in section 2.1.6.

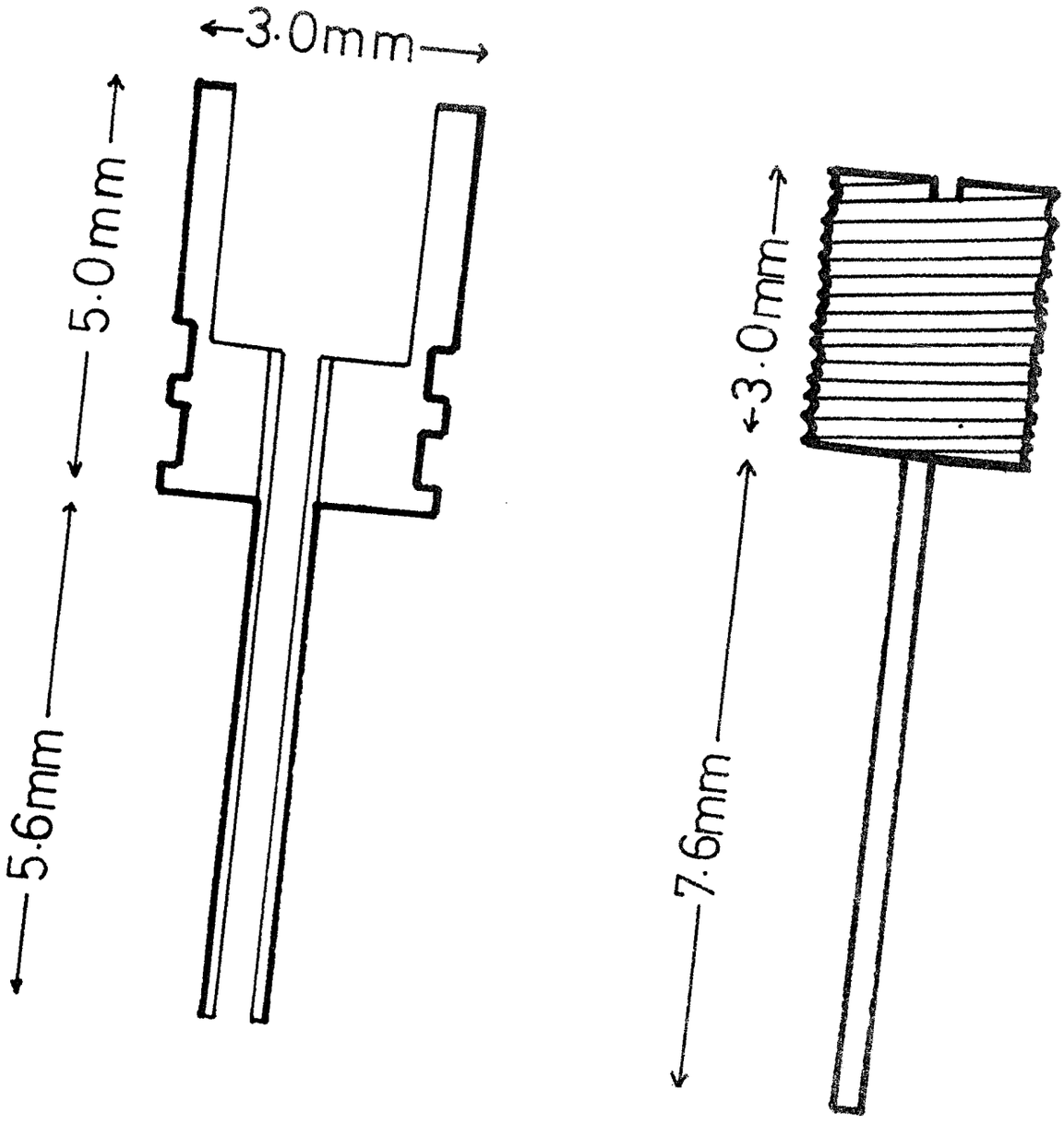


Figure (2.6). Cross section of the cannula used in both VTA and nucleus accumbens cannulated animals.

CHAPTER 3. RESULTS

3.1. Electrophysiology and iontophoresis

When recording from the substantia nigra it was usually unnecessary to use either glutamate or DLH filled electrodes in order to excite cells as most neurones were found to be spontaneously active. The firing rates of cells varied quite markedly. Cells in the zona compacta thought possibly to be dopaminergic (SNCD cells) fired at between 2 and 8 Hz and had wide action potentials up to 5msec in duration as previously reported (1.5.2.). In the mesencephalic reticular formation immediately above the nigra and in the zona reticulata itself neurones had activities between 0.1 and 60Hz, these cells had a shorter action potential and a much faster rise time than compacta neurones. In spite of the high density of cells in the zona compacta, it was usually only possible to record from one or two compacta cells in a single electrode tract. However, these compacta neurones always gave a very stable baseline firing rate which rarely varied by more than 10% in the absence of drugs. Reticulata neurones also gave stable baseline firing rates but these were more variable.

In the caudate nucleus and nucleus accumbens spontaneously active units were rarely encountered, making up less than 5% of units used. In these two areas an iontophoretic channel releasing either glutamate or DLH continuously or in brief pulses was a prerequisite for extracellular recordings. Frequently neurones would become overdepolarized if either excitant was continuously applied and cells would disappear. If this occurred excitants were applied in brief pulses and a reduction of the size of a glutamate or DLH induced burst of firing was taken as an indication of an inhibitory action by another drug such as dopamine. When this technique was used, the timing of the pulses of excitants applied to the neurones had to be at regular intervals. During the continuous application of excitants the inhibitory responses to drugs such as dopamine were usually of fast onset and short duration compared to a more leisurely action on spontaneously active neurones in the reticular formation and substantia nigra. Different types of neurones in specific areas of the caudate and acuumbens were not readily apparent, thus all neurones in their respective nuclei were considered together.

3.1.1. Iontophoresis of substance P and analogues

Substance P had an excitatory effect which was of slow onset and long duration on neurones in the substantia nigra. When applied with positive ejecting currents from 20mM Acetic acid solutions a total of 40 out of 115 cells

in the substantia nigra were excited by substance P. In the nucleus accumbens of ten cells on which substance P was tested five were not affected. Of the remaining five neurones in this area it is possible that the firing rate of three was increased while the other two were inhibited. The uncertainty over these results is due to their being obtained from neurones with poor spike size in which responses were either mimicked by current controls or were not reproducable. Substance P was not tested on neurones in the caudate nucleus. A summary of these results is given in table 3.1.

The substance P analogues were only tested iontophoretically in the substantia nigra. Of the analogues, only one was seen to possess similar activity to substance P. Thus it was found that substance P 1-9 excited 29 neurones while 70 were unaffected. Only neurones excited by substance P were similarly affected by substance P 1-9 (fig. 3.1), this was tested on 12 neurones. A cross tachyphylaxis between substance P and substance P 1-9 was seen on three cells on which this was tested. The large number of neurones on which substance P and the 1-9 fragment gave positive results but were not tested against each other is due to both of these drugs being used as controls in the testing of the other fragments of substance P and the putative antagonists.

When testing any substance P fragment either substance P or substance P 1-9 was included in one of the iontophoretic barrels to ensure that the cells on which fragments were found to be inactive would actually respond to the parent molecule. Thus it was found that the N-terminal fragments of substance P, substance P 1-2, 4-9 and 5-9 were without effect on 20, 31 and 18 neurones respectively, (see figure 3.2). The C-terminal fragment of substance P, substance P 6-11 and its pyroglutamyl analogue were found to be inactive on 5 and 4 cells respectively. Similarly an eledoisin related peptide containing a similar C-terminal sequence to substance P, Arg-Ala-Phe-Ile-Gly-Leu-Met-NH_2, was also inactive on 20 cells, and while it did excite one cell this cell was not excited by substance P. A pyroglutamyl analogue of substance P, 140143, modified to give the following structure, pyroGlu-Phe(pNH_2)-Phe-Gly-Leu-Met-NH_2, was tested on 9 cells as an antagonist of substance P. Not only did it fail to change the response to substance P on any of these cells but it did not produce any response on its own. Similarly a modified amino acid, 140975, which has antagonist properties on the guinea pig ileum was without effect on the response to substance P or its 1-9 fragment on 5 cells. However, it did cause a similar excitation to

substance P on three cells after which the response to substance P was slightly reduced. A summary of the results obtained with fragments of substance P is given in table 3.2.

Substance P has been found to cause behavioural effects suggesting an activation of the ascending nigrostriatal and mesolimbic dopamine pathways. However, when applied to neurones in the SNC thought to be the cell bodies of the dopamine pathways (SNCD cells) no consistent effects were observed. Of the 4 cells on which dopamine had an inhibitory effect, 2 were excited by substance P, 70nA, 60 seconds and 2 were not affected by long applications. Additionally, on these latter 2 neurones substance P 80nA applied for 540 seconds failed to modify the response to a submaximal dose of dopamine, illustrated in figure 3.3.

3.1.2. Substance P behavioural studies

10 µg (6.6 nmole) substance P was dissolved in 0.5 µl artificial CSF and injected bilaterally into the ventral tegmentum (VTA) of chronically cannulated rats over a 60 second period. The animals were then returned to their activity cages. The activity counts obtained during the first five minutes were discarded. Substance P was found to produce a short term increase in locomotor activity when compared with CSF treated control animals. This is illustrated in figure 3.4. Thus when comparing the potencies of substance P fragments (using the students t-test) locomotor counts measured during the first twenty minutes were added together and plotted as a histogram. Thus it can be seen (fig. 3.5) that 3 and 10 µg (2 and 6.6 nmoles) of substance P cause a significant increase in locomotor activity while 1 µg (0.66 nmoles) does not. 10 µg (7.4 nmoles) of substance P 1-9 also produces a significant increase in locomotor activity while substance P 4-9 (10 nmoles) and substance P 5-9 (12 nmoles) do not. The response to substance P and substance P 1-9 was still present after a series of seven different injections in the same group of rats, although it appears to be slightly reduced, see figure 3.5, last two columns.

Bilateral injections of 5 µg of the modified C-terminal fragment of substance P, 140143, (5 nmoles) did not change the activity of any of the rats. The modified amino acid 146125 was injected intraperitoneally (I.P.) as it dissolved only in absolute ethanol, 40 mg/kg caused a slight non significant reduction in the locomotor response to bilateral injections of substance P, 10 µg/Side.

To summarise, it was found in both behavioural and iontophoretic studies that substance P 1-9 had similar actions to substance P, while substance P 1-2, 4-9 and 5-9 were without effect. Of the putative antagonists, the modified peptide 140143 was devoid of activity while the amino acid 146125 caused a non significant reduction in substance P hyperactivity and the amino acid 140975 had a partial agonist action when iontophoretically applied to neurones in the substantia nigra.

3.2.1. Effects of MIF on the firing rate of neurones

The tripeptide MIF was dissolved in either distilled water, 20 mM acetic acid or 3 mM tartaric acid and the pH adjusted to between 4.5 and 7. The final concentration of MIF was 55 mM. Iontophoresis of MIF onto neurones was carried out in the substantia nigra, caudate nucleus and nucleus accumbens as these are areas where dopamine is thought to have a physiological function. In the substantia nigra recordings were made from spontaneously active neurones. These included cells in the reticulata (SNR), compacta (SNC) and also in the mesencephalic reticular formation (MRF) immediately above the SNC.

MIF was without effect on cells in the SNC which responded to dopamine, as is clearly seen in figure 3.6. Similar results were obtained in the SNR and MRF where neurones do not respond to dopamine but aresensitive to GABA. MIF was tested on a total of 57 cells in the SNC, SNR and MRF with currents up to 100 nA for periods of between 20 and 180 seconds and in no cases did it produce consistent or reproducable effects.

In the caudate nucleus and nucleus accumbens most of the cells encountered were driven by DLH or glutamic acid applied with negative currents of between zero and 50 nA. MIF was tested on 22 and 35 units in the caudate and accumbens respectively. Using similar currents for similar periods of time to those used in the substantia nigra no consistent or reproducable changes in the firing rate of neurones in either the caudate or accumbens were observed. This is clearly illustrated in figure 3.7 which is a ratemeter recording from a cell in the caudate nucleus driven by DLH. This neurone like many others was inhibited by dopamine.

To see whether MIF has an indirect action on dopamine responses MIF was continuously applied with high currents of 80 to 100 nA during pulses of dopamine which had been previously found to be threshold doses. MIF was without effect on a threshold dose of dopamine on four cells in the caudate nucleus and five cells in the nucleus accumbens. This is illustrated in figure 3.8 which is a rate meter recording from a spontaneously active neurone in the nucleus accumbens.

Drugs which cause a change in the metabolism of dopamine in the brain have been shown to change the firing rate of neurones in the SNC (see section 1.5.). To see if MIF is acting indirectly on the dopamine systems, it was

injected intravenously while recording from a cell presumed to be a cell body of the ascending dopaminergic nigro striatal tract (SNCD cell). No change in the firing rate of these neurones was observed over a wide range of doses of MIF although apomorphine still inhibited these neurones and fluphenazine or chlorpromazine reversed this inhibition. This is illustrated in figure 3.9. A similar lack of effect with intravenous MIF was seen on two other SNC neurones.

To ensure that the apparent inactivity of iontophoretically applied MIF was not due to its being broken down after release before it reached a receptor, three metabolically stable α-aza analogues of MIF were tested on 14, 6 and 11 neurones in the substantia nigra, caudate nucleus and nucleus accumbens respectively. This is illustrated in figure 3.10 which is a ratemeter recording from a SNC neurone which is inhibited by dopamine. A summary of the results obtained with MIF and the three analogues in the substantia nigra, caudate nucleus and nucleus accumbens is given in table 3.3.

Therefore we can conclude that in the experimental conditions we used MIF is released with positive currents from microelectrodes. However, it does not appear to have an acute effect on the firing rate of neurones in the substantia nigra, caudate nucleus and nucleus accumbens. Neither does it have an acute indirect effect on dopamine responses or neurones and this lack of effect is unlikely to be due to rapid metabolism of the peptide.

3.2.2. MIF, behavioural studies

To observe whether the behavioural effects reported after peripheral administration of MIF were mediated by systems in the brain intimately involved with catecholamines, MIF was given to animals which had been pretreated with amphetamine. To see if the nucleus accumbens was involved in the response MIF was also administered to rats in which a hyperactive response had been induced by bilateral intraaccumbens injections of amphetamine.

A 2 mg/kg dose of amphetamine in 0.2 ml of 0.9% saline given intraperitoneally (I.P.) was found to produce a significant hyperactivity without much stereotypy. MIF was administered subcutaneously (S.C.) 20 minutes after injection of amphetamine, at this point the amphetamine hyperactivity had passed its peak. Thus any amphetamine potentiating property of MIF would be manifest as a partial or complete return to peak hyperactivity values. SC administration of 100 mg/kg MIF in 0.4 ml saline failed to change the shape

of the hyperactivity curve produced by 2 mg/kg amphetamine. Neither did MIF change the activity of control animals treated with 0.2 ml of saline instead of amphetamine.

10 µg amphetamine per side injected bilaterally into the nucleus accumbens in a 1 µl vol of 0.9% saline was found to produce a submaximal locomotor response which was significantly different to that produced by identical volumes of saline (see figure 3.11). SC injections of 100 mg/kg MIF given simultaneously with the intraaccumbens injections of amphetamine produced no change in the shape of the hyperactivity curve produced by amphetamine (see figure 3.12).

MIF is reported to be effective in the treatment of Parkinsonism, one of the side effects of neuroleptic drugs is iatrogenic Parkinsonism (section 4.5.1.). In experimental animals one of the effects of neuroleptics is sedation, thus the spontaneous exploratory activity of rats placed in a new cage can be reduced by neuroleptics such as haloperidol. We attempted to abolish this sedation with MIF to see if this has a common mechanism of action to the Parkinsonian side effects of neuroleptics.

Rats pretreated with 0.5 mg/kg haloperidol I.P. half an hour before being placed in the activity cages exhibited no spontaneous exploratory activity (see fig 3.13) while those treated with 0.25 mg/kg showed a slight non significant reduction in activity. The loss of exploratory activity was not affected by simultaneous injection of 1, 3, 30 or 100 mg/kg MIF given SC. A summary of the results is given in table 3.4.

We can conclude that MIF at the doses we used has no effects on the hyperactivity produced by amphetamine given either I.P. or bilaterally into the nucleus accumbens. Furthermore, over a wide range of doses over which it was reported to be behaviourally active (section 1.2) it failed to reverse the hypoactivity caused by haloperidol.

3.3.1. TRH, iontophoretic studies

The tripeptide TRH was applied to neurones in the substantia nigra, caudate nucleus and nucleus accumbens as there is some evidence that it interacts with dopamine in these areas as well as possessing actions in other areas of the brain (section 1.3.2.). The peptide was dissolved in distilled water, 20mM acetic acid or 3mM tartaric acid and the pH adjusted to 4.5. The final concentration of TRH in the microelectrode was 48mM.

In the substantia nigra TRH was applied to cells in the zona compacta (SNC), zona reticulata (SNR) and mesencephalic reticular formation (MRF) immediately above the SNC. Currents of up to 100nA for periods of 180 seconds failed to change the firing rate of 40 cells in these areas, although other drugs such as dopamine and GABA did have effects suggesting the microelectrodes were intact and functioning correctly.

As previously mentioned, in the caudate nucleus and nucleus accumbens most cells were silent and had to be driven by continuous application of amino acid excitants. TRH was without effect on 33 neurones in the caudate nucleus and 42 neurones in the nucleus accumbens. This is illustrated in figure 3.7 which is a ratemeter recording from a silent cell in the caudate nucleus. The effect of long applications of TRH, 80-100nA for 180 seconds was tested on the response to a threshold iontophoretic dose of dopamine on two neurones in the caudate nucleus and four neurones in the nucleus accumbens. Again no effects with TRH were observed, this is illustrated in figure 3.8.

A similar procedure to that used with MIF was used to record the effects of intravenous (I.V.) TRH on the firing rate of presumed dopamine neurones in the compacta of the substantia nigra and ventral tegmentum (VTA). This was carried out on only one cell in each area due to the scarcity of TRH. A slight increase in the firing rate of neurones in both areas was seen which was of relatively short (two minutes) duration. These cells responded to dopamine agonists and antagonists in the usual manner. It has been suggested that a methylated TRH derivative, CRM221/3, or a dipeptide breakdown product of TRH, $\overline{\text{His-Pro}}$, might have actions in the CNS. These two compounds and TRH were iontophoretically applied to eight neurones in the substantia nigra of which three responded to dopamine and were identified as being nigrostriatal neurones by antidromic spikes following electrical stimulation of the ipsilateral caudate nucleus. The three drugs were also

applied to 11 and 13 neurones in the caudate nucleus and nucleus accumbens respectively with high currents of 100nA for up to 180 seconds. None of these drugs, TRH, CRM221/3 or [His-Pro] affected the firing rate of neurones in any of the areas in which they were tested, this is illustrated in figure 3.15, and a summary of the results is given in table 3.5.

In addition to injecting TRH intravenously (10 mg/kg), CRM221/3 was also injected intravenously (10 mg/kg) while recording from a presumed dopamine containing cell in the SNC. A similar result to that obtained with TRH was obtained, that is a short duration increase in firing rate, illustrated in fig. 3.14. The increase in firing rate of the neurone was also accompanied by a visible rise in respiration rate which was not quantified.

Thus we can conclude from the electrophysiological studies that we have carried out that iontophoretic application of TRH, a stable methylated analogue CRM221/3, and a breakdown product [His-Pro] does not affect the firing rate of neurones in either the substantia nigra, caudate nucleus or nucleus accumbens. Additionally, the response to dopamine in the latter two areas was not affected by TRH. However, an increase in firing rate of presumed dopamine neurones in the SNC is seen following I.V. injections of TRH and CRM221/3.

3.3.2. TRH, behavioural studies

TRH is reported to have behavioural effects after peripheral administration. To see if these are mediated via catecholamine systems TRH was injected into animals pretreated with the catecholamine releasing agent amphetamine. An identical procedure to that used in the behavioural experiments with MIF was used. After the response to a peripheral injection of amphetamine (2mg/kg) had passed its peak TRH was injected subcutaneously. TRH was also injected peripherally into animals which had been stimulated by bilateral injections of amphetamine (10 µg/side) into the nucleus accumbens to see if this nucleus was important in the response.

In these experiments we found that TRH failed to modify the response to amphetamine, injected into the nucleus accumbens. However it did cause a return to peak amphetamine values after peripheral injection. This is illustrated in figure 3.16. However, in contrast to previous reports (Miyamoto and Nagawa, 1977) we could find no stimulation of locomotor activity after peripheral administration of TRH alone.

3.4. Angiotensin II, iontophoretic studies

High levels of angiotensin converting enzyme have been detected in the substantia nigra (section 1.1.7. and 4.1.) and there appears to be similarities in the metabolism of angiotensin and substance P (section 4.1.). In view of these reports angiotensin II was iontophoretically applied to neurones in both the zona compacta (SNC) and zona reticulata (SNR) of the substantia nigra from 0.02M solutions of acetic acid. Of 15 neurones on which angiotensin II was tested 14 were not affected by high (100 nA) currents for long (180 second) periods. While one neurone was excited the result was poor and could possibly be due to electrical artifacts. Cells on which angiotensin II was inactive responded to dopamine, acetylcholine and GABA implying that the electrodes were functioning normally. A summary of the results is given in table 3.1.

3.5.1. Opiod peptide, behavioural studies

Injection of opiate drugs into either the nucleus accumbens or ventral tegmentum (VTA) has been shown to cause hyperactivity (section 1.4.1.). There is some evidence that the pentapeptide (BW180C) is a more potent agonist at δ than μ receptors in peripheral preparations (section 4.4.) and that the modified peptide 139462 is a fairly selective antagonist at δ receptors. In addition it has been proposed that ethylketocyclazocine is a potent agonist at k receptors.

The chronically cannulated rats used in this study were placed in their activity cages half an hour before commencing the experiment to familiarise them with their new surroundings. Bilateral injections of BW180C, 5 μg/side (10 nmoles), in 0.5 μl artificial CSF were made into the VTA. The locomotor stimulation that followed appears to consist of two components. Immediately after injection there is a short duration hyperactivity. This is followed by a period of hypoactivity which gives way to a low level but continuous hyperactivity starting some 40 minutes after the BW180C injection and continuing for a further 60 minutes. This is illustrated in figure 3.17.

Therefore when analysing the data, activity during the first 15 minutes was summed and compared with CSF values. A similar procedure was adopted for the activity between 50 and 100 minutes. Injections of naloxone (2mg/kg SC) were made immediately before BW180C injection and 40 minutes after as the antagonist actions of this opiate antagonist are reported to be brief. The modified peptide 139462 (5 μg/side) was injected simultaneously in the same volume of CSF as the BW180C. Neither naloxone nor 139462 changed the initial hyperactive response to BW180C. Both naloxone and BW180C caused a non-significant reduction in the second slow onset long duration period of hyperactivity. Peripheral administration of naloxone or 139462 alone caused no change in the spontaneous locomotor activity of the rats. A summary of the results is given in table 3.6.

Bilateral injections of BW180C (10 μg/side (20 nmoles) in 0.5 μl of artificial CSF) into the nucleus accumbens resulted in a potent stimulation of locomotor activity. This commenced some 20 to 30 minutes after the injection of BW180C and continued for a further 70 minutes. This is illustrated in figure 3.18. Simultaneous administration of 139462 (5 μg/side) bilaterally into the accumbens or peripheral injections of naloxone (2 mg/kg) failed to change the shape of the hyperactivity curve. Activity was summed between

30 and 50 minutes to make statistical comparisons with CSF control injections. A histogram of the results is given in table 3.7. Ethylketocyclazocine which has been proposed as a k receptor agonist produced no effect on locomotor activity after bilateral injections of 10 µg/side in 1 µl of artificial CSF into the nucleus accumbens.

3.5.2. Opiod peptides, iontophoretic studies

In a continuation of the above study we attempted to block the iontophoretic effects of BW180C on neurones in the nucleus accumbens with concurrent iontophoresis of the modified peptide 139462. BW180C was tested on 26 neurones of which 20 were inhibited. The inhibition was fast in onset and of short duration, the inhibition ending soon after ending application of the drug. All 10 of the cells inhibited by BW180C on which dopamine was also tested were also inhibited by dopamine. However, four cells inhibited by dopamine were not inhibited by BW180C. Long applications (240 seconds) of the modified peptide 139462 with high currents (100 nA) failed to change the inhibitory response to BW180C on all 20 cells on which it was tested. An example of this failure to antagonise BW180C is given in figure 3.19.

There is some behavioural evidence that opiates interact with dopamine systems. Changes in the activity of the dopamine systems can be monitored by measuring the firing rate of cells in the substantia nigra presumed to be the cell bodies of the ascending dopaminergic nigro striatal tract. The effect of intravenous ethylketocyclazocine, a drug said to be a potent k receptor agonist, was tested on these cells. Three different doses, 1, 3 and 10 mg/kg, were tested consecutively on three separate neurones in the substantia nigra compacta. No changes in firing rate were seen while morphine produced the expected increase in firing rate which has been previously reported (sections 1.4 and 4.4.).

To briefly summarise the results obtained in the studies with opiates. The peptide BW180C was found to produce hyperactivity when injected bilaterally into the nucleus accumbens or ventral tegmentum (VTA). Ethylketocyclazocine had no such effects when injected bilaterally into the nucleus accumbens. The putative peptide antagonist 139462 and naloxone caused a non significant reduction in the hyperactivity produced by BW180C in the VTA, however no such effect was seen on the hyperactivity produced by BW180C in the nucleus accumbens. BW180C inhibited cells in the nucleus accumbens, this inhibition was not affected by 139462. Ethylketocyclazocine was without effect on the firing rate of cells presumed to be dopaminergic while morphine increased their firing rate.

3.6.1. Iontophoretic studies with dopamine agonists and antagonists in the substantia nigra

In the zona compacta of the substantia nigra there are a group of neurones which can be readily distinguished from surrounding cells by their unusually long action potential duration. These have been suggested as the cell bodies of the ascending nigrostriatal dopamine pathway (section 1.5 and 4.5). They have been abbreviated in this study to SNCD cells on some occasions. In addition they can be antidromically identified after electrical stimulation of the ipsilateral striatum. Iontophoretic or intravenous administration of dopamine agonists depresses the firing rate of these cells, an effect which is reversed by antagonists such as haloperidol.

The effects of several drugs known to act on dopamine systems was tested on these neurones. Dopamine was tested on 65 of these neurones. Of these, 60 were inhibited in a dose dependant manner (figure 3.20). Five were not affected by dopamine even at high currents (100 nA, 120 seconds). Dopamine never excited this type of neurone. Dopamine had been tested many times in the course of our peptide studies and these cells are not included in this section.

The substituted benzamide sulpiride was tested on the inhibitory response to dopamine on 12 neurones. Sulpiride was applied with currents of 20-50 nA. It showed dopamine antagonistic activity which was apparent 2-4 minutes after commencing application. This was rapidly reversible, complete recovery of the dopamine response returning within 10 minutes of ending sulpiride application. During the antagonism of the dopamine response the baseline firing rate of the cell increased slightly, returning to normal with the return of the response to dopamine. This is illustrated in figure 3.21. There are two optical isomers of sulpiride, d or + and l or -. Both were tested on neurones using similar currents and application times as were used for the racemate. The d isomer of sulpiride antagonised the response to dopamine on seven of the 11 cells on which this was tried. l-sulpiride antagonised the response to dopamine on all 11 cells on which it was tested. On four cells on which l-sulpiride completely reversed the response to dopamine, d-sulpiride was inactive. Additionally the inhibitory response to GABA on these neurones was not affected by either isomer of sulpiride, figure 3.22.

GABA (20-80 nA, 15-30 seconds) inhibited all eight cells on which it was

tested. The response remained during the application of sulpiride which antagonized dopamine in all these neurones.

Noradrenaline (30-60nA, 15-30 seconds) was applied to nine neurones which responded to dopamine. Noradrenaline produced a similar reduction in firing rate to dopamine, however the inhibitory effect was of shorter duration (figure 3.23). One cell identified by antidromic stimulation to be a SNCD cell was unaffected by either dopamine or noradrenaline. Both (-)-sulpiride and the racemate antagonised the noradrenaline response in a total of four cells.

Iontophoretic application of p-tyramine inhibited six cells which also responded to dopamine but failed to affect two other cells which were affected by dopamine. The response to p-tyramine was weak with low quantities (60nA, 20 seconds) (figure 3.23). However when higher currents were applied for longer periods of time (100nA, 90 seconds), a weak but long lasting inhibition of firing rate was apparent. Sulpiride antagonized the effects of p-tyramine on all four cells on which it was tested. A summary of the results is given in table 3.8.

It is of interest that all drugs which caused a decrease in the firing rate of the cells frequently caused an increase in the extracellularly recorded spike size (fig. 3.24). Excitants often cause a reduction in spike size.

2-amino,6,7-dihydroxy,1,2,3,4-tetrahydronaphthalene (ADTN) has been proposed as a dopamine agonist (section 1.5.). After obtaining a standard response to iontophoretically applied dopamine (60nA, 20 seconds), an empty barrel of the microelectrode was filled with ADTN while applying a 10nA retaining current. ADTN was then applied (60nA, 60-120 seconds) onto the neurones. On all six cells on which ADTN was tested it caused a 50-100% depression of firing rate accompanied by an increase in the spike size. The response to dopamine was immediately lost. The depression of firing rate slowly recovered to about 70% of its original value over an hour. However, no further responses to either dopamine or ADTN could be obtained using large currents (100nA, 120 seconds). This is illustrated in figure 3.25.

GABA was only tested on two cells on which ADTN was tested. On these cells the response to GABA appeared to be unaffected by ADTN. Sulpiride (60nA for 4 minutes) applied immediately before ADTN on two cells did not appear

to antagonise the ADTN response.

3,4-dihydroxy nomifensine has also been proposed as a dopamine agonist. It was tested on 10 dopamine type cells in the SNC. Of these six were not affected while the remaining four were only very weakly inhibited by large currents of 100nA for 120 seconds. Furthermore, there was no loss of the response to dopamine.

Nomifensine, 80nA for 120 seconds, produced no response on all seven cells on which it was tested. It did not appear to potentiate the response to dopamine on three cells on which this was tested, suggesting it does not block dopamine reuptake in this brain region. However when injected intravenously (I.V.) while recording from three dopamine containing cells it did appear to inhibit the firing rate. The ED50 being 1.47 ± 0.24 mg/kg (illustrated in figure 3.26).

It is well documented that drugs which may act on dopamine receptors will change the firing rate of neurones in the substantia nigra compacta thought to be dopaminergic (SNCD cells) (sections 1.5 and 4.5). Apomorphine was injected in logarithmically increasing doses during stable recordings from three SNCD cells. A dose response relationship was obtained (fig. 3.27) with the ED50 being between 3 and 50 µg/kg (15.4 ± 8.3 µg/kg). Many more of these SNCD cells were tested with single 10.30 or 100 µg/kg doses in other experiments to confirm that the cells being examined would respond to intravenous dopaminergic drugs. During the inhibition caused by any dose of apomorphine the spike size often increased, returning to normal as the ratemeter recording returned to its original baseline level.

Isoapomorphine was tested intravenously over a wide range of doses on three cells. No change in the firing rate of these cells was seen while apomorphine produced its expected inhibition of firing (Fig. 3.28).

The neuroleptics chlorpromazine and fluphenazine (0.5 mg/kg) reversed the inhibitory effect of 100 µg/kg apomorphine on three cells on which they were tested (fig. 3.9). However, no reversal of the effects of apomorphine could be achieved by 40 mg/kg of sulpiride, a dose which antagonises the behavioural effects of apomorphine, or other dopamine agonists.

After iontophoretic application of ADTN to SNCD cells they were insensitive

to further iontophoretic application of dopamine or ADTN. IV apomorphine (100 µg/kg) produced the usual depression of firing in two of four cells on which this was tested. On the other two cells a slight increase in firing rate was seen as the dose of apomorphine was logarithmically increased (fig. 3.29).

3.6.2. Iontophoretic action of sulpiride in the caudate nucleus

The majority of the cells in this area of study were silent. With continuous application of excitants such as DLH the cells tended to become over-depolarized and would go into "depolarization block". Thus it was necessary to apply excitants in pulses. Reduction in the size of the resulting burst of firing induced by simultaneous administration of another drug was taken as an inhibitory effect of this other drug. While most of the neurones were in the rostral caudate, some were in the dorsoventral accumbens. However no distinction has been made between them in this study.

0-20 nA of DLH or glutamate was found sufficient to drive most silent cells. Twenty-nine cells were found which did not respond to dopamine. Three of these were orthodromically activated following electrical stimulation of the ipsilateral substantia nigra. The remaining 37 cells encountered were inhibited by iontophoretic application of 30-80 nA dopamine applied for 30-60 seconds. 30-60 nA sulpiride antagonised the response to dopamine on five cells but was without effect on the response to dopamine on 14 neurones. The antagonism followed a similar time course to that in the substantia nigra, being apparent within four minutes of starting the sulpiride application and rapidly reversible.

On three neurones the response to dopamine was blocked by both sulpiride and fluphenazine (50 nA, 5 minutes) while on a further two neurones neither antagonised dopamine. Thus there appears to be an iontophoretic action of dopamine which is insensitive to neuroleptics.

The (+)-isomer of sulpiride at similar currents to the racemate reversed the response to dopamine on one out of five neurones, (-)-sulpiride antagonised dopamine on two out of six neurones (fig. 3.30). These preliminary results indicate that the (-) enantiomer is the more potent of the two.

A summary of the results with sulpiride is given in table 3.9.

3.7.1. Electrical stimulation of the substantia nigra

Electrical stimulation of the substantia nigra 10-60V, 0.1-0.5 msec duration, 0.1-50 Hz) produced a single spike excitation in the 11 cells in the ipsilateral striatum (fig. 3.31). Similar results were obtained with all the different stimulus parameters used. The latency of the spike was some 15 msec, suggesting a conduction velocity of 1.0 ms^{-1}. The single spike excitation was of remarkably constant latency not usually varying by more than 1-3 msec. However it was presumed to be orthodromic because collision of spikes did not occur when these normally silent cells were induced to fire by DLH. None of these cells could be inhibited by iontophoretic dopamine. The average conduction velocity of the spikes was 1.1 ± 0.07 ms.

Occasionally other cells were seen to be affected by stimulation of the substantia nigra. A slow latency inhibition was seen on two cells which also responded to dopamine, however it is probable that many more could have been found using a post stimulus time histogram (PSTH).

3.7.2. Electrical stimulation of the striatum

Following electrical stimulation of the striatum , four different effects were seen. In the zona compacta of the nigra (SNC) two types of neurone were found to be activated. The first type were rarely encountered (two cells). These exhibited a constant latency spike. The latency was between 2 and 3 msec. However, these could not be identified as antidromic spikes as they were silent cells (fig. 3.32). The second type of cell was more frequently found. These have a firing pattern and action potential shape typical of those neurones said to be the soma of the ascending dopaminergic nigrostriatal tract (SNCD cells). Some 30 of these neurones were found which could be activated by stimulating the ipsilateral striatum. They have a slow latency of between 15 and 30 msec, the conduction velocity being < 1.0 ms^{-1}, typical of small diameter unmyelinated fibres. The simulus parameters usually required to activate these neurones were a stimulus of 0.3-0.5 msec duration, delivered at less than 50 Hz with voltages of 10-60 V (0.5-3.0 mA). Frequently only the initial segment of the spike was apparent (fig. 3.33). The spikes were shown to be antidromic by their absence through collision if a spontaneous action potential occurred in a period before or after the striatal stimulus equal to or less than the latency of the antidromic spike (fig. 3.34). Rapid repetitive stimulation (50 Hz) of the striatum caused a slight reduction in the conduction velocity on the four cells on which this was tried (fig. 3.35). On a further 2 cells two extracellularly recorded antidromic spikes after one stimulus pulse had been

applied to the striatum were seen. This did not occur after every stimulus, however when it did occur these double spikes were present only as the initial segment (fig. 3.36). Both these and single antidromic spikes were absent on some occasions due to collision with spontaneous action potentials if these occurred in the period immediately before or after the stimulus which was less than the latency of the antidromic spikes. In addition to antidromic spikes, two orthodromic spike patterns are seen after electrical stimulation of the ipsilateral striatum. In the zona reticulata (SNR) an inhibition of spontaneous firing is seen between 10 and 100 msec after stimulation of the striatum. This was found on nine cells (fig. 3.37).

In the mesencephalic reticular formation immediately above the SNC more than 30 neurones were found which were excited by striatal stimulation. The latency of this excitation varied between five and 12 msec (fig. 3.38) and was usually followed by a period of inhibition lasting some 100 msec. No statistical analysis was carried out on the latencies of excitation or inhibition on this particular group of neurones. The stimulus parameters for these orthodromic effects were similar to those for antidromic stimulation of the SNCD cells.

3.7.3. Position of stimulating and recording electrodes in the brain

At the end of an experiment a 5 µA current was passed through the recording electrode to deposit Pontamine Sky Blue dye at the position of the neurone which was last recorded from. The animals were then perfused through the aorta with 10% formal saline and sectioned. Histological examination confirmed that the electrode was at the expected co-ordinates. This is illustrated in fig. 3.39 and 3.40. To mark the position of the stimulating electrode a 10 mA current was passed across its tip. The position was marked by a small hole in the brain tissue (fig. 3.39 and 3.40).

3.7.4. Position of cannulae in the rat brain

The animals were killed and the brains examined by a similar procedure to that for the stimulating electrode. The cannulae can be seen to be in either the nucleus accumbens or the ventral tegmentum (fig. 3.41 and 3.42).

3.8.1. Release of drugs from micropipettes

Amino acid analysis of peptide released from multibarrel electrodes containing MIF found it was released with positive currents. At pH 4.5, 0.3 pmol/nA/min and at pH 7.0, 4.5 pmol/nA/min of MIF was released.

Measurement of tritiated noradrenaline and dopamine from electrodes found that 1.02 $\pm$ 0.14 pmol/nA/min of dopamine and 1.25 $\pm$ 0.13 pmol/nA/min of noradrenaline were released with positive currents. There is no significant difference between these two quantities.

3.9.1. Potency of substance P fragments and putative antagonists

This work was carried out by J. Growcott, I.C.I. Pharmaceuticals Ltd. The putative peptide antagonist 140143 has an IC50 value of 3.0×10^{-8}M. IC50 values for the two amino acid derivatives 146125 and 140975 are 7.4×10^{-7} and 3.2×10^{-5}M respectively. These values were obtained from experiments on the isolated guinea pig ileum.

The eledoisin related peptide 81495 has 0.1% of the potency of substance P in the ileum. Substance P fragment 134135 (SP1-9) was tested on three peripheral assays. It was found to be at least 1,000 times less active than substance P on all three. See table 3.10.

3.9.2. Potency of opiate agonists and antagonists on peripheral preparations

This work was carried out by J. Shaw, I.C.I. Pharmaceuticals Ltd. A summary of the results is given in table 3.11.

These values were obtained from experiments on isolated mouse vas deferens and guinea pig ileum.

PEPTIDE	AREA OF BRAIN								
	Substantia nigra			Caudate nucleus			Nucleus accumbens		
	+	0	-	+	0	-	+	0	-
Substance P	40	75	0	NT 0	0	0	3?	5	2?
Angiotensin II	1?	14	0	NT 0	0	0	NT 0	0	0
TRH	0	40	2?	0	33	1?	0	42	2?
MIF	0	57	3?	0	22	2?	0	35	0
TRH + DA	NT 0	0	0	0	2	0	0	4	0
MIF + DA	NT 0	0	0	0	4	0	0	5	0

Table (3.1). Summary of the results of the iontophoretic studies with different types of peptides in different brain areas.
+ = increase in firing rate
- = decrease in firing rate
0 = no change in firing rate.
Cells were in both the compacta and the reticulata of the substantia nigra, mainly the rostral part of the caudate nucleus and the medial part of the nucleus accumbens.
? indicates that these results are of poor quality due to poor action potentials, mimicking of the response by current controls, additionally they were not confined to the particular region of the area under study.
NT : Not tested

Peptide fragment	Response		
	-	0	-
SP	40	75	0
SP 1-2	0	20	0
SP 4-9	0	31	0
SP 5-9	0	18	0
SP 1-9	29	70	0
SP 6-11	0	5	0
SP 6-11 pyroglu	0	4	0
ERP (81495)	1	20	0

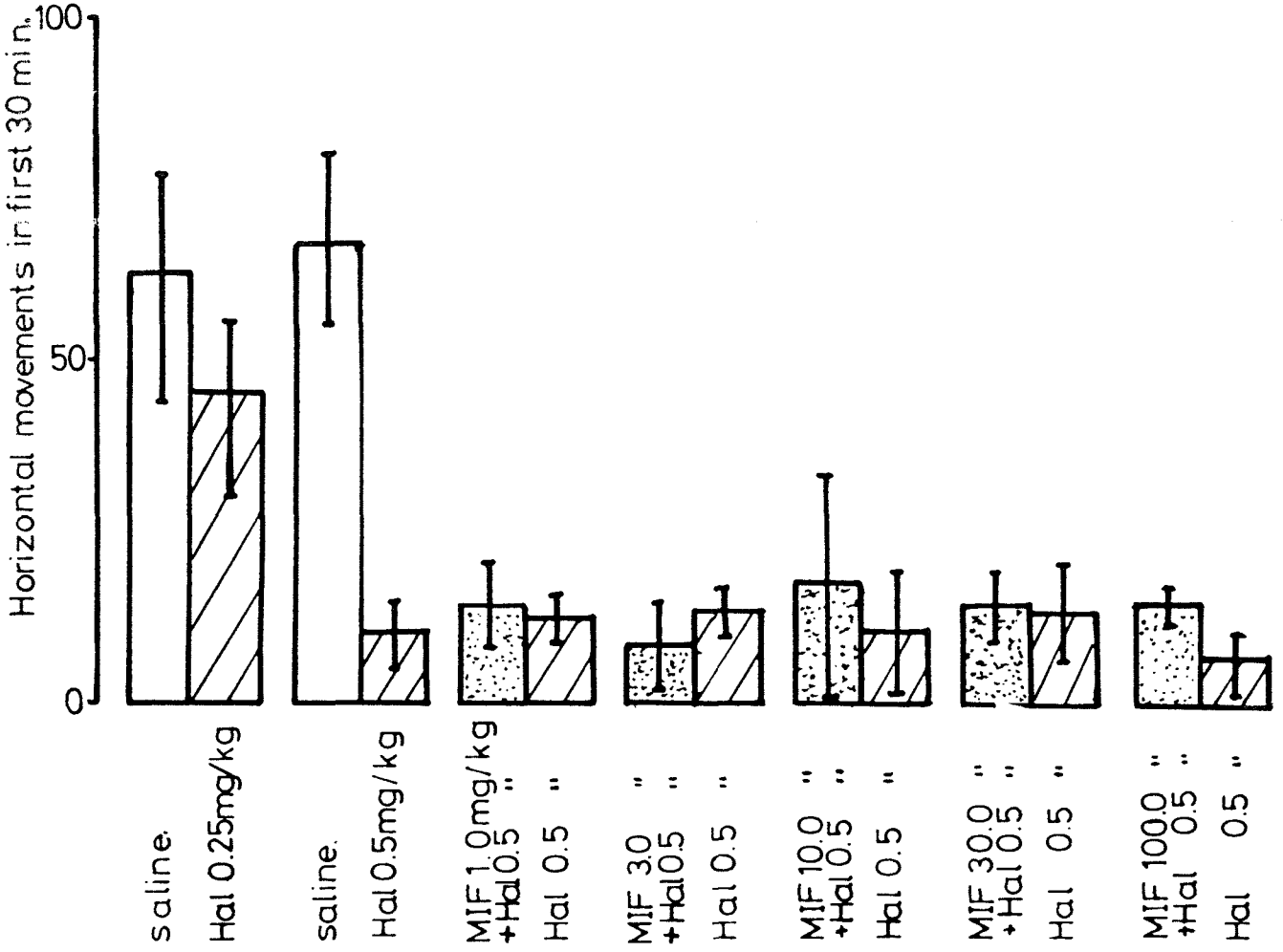
Table (3.2). Summary of the number of cells on which the substance P fragments were iontophoretically tested.
- : increase,
0 : no change
- : decrease in firing rate.

Peptide	Area of brain: Substantia nigra			Caudate nucleus			Nucleus accumbens		
	+	0	-	+	0	-	+	0	-
MIF	0	57	3?	0	22	1?	0	29	2?
96955	2?	8	0	0	4	1?	0	11	0
96956	0	14	0	0	3	0	0	8	0
96957	0	9	0	0	6	0	0	4	0

Table (3.3). Table of results with alpha-aza analogues of MIF in the substantia nigra, caudate nucleus and nucleus accumbens.
+ : increase in firing rate,
0 : no change in firing rate,
- : decrease in firing rate,
? : indicates uncertain results, due to poor potentials, these were not repeatable.
Cells were in both zona compacta and reticulata of the s. nigra, the rostral part of the caudate n., and the medial part of the n. accumbens.

the effect of

Table (3. 4). Histogram to show that a threshold dose of haloperidol (Hal 0.5mg/kg) is not reversed by between 1, 3, 10, 30 and 100 mg/kg MIF (both drugs given 30 minutes before placing the animals in their activity cages.



Drug	Area of Brain					
	Substantia Nigra			Caudate and Accumbens		
	+	0	-	+	0	-
TRH	0	40	0	0	75	0
-His-Pro-	0	8	0	0	19	0
CRM221/3	0	8	0	0	22	2
Dopamine	0	5	3	-	-	-
DLH	-	-	-	24	0	0

Table (3.5). Summary of the results of TRH and analogues in different brain areas.
+ : increase in firing rate,
0 : no change,
- : decrease in firing rate.

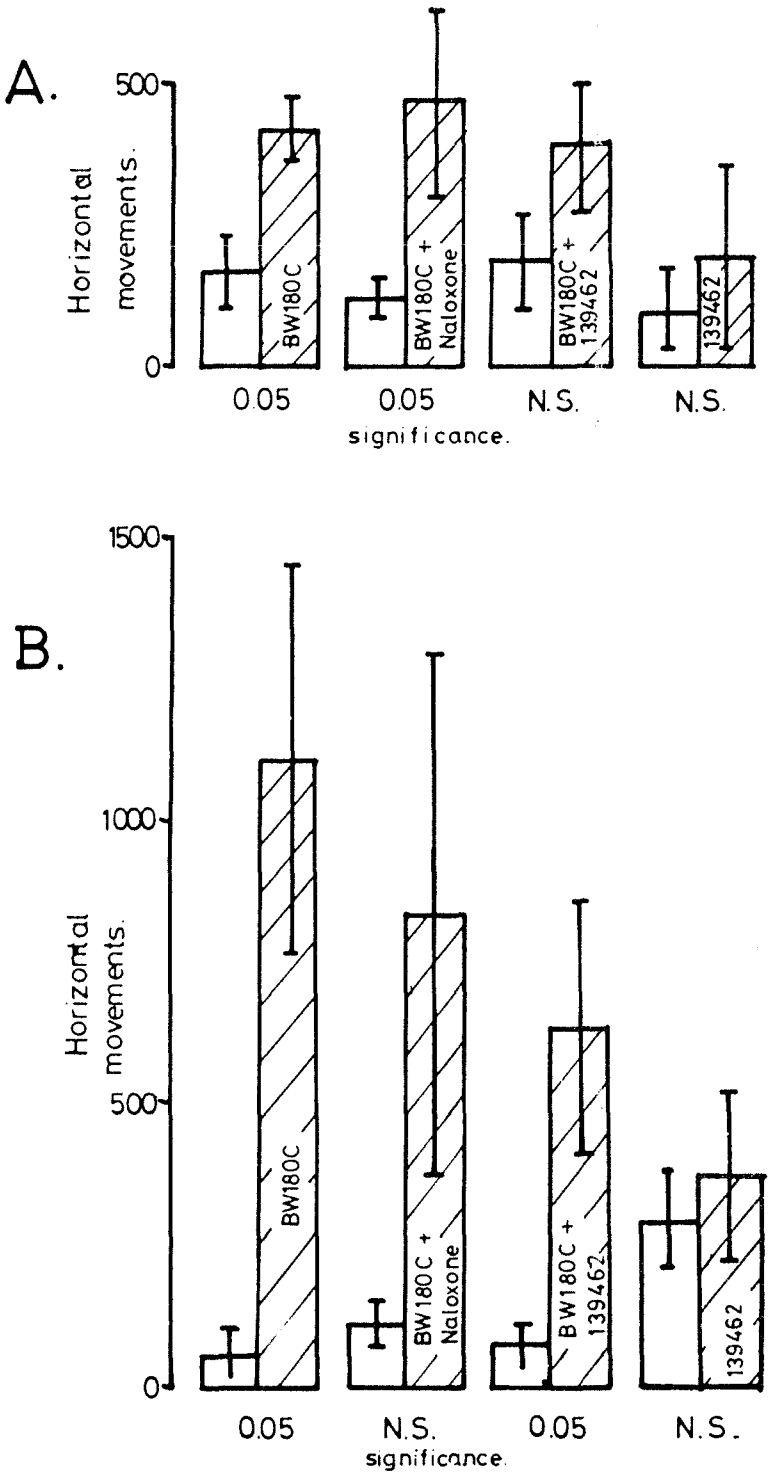


Table (3.6). Locomotor activity of rats between 0 & 15 minutes (A),50 & 100 minutes (B), which had been treated with different combinations of BW180C (10ug/side),139462 (5ug/side) (both into the VTA) and naloxone (2mg/kg I.P.).Blank columns are CSF controls,the statistical difference between these values and thr drug treated animals is given below the respective columns.Each bar is the mean and S.E. of three animals.

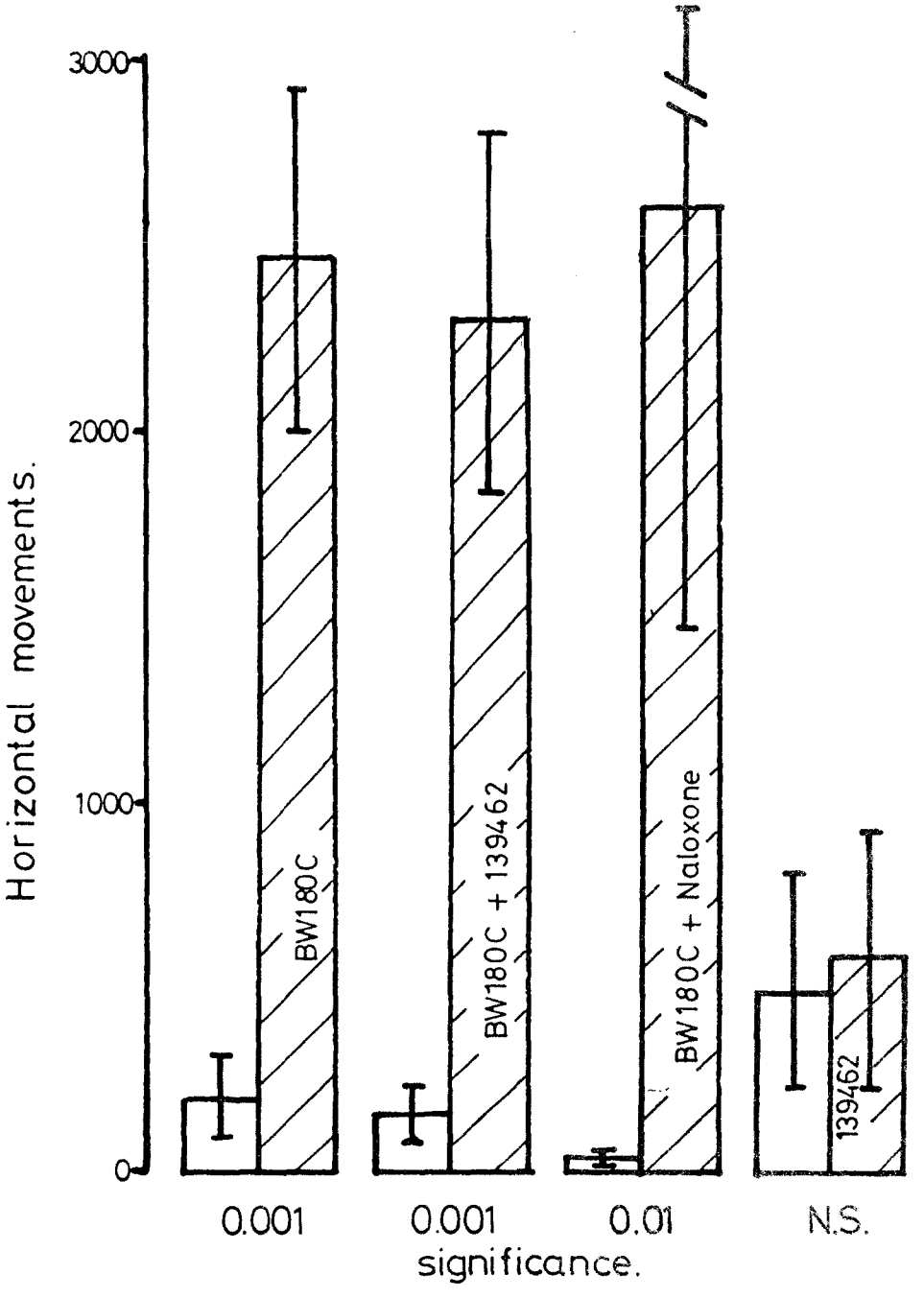


Table (3.7).Locomotor response to bilateral injection of BW180C (10ug/side) into the nucleus accumbens of thrre rats.Naloxone (2mg/kg) was injected I.P. immediately before BW180C.The peptide 139462 (5ug/side) was injected in the same solution as the BW180C.The difference between drug (hatched columns) and CSF (blank columns) treated animals is given below the respective pairs of columns.

Drug	Number of cells			Antagonised by sulpiride		
	-	0	+	(±)	(+)	(-)
Dopamine	37	4	0	$12/_{12}$	$7/_{11}$	$10/_{10}$
GABA	8	0	0	-	$0/_{8}$	$0/_{8}$
Noradrenaline	9	1	0	$2/_{2}$	-	$2/_{2}$
p-tyramine	6	2	0	$4/_{4}$	-	-

Table (3.8). Summary of the number of cells in the substantia nigra compacta inhibited by dopamine, GABA, noradrenaline and p-tyramine.
- : inhibition of firing,
0 : no change,
+ : increase in firing rate.
The right hand section of the table indicates the effects of the different enantiomers of sulpiride on the inhibitory responses to the different drugs.

	excite	no change	inhibition
Effect of dopamine iontophoresis	0	29	37
Electrically excited from s. nigra	-	10	0

	antagonism	partial block	no change
Effect of sulpiride on dopamine response	5	1	14
Effect of sulpiride and fluphenazine on the same dopamine response	2	0	2
Effect of d-sulpiride on dopamine response	1	0	4
Effect of l-sulpiride on dopamine response	2	0	4

Table (3.9). Summary of the results with dopamine antagonists in the caudate nucleus. The top part of the table indicates how many cells were affected by dopamine and if they were excited by electrical stimulation of the ipsilateral substantia nigra. The lower half of the table indicates the number of cells on which the inhibitory response to dopamine was antagonised by sulpiride and fluphenazine and which isomers were tested.

Assay	SP 1-9	SP
Guinea pig ileum EC 50	> 10^{-5} moles	4.8 x 10^{-10} moles
Rat salivation ED 50	> 2.5mg/kg I.V.	7µg/kg I.V.
Hypotension ED 50	> 10mg/kg I.V.	1.3µg/kg I.V.

Table (3.10). Summary of the results obtained with substance P (SP) and substance P 1-9 (SP 1-9) on three different substance P sensitive peripheral assays.

Comparison of Guinea Pig Ileum and Mouse Vas Deferens Preparations

	Agonist Potency (x Leu-enkephalin)		Antagonism by Naloxone (Ke.nM)	
	Ileum	Vas	Ileum	Vas
Leu-enkephalin	1	1	1.74	21.4
Met-enkephalin	2.1	0.28	1.94	28.3
B-Endorphin	8.9	0.14	2.53	30.5
Normorphine	4.5	0.07	1.83	4.75

In vitro comparison of naloxone and ICI 139462

Agonist	Mouse vas deferens	Guinea pig ileum
	Naloxone	
Leu-enkephalin	21.4 ± 4.04	1.74 ± 0.21
B-endorphin	30.5 ± 2.11	2.53 ± 0.27
Normorphine	4.75 ± 0.59	1.83 ± 0.18
	ICI 139462	
Leu-enkephalin	234.7 ± 42.5	1560 ± 230
B-endorphin	1050 ± 200	2200 (n = 2)
Normorphine	3600 ± 560	3270 ± 570

Figures represent Ke values (nM)

Table (3.11). Summary of data on peripheral assays for opiate agonists and antagonists.

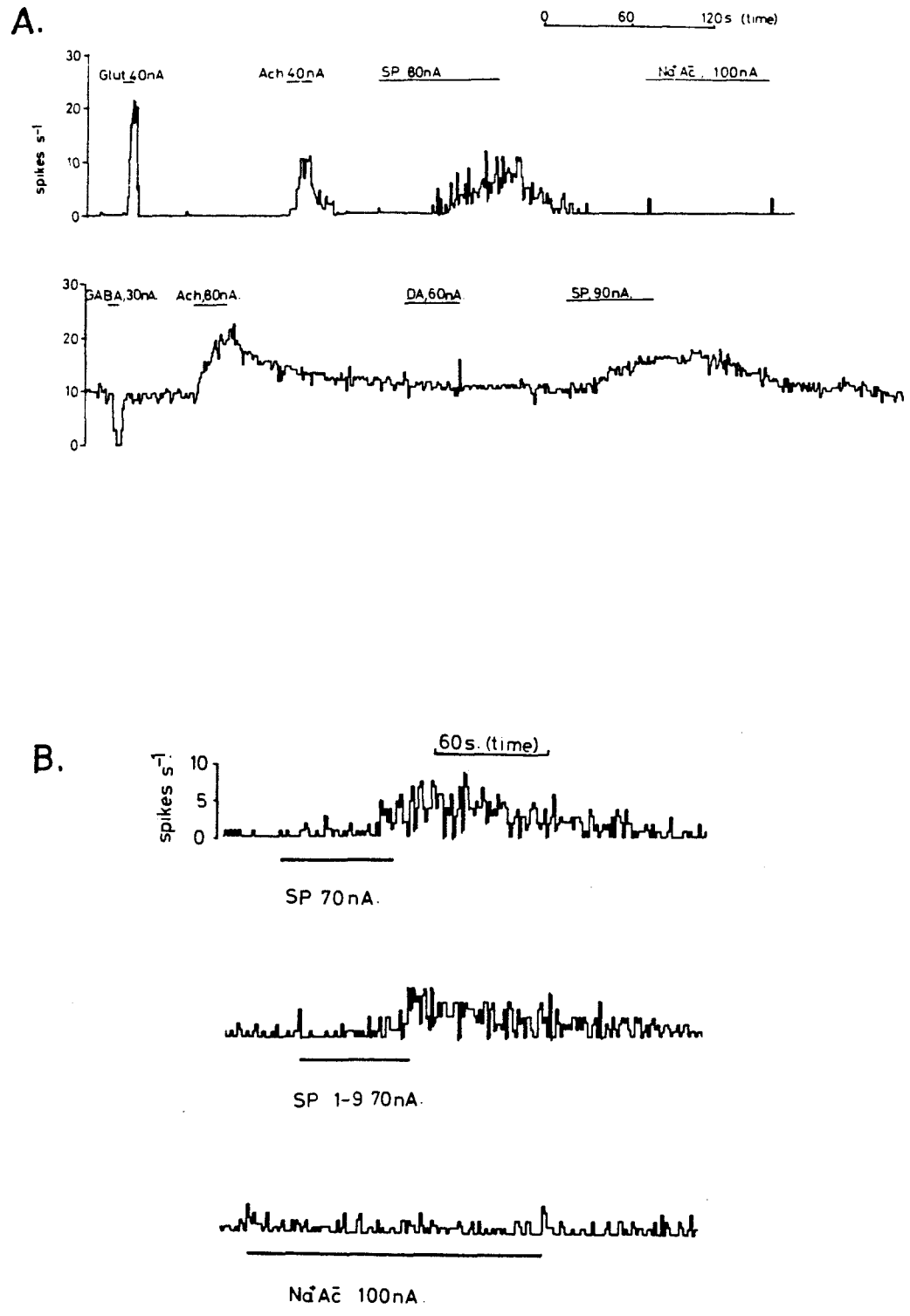


Figure 3.1. A: Effects of glutamate, acetylcholine, GABA and substance P on the firing rate of two neurones in the substantia nigra reticulata.
B: Effect of substance P and the 1-9 fragment on the same cell in the substantia nigra.

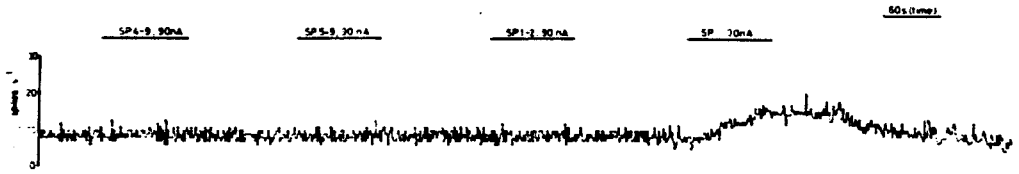


Figure 3.2. Excitatory effect of substance P on a substantia nigra reticulata neurone which is not affected by substance P 1-2, 4-9 and 5-9.

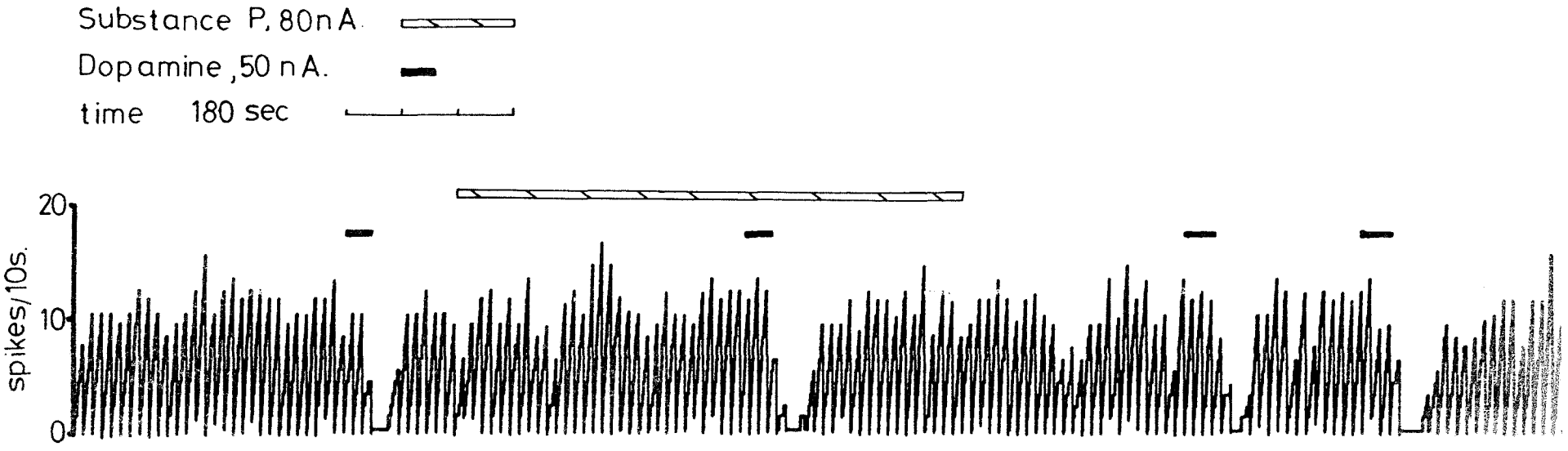


Figure (3.3). Ratemeter recording from a zona compacta neurone of the substantia nigra. The cell is inhibited by 50nA of dopamine (DA), but unaffected by 80nA of substance P applied for 540 seconds. The response to dopamine does not appear to be modified by substance P.

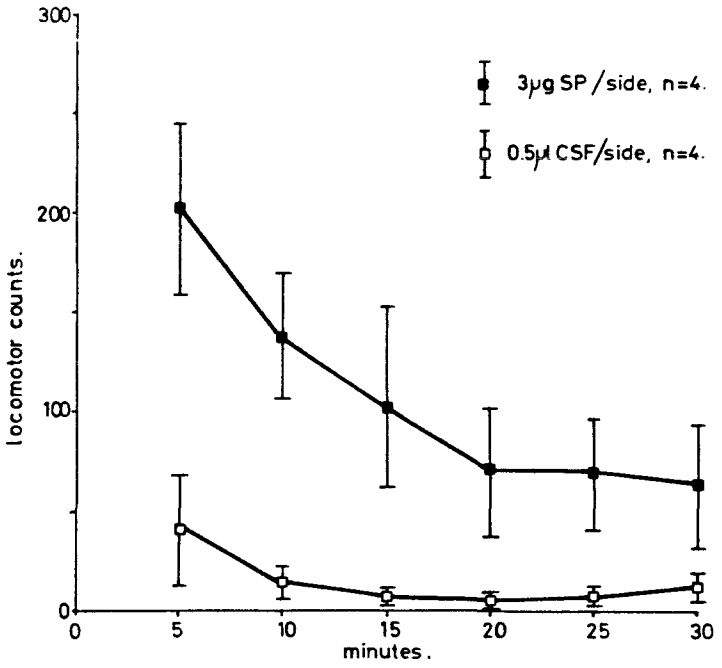


Figure 3.4. Effect of bilateral injections of substance P into the VTA on the spontaneous locomotor activity of conscious rats.

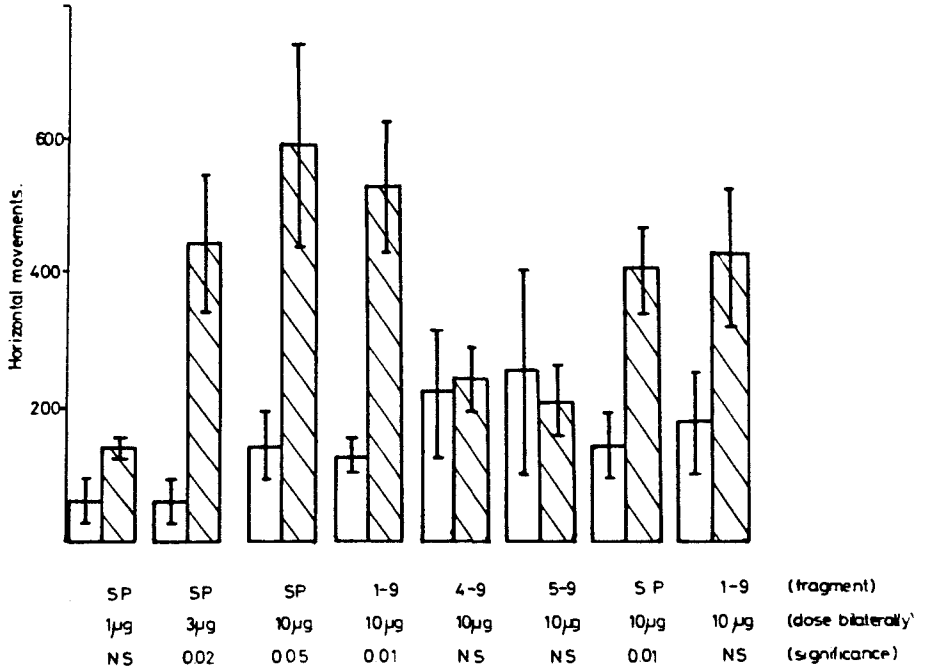


Figure 3.5. Histogram of locomotor activity after various substance P analogues compared with saline controls.

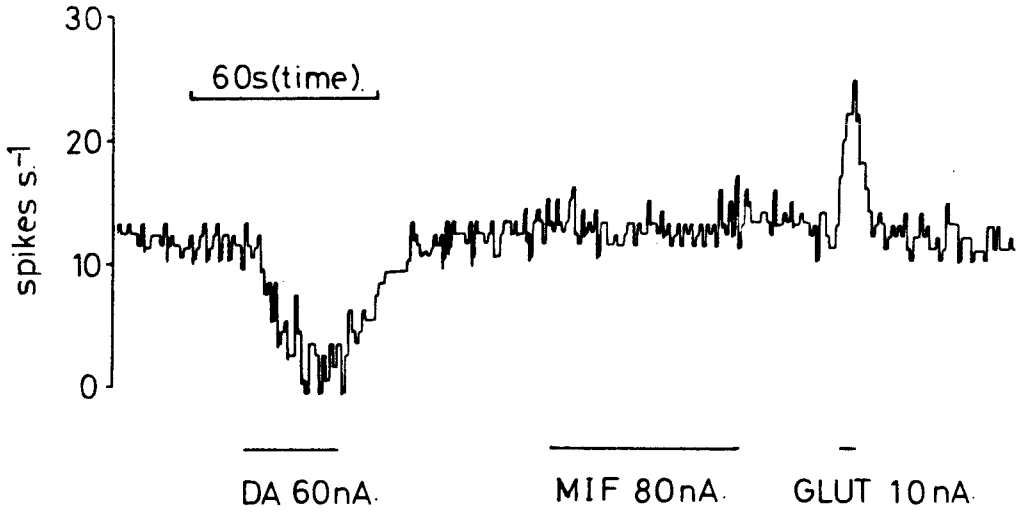


Figure 3.6. Failure of MIF to affect a substantia nigra compacta neurone which is inhibited by dopamine (DA) and excited by glutamate (glu).

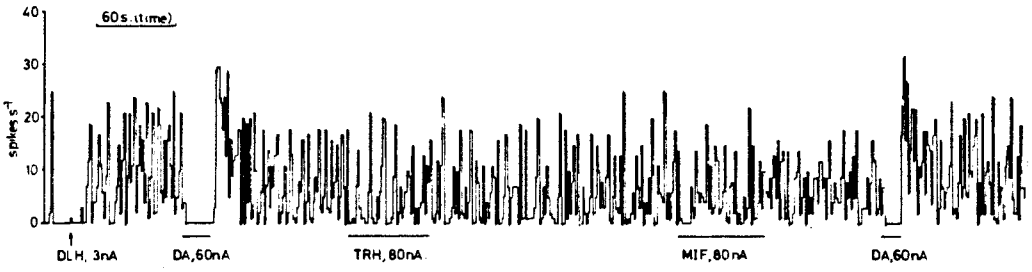


Figure 3.7. Failure of TRH and MIF to affect a neurone induced to fire by continuous application of DLH which can be inhibited by dopamine (DA).

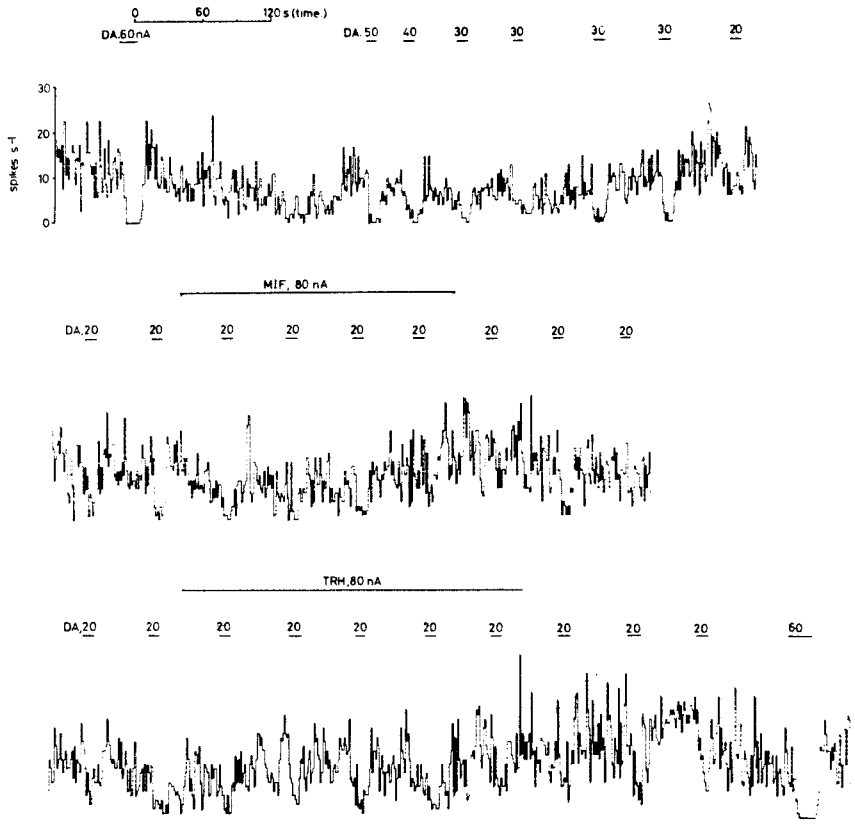


Figure 3.8. Failure of MIF or TRH to alter the threshold inhibitory response to dopamine (DA) on a nucleus accumbens neurone.

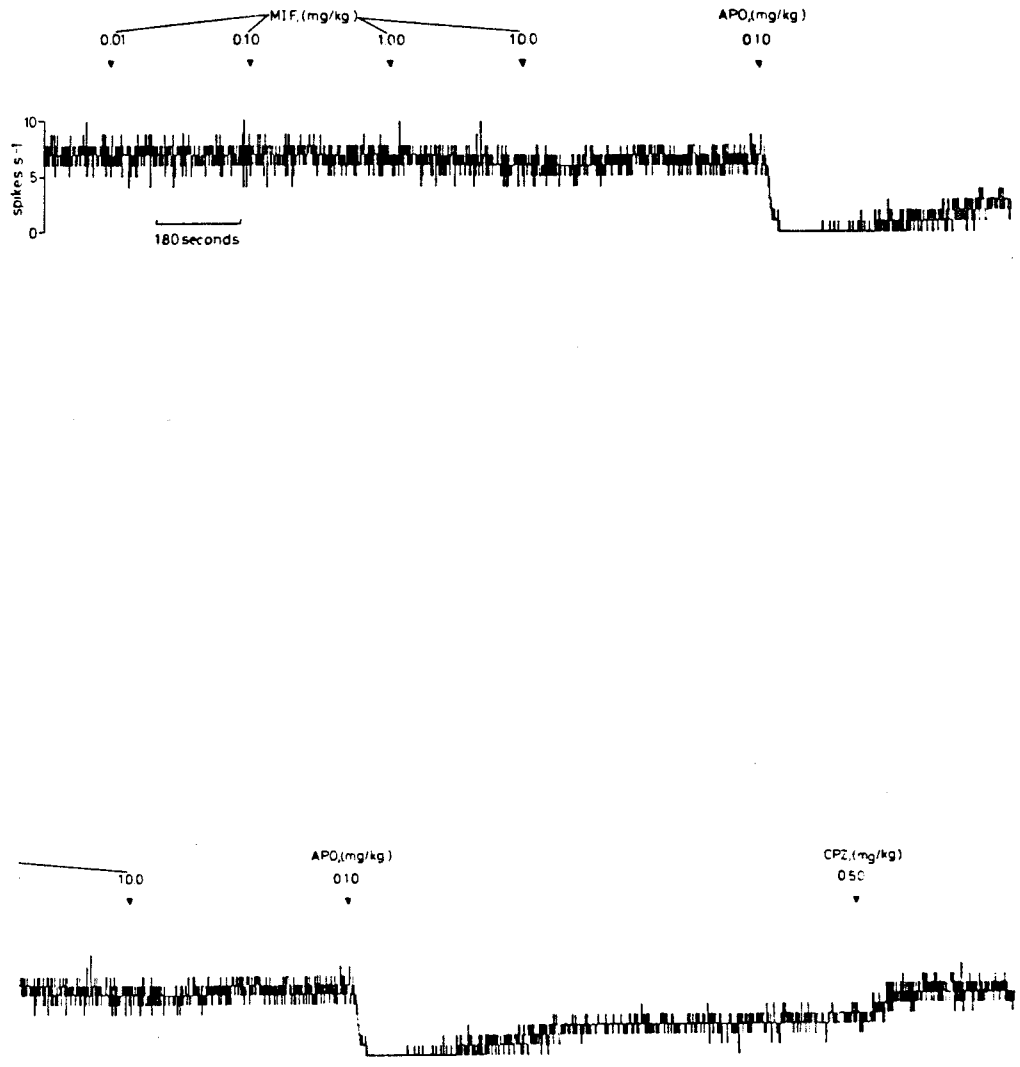


Figure 3.9. Failure of intravenously administered MIF to change the firing rate of a nigral compacta neurone, inhibition occurs with apomorphine (APO) and can be reversed by chlorpromazine (CPZ).

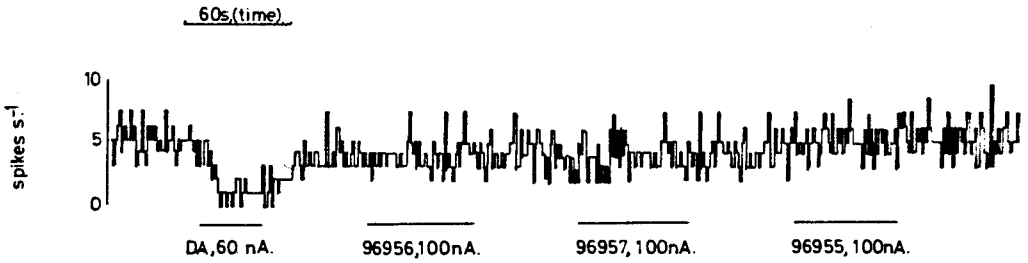
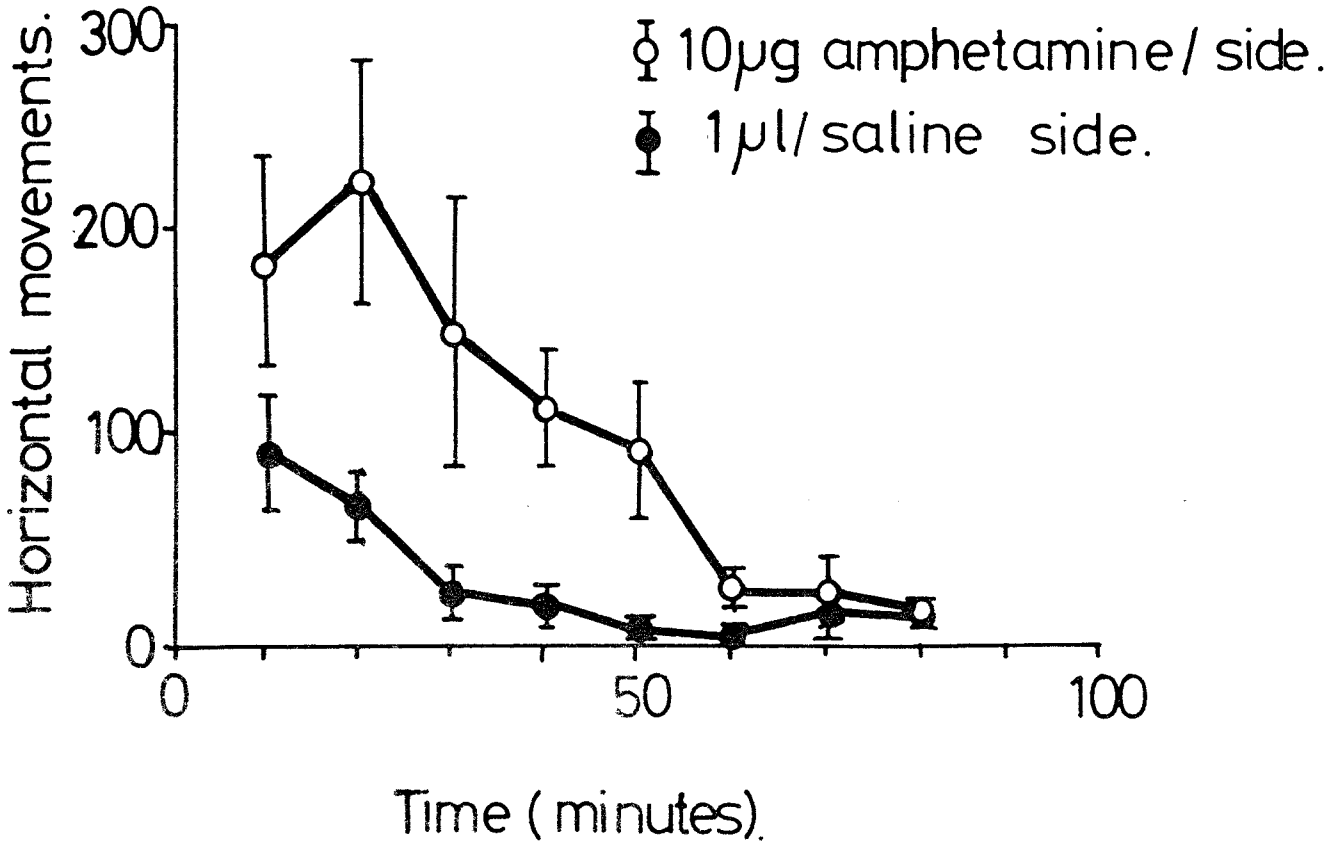


Figure 3.10. Ratemeter recording from a substantia nigra compacta neurone which is inhibited by dopamine (DA), but is unaffected by three MIF analogues.



Figure(3-11)Effect of bilateral intra accumbens injections of d-amphetamine.(n= 3.).

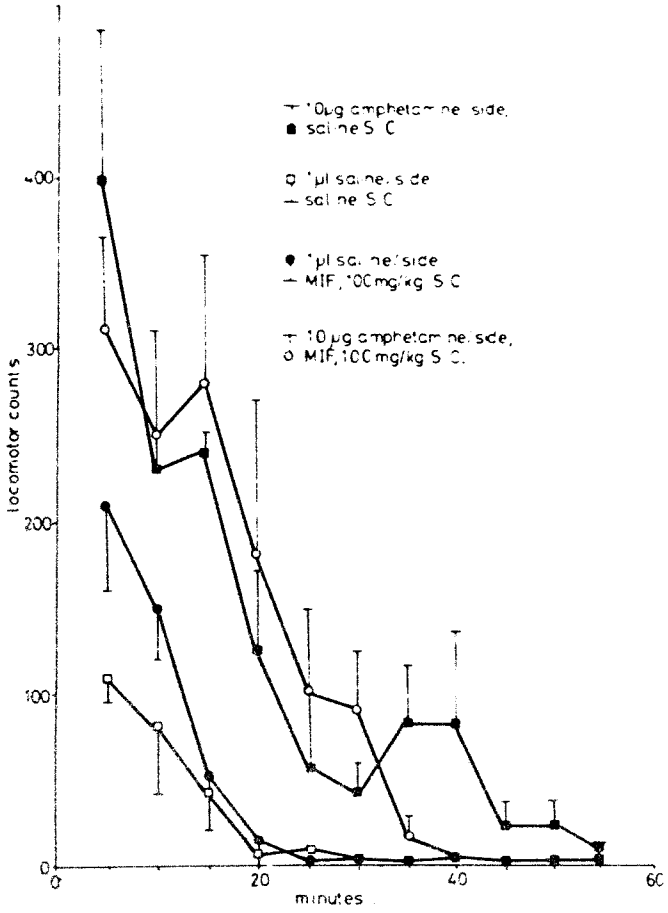
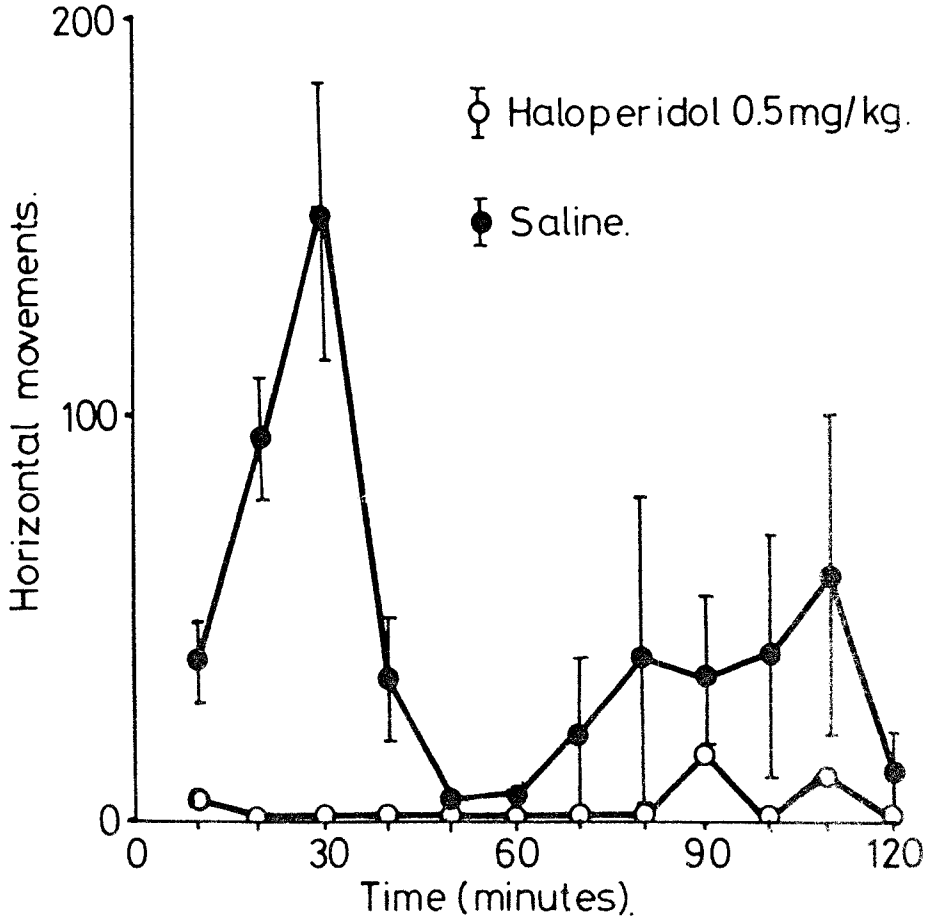
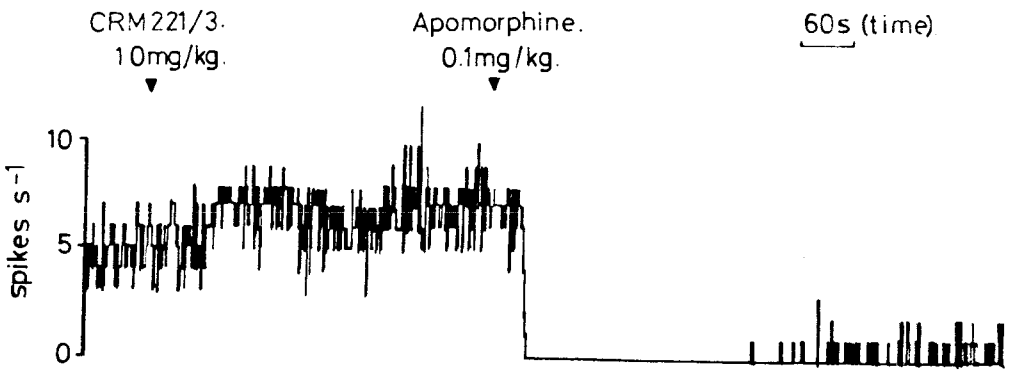


Figure (3.12) Effect of MIF on amphetamine hyperactivity.



Figure(3.13).Effect of haloperidol on spontaneous exploratory activity. n=6.



Ratemeter recording from a SNC neurone showing the effect of I.V. CRM 221/3 and apomorphine.

Figure 3.14. Weak excitant effect of CRM221/3 on the firing rate of a substantia nigra compacta neurone after intravenous injection.

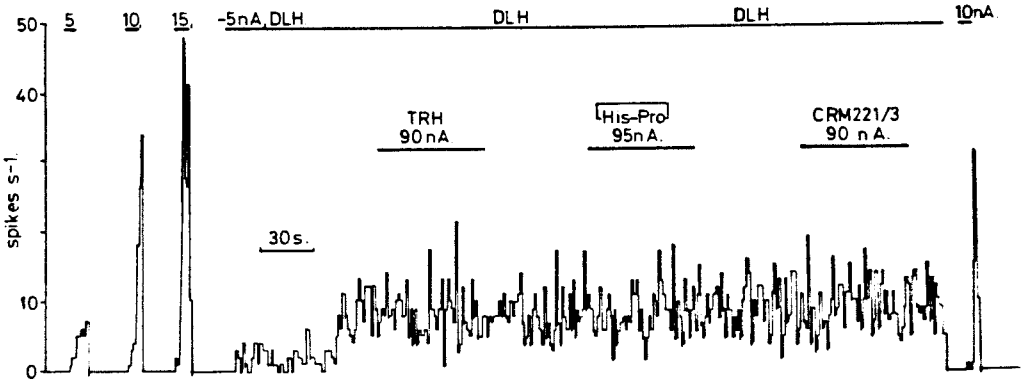


Figure 3.15. Failure of iontophoretic TRH, His-Pro and CRM221/3 to change the firing rate of an accumbens neurone driven by DLH.

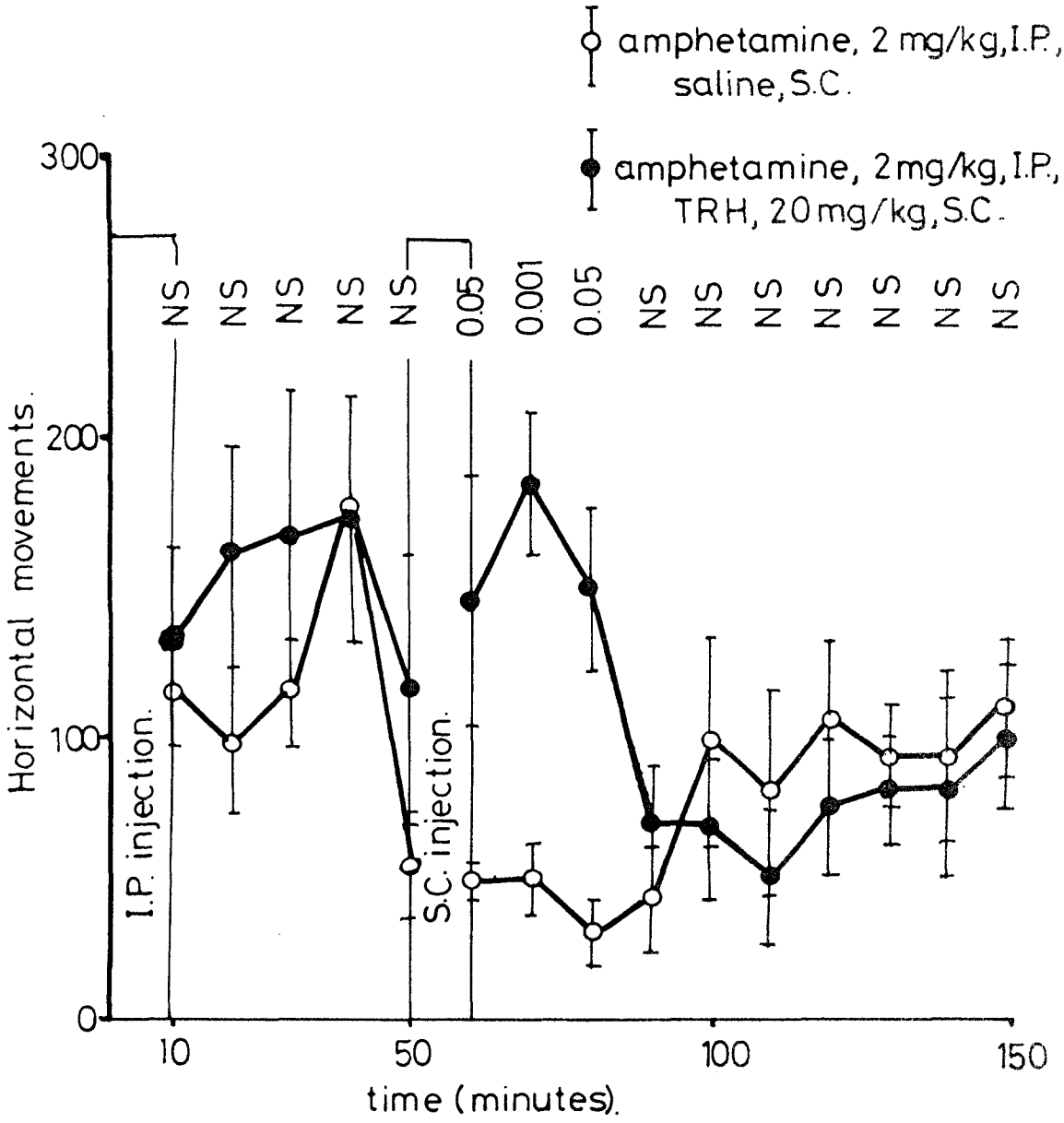


Figure (3.16). Potentiating action of TRH on the locomotor response to amphetamine.

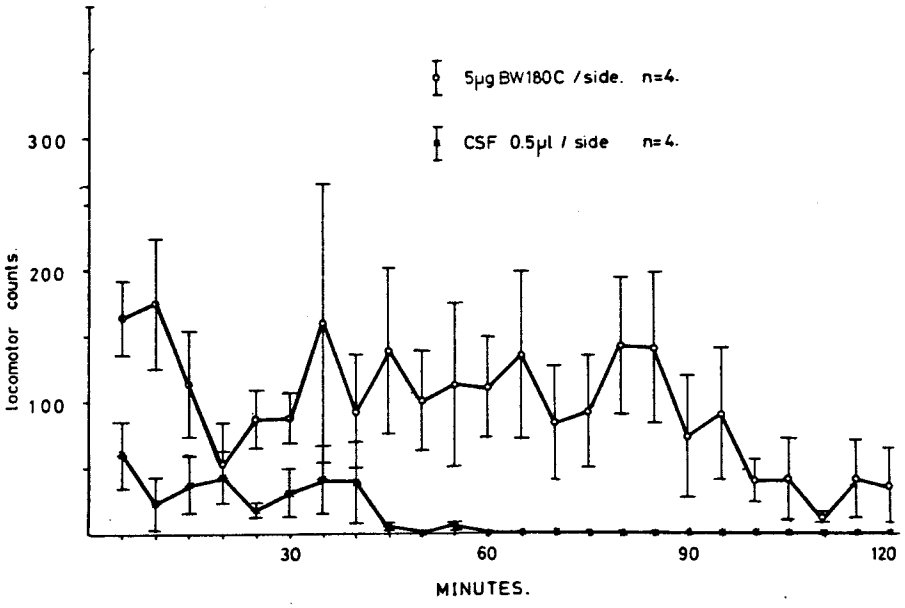


Figure 3.17. Locomotor stimulant effect of bilateral injections of BW180C into the VTA.

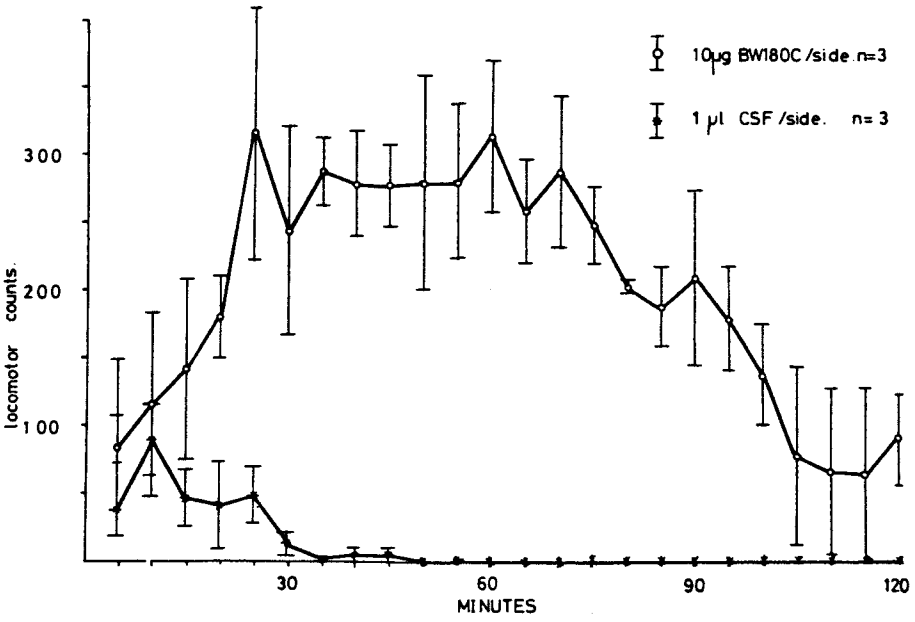


Figure 3.18. Locomotor stimulant effect of bilateral injections of BW180C into the nucleus accumbens.

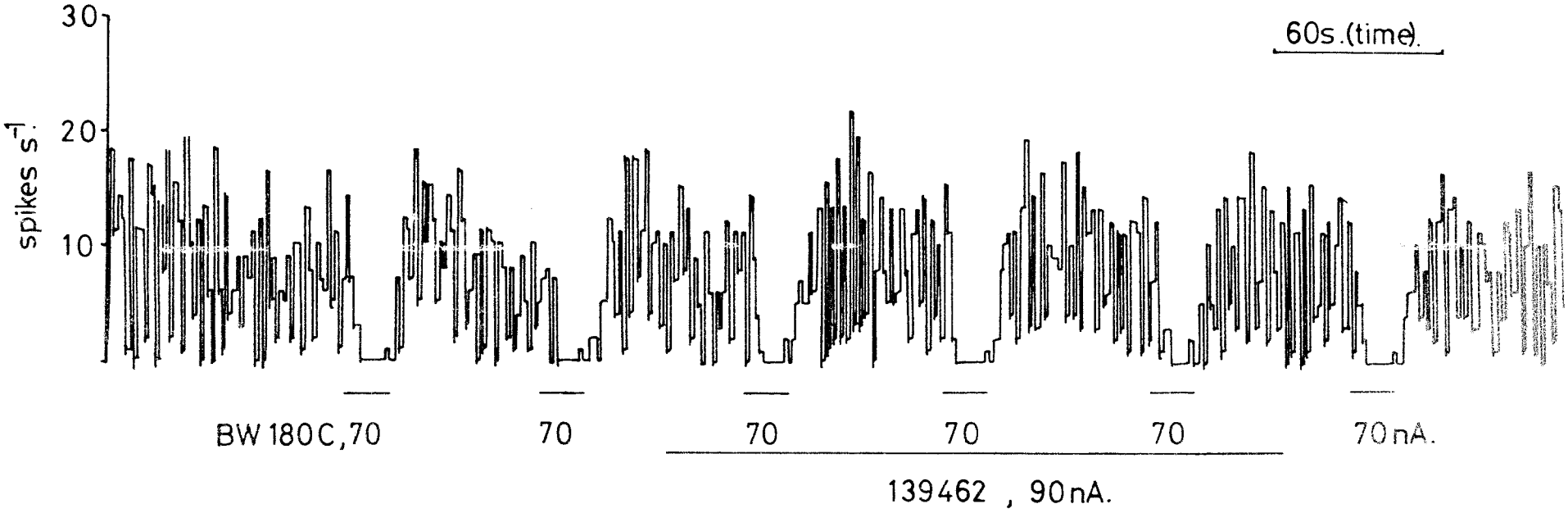


Figure (3.19).Ratemeter recording from a spontaneously active neurone in the nucleus accumbens. The cell is inhibited by BW180C (70nA) but unnafected by the putative opiate antagonist peptide 139462 (90nA) applied for three minutes.

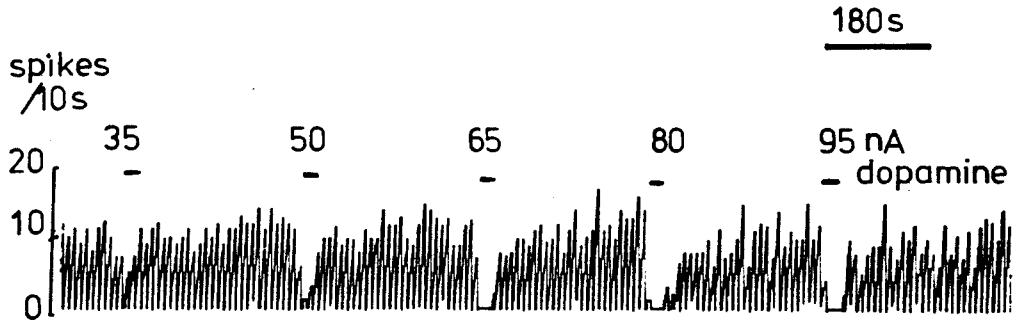


Figure 3.20. Dose related inhibition of a substantia nigra compacta neurone by iontophoretic dopamine.

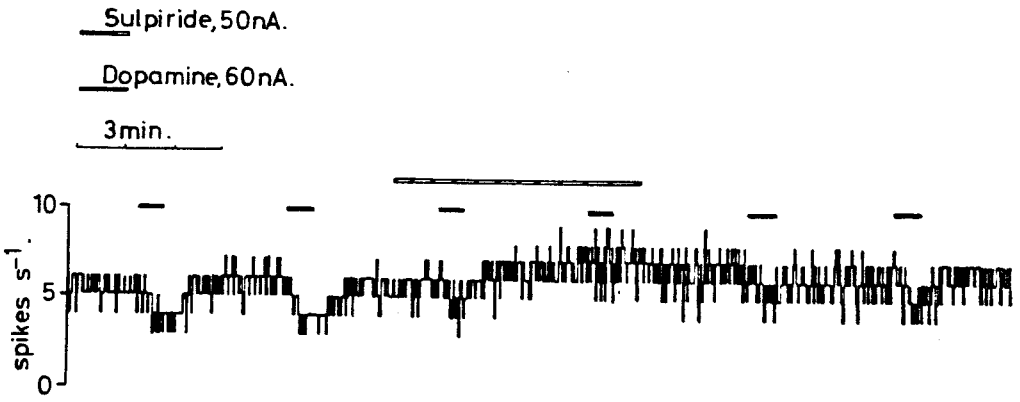


Figure 3.21. Antagonism of the dopamine induced inhibition of firing of a substantia nigra compacta neurone by iontophoretic sulpiride.

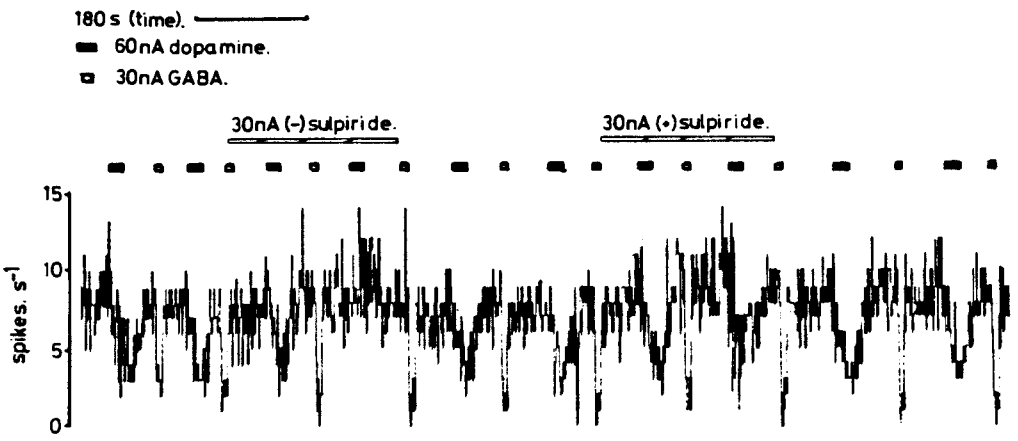


Figure 3.22. Effect of the + and - enantiomers of sulpiride on the response to dopamine on a substantia nigra compacta neurone.

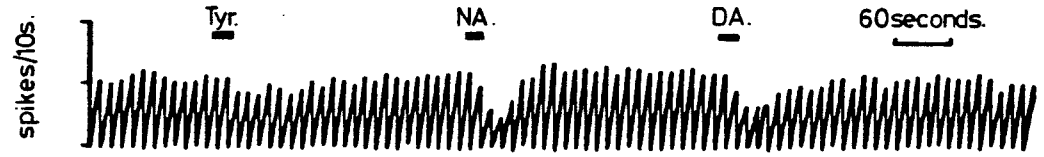


Figure 3.23. Inhibitory effect of 60nA of tyramine (Tyr), dopamine (DA) and noradrenaline (NA) on the firing rate of a substantia nigra compacta neurone.

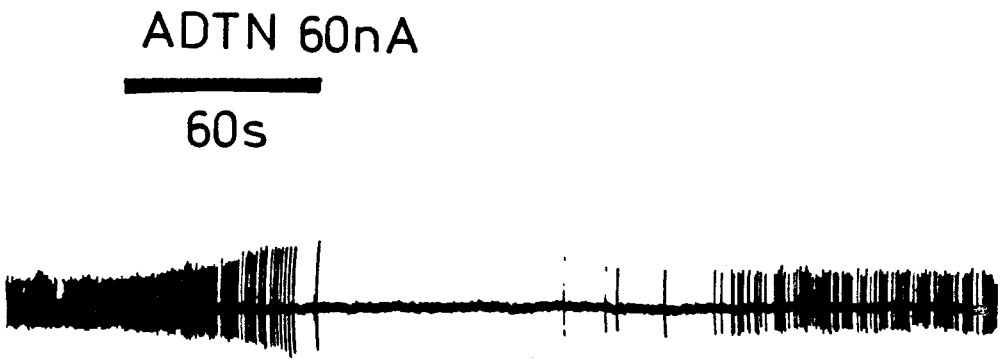


Figure 3.24. Spike record from a substantia nigra compacta neurone inhibited by ADTN.

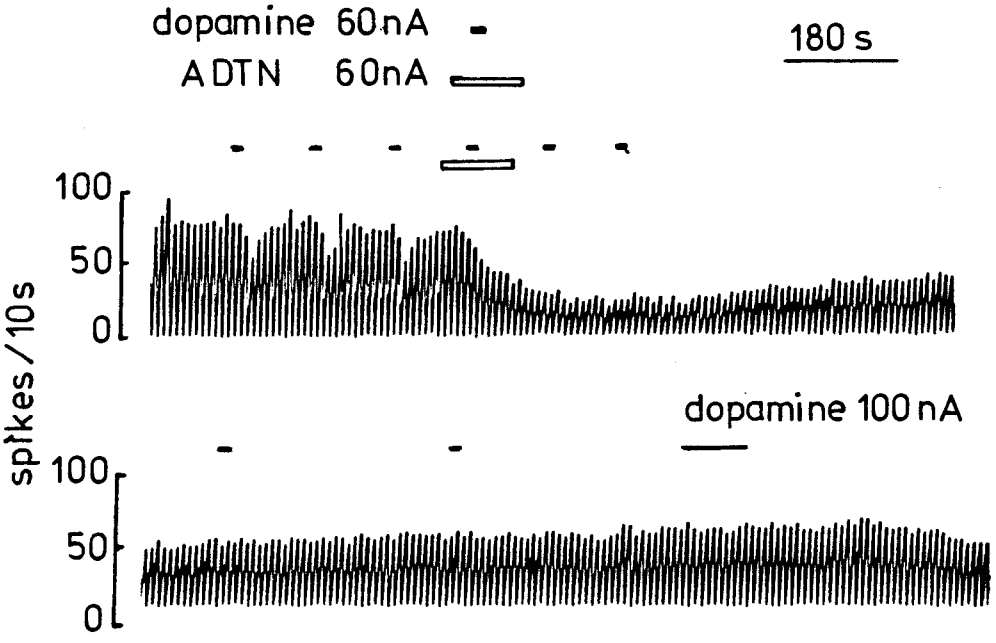


Figure 3.25. Ratemeter recording from a substantia nigra compacta neurone inhibited by ADTN, following which the response to dopamine is also lost.

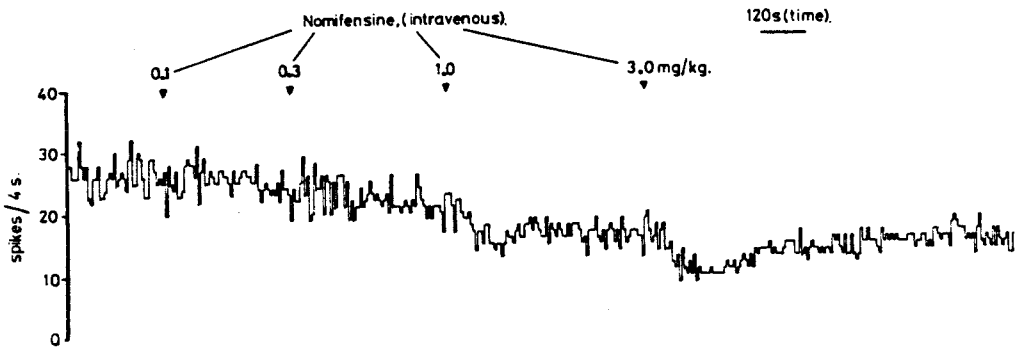


Figure 3.26. Effect of intravenous nomifensine on the firing rate of a compacta neurone.

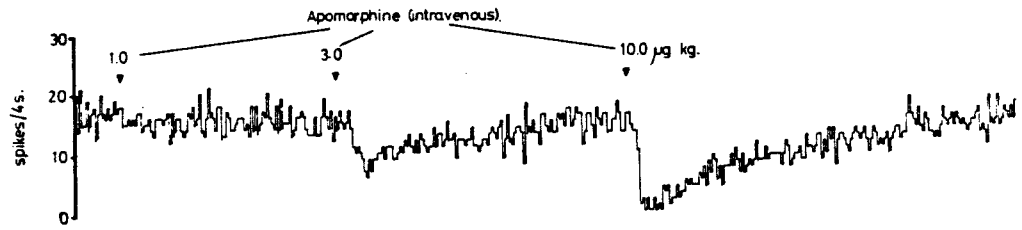


Figure 3.27. Effect of intravenous apomorphine on the firing rate of a compacta neurone.

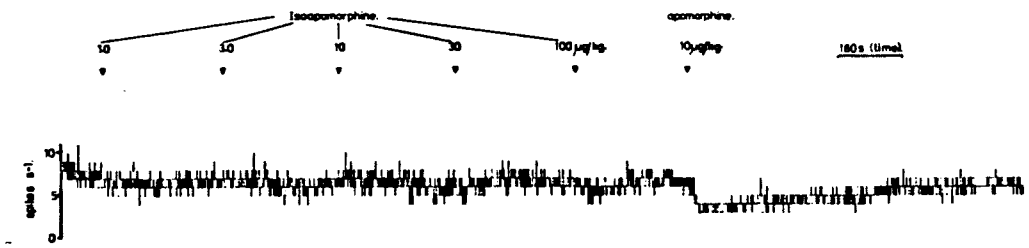


Figure 3.28. Failure of intravenous isoapomorphine to change the firing rate of a compacta neurone. Apomorphine is still active.

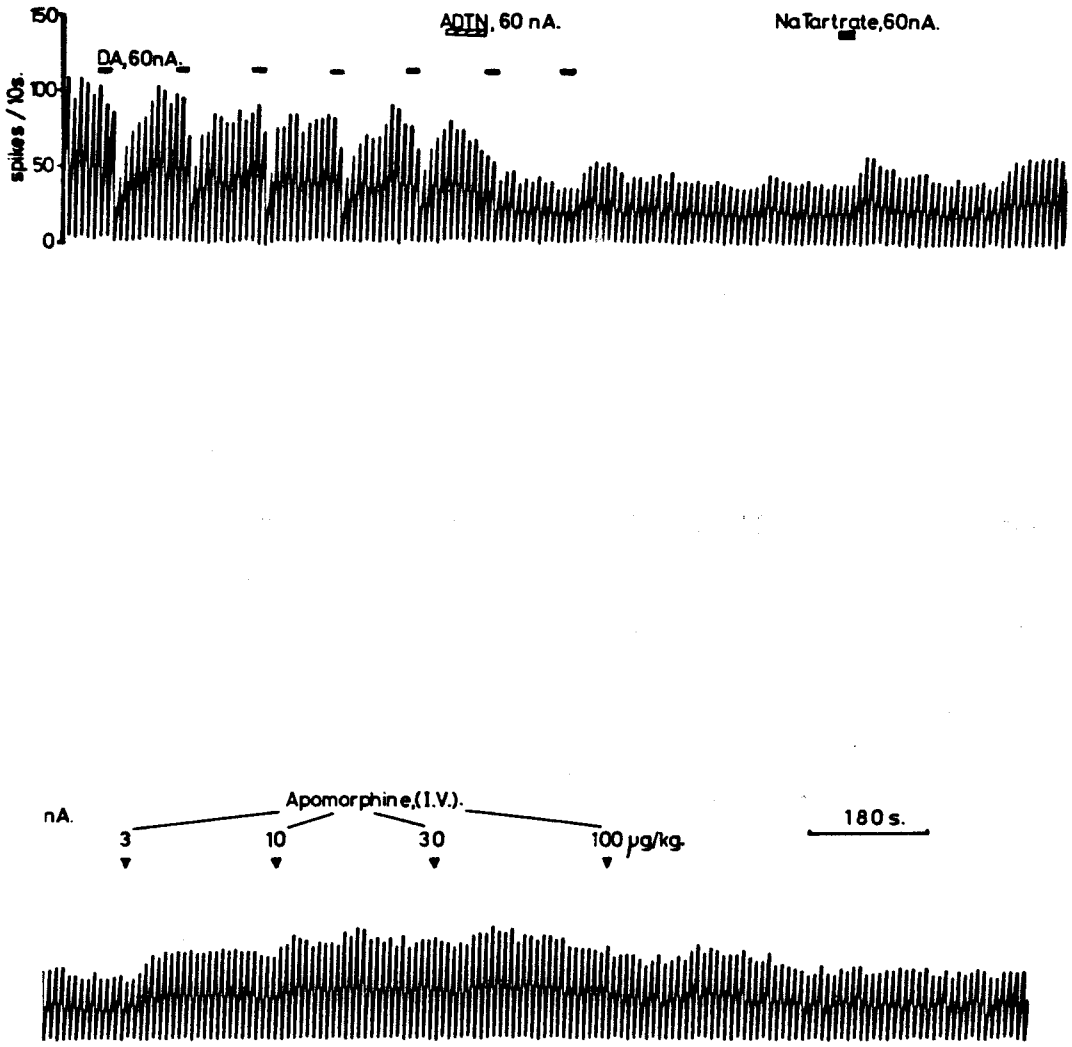


Figure 3.29. Excitatory effect of intravenous apomorphine on a neurone in the substantia nigra compacta which has been desensitised to dopamine by ADTN.

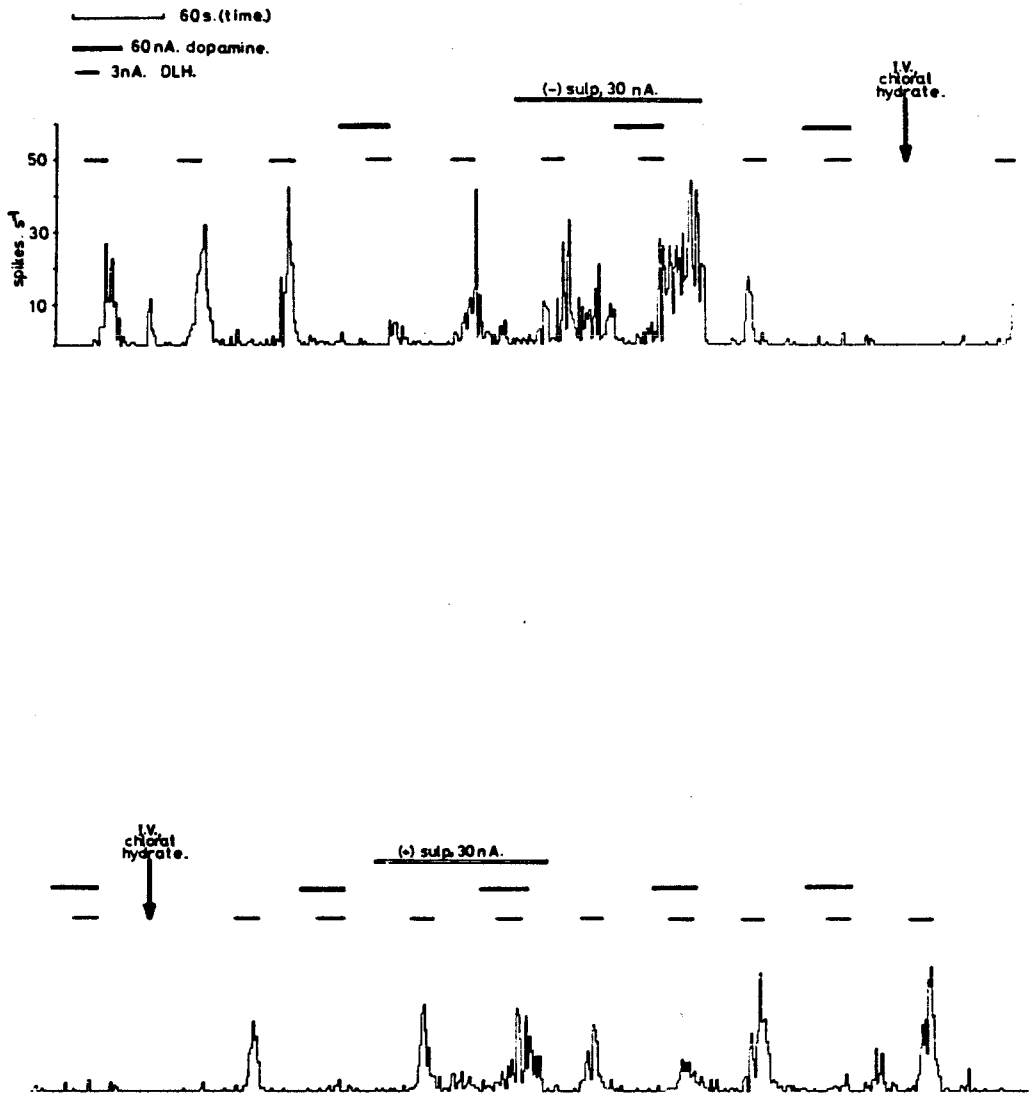


Figure 3.30. Antagonism of the inhibitory effects of dopamine by the - and + enantiomers of sulpiride on a caudate neurone. The neurone was not induced to fire continuously as this resulted in a depolarization block of firing.

Figure (3.31). Single spike excitation in the caudate nucleus following electrical stimulation of the ipsilateral substantia nigra. The stimulus, 20V, 0.2msec duration was given 50msec after the oscilloscope beam had started its sweep. Each trace is a single sweep.

Figure (3.32). Single spike in a substantia nigra compacta neurone of the non dopaminergic type following electrical stimulation of the ipsilateral caudate nucleus. The stimulus, 25V, 0.5msec duration was delivered 3msec after the beam had started its sweep. Each trace is a single sweep.

Figure (3.33). Antidromic spike in a dopamine type of neurone in the compacta of the substantia nigra following electrical stimulation of the ipsilateral striatum. The stimulus, 30V, 0.5msec, was delivered 5msec after the beam had started its sweep. Each trace is a single sweep. The top trace shows a full spike, the bottom trace an initial segment only.

Figure (3.34). Collision of ortho- and anti-dromic spikes in a dopamine neurone in the compacta of the substantia nigra following electrical stimulation of the ipsilateral striatum. The antidromic spikes are initial segments only. The stimulus, 30V, 0.5msec, was delivered 50msec after the beam had started to sweep. Each trace is a single sweep. The antidromic spike is absent if an orthodromic spike occurs in a period before or after stimulation which is less than the latency of the antidromic spikes.

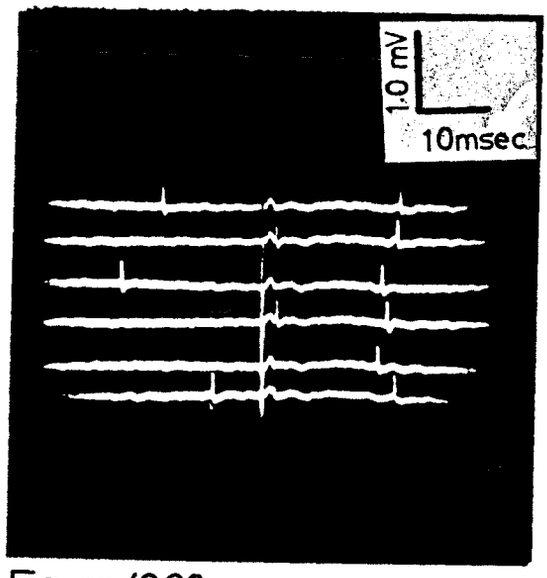


Figure (3.31).

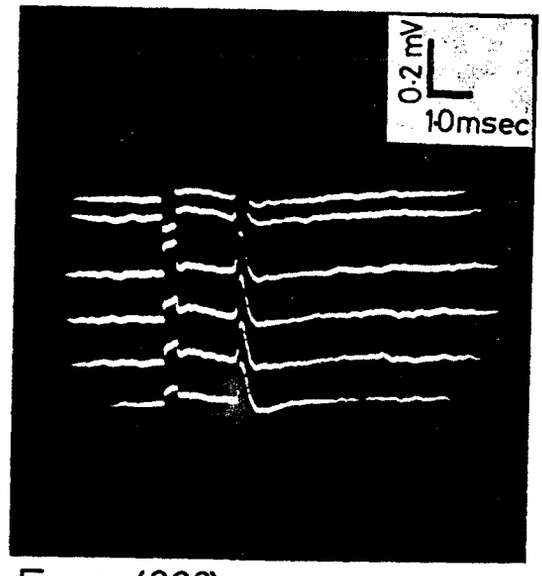


Figure (3.32).

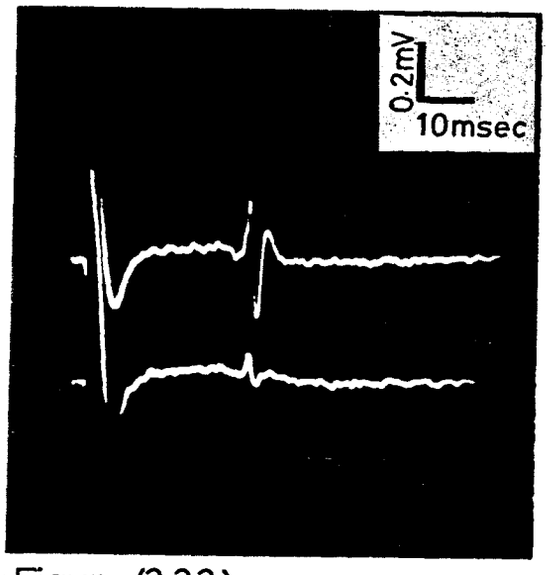


Figure (3.33).

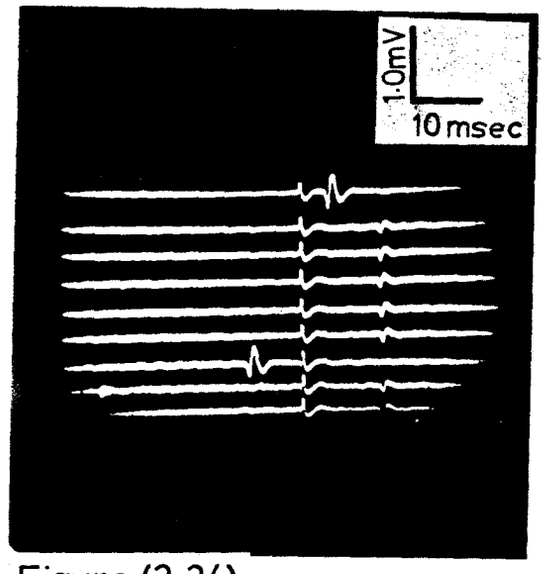


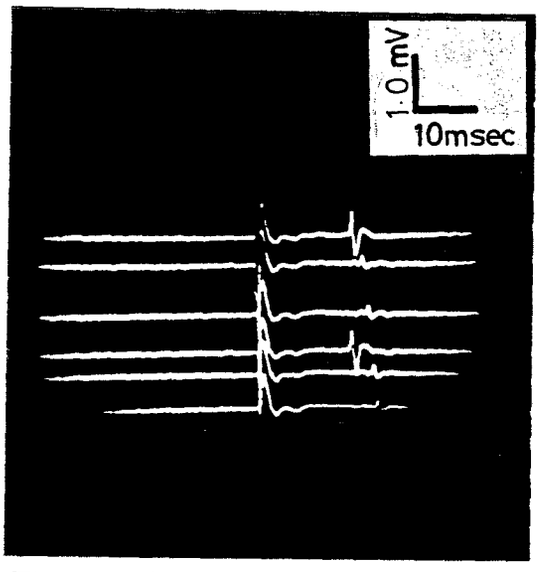
Figure (3.34).

Figure (3.35). Increasing latency of antidromic spikes in a dopamine type neurone in the compacta of the nigra during rapid repetitive (200Hz) stimulation of the ipsilateral striatum. The stimulus, 40V, 0.5msec was given 50msec after the beam had started to sweep, each trace is an individual sweep. The latency of the antidromic spike increased by 3msec. Only every tenth stimulus was recorded.

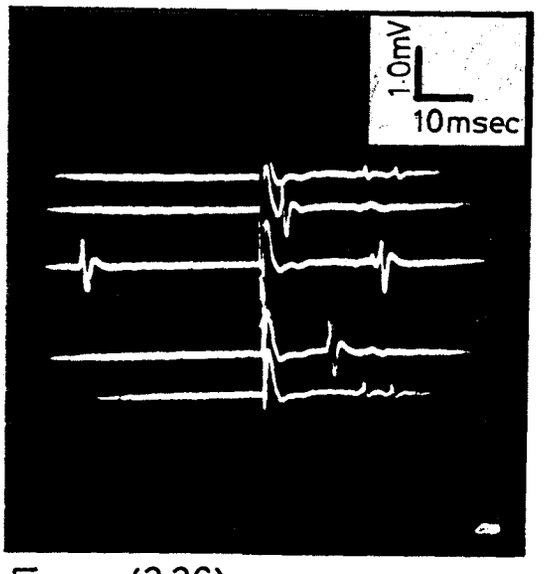
Figure (3.36). Double antidromic spikes in a dopamine type neurone in the compacta of the nigra following stimulation of the ipsilateral striatum. Details of the stimulus are identical to those for the previous figure.

Figure (3.37). Inhibition of a neurone in the reticulata of the substantia nigra following electrical stimulation of the ipsilateral striatum. The stimulus, 50V, 0.1msec was given 1msec after the beam had started to sweep. Twenty sweeps are superimposed on top of each other. The inhibition is apparent 7msec after stimulation and lasts some 20msec.

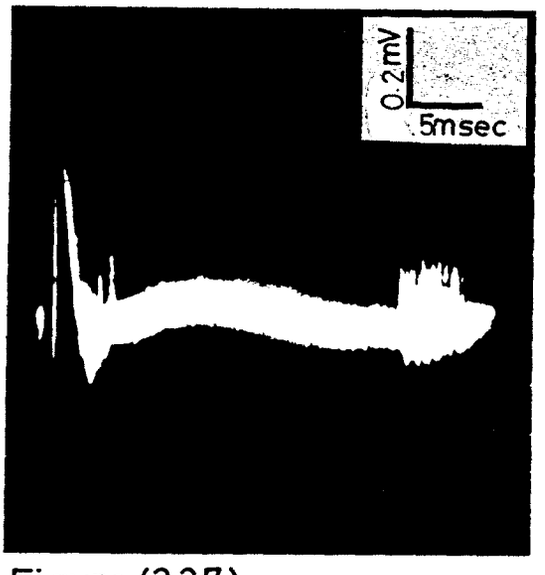
Figure (3.38). Excitation of a neurone in the MRF immediately above the substantia nigra following electrical stimulation of the ipsilateral striatum. The stimulus, 25V, 0.1msec was delivered 3msec after the beam had started to sweep. Ten sweeps are superimposed on top of each other.



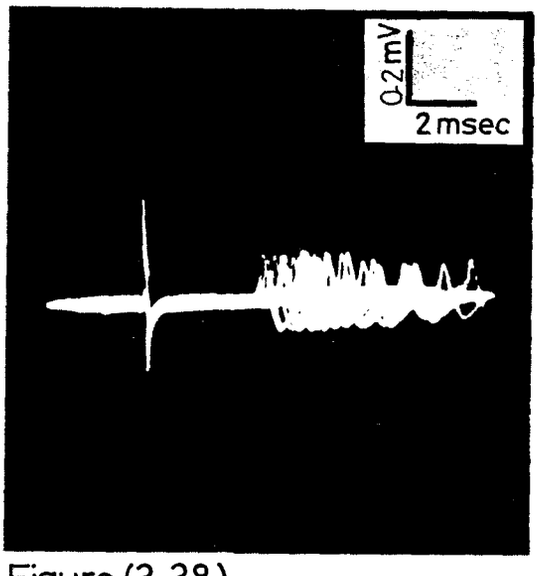
Figure·(3·35)·



Figure·(3·36)·



Figure·(3·37)·



Figure·(3·38)·

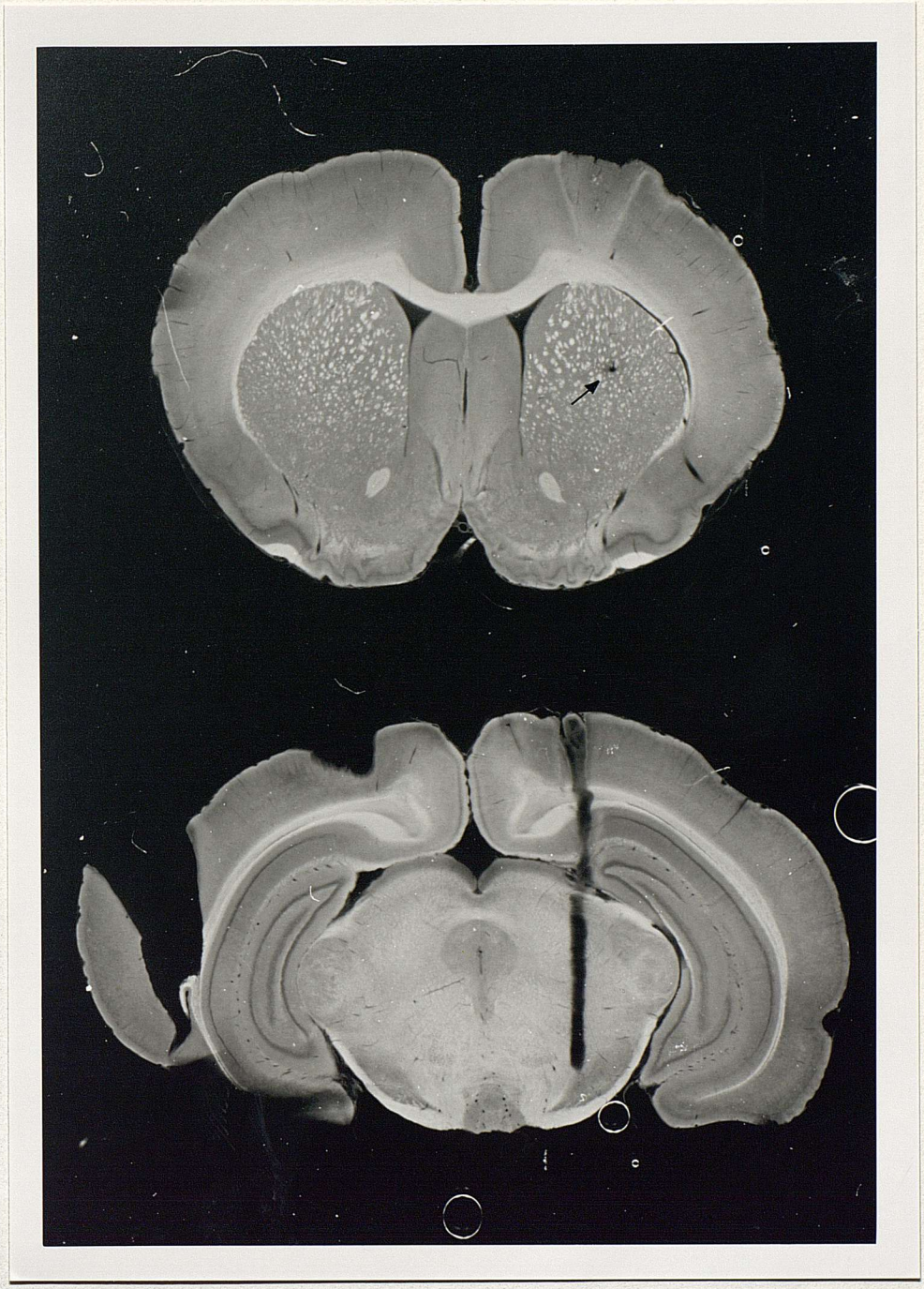


Figure (3.39). Coronal sections through a rat brain. The pontamine sky blue dot marking the recording electrode position can be seen (arrow) in the centre of the caudate nucleus. The tip of the stimulating electrode at the end of the electrode tract is located in the compacta of the substantia nigra.

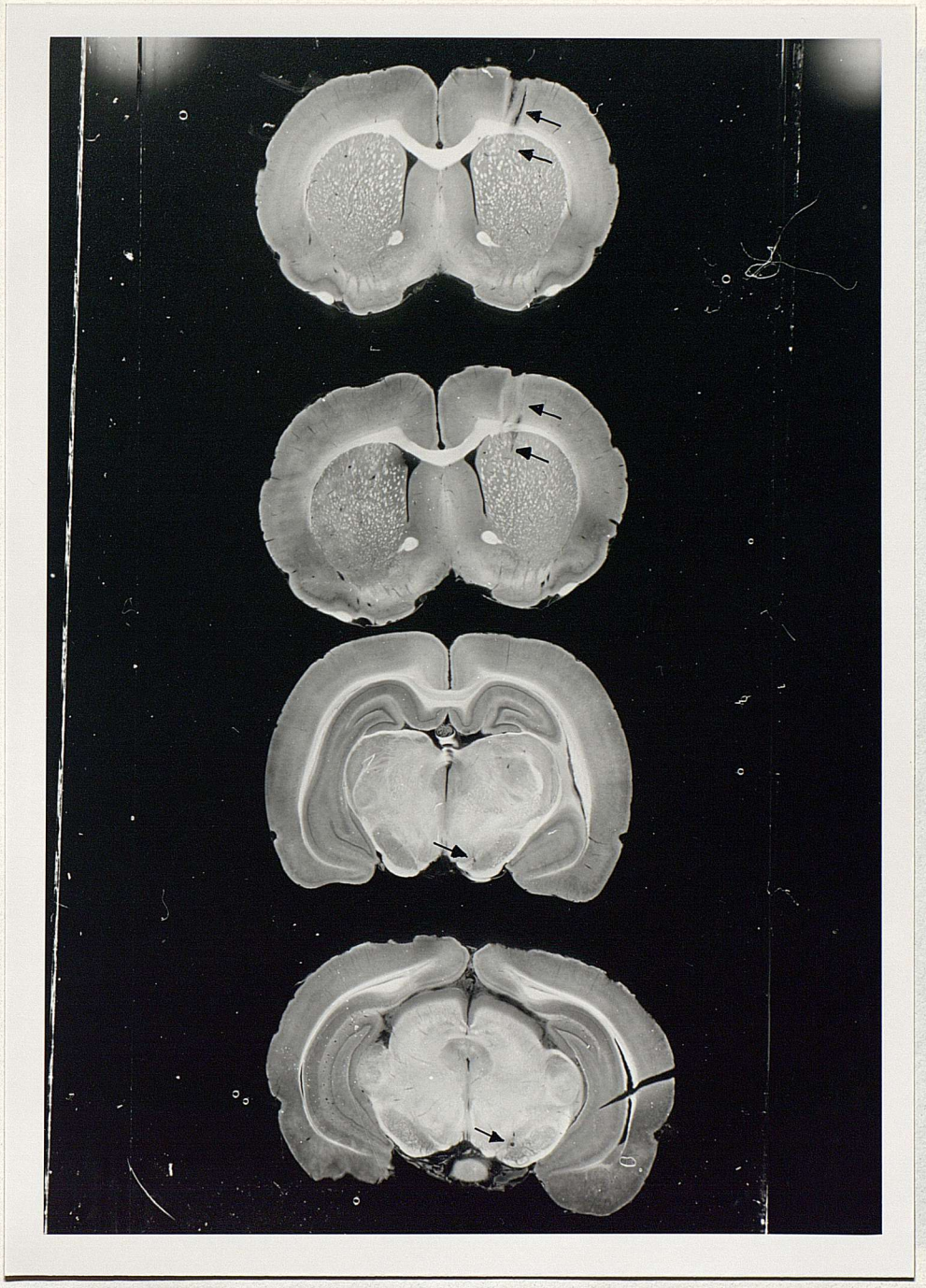


Figure (3.40). Coronal sections through two different rat brains. The recording electrodes were located in the zona compacta of the substantia nigra (arrows). The stimulating electrodes were in the centre of the caudate nucleus (arrows mark the electrode tracts).

Figure (3.41). Coronal section through an accumbens cannulated rat. The tips of the cannulae can be seen to terminate immediately above the nucleus accumbens.

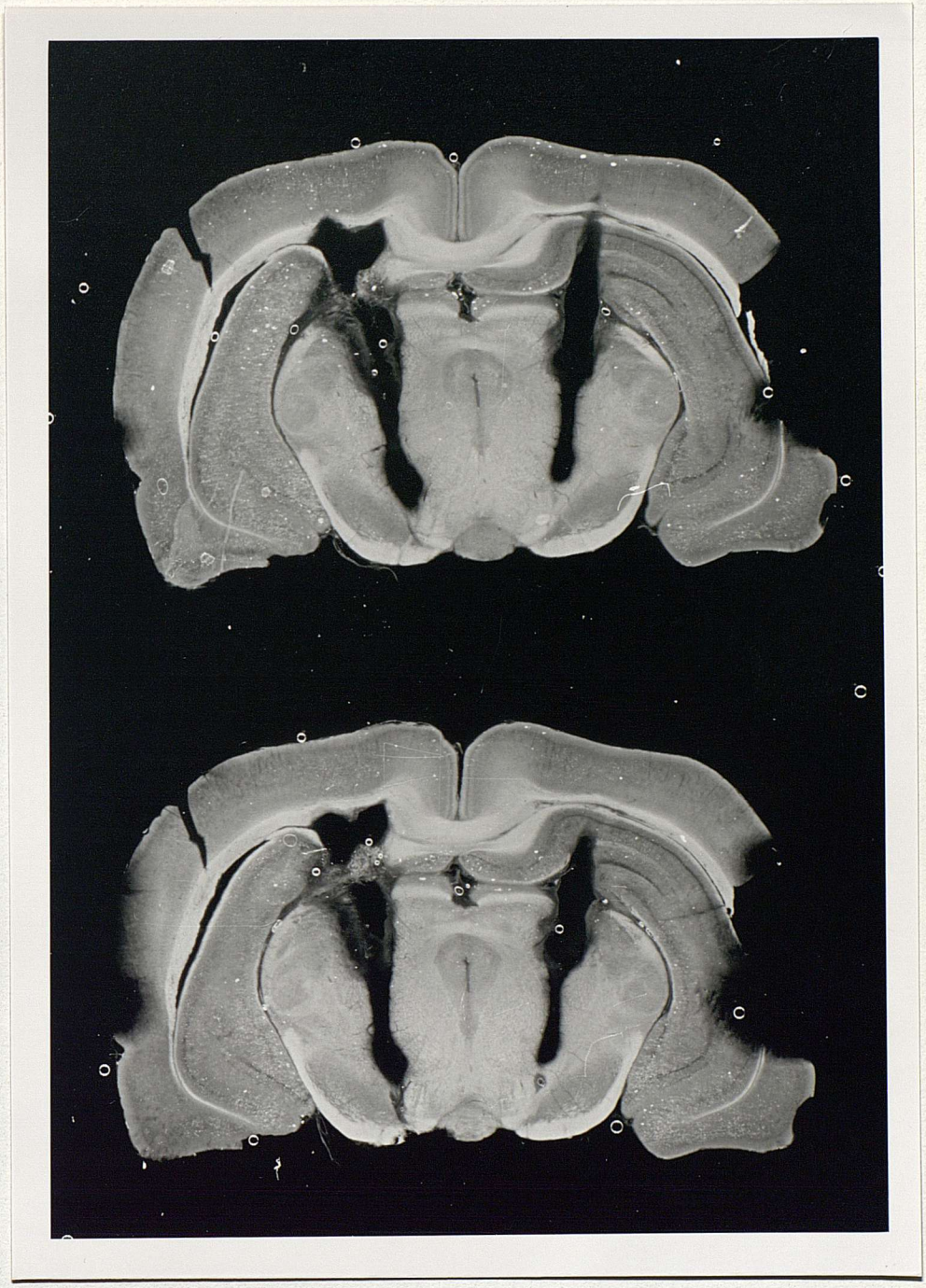


Figure (3.42). Coronal section through a VTA cannulated rat brain. As the sections are not exactly vertical the cortex through which the cannulae passed appear to be intact.

CHAPTER 4. DISCUSSION

4.1. Substance P

Iontophoresis of substance P produced a characteristic increase in the firing rate of cells in both the substantia nigra and the mesencephalic reticular formation (MRF) immediately above the nigra. These observations are similar to those obtained in 1976 by Davies and Dray. On the other hand, cells in the nucleus accumbens were not excited by substance P. Although the number of cells tested in this area was small this result is consistent with the small quantitiy of peptide present in the accumbens terminals.

The substantia nigra can be divided into two regions, the zona compacta and the zona reticulata. Electrophysiological evidence suggests the slow firing cells in the compacta are the soma of the ascending nigrostriatal dopamine neurones (Guyenet and Aghajanian, 1978), although the dendrites penetrate into the reticulata (Juraska, Wilson and Groves, 1977) see fig. 1.3. While substance P excited cells in the MRF and reticulata it did not have a consistent effect on the dopamine containing cells in the compacta either on its own or on the response to dopamine. Some consistent effect of the peptide might have been expected if substance P was acting directly on dopamine cells, as is suggested by the behavioural (Kelley and Iversen, 1979, Olpe and Koella, 1977, James and Starr, 1977, Diamond, et al. 1979) and biochemical evidence (Cheramy, et al. 1978). In these reports rotation, stereotypy and an increase in ipsilateral striatal dopamine release are caused by infusions of substance P into the nigra. Thus it is unclear how substance P causes these effects, or by analogy the locomotor stimulation after infusion of substance P into the VTA (Kelley, Stinus and Iversen, 1979) whose dopamine cells are morphologically part of the same structure as the nigral dopamine cells (Fallon and Moore, 1978).

Iontophoresis of the 1-2, 4-9 and 5-9 fragments of substance P produced no effects on any cells, which is consistent with the activity residing in the C-terminal hexapeptide. However, iontophoresis of the 1-9 nonapeptide did produce a very similar effect to substance P on cells which were substance P sensitive. A cross tachyphylaxis was seen between substance P and substance P 1-9 suggesting a common effector for the response. A similar effect has recently been reported for the 1-10 fragment of substance P in the spinal cord (Piercey and Einspahr, 1980). These results are surprising since neither fragment (1-9 and 1-10) contain the methionine residue which was found to be essential for tachykinin activity (Yanaihara, et al. 1979).

The significant increase in locomotor activity caused by injection of 3 µg of substance P bilaterally into the VTA is in accord with previously reported work which suggests activation of the ascending dopamine systems (Kelley, Stinus and Iversen, 1979). Substance P 1-9 also produced a similar increase in locomotor activity, while 1-2, 4-9 and 5-9 fragments were without effect at a similar dose to substance P.

There are several possible explanations for the unexpected activity of the 1-9 N-terminal fragment. For example substance P receptors in the nigra may not require the C-terminal for activity. This seems unlikely as in a behavioural study it was reported that substance P or ERP caused similar rotation (Olpe and Koella, 1977). A mixed population of receptors is possible as it has been reported from an iontophoretic study in the amygdala that while desensitization occurs after repeated applications of substance P or ERP, cross desensitization does not occur if ERP was applied after substance P desensitization had occurred (Ben Ari, et al. 1978). The N-terminal end of the substance P molecule does have activity, as in cultured neuroblastoma tissue addition of substance P or its 1-4 fragment increased cAMP levels within 20 minutes and caused neurite extension within 24 hours (Narumi and Maki, 1978). One of the most feasible explanations is that the 1-9 and 1-10 fragments are acting indirectly to inhibit a degradative enzyme, preventing the breakdown of endogenous substance P. Studies on the inactivation of substance P in which the C-terminal octapeptide and substance P are degraded at a much slower rate than the C-terminal hepta and hexapeptide, suggest that this N-terminal tetrapeptide stabilizes the rest of the molecule, protecting it from degradation by aminopeptidases (Blumberg and Teichberg, 1979). A recent report by the same authors on the kinetics of a "post-proline cleaving enzyme" isolated from cow brain shows that substance P interacts with this enzyme with a high affinity and is cleaved at the Pro^{4}-Gln^{5} bond to yield the C-terminal hexapeptide and the N-terminal tetrapeptide. Substance P is a potent competitive inhibitor of this enzyme (Blumberg, et al. 1980). These authors suggest that "breaking the Pro^{4}-Gln^{5} bond is the first step of a mechanism to regulate the synaptic lifetime of the peptide". A summary is shown in figure 4.2. Some support is given to this as while the 1-9 fragment of substance P does have an effect in the isolated rat spinal cord preparation (I. Briggs, ICI personal communication) this effect has not been reported in several studies, possibly due to the presence of protease inhibitors and also because these invitro systems (Otsuka and Konishi, 1977) may not be tonically active.

However if such a metabolic effect is the total explanation of the activity of the 1-9 and 1-10 activity it would be anticipated that iontophoretically or behaviourally substance P 4-9 would have activity. This was not found to be the case. In addition when applied iontophoretically we have found the peptides TRH, MIF and angiotensin II were inactive in the substantia nigra and surrounding areas. All of these peptides would be substrates for the degradative enzyme. The broad specificity of the degradative enzyme was shown in the study of Hersh and McKelvy (1979) who demonstrated that the enzyme from bovine and rat brain would metabolise substance P, TRH and LHRH and is potently inhibited by TRH (Rupnow, Taylor and Dixon, 1979). The properties of the post proline cleaving enzyme are very similar to those found in renal enzyme which cleaves the post proline bond in insulin, oxytocin, vasopressin and angiotensin I and II (Koida and Walter, 1976) (see figs. 4.2 and 4.3) suggesting the ubiquitous nature of proteolytic enzymes. It must be stressed that the mechanisms for peptide metabolism are far from clear. For example the snake venom peptide SQ20881 is an inhibitor of angiotensin converting enzymes (Oparil, 1977) and inhibits substance P degradation (Lee, Arregui and Iversen, 1979) while substance P inhibits angiotensin converting enzyme (McGeer and Singh, 1979). Angiotensin converting enzyme is decreased in both the striatum and nigra in Huntingtons Chorea (Arregui, Emson and Spokes, 1978) as is substance P (Kanazawa, et al. 1979), and angiotensin converting enzyme is also reduced in some types of schizophrenia (Arregui, et al. 1978). This may suggest a link between angiotensins and substance P or their metabolism. Infusions and iontophoresis of other N-terminal fragments of substance P and enzyme inhibitors such as SQ20881 might throw some light on the role of the substance P N-terminal.

Structure activity studies (1.1.3) suggest that the C-terminal hexapeptide is the essential fragment of the substance P molecule. Therefore it was some surprise to find that this peptide, its pyroglutamyl analogue and an eledoisin analogue (81495, see fig. 4.3) were without effect on any cells in the substantia nigra when applied by iontophoresis. This result is in contrast to an iontophoretic study in the spinal cord where the 6-11 fragment is equiactive to substance P (Piercey and Einspahr,1980). Other studies in the locus coeruleus have found ERP and physalaemin are as active as substance P (Guyenet and Aghajanian, 1977). Additionally, the behavioural effects of substance P are the same as those of substance P 6-11 (Olpe and Koella, 1977, Kubicki, 1978). However a likely explanation of this lack of activity is apparent when considering the limitations of the iontophoretic technique.

The technique is dependant upon the production of an ionizable current carrying molecule being expelled from the micropipette into the brain tissue. Substance P is a triacetate which will dissociate to give charged molecule, see fig. 4.4. At pH 4-5 it has been shown to be expelled from micropipettes with positive currents (Henry, Krnjevic and Morris, 1974, Guyenet and Aghajanian, 1978). At higher pH, electrophoretic mobility studies show it smears rather than moves in a discrete band (Mroz and Leeman, 1978). The charge is carried at the N-terminal. Hence peptides lacking the N-terminal tripeptide carry considerably less charge and while they may contain polar groups, are less soluble, than substance P by a factor of 10-100. The pyroglutamyl 6-11 fragment is soluble only after first dissolving in absolute alcohol. Thus it seems probable that in these cases where C-terminal sequences were inactive, sufficient quantities of the peptides were not expelled from the electrodes, most of the charge being carried by hydrogen ions. The currents in our system required to produce a substance P response are in the order of 50-100nA, hence a decrease in potency of 10 would require currents of 500-1000nA, which is beyond the capacity of the present system and furthermore, at the lower end of the range of electrical currents required to stimulate whole brain nuclei. For example the eledoisin related fragment (81495) was 1,000 x less potent than substance P on the ileum (J. Growcott personal communication) even though it was water soluble, hence a current of 10,000nA would be required to produce an effect comparable to substance P, thus it was no surprise to find no effect in our experiments.

It must be added that some authors do not find charge of the molecule of great importance, both ERP and substance P being effectively ejected from 16mM NaCl solutions (Piercey and Einspahr, 1980, Wright and Roberts, 1978, Le Gal La Salle and Ben Ari, 1977) although Guyenet, et al. (1979) find this causes the transport number to fall by a factor of 10.

It has been suggested that as shorter analogues are degraded faster than longer ones (Blumberg and Teichberg 1979) they never reach a receptor after being ejected. This seems unlikely in view of Piercey and Einspahr's results (1980) which found that the 6-11 fragment has a similar potency and time course of action to substance P. However the extremely poor solubility of this fragment in acetate solutions prevented us from achieving any meaningful results. Guyenet and Aghajanian (1977) found substance P and ERP, an analogue of the C-terminal hexapeptide, have similar iontophoretic

effects in the locus coeruleus. The fact that most substance P solutions may contain up to 100% Substance P sulfoxide (Floor and Leeman, 1980) and substance P breaks down in microelectrodes (Gozlan, et al. 1977) means that quantitative interpretations are difficult with the iontophoretic technique.

The low solubility of the C-terminal 6-11 fragment and low potency of the eledoisin peptide 81495, made testing of these analogues in the behavioural system impractical. The volume of CSF required as solvent was greater than the volume of the VTA itself leading to the possibility that structures outside the VTA may be affected by the analogues. The use of strong acids, bases or organic solvents would increase the solubility of the fragments but would then be expected to produce side effects. For these reasons it was decided not to test the fragments in this system.

A substance P antagonist may have analgesic activity and would be of use in studying the function of substance P in the brain. Studies with putative antagonists on peripheral preparations have been inconclusive. Pinas, Poulos and Theodro (1979) have suggested that the N5 dimethyl glutamine analogue of the C-terminal hexapeptide is an antagonist or partial agonist. However, Growcott and Petter (1980) found from their studies with D-Phe^{7}-Substance P that tachyphylaxis rather than antagonism is a more likely explanation of the putative antagonists. A modified C-terminal analogue of substance P, 140143 (structure in appendix) and two modified amino acids have been found to reduce or abolish substance P responses in the GPI (J. Growcott personal communication). If a substance P response can be blocked by iontophoretic application of one of these antagonists, it should be possible, by stimulating the appropriate substance P pathway, to satisfy one of the criteria for identification of putative neurotransmitters. That is, the antagonism of an evoked response. Therefore we used all three putative antagonists even though the structures of the amino acids are not known.

However, the peptide 140143 was inactive in both behavioural and iontophoretic studies and the amino acids produced inconsistent effects. Thus no firm conclusions can be drawn. Again it must be stressed that any partial or even complete antagonist effect would be barely detectable unless it produced more than a 25% reduction in substance P response.

We have confirmed the previously described behavioural (Kelley, Stinus and

Iversen, 1979) and iontophoretic effects of substance P (Davies and Dray, 1976). In spite of the scarcity of peptides and their poor solubility, we have evidence, through the use of N-terminal fragments of substance P, that there is a difference between the central and peripheral pharmacology of substance P. This may involve receptor specificity, metabolism or a combination of both.

4.2. MSH Release inhibiting factor. MIF

The electrophysiological and behavioural changes observed after iontophoretic application and peripheral injection of MIF were no different from controls. Direct effect of the tripeptide on neurones would be very easily seen in our iontophoretic studies in any of the three areas in which these were tried, (substantianigra, caudate nucleus and nucleus accumbens), as would a modulating effect on the postsynaptic dopamine reponse if these occurred with acute administration. Neither electrophysiological nor behavioural changes occurred. This disagrees with reports which suggest a separate postsynaptic site for MIF, as the peptide had no effect on dopamine metabolism, turnover or uptake (Spirtes, et al. 1976, Plotnikoff, Minard and Kastin, 1979). Chiu, Wong and Mishra (1980) describe the binding of ^{3}H-MIF to calf caudate membrane preparations while Kostrzewa, et al. (1976) found that MIF did not alter the binding of ^{3}H-dopamine. This suggests a separate MIF receptor. If this was so our studies would have shown at least a small percentage of the cells to respond to direct application of the peptide in at least one of the areas, in spite of the fact that the number of cells encountered in the caudate nucleus and nucleus accumbens was small. It is possible that while applying an excitatory substance such as DLH to silent cells a variable to the normal neuronal function may be introduced. The number of spontaneous cells encountered in the caudate and accumbens was so low that they may represent a select population and hence introduce a bias problem. Thus it is possible that a "MIF effect" is masked. A further possibility is that MIF acts in another brain area not examined in this study. Kostrzewa, Kastin and Spirtes (1975) for example, reported raised noradrenaline levels in the mid brain but not hypothalamus or striatum after MIF treatment.

Some authors have reported changes in dopaminergic function following MIF treatment, for example Friedman, Friedman and Gershon (1973) found changes in dopamine levels after MIF administration. In view of literature which suggests that MIF might interact with dopamine systems (1.2.2.) the lack of reponse to MIF reported in this thesis is very surprising. It is possible that the failure to observe a response might be due in part to experimental design and procedure. If MIF were acting presynaptically to modulate the release of dopamine our inability to obtain any effects could be due to dopamine pathways being inactivated by the anaesthetic we used.

If the activity of the dopamine pathways was depressed a presynaptic effect would be masked as our electrophysiology system measures changes in

firing rate at the post synaptic membrane. However this is unlikely since using the same anaesthetic both ourselves and others (Bunney, et al. 1973) have demonstrated an increase in the firing rate of the dopamine neurones with increasing doses of anaesthetic. Additionally, in the behavioural experiments the animals were treated with a submaximal dose of amphetamine, to ensure active dopamine metabolism during MIF administration. Furthermore in untreated conscious animals showing none of the classical symptoms of dopaminergic depression such as catatonia or Parkinsonian like symptoms again MIF was without effect. When a recording is made from the dopamine cell bodies of the nigro-striatal tract, a very stable baseline firing rate is obtained. The activity of these cells is extraordinarily sensitive to drugs which interact with dopamine (section 1.5.4.). A wide range of MIF doses injected intravenously while recording from such neurones produced no change in firing rate. Using this experimental approach the system is sensitive to 3 µg/kg apomorphine, a dose which is at least ten times lower than that required to produce a stereotyped behavioural effect. It seems fair to conclude that a compound which has similar actions would produce at least some effect at 10,000 times this dose of apomorphine. If the MIF effect is long term rather than short term as is suggested by the experiments in which MIF is given chronically, it would be hard to detect in our system, as it is unusual to record from a neurone for periods of more than one hour and results over such a period are difficult to interpret.

There seems to be an increasing volume of literature which demonstrates the inability of MIF to affect systems other than those involved in MSH release. It has been observed that in the original clinical trials where MIF had therapeutic actions, the effects may be largely placebo. This is suggested from a study using identical conditions to those originally used by Barbeau in 1975. In this latter study no change was seen in the total disability score, intellectual function and dopa-induced excretion of anterior pituitary hormones in Parkinsonian patients concurrently receiving L-dopa therapy (Carenceni, 1979). However, Gonce and Barbeau (1978) have been able to repeat their original results. It has also been found that neither MSH, melatonin or MIF caused a change in general locomotor activity in hypophysectomized or normal rats, nor any change in food or water intake (Kastin, et al. 1973). Sandman and Kastin (1978) found that B-MSH but not MIF potentiated the effect of amphetamine in rats. In a very thorough study, Cox, Kastin and Schnieden (1976) found that in addition to its inability to potentiate amphetamine or apomorphine, MIF failed to produce stereotyped behaviour in

the same way as amphetamine, apomorphine or amantadine, neither did it have the same effect as L-dopa in rats treated with MAO inhibitors, nor did it antagonise chlorpromazine induced loss of activity, (a similar result to ours with haloperidol) which could be reversed by amphetamine, apomorphine or amphetamine. The only effect produced by MIF seemed to be a slight antagonism of apomorphine. Similar results to these have been found in other laboratories (T.P. Blackburn personal communication). MIF also failed to abolish TRH induced tremor in rats (Schenkel-Hulliger, et al. 1974) and recent reports have cast doubts on the reproducibility of experiments in which MIF reversed oxotremorine tremor, fluphenazine catalepsy and potentiated amphetamine hyperactivity (Bjorkman, Lewander and Zetterstrom, 1980). Electrophysiological experiments in a sensitive invertebrate system under control of a dopaminergic cholinergic balance (which shows a striking similarity to the extrapyramidal system) found that neither acute nor chronic administration of MIF has either dopaminergic or anticholinergic properties (Carolei, Margotta and Palladini, 1977). Iontophoretic application of MIF to neurones has been attempted in the cortex where it was without effect (Yarbrough, 1978). It is possible that strain differences in animals could account for these negative results, for example we use an albino rat which has a defective pigmentation system. However it is significant that there are very few reports on MIF release, uptake, microscopic distribution or mimicry of an electrophysiological event, all essential criteria for putative transmitters. It is possible that MIF only has effects in the Parkinsonian state, as it is known that the substantia nigra is only pigmented in primates (quoted in review by Dray, 1979). Thus we have found that MIF has no effect when applied iontophoretically to neurones in the substantia nigra, caudate nucleus and nucleus accumbens. This was not due to a failure to release MIF from micropipettes. Peripheral administration of MIF did not potentiate the locomotor response to a submaximal dose of amphetamine administered either peripherally or into the accumbens, nor does it change the firing rate of dopamine containing cells. Finally it does not antagonise the effect of haloperidol on spontaneous exploratory behaviour in rats.

4.3. Thyrotropin releasing hormone (TRH)

Another tripeptide which is thought to have extrahypothalamic actions is TRH. Previously discussed literature (1.3.2.) has suggested that there is a link between this tripeptide and dopaminergic function. Thus it was surprising that it had no effects either applied iontophoretically or in behavioural experiments. When recordings were made from spontaneously active dopamine containing cells in the substantia nigra or VTA both TRH and a stable methylated analogue CRM 221/3 (Structure in the appendix) produced a slight increase in baseline firing rate of the dopamine cells. This may be in accord with a regulation of dopamine release as suggested by Heal and Green (1979). However, a drug which causes dopamine release, as these authors propose, would be expected to decrease rather than increase the firing rate of presynaptic neurones. It is possible that TRH could be acting at some other site either centrally or peripherally as the increase in neuronal firing was accompanied by a rise in respiration rate. Other laboratories have found that peripheral stimuli changed the firing rate of these neurones (Chiodo, et al. 1980). The negative results with iontophoretic TRH may be caused by several factors. Although Renaud, Martin and Brazeau (1976) have shown that TRH is released iontophoretically, we did not. It is of course possible that the iontophoretically released TRH could be metabolized before reaching its receptors. It should be noted, however, that the stable TRH analogue, CRM 221/3 was similarly inactive.

While a negative result with TRH from the substantia nigra and caudate nucleus was not unexpected, the lack of effects in the accumbens is, in view of the stimulatory role suggested from the literature previously discussed (1.3.2.). Previous literature reports have found TRH inhibits most cells with a very rapid onset and short duration of action similar to that of GABA in the preoptic area and septum. These areas are densely innervated by dopamine fibres. In these experiments no potentiation of dopamine response by TRH was observed (Winokur and Beckman, 1978). This is similar to our results where no dopamine potentiation was apparent.

It has been suggested that the effects of TRH are caused by $\overline{\text{His-Pro}}$, a breakdown product of the tripeptide. This metabolite is produced by the action of pyroglutamyl peptidase rather than by post-proline amidase which yields the acid-TRH metabolite (Prassad, Matsui and Pelerkofsky, 1977). However, these reports have not been substantiated (B. Morgan personal communication). In our hands $\llcorner$His-Pro$\lrcorner$ was inactive also.

Other authors have found TRH to be active when applied iontophoretically into many brain areas which we did not examine. For example Renaud, Martin and Brazeau (1976) found it to be inhibitory, even in the cerebellum, an area devoid of TRH-like material (Hokfelt, et al. 1975). Cortical cell firing induced by glutamate but not acetyl choline was found by Renaud, et al. (1979) to be depressed by TRH. On the other hand, Yarbrough (1978) reported that TRH and the TRH analogue MK 177, caused a potentiation of Ach responses, but had no effect on glutamate evoked excitations. However, it is important to note that the experiments of Yarbrough (1978) were performed in animals anaesthetized with barbiturates. These are known to supress Ach responses in other brain areas (Bloom, Costa and Salmoiraghi 1965).

There is also some question over the action of MK 177. In common with TRH it produces an increase in rat fore and hind limb EMG valves after intracisternal injection. However, when dose response curves of the two agonists are plotted, the lines are not parallel (Yarbough, McGuffin and Clineschmidt, 1979). It has been suggested that the barbiturate reversing properties of TRH reported by Prange, et al. (1974) are due to the excitatory effects of the peptide on medullary neurones (Briggs, 1979). These arousing properties are centainly not mediated via the locus coeruleus neurones where iontophoretic TRH is inactive, although both substance P and enkephalins have effects (Guyenet and Aghajanian, 1977).

Although TRH has been reported to cause hyperactivity after peripheral or intra-accumbens injection (Miyamoto and Nagawa, 1977, Heal and Green, 1979, Miyamoto, et al. 1979) it was inactive in our hands. Others have been unable to repeat these observations (Costall, et al. 1979). Other negative results with TRH have been obtained in experiments in which the substance was tested for its ability to potentiate amphetamine or reverse motor depression caused by neuroleptics (Costall, et al. 1979). This is in agreement with behavioural studies reported in this thesis. Here TRH was found to have no effect on hyperactivity, and did not potentiate a submaximal dose of either peripherally administered or intra accumbens amphetamine.

It is possible that our failure to find TRH responses in either behavioural or electrophysiological tests could be caused by the hormonal state of the animal. It has been reported that after oestradiol pretreatment, a reduced response to TRH on medial preoptic neurones occurs (Moss and Dudley, 1978). In addition, TRH decreased MAO activity in the median eminance of male rats

while it increases it in female rats (Wirz-Justice and Lichtsteiner, 1977). Other evidence indicates there may be different species responses to TRH. For example TRH causes a change in REM sleep onset in rats (Korany, Whitmoyer and Sawyer, 1977) while no changes are seen in man (Nakazawa, et al. 1979). Furthermore, there are now doubts whether the therapeutic effects of TRH originally reported in depression (Schalch, et al. 1972) "were merely a placebo effect" (Benkert, Gordon and Martichke, 1974). This is supported by evidence where it was found that TRH will not cross the blood brain barrier in significant quantities (Dupont, et al. 1972). Finally and more significantly, there is evidence that immunoreactive TRH is not the same as true TRH. Molecular species other than TRH were capable of displacing tracer TRH, a common problem with the immunohistochemical techniques. Lipids have been shown to interfere non competitively with the tracer TRH (Youngblood, Lipton and Kizer, 1978). This has important implications for distribution studies which show over 70% of the immunoreactive TRH is extrahypothalamic (Hökfelt, et al. 1975).

We have found that iontophoretic application of TRH, CRM 221/3 a stable methylated analogue and His-Pro produced no change in the firing rate of cells in the substantia nigra, caudate nucleus and nucleus accumbens. The inhibitory response to iontophoretically applied dopamine was unchanged by TRH in either the caudate or the accumbens. A slight increase in the firing rate of dopamine cells in the VTA and substantia nigra was seen after intravenous injections of TRH or CRM 221/3. Neither peripheral nor intra accumbens amphetamine induced hyperactivity was changed by TRH. These results suggest that the stimulant actions of TRH may not be intimately involved with dopamine.

4.4. Opiod peptides

It is thought that enkephalins act more selectively on δ than μ receptors (reviewed by Lord, et al. 1977). A particularly potent δ receptor agonist is D-Ala^2-d-Leu^5-enkephalin (BW180C) (J. Shaw personal communication). The classical opiate antagonists such as naloxone, are thought to act more selectively on μ receptors than δ receptors, however, little is known about δ receptor antagonists. A pentapeptide protected at its N-terminal by blocking groups, 139462, whose structure is not known, has been found to be the most selective δ antagonist on δ receptors in the mouse vas deferens (MVD). It has been reported that bilateral injections of d-Ala^2-met^5-enkephalin (DALA-enk) into the VTA cause a naloxone reversible hyperactivity (Broekkamp, Phillips and Cools, 1979).

We repeated these experiments and tried to block the hyperactive response with either naloxone or 139462, in an attempt to determine whether μ or d receptors are the predominant forms in this area. Similar experiments were carried out in the nucleus accumbens where opiates induced hyperactivity after bilateral injection (Pert and Sivit, 1977). In the VTA it was found that BW180C produced an initial short latency and duration hyperactivity, followed by a period of quiescence. This was followed by a long lasting but relatively low intensity hyperactivity. This result is not entirely in agreement with previously reported enkephalin effects where 2μg/side (DALA-end) produced a continuous effect lasting for over 90 minutes (Broekkamp, Phillips and Cools, 1979). This descrepancy may be due to the dose injected, as Kelley, Stinus and Iversen (1980) found 2μg/side produced a maximum response, while increasing the dose to 5mg/side produced a period of inactivity followed by a lower level hyperactivity, peaking some 90 minutes after injection (Kelley, Stinus and Iversen, 1980). A similar effect was observed with high doses of β-endorphin, where a period of immobility is followed by hyperactivity, in contrast to the continuous locomotor activity seen with low doses (Stinus, et al. 1980). Although both short and long duration locomotor effects after BW180C injections into the VTA were partially reduced by 2mg/kg naloxone or 5μg/side 139462 the effects were not statistically significant. Neither naloxone nor 139462 had any effects when administered alone. The failure to observe antagonism may be due to the proportionately low doses of antagonists used. It has been found that 1mg/kg naloxone will block hyperactivity caused by 0.06 nmol β-endorphin while it is without effect on 1.5 nmol (Stinus, et al. 1980). It seems a wide range of both antagonists and agonists must be tried if this study is

to be extended.

These results are different from our results with substance P, where a short duration hyperactivity is produced. There appears to be a long term interaction between opiates and dopamine neurones in that the response to amphetamine is reduced for some hours following DALAenk infusions into the VTA (Kelley, Stinus and Iversen, 1980). They suggest that opiates have a presynaptic inhibitory action on the terminals of inhibitory neurones, thereby causing an increase in the firing of dopamine containing cells, as opposed to the direct stimulant action they propose for substance P. Certainly these effects are different from those at the "pain gate" in the spinal cord.

A slow onset hyperactivity of greater magnitude than that already described was observed following infusion of BW180C into the nucleus accumbens. This is in agreement with the previously reported work of Pert and Sivit (1977). The hyperactivity commences some 20-30 minutes after injection and continues for a further 70 minutes. It is unlikely that the effect is caused by the peptide diffusing from the injection site to a different brain area as injection of 0.5µl of pontamine sky blue into the VTA and nucleus accumbens three hours before fixing with 10% formol saline indicates the drugs remain in the area of injection. However in contrast to the previous report we were unable to show any decrease in locomotor activity with naloxone or 139462. Again neither antagonist had any effect when given alone. As already discussed larger dose ranges must be employed if a distinction between different receptors proposed in the VTA and nucleus accumbens are to be detected.

Iontophoretic application of BW180C inhibited both caudate and accumbens neurones in a similar manner to that reported for leu and met-enkephalin (McCarthy, Walker and Woodruff, 1977). The peptide 139462 was without effect on its own or on the inhibition produced by BW180C. Naloxone was similarly without effect. The enkephalin effects were shown by McCarthy, Woodruff and Walker (1977) to be reversed by naloxone but only after long application. Additionally, the possibility that 139462 may not be released from the electrodes was not checked in these experiments. It is also of interest that Naloxone has been reported to have non specific GABA antagonising properties under iontophoretic conditions (Dingledine, Iversen and Breuker, 1978).

While there are k receptors in the periphery there do not appear to be any in the brain or spinal cord. The k receptor agonist ethylketocyclazocine has a similar binding distribution to the other opiates (Hiller and Simon, 1979). Additionally, it does have antinociceptive activity even though it does not substitute for morphine in the morphine dependant monkey (reviewed by Lord, et al. 1977). Thus it was somewhat surprising to find that bilateral infusions of 10μg of ethylketocyclazocine bilterally into the nucleus accumbens produced no increase in spontaneous locomotor activity, although as previously discussed enkephalin analogues will do so. Intravenous injections of 0.1 - 10mg/Kg ethylketocyclazocine had no effect on the firing rate of substantia nigra compacta dopamine neurones, while morphine increased the firing rate in a similar manner to that reported by Iwatsubo and Cluoet (1977). These results suggest that ethylketocyclazocine does not act on these systems in the same way as some other opiate drugs.

The experiments presented here were carried out in an attempt to discriminate between the three proposed opiate receptors, μ, δ and k. While the preliminary results of this study suggest there are no k receptors and that the identity of the receptor mediating either hyperactivity or cell inhibitory responses is unclear it must be noted that the study is incomplete. Wide ranges of both agonists and antagonists need to be used. Finally, it is possible that the receptors in the peripheral assays may not be the same as those in the brain.

4.5. Dopamine

Dopamine is thought to have an important neurotransmitter role in the basal ganglia and mesolimbic system. In addition it may have a function in the retina and control of pituitary hormone release.

There is good evidence that dopamine dysfunctions are involved in the pathology of Parkinsonism and Schizophrenia (1.5.6.). Side effects of any drugs limit their therapeutic value, thus L-Dopa therapy, while alleviating Parkinsonian symptoms can produce hyperkinesia and hypotension. Neuroleptics on the other hand may lead to iatrogenic Parkinsonism (reviewed by Hornykiewicz, 1979).

One of the problems in the testing of dopamine agonists and antagonists is the lack of an easily studied peripheral preparation which contains specific dopamine receptors. The most commonly studied peripheral preparation is the dog renal vasculature which was first used by Goldberg, et al. in 1968. CNS dopamine preparations are most numerous and have received most attention. These include the dopamine sensitive adenylate cyclase (1.5.2.) and receptor binding techniques using labelled dopamine receptor agonists or antagonists (1.5.2.) and review by Titeler and Seeman (1979). Behavioural models include rotation in rats with unilateral lesions of the nigrostriatal tracts and locomotor stimulation after bilateral drug injections into the nucleus accumbens. However, these techniques have several associated problems. For example, the adenylate cyclase is thought to be linked to a post synaptic receptor (reviews by Iversen, 1975, and Krueger, et al. 1976) thus presynaptic effects are ignored. The binding of ligands to dopamine receptors is complicated by factors such as the specificity of the ligand, the association rate of the receptor-ligand complex (Titeler and Seeman, 1978) and most importantly the use of crude preparations containing pre and post synaptic binding sites. Behavioural responses to drugs are extremely complex as generally the end response measured is far removed from the initial receptor response and mediated by other factors. For example, drugs such as opiates can produce similar behavioural results, such as catalepsy and hyperactivity, which are produced by dopamine antagonists and agonists, indicating the complexity of the responses.

Electrophysiology is a good alternative for studying the effect of drugs at the cellular level. However, the techniques have inherent problems. One of the most satisfactory models originally used for dopamine structure activity studies by Woodruff and Walker in 1969 was cells in the brain of the

snail Helix Aspersa. A mammalian system has certain advantages, hence the use of iontophoresis. Unfortunately, the areas of the mammalian brain innervated by dopamine, such as the caudate nucleus and nucleus accumbens, contain a largely silent population of neurones in the anaesthetized animal (Bloom, Costa and Salmoiraghi, 1965, Feltz and McKenzie, 1969). Additionally, the extracellular recording techniques which the majority of studies have used cannot distinguish between a pre and post synaptic drug effect very easily.

In the substantia nigra the dopamine containing cell bodies of the ascending nigrostriatal tract can be easily distinguished from other neurones by their characteristic action potential shape and duration (Bunney, Aghajanian and Roth, 1973) and by antidromic activation from the ipsilateral striatum (Guyenet and Aghajanian, 1978). The firing rate of these cells in chloral hydrate or halothane anaesthetised rats is exceptionally stable. The cells fire at 2 to 10 Hz, this rate rarely changing by more than 10% over many minutes. This property makes them ideal for extracellular recording and iontophoresis. These cell bodies are thought to have dopamine receptors on them and these receptors may be the same as the presynaptic receptors on the terminals. The evidence for this came from both electrophysiological and biochemical studies. While a dopamine sensitive adenylate cyclase was found in the nigra, it was thought to be present on cellular elements other than dopamine neurones. Thus 6OHDA lesions of the nigro striatal tract did not affect the cyclase activity in either the nigra (Kebabian and Saavedra, 1976) or the striatum (Kreuger, et al. 1976). Intrastriatal kainate lesions did not reduce striatal dopamine content (Di Chiara, et al. 1977) nor did it affect the inhibitory response of nigral neurones to iontophoretic dopamine or intravenous apomorphine. However, it did significantly reduce the response to the dopamine releasing drug amphetamine (Bunney and Aghajanian, 1978, Baring, Walters and Eng, 1980). Further support for dendro-dendritic synapses rather than an interneuronal involvement came from autocorrelation studies on spike trains of substantia nigra neurones (Wilson, Young and Groves, 1977). Anatomical evidence found that the only post synaptic elements engaging in monoaminergic synapses in the substantia nigra were dendrites of medium sized pars compacta neurones (Wilson, Groves and Fifkova, 1977). Additionally, an autoradiographic electron microscopic study found ^{3}H-dopamine localized on the dendrites of nigral compacta neurones (Cuello and Kelly, 1977).

Thus when using the substantia nigra compacta neurones we are probably looking at the effects of drugs on a specific population of dopamine receptors. It is probable that both dendrodendritic or "autoinhibition" and a striatonigral feedback control the firing rate of dopamine containing neurones. Certainly, both have a relevance in the clinical treatment of dopamine disorders as post firing inhibition was found to be decreased by haloperidol (Wilson, Fenster, Young and Groves, 1979) and iontophoretic trifluoperazene reduced the post antidromic spike inhibition usually seen (Nakamura, et al. 1979). Striatal lesions with kainic acid also produced a decrease in post stimulus firing rate (Kondo and Iwatsubo, 1980, Nakamura, et al. 1979). Feltz (1971) and Crossman, Walker, Woodruff (1974)b have both reported an inhibition of cell types corresponding to reticulata cells after electrical stimulation of the ipsilateral striatum.

In the present study it was found that iontophoretic application of noradrenaline to dopamine containing cells in the nigra caused a similar decrease in firing rate as dopamine. This is in agreement with previously reported literature (Aghajanian and Bunney, 1977, Collingridge and Davies, 1980). However, in contrast to these reports the duration of noradrenaline action was much shorter than that of dopamine. This discrepancy cannot be accounted for as we have also shown that the amount of tritiated noradrenaline or dopamine released with identical iontophoretic currents was very similar. p-tyramine had little effect when applied to neurones at the same current for the same length of time as dopamine, but when applied with high currents for long periods it had a weak but long lasting effect. The structure activity requirements for dopamine at its receptor site were first systematically tested on two preparations. These were the dog renal vasculature (Goldberg, Sonneville and Mc Nay, 1968) and neurones in the brain of the snail Helix Aspersa (Woodruff and Walker, 1969). In both of these preparations L-noradrenaline and p-tyramine had very weak or no dopamine-like activity. These requirements have subsequently been shown to be very similar to those for stimulation of the dopamine sensitive adenylate cyclase in both the striatum (Munday, Poat and Woodruff, 1976) and nucleus accumbens (Munday, et al. 1977, Watling, Woodruff and Poat, 1979). Thus the tyramine and noradrenaline effects may have been due to a releasing action on the tonically active system rather than a direct effect on dopamine receptors. The use of tyrosine hydroxylase inhibitors, uptake inhibitors and substitution of calcium ions could possibly throw some light on this. Dopamine, noradrenaline and tyramine effects were blocked by iontophoretically applied

sulpiride (discussed later), while the response to GABA was not changed, which suggests a common effector for the response to amines.

On the basis of the original studies with the snail preparation it was proposed that the molecule 2 amino, 6, 7-dihydroxy, 1, 2, 3, 4-tetrahydronaphthalene (ADTN) would contain the active form of dopamine (Woodruff, 1971). As previously mentioned the dopamine molecule can reside in extended and folded conformations. In the extended conformation, rotation about the dihydroxyphenyl moiety can lead to the possibility of two rotomeric extremes which have been designated α and β by Cannon (1975). The α rotomer corresponds to 2-amino, 5, 6- dihydroxy 1, 2, 3, 4, tetrahydronaphthalene (5, 6 ATN) while the β rotomer corresponds to ADTN. There are some similarities between 5, 6 ATN and apomorphine which has been suggested as a potent dopamine agonist on the basis of behavioural tests. In the present study the effects of ADTN were tested with dopamine on substantia nigra dopamine neurones identified by antidromic spikes, and compared with the effects of intravenous apomorphine. It was found that iontophoretic application of ADTN produced a very potent (50-100%) and longlasting inhibition of firing rate during which the extra-cellularly recorded spike size increased. The response to dopamine was lost after application of ADTN and did not return after one hour. By this time the firing rate of the cell had returned to some 70% of its baseline value. This result could be explained by a desensitisation of the dopamine receptors by ADTN application. The desensitisation caused initial technical problems in the testing of ADTN. For example if the iontophoretic barrel was prefilled with ADTN in the normal fashion, many of the dopamine cells located were very slow firing or silent (idenitified by antidromic stimulation) and did not respond to iontophoretic dopamine. This is presumably due to spontaneous leakage of ADTN and subsequent desensitisation of the cells, even with the normal 10nA retaining currents. This was overcome to some extent by increasing the retaining current to 20nA. Even so, the response of the cells to dopamine was very weak. This problem was eventually solved by leaving one barrel of the microelectrode empty. A cell producing consistent responses to dopamine was then located. After this had been achieved the ADTN was injected into the empty barrel via a previously inserted length of PP10 portex tubing. A 10nA retaining current was applied throughout filling. In this way the cell recording was not disturbed. Then a pulse of ADTN, usually 60nA for 60 seconds was applied during the sequence of dopamine pulses (60nA 15 sec at 2 or 3 min intervals). The inhibition produced by ADTN was 100 to 1000 times greater than that

produced by dopamine. However, quantitative comparisons are difficult as the amount of ADTN released must be small. Even using a prefilled ADTN electrode quantitization would be difficult as to retain the drug a high current is needed and hence the tip concentration is low. This means that several ejection pulses are needed before a constant quantity of drug is released. Only one pulse of drug can be given during the experiment as the effect is so long lasting and it is unusual to record from one cell for more than 120 minutes. For these reasons relative potencies of different ADTN derivatives would have to be made on different cells in different rats. In fact our own results show that the iontophoretic application of ADTN produces a similar inhibitory effect on the firing rate of dopamine SNC cells as 3-30 µg/Kg apomorphine. Interestingly it has been found that iontophoresis of apomorphine will produce a long lasting inhibition of dopamine containing neurones (Aghajanian and Bunney, 1977). This inhibition was of shorter duration than that found with ADTN suggesting that apomorphine is less potent on dopamine receptors than ADTN.

The long lasting effects of ADTN are in agreement with other work which shows that ADTN is a potent dopamine agonist in receptor binding, stimulation of adenylate cyclase and behavioural tests (Woodruff, et al. 1977, Munday, et al. 1977, Woodruff et al. 1979, Watling, Woodruff and Poat, 1979). In addition ADTN has been shown to be more potent than 2 amino 5, 6 dihydroxy 1234 tetrahydronaphthalene in hyperpolarizing snail neurones (review by Woodruff, 1979) and depolarising other snail neurones (Batta, Woodruff, Walker unpublished). ADTN has also been found to be a potent dopamine agonist in decreasing G.P. blood pressure (Woodruff, Elkhawad and Pinder, 1974) and in dilating renal and mesenteric blood vessels (Crumly, et al. 1976). ADTN contains an assymetric carbon atom at the 2 position of the tetrahydronaphthalene nucleus. Thus optical isomers exist. Recently it has been found that R-(+)-ADTN is 10 times more potent than S-(-)-ADTN in both binding assays and in stimulating the dopamine sensitive adenylate cyclase. In stimulating locomotor activity after intra accumbens injections the R(+) enantiomer was again more potent than the S(-)-enantiomer but only by a factor of 13 (Andrews, et al. 1978, Woodruff, et al. 1979). The specificity was also apparent in invertebrate preparations where there is a 100 fold difference in the relative potency of the two enantiomers (Batta, Woodruff and Walker, 1979).

It is known that as amino tetralins have a substituted α-carbon atom they

are not substrates for MAO. Additionally, they do not readily pass through the blood brain barrier and have been shown to remain within discrete brain areas for several hours (Westerink, et al. 1980). The long duration of action of ADTN seen both in our iontophoretic experiments and in producing locomotor stimulation (Woodruff, Elkhawad and Pinder, 1974) may be caused by reuptake and re-release, as has been reported by Horn (1974) and Davis, Roberts and Woodruff (1978). Although 6OHDA lesions do not affect the duration of the hyperactivity response (C.D. Andrews personal communication).

This impermeability of the blood brain barrier to aminotetralins also may be the reason why several substituted aminotetralin derivatives tested as antagonists, do not affect apomorphine rotation in rats while they will abolish emesis in dogs (Rusterholz, et al. 1978). While a logical step would be the quantitative assessment of several aminotetralins the problem of desensitization limits the use of iontophoresis to one test compound per electrode. However, a prodrug of ADTN, the dibenzoylester has been found to pass from the systemic circulation into the brain (Horn, et al. 1978). Intravenous injections of this and analogues while recording from dopaminergic compacta neurones similar to Bunney and Aghajanian (1973) might prove a useful method of comparing the potencies of different amino tetralins. In addition to ADTN, nomifensine and a dihydroxy derivative were tested on the firing rate of dopamine containing neurones in the nigra. Nomifensine is a recently introduced anti-depressant drug which has been found to have potent inhibitory effects on the neuronal uptake of noradrenaline and dopamine (Samanin, et al. 1975). Nomifensine was shown to possess dopamine-like behavioural effects including the ability to induce ipsilateral rotation in rats with unilateral 6OHDA lesions of the nigro striatal tract (Costall and Naylor, 1975). Nomifensine iontophoretically administered to dopamine containing cells did not change the firing rate, nor did it potentiate the inhibitory response to dopamine. This may be partly due to the poor solubility of the drug. At the low pH required to dissolve nomifensine more protons rather than drug may be released, hence insufficient drug is reaching the neurones. Furthermore, intravenous injections of nomifensine did cause relatively short lasting inhibition of firing of dopamine neurones at similar doses to those produced by amphetamine (Aghajanian and Bunney, 1977). Thus it is possible that nomifensine has a greater dopamine releasing effect at dopamine terminals rather than at dendrites.

Some metabolites of nomifensine are known, these arise from hydroxylation and methylation of the phenyl ring. The 4-hydroxy phenyl (M1) and the 3 methoxy-4-hydroxyphenyl (M2) metabolites of nomifensine produced sterotypy in a similar manner to nomifensine while the 3-hydroxy-4 methoxyphenyl (M3) derivative was inactive. Following direct intracerebral injections only the M1 metabolite showed dopamine-like activity (Costall and Naylor, 1977). It was suggested by Woodruff (1977) that a dihydroxy derivative of nomifensine, 3, 4-dihydroxynomifensine, may have direct effects on dopamine receptors since it bears a striking resemblance to the dopamine structure contained in ADTN. This was found to be the case, with dihydroxynomifensine having a similar potency to dopamine (Woodruff, et al. 1979, Poat, Woodruff and Watling, 1978) in biochemical tests. Thus it was some surprise to find that iontophoresis of 3, 4-dihydroxynomifensine produced a very weak or no effect on the firing rate of dopamine sensitive cells in the substantia nigra. This is in contrast to the potent effects one would expect if the compound was acting in a similar manner to ADTN which we have shown to be very active on these cells.

It is of interest that when injected bilaterally into the nucleus accumbens, 3, 4-dihydroxynomifensine has a much weaker effect than ADTN although it is still a fairly potent drug (Woodruff, et al. 1979). Furthermore, on a peripheral preparation which is thought to have similar structure activity requirements for dopamine at the adenylate cyclase and snail brain preparation (Goldberg, Sonneville and McNay, 1968) it was found that 3, 4-dihydroxynomifensine had partial agonist properties which resembled those of apomorphine (Kohli and Goldberg, 1980).

The firing rate of dopamine containing neurones in the substantia nigra has been shown to be inhibited by intravenous injections of dopamine agonists (Bunney, et al. 1973). As little as 3 µg/Kg apomorphine produces a significant depression of firing rate (Guyenet and Aghajanian, 1978). In this study we found that 3-30 µg/Kg apomorphine produced a 50% inhibition while large doses of isoapomorphine were without effect. The fact that apomorphine is a dopamine agonist in some systems (Bunney, et al. 1973) is surprising since it does not have the same configuration as ADTN. It has been suggested that apomorphine binds preferentially to presynaptic dopamine receptors (Titeler and Seeman, 1979) although others disagree (Snyder, et al. 1978). There is some electrophysiological evidence to suggest that apomorphine may have more potent actions on pre than post synaptic receptors

(Skirboll, Grace and Bunney, 1979). Our own results are not inconsistent with this suggestion. For example after iontophoretic administration of ADTN the dopamine containing cells in the nigra were inhibited, the firing rate gradually increased but never reached more than 70% of the original baseline. The inhibitory response to dopamine or further application of ADTN was lost. If apomorphine was given intravenously during this partial recovery phase on 50% of cells an increase in firing rate was observed rather than an inhibition. On the other 50% of cells the usual inhibition of firing with a low dose of apomorphine was encountered. The excitatory effect of apomorphine could possibly be explained in the following way. If apomorphine has more effect on presynaptic rather than post synaptic receptors then at low doses intravenous apomorphine would cause a decrease in dopamine release at the terminals. The feedback pathways from the caudate would tend to cause the dopamine neurones to fire faster to compensate for this lack of dopamine release. In the normal animal this effect would be masked by the direct inhibitory action of apomorphine in the cell body dopamine autoreceptors. However, where ADTN has been applied to the cell the dopamine receptors are densensitised and the cell will not respond directly to apomorphine hence an increase in firing is seen. In the case of a cell which has had ADTN iontophoretically applied but still responds to apomorphine, it is possible that the ADTN has not densensitised the whole receptor population as they cover the dendrites which extend far into the reticulata of the nigra. Thus intravenous apomorphine which reaches all of the receptors can cause inhibition of the neurone by acting on receptors not desensitised by ADTN. This does not imply that apomorphine is more potent than ADTN on these receptors, it may suggest apomorphine is more potent on pre than post synaptic receptors.

Apomorphine has behavioural actions which are believed to be due to a direct stimulation of postsynaptic dopamine receptors. It has been found that intra accumbens injections produce a stimulation of locomotor activity (Grabowska and Anden, 1976) sometimes or depression (Pijnenberg, et al. 1976). When the dopamine terminals have been destroyed with 6OHDA (Kelly, Seviour and Iversen, 1975) if the level of dopamine depletion was sufficient to abolish amphetamine induced responses the response to apomorphine was potentiated. However, on the dopamine sensitive adenylate cyclase in the striatum and nucleus accumbens, apomorphine behaves as a partial agonist producing a maximum response that is only about 50% of that produced by dopamine (Kebabian, Petzhold and Greengard, 1972, Watling, Woodruff and Poat,

1978). Furthermore, on the dog renal vasculature which as already discussed has similar structural requirements for dopamine to the adenylate cyclase apomorphine behaves as a partial agonist (Crumly, et al. 1976). It has been suggested that in these peripheral dopamine receptors apomorphine and 3, 4-dihydroxy nomifensine are acting in a similar manner (Kohli and Goldberg, 1980).

Several studies have shown that neuroleptic drugs are potent antagonists of the effects of dopamine on the dopamine sensitive adenylate cyclase. There are some discrepancies between the clinical efficacy of these drugs and their ability to inhibit the dopamine sensitive cyclase. Thus the correlation is good for phenothiazines and thioxanthenes. The butyrophenones are much weaker than would be expected (Seeman, 1977). Another class of drugs some of which have antipsychotic properties are the substituted benzamides. One of the most studied is sulpiride (Haase, Floru and Ulrich, 1974). The major difference between sulpiride and the classical neuroleptics is that sulpiride does not block the effects of dopamine (Trabucchi, et al. 1975, Elliot, et al. 1977) or of ADTN (Woodruff, et al. 1979) on rat brain adenylate cyclase. Sulpiride is also particularly potent in stimulating the release of prolactin, whose secretion is controlled by a dopamine receptor system not coupled to adenylate cyclase (review by Kebabian and Calne, 1979).

Partly on the basis of differences in behavioural phenomena (Cools and Van Rossum, 1976) differences in the binding of agonists which have monophisic and antagonists which have biphasic curves, (Titeler, et al. 1978) and on the ability of atypical neuroleptics such as sulpiride to block dopamine stimulated adenylate cyclase (Kebabian and Calne, 1979) different types of receptors for dopamine have been proposed. Kebabian and Calne have suggested two, which are DA1 for postsynaptic receptors, thought to be linked to an adenylate cyclase, and DA2 for presynaptic receptors which are not cyclase linked. DA2 or presynaptic receptors could be located in three places in the nigro-striatal system. These are, the terminals of the cortical afferents to the striatum (Schwarcz,et al. 1978), the terminals of the nigro striatal neurones and on the cell body of the nigro striatal neurones (Kebabian and Calne, 1979) illustrated in Fig. 1.4. Recording from the soma of the nigro striatal neurones and iontophoresis of drugs is thus a good system for looking at autoreceptors presumed to be DA2 receptors while iontophoresis in the caudate nucleus and nucleus accumbens is more appropriate

for examining DA1 receptors. It must be added that both receptor types are apparent in both areas even though they are on different neural elements. We found (±)-sulpiride to be a potent antagonist of the inhibitory response produced by dopamine in the substantia nigra. The antagonism took several minutes to appear and persisted for a short period after application. GABA inhibitions on these cells were not affected by sulpiride.

Sulpiride is an optically active molecule. A wide range of potencies for the enantiomers have been reported. (-)-sulpiride has been found to be 100 times more potent than (+)-sulpiride in the displacement of haloperidol binding (Spano, Govoni and Trabucchi, 1978). Given directly into the nucleus accumbens (-)-sulpiride is more potent than (+)-sulpiride and fluphenazine (Woodruff, et al. 1979). Clinical trials also suggest the (-)-enantiomer is also more active (Hoffman, et al. 1979) and while the (+)-enantiomer has not been tested, (-)-sulpiride blocked the inhibitory effects of dopamine on snail neurones (Batta, Woodruff and Walker, 1979). In agreement with these results we found that (-)-sulpiride was more potent than (+)-sulpiride. However, the difference between the two enantiomers in blocking the inhibitory effects of dopamine in the substantia nigra was less than a factor of 10. In the striatum the inhibitory response to dopamine was only blocked by iontophoretically applied sulpiride on less than 20% of neurones. Fluphenazine was active on the same neurones as sulpiride. The failure to antagonise dopamine effects on all cells is unclear. However, a similar effect has been found by other workers, who could find no effect of intravenous haloperidol on dopamine inhibitions in the striatum (Skirboll and Bunney, 1979). On a few cells it was possible to test the two enantiomers of sulpiride. Similar results to those in the substantia nigra were obtained with the difference in potency being less than a factor of 10. Thus our results are not entirely in agreement with biochemical studies since difference in potencies between the two enantiomers is not great and sulpiride has actions in the striatum where dopamine has both pre and post synaptic action. These results are similar to those on the renal vasculature (Munday, et al. in press). Furthermore, other evidence suggests that sulpiride is an extremely potent antagonist of ADTN-induced hyperactivity, a response which is believed to be mediated by postsynaptic receptors (Woodruff and Andrews, 1979, Woodruff, et al. 1979). Additionally on the renal vascular dopamine receptor (which has very stringent agonist requirements) sulpiride is more potent than other neuroleptics (Goldberg, et al. 1978). Hypotension in the guinea pig produced by ADTN was also found to be reversed to sulpiride

(Woodruff and Andrews, 1979).

The inability of substituted benzamides to antagonise dopamine sensitive adenylate cyclase has been interpreted as evidence for two dopamine receptors. One which is sulpiride sensitive and not linked to the cyclase and one which is sulpiride insensitive and cyclase linked. There is an increasing body of evidence to suggest that such a classification needs re-evaluation. Sulpiride binding shows a similar specificity towards agonists and neuroleptics as classical neuroleptic binding (Freedman, Poat, & Woodruff, 1980). Furthermore nucleotides such as GTP decrease the affinity of agonists for sulpiride binding sites (Freedman, Poat and Woodruff, 1980) an effect usually suggestive of cyclase linkage. It has been suggested that other factors such as lipid solubility (Woodruff, Freedman and Poat, 1980) or linkage to an ionophore (Batta, Walker and Woodruff, 1979) might in part explain the lack of cyclase sensitivity to sulpiride. Intracellular studies and examination of reversal potentials in mammalian systems could make this clearer.

A useful model for predicting the therapeutic efficacy and incidence of extrapyramidal side effects of antipsychotics has been proposed by Bunney and Aghajanian (1979). They found that mepazine, a drug which has extrapyramidal side effects but no antipsychotic activity, would reverse the inhibitory effects of intravenous amphetamine on the substantia nigra but not the VTA dopamine containing neurones. Classical neuroleptics, which have side effects, increase the baseline firing of these neurones. Drugs which have few side effects, such as clozapine, do not do so. Both drug types will reverse the effects of amphetamine. Thus while sulpiride did not affect the baseline firing of dopamine containing cells as expected for a neuroleptic with few side effects, it failed to block the effects of low doses of apomorphine, although both fluphenazine and chlorpromazine did so at similar doses to these used by Bunney, et al. (1973). This failure is most surprising as the doses used are similar to those found necessary to reduce ADTN hyperactivity (Woodruff and Andrews, 1978). It must be emphasised that many drugs and stimuli other dopamine agonists or antagonists will also affect the firing rate of the dopamine containing neurones (Chiodo, et al. 1980, Raja and Guyenet, 1980). Thus we observed that increasing the concentration of halothane in the anaesthetic gas increased the firing rate of some dopamine containing neurones in the substantia nigra.

Concluding remarks on Dopamine Receptors

While there is much evidence which is difficult to rationalize in terms of one dopamine receptor, the evidence for two receptors still rests on incomplete and increasingly confusing biochemical evidence, particularly with respect to the action of sulpiride (Kebabian and Calne, 1979). Neuroleptics are notorious for their properties aside from dopamine antagonism such as antimuscarinic and anti alpha adrenergic blocking activity (reviews by Snyder, et al. 1974, Carlsson, 1978), hence studies using them usually require cautious interpretation. Additionally, sulpiride has been found to block dopamine in preparations which have similar structure activity requirements to the adenylate cyclase such as Helix Aspersa (Munday, Poat and Woodruff, 1974) and the dog renal vasculature (Goldberg, et al. 1978). The ADTN hyperactivity in 6OHDA treated animals is also abolished by sulpiride suggesting a post synaptic action. Our results of inhibition of dopamine response in the striatum support a post synaptic effect and although sulpiride blocked dopamine at the autoreceptors in the substantia nigra as would be expected for a non cyclase linked receptor, the autoreceptor may well have very similar structure activity requirements to the cyclase. The evidence for this is incomplete, however, while we have found intravenous injections of apomorphine potently inhibit the firing of dopamine containing neurones in the substantia nigra, the iontophoretic effect of ADTN we obtained is far greater (10-100 times) than the response obtained with iontophoretic apomorphine by other workers (Aghajanian and Bunney, 1977). The fact that intravenous isoapomorphine and 3, 4-dihydroxynomifensine were inactive could possibly be explained by steric hindrance, as suggested by Goldberg, et al. (1978). In conclusion, this study using the benzamide sulpiride has produced little evidence to support the classification of two dopamine receptors on the nigro striatal tract and it has further shown ADTN to be a very potent dopamine agonist at the cellular level on the autoreceptors in the substantia nigra.

4.6. Identification of the nigrostriatal and striatonigral neurones

Electrical stimulation of the substantia nigra has been shown to produce a single spike excitation in silent cells in the ipsilateral striatum. This excitation was suggested to be orthodromic because its latency increased during rapid stimulation and an epsp was seen if intracellular recordings were made. These cells are not affected by iontophoretic dopamine. There is another population of neurones, encountered mostly in the lateral and more superficial parts of the head of the caudate nucleus. This second population of neurones is spontaneously active and inhibited by electrical stimulation of the ipsilateral substantia nigra with a slow onset latency (Feltz and Albe-Fessard, 1972).

In the present study we have found similar excitations on silent cells which do not respond to iontophoretic dopamine. The inhibitory responses to stimulation were difficult to detect without a PSTH although they are sometimes apparent in spike records. While short latency excitations following electrical stimulation of the ipsilateral substantia nigra, which are said to be mimicked by short pulses of dopamine have been reported (Kitai and Koscis, 1978) we could detect none of these. Furthermore it has been suggested by Moore and Bloom (1978) that the iontophoretic currents used by Kitai and Koscis (1978) were too short to release sufficient quantities of dopamine. Furthermore, the dopamine neurones of the nigrostriatal tract have been shown to have a conduction velocity of less than 1m/s (discussed below), hence the orthodromic spikes reported by Kitai and Koscis (1978), which have a conduction velocity of more than 1 m/s are unlikely to be the same neurone population. These faster conducting fibres could be another nigrostriatal pathway or a pathway whose axons pass through the nigra, a detailed review has been written by Dray (1979).

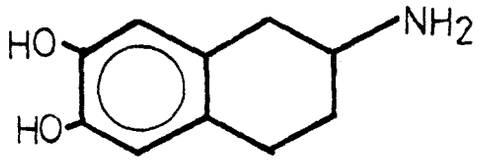
The dopamine containing neurones in the substantia nigra were identified as such by antidromic spike patterns following electrical stimulation of the ipsilateral striatum (Guyenet and Aghajanian, 1978). We have obtained similar results in this study. As previously found, frequently only the initial segment of the spike appears. This is probably the axonal component of the spike. Best results were obtained by stimulating the rear or caudal parts of the striatum, these parts probably contain more axons than terminals of the dopamine tract. The conduction velocity was slow, less than 1m/s, typical of unmyelinated axons. These antidromically identified neurones were inhibited by iontophoretically applied ADTN, dopamine and intravenous

apomorphine. The antidromic spikes were not abolished, nor was the latency changed by any of these agents. These results are in accord with an event which does not involve dopamine receptors.

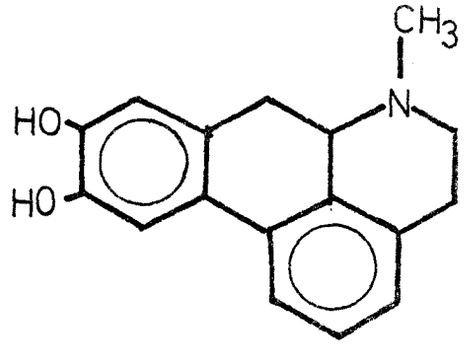
There was some evidence of branching of neurones within the caudate nucleus from collision experiments. This has previously been reported by Deniau, Thierry and Feger (1980). These authors, using similar electrophysiological techniques have found that occasionally a single neurone from either VTA or substantia nigra innervates areas of both the caudate nucleus and nucleus accumbens.

Two other phenomena were occasionally noted in our study. One was a variable latency to the antidromic spikes during rapid repetitive stimulation. A similar increase in latency has been seen in the noradrenergic systems in the brain and is thought to be caused by a change in ion concentration along unmyelinated fibres (Aston-Jones, Segal and Bloom, 1980). The other observation was that occasionally one stimulus pulse produced two antidromic spikes of slightly different latencies simultaneously. When this occurred both spikes were recorded as initial segments only. As it is not possible without intracellular recordings to see if both spikes originate from the same neurone the following interpretations are highly speculative. It is possible that with these neurones the axon divides immediately after the axon hillock sending two or more parallel axons to the target area. Thus action potentials evoked in this target area travelling antidromically would not collide and cancel out until they reached the coll body, where they would appear extracellularly as two slightly different latency axonal spikes.

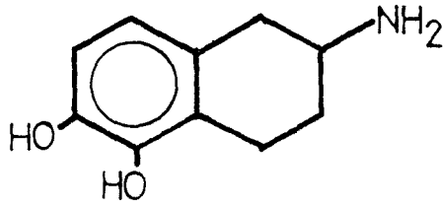
Thus we have confirmed that there is an excitatory response in the caudate nucleus following electrical stimulation of the ipsilateral substantia nigra. Neurones in the pars compacta of the substantia nigra which are thought to be the cell bodies of the ascending nigro striatal tract can be antidromically identified by electrical stimulation of the ipsilateral striatum. Thus when looking at drug responses on cells in both areas it is possible to include or exclude them from identified populations of neurones.



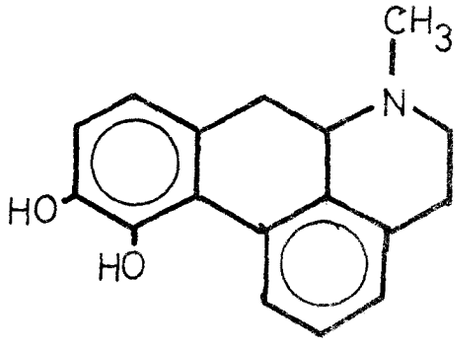
1: ADTN.



2: Isoapomorphine.



3: 5,6dihydroy,1,2,3,4 tetrahydronaphthalene.



4: Apomorphine.

Figure (4·1)· Dopamine is contained in 1&2 in the β rotomeric extreme and the α rotomeric extreme in 3 &4.

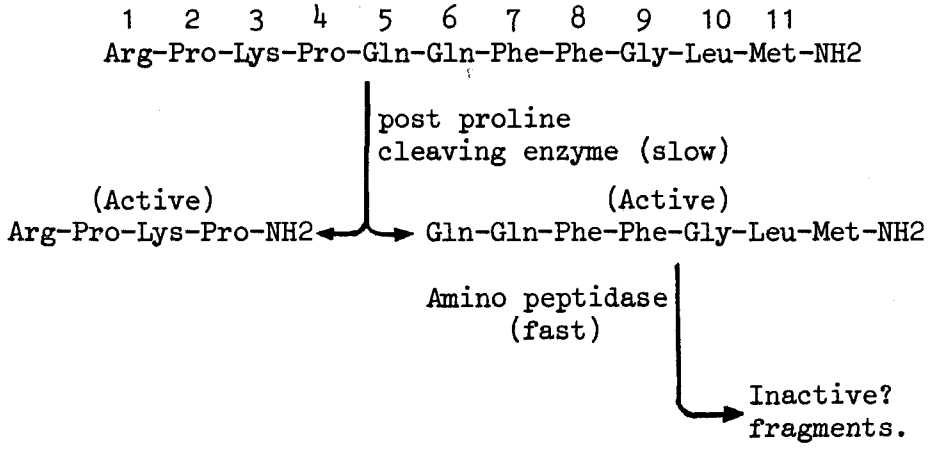


Figure (4.2). Proposed substance P degradation by "post proline cleaving enzyme" and amino peptidases.

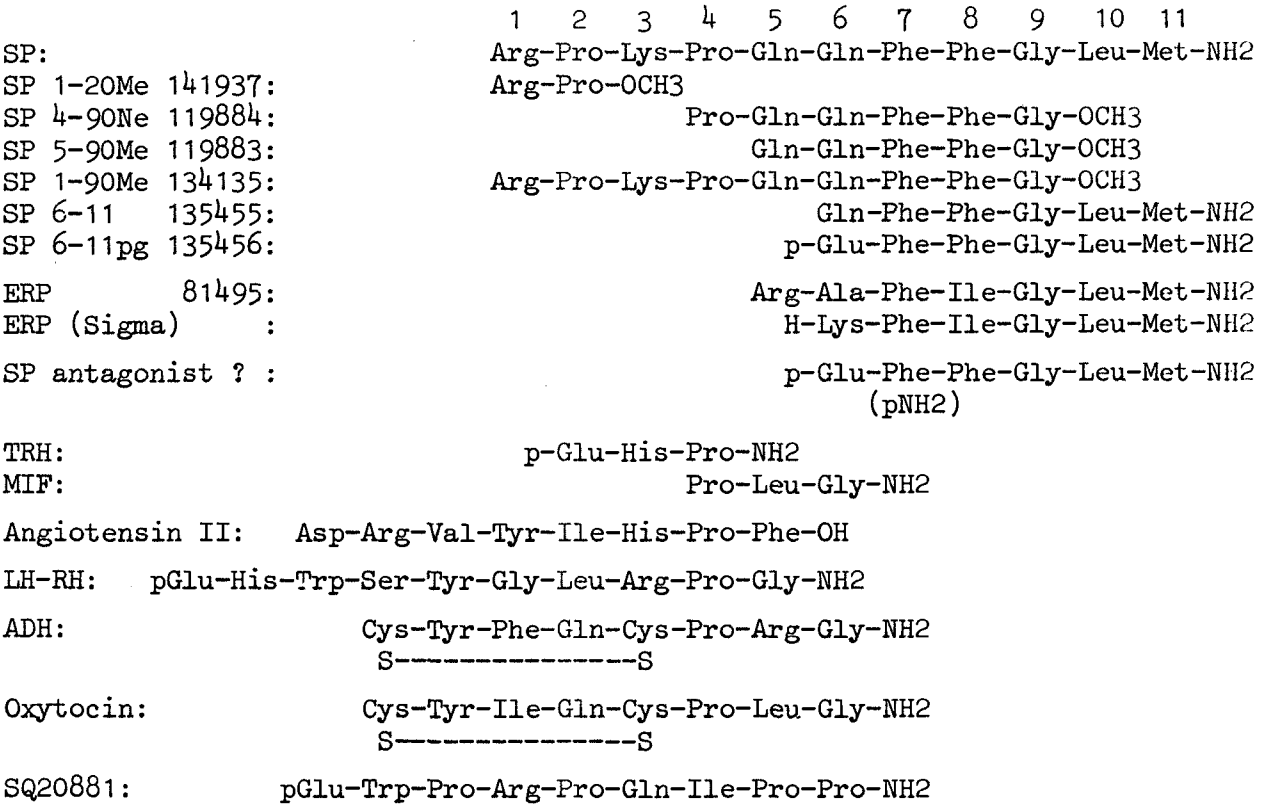


Figure (4.3). Amino acid sequence of the peptides used in this study and a sequence of substrates for post-proline cleaving enzymes.

H_2N-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-$CONH_2$

\+ + H + H p p H H H H H p

Figure (4.4). Probable distribution of charge on the substance P molecule in 20mM acetic acid solution.
\+ : positive charge
p : polar non charged
H : hydrphobic non charged.

SUMMARY

1. Extracellular recordings were made from neurones in the mesencephalic reticular formation, substantia nigra, caudate nucleus and nucleus accumbens of the anaesthetised rat. Drugs were applied by iontophoresis to single neurones or intravenously via a femoral cannula.
2. Drugs were bilaterally injected via chronically implanted cannulae into either the nucleus accumbens or ventral tegmentum of conscious animals. The locomotor activity of these animals was monitored immediately after injections. The actions of peripherally administered drugs was also studied.
3. Iontophoretic application of the tripeptide MIF was without effect on the firing rate of neurones in the substantia nigra, caudate nucleus and nucleus accumbens. The response to a threshold iontophoretic dose of dopamine was not changed by MIF. Intravenous injections of MIF did not change the firing rate of identified neurones of the nigro striatal tract. Peripheral injections of MIF failed to change the hyperactivity caused by amphetamine given into the nucleus accumbens or peripherally and the hypoactivity produced by haloperidol. Iontophoretic application of metabolically protected analogues showed the negative results were not due to rapid breakdown and measurement of release from microelectrodes confirmed positive currents were necessary.
4. TRH was similarly inactive to MIF when iontophoretically applied to neurones in the substantia nigra, caudate nucleus and nucleus accumbens. A more stable analogue, CRM221/3, and a metabolite -His-Pro- were similarly inactive iontophoretically. Peripheral injection of TRH potentiated the effect of peripheral but not intra accumbens amphetamine.
5. The putative δ opiate agonist BW180C was found to produce a long lasting hyperactivity after bilateral injection into the nucleus accumbens. Bilateral injection into the ventral tegmentum caused a short and long term hyperactivity. A non significant reduction in this last hyperactivity was apparent after peripheral injection of the μ receptor antagonist naloxone or the central injection of a putative peptide δ antagonist 139462. The other hyperactivity responses were completely unaffected by these treatments. The opiate k receptor agonist ethylketocyclazocine produced no hyperactivity when injected into the nucleus accumbens. These results are discussed with respect to multiple opiate receptors.

6. Substance P was found to excite neurones in the substantia nigra. Neurones in the compacta identified as dopaminergic were not consistently affected by substance P and responses to dopamine were not modified. Neurones in the nucleus accumbens were unaffected by substance P. Bilateral injections of substance P into the ventral tegmentum produced a short term hyperactivity. These results are discussed with respect to possible dopamine, substance P interactions.

7. Using a series of substance P analogues it was found that the N 1-9 fragment was equipotent with substance P in both behavioural and electrophysiological systems while other N-terminal fragments were not. C-terminal fragments were unexpectedly inactive. The putative peptide antagonist 140143 was without effect in either system. The putative amino acid antagonists produced inconsistent results. These results are discussed with regard to the metabolism of substance P and to problems in quantifying release of uncharged molecules from microelectrodes.

8. Iontophoretic application of noradrenaline to identified nigrostriatal neurones in the substantia nigra compacta caused similar but shorter lasting inhibitions of firing. p-Tyramine was much weaker than dopamine. The inhibitory responses to these amines was antagonised by sulpiride while the inhibitory response to GABA was unaffected. The rigid dopamine analogue ADTN produced a very potent long lasting inhibition of firing with densensitization to dopamine. 3, 4-dihydroxy nomifensine and nomifensine were both inactive when iontophoretically applied. Intravenous injections of nomifensine or apomorphine inhibited cell firing while isoapomorphine failed to do this. These results are discussed with respect to the structural requirements for dopamine.

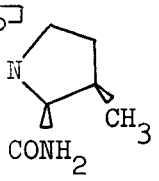
9. Inhibitions produced by dopamine on identified neurones in the substantia nigra compacta were antagonised by both - and - enantiomers of sulpiride. The - enantiomer was 2 times more potent than the - isomer. In the caudate nucleus sulpiride and fluphenazine antagonised dopamine inhibitions on the same cells. However a large population of neurones could be inhibited by dopamine although the responses were not blocked by either sulpiride or fluphenazine. These results are discussed in terms of a cyclase and a non cyclase linked dopamine receptor.

10. Neurones in the zona compacta of the substnatia nigra were identified by antidromic spikes following electrical stimulation of the ipsilateral striatum. Some cells in the zona reticulata could be identified by orthodromic inhibition following similar stimulation. Cells in the caudate nucleus which did not respond to dopamine were orthodromically

excited following electrical stimulation of the ipsilateral nigra. Neurones in the nucleus accumbens were not identified by these methods.

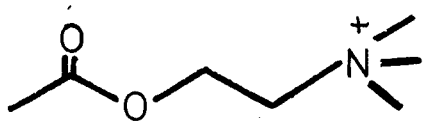
APPENDIX

1. Amino acid sequence of peptides

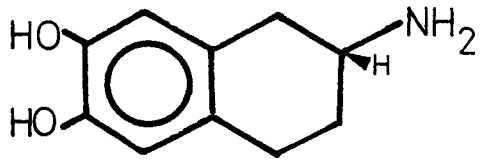
Angiotensin II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH
BW180C	Tyr-DAla-Gly-Phe-DLeu
ERP (81495)	Arg-Ala-Phe-Ile-Gly-Leu-Met-NH_2
MIF	Pro-Leu-Gly-NH_2
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
SP 1-20Me, 141937	Arg-Pro-OCH_3
SP 4-90Me, 119884	Pro-Gln-Gln-Phe-Phe-Gly-OCH_3
SP 5-90Me, 119883	Gln-Gln-Phe-Phe-Gly-OCH_3
SP 1-90Me, 134135	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-OCH_3
SP 6-11, 135455	Gln-Phe-Phe-Gly-Leu-Met-NH_2
SP 6-11pg, 135456	pGlu-Phe-Phe-Gly-Leu-Met-NH_2
SP antagonist 140143	pGlu -Phe(pNH_2)-Phe-Gly-Leu-Met-NH_2
TRH	pGlu-His-Pro-NH_2
His-Pro	His-Pro
CRM221/3	Pyr His 

2. Structures of non peptide drugs.

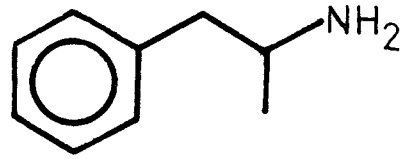
ACETYLCHOLINE



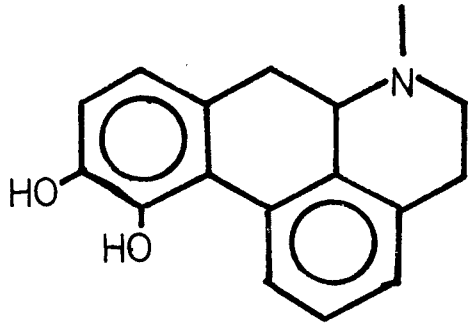
ADTN



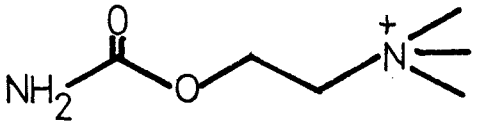
AMPHETAMINE



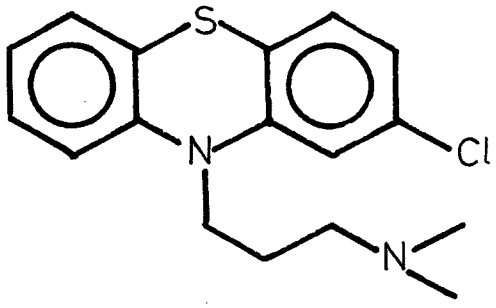
APOMORPHINE



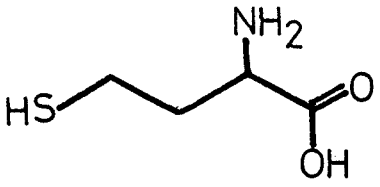
CARBACHOL



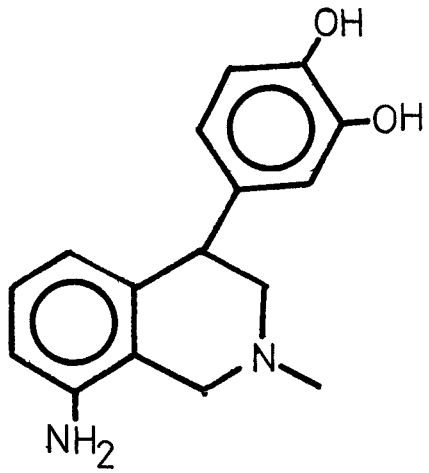
CHLORPROMAZINE



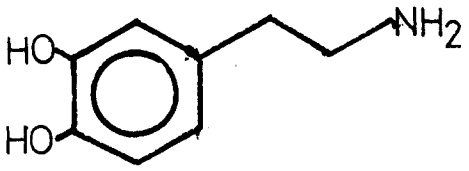
DL HOMOCYSTEIC ACID



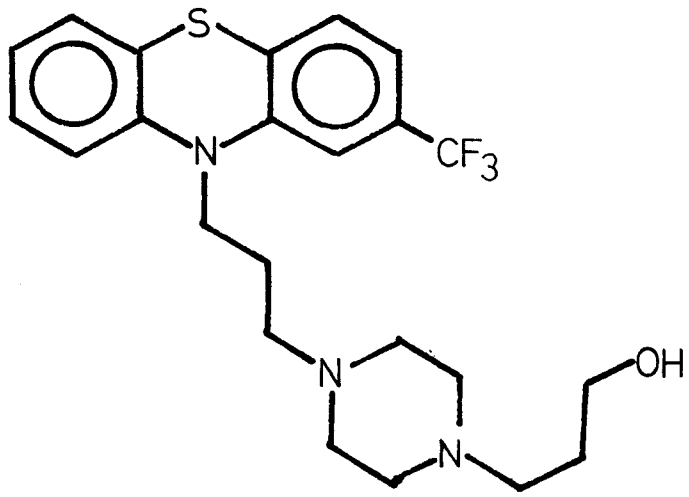
3,4 DIHYDROXY NOMIFENSINE



DOPAMINE

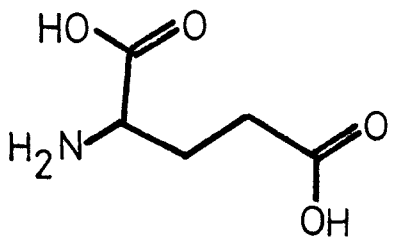


FLUPHENAZINE

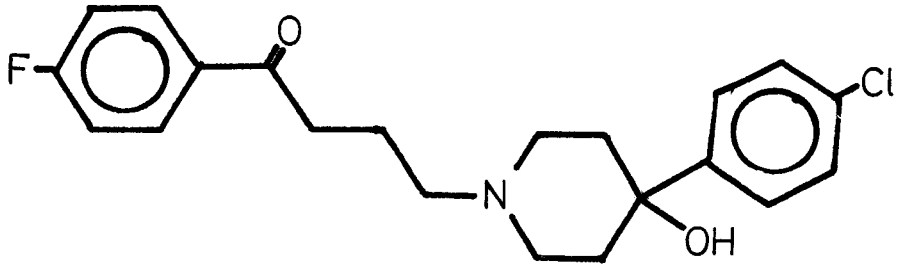


GABA

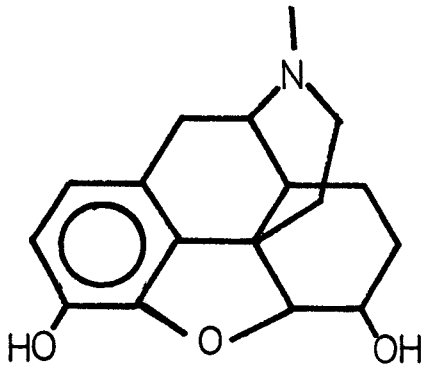
GLUTAMATE.



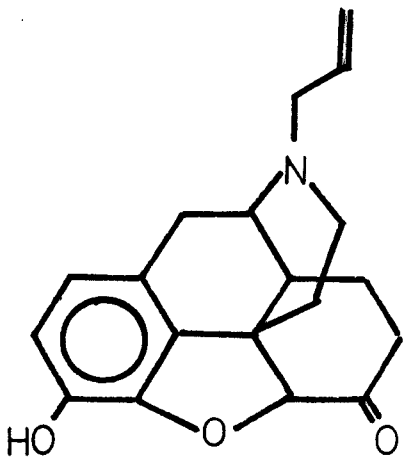
HALOPERIDOL



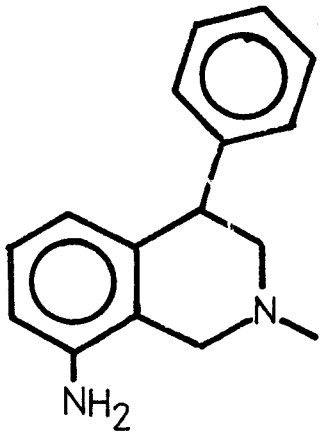
MORPHINE



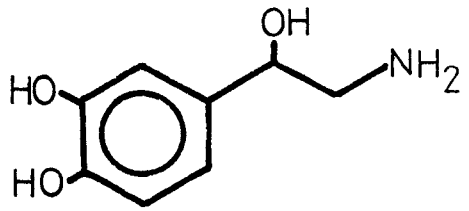
NALOXONE



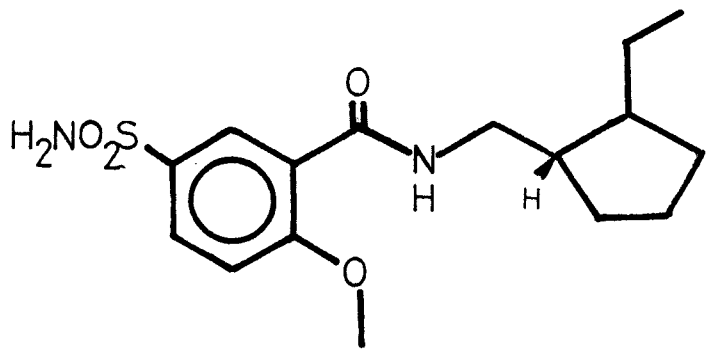
NOMIFENSINE



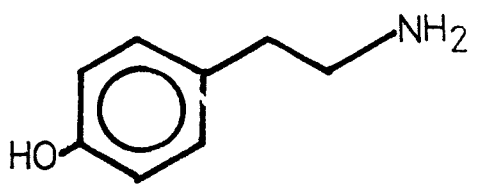
NORADRENALINE



SULPIRIDE



TYRAMINE



BIBLIOGRAPHY

Agarwal, R.A., R.B. Rastogi, and R.L. Singhal, 1977.
Enhancement of locomotor activity, catecholamine and 5HT metabolism by TRH
Neuroendocrinology.

Aghajanian, G.K., and B.S. Bunney, 1977.
Dopamine "autoreceptors": pharmacological characterization by microiontophoretic single cell recording studies
Naunyn-Schmiedeberg's Arch. Pharmacol. 297, 1-7.

Andrews, C.D., A.Davis, H.S. Freeman, D. McDermed, J.A. Poat, and G.N. Woodruff, 1978.
Effect of the (+) and (-) enantiomers of 2-amino-6, 7-dihydroxy-1, 2, 3, 4-tetrahydronaphthalene on dopamine receptors and on dopamine uptake
Br. J. Pharmacol. Vol. 64, 433P.

Andrews, C.D., and G.N. Woodruff, 1978.
Effect of the (+) and (-) enantiomers of sulpiride on ADTN-induced hyperactivity in the rat
Br. J. Pharmacol. 64, 434P.

Arregui, A., A.V.P. MacKay, L.L. Iversen, and E.G. Spokes, 1978.
Reduction of Angiotensin-converting enzyme in substantia nigra in early onset Schizophrenia
The New England Journal of Medicine, 300, 502-503.

Arregui A., P.C. Emson, and E.G. Spokes, 1978.
Angiotensin-converting enzyme in substantia nigra : reduction of activity in Huntington's disease and after intrastriatal kainic acid in rats
Eur. J. Pharmacol. 52, 121-124.

Aston-Jones, G., M. Segal, and F.E. Bloom, 1980.
Brain aminergic axons exhibit marked variability in conduction velocity
Brain Res. 195, 215-222.

Barbeau, A, 1975.
Potentiation of L-dopa by MIF
Lancet, II, 683-684.

Baring, M.D., J.R. Walters, N. Eng, 1980.
Action of systemic morphine on dopamine cell firing after neostriatal kainic acid lesion
Brain Research 181, 214-218.

Batta, S., R.J. Walker, and G.N. Woodruff, 1979.
Further studies on dopamine receptors in the brain of Helix Aspersa
Br. J. Pharmacol. 66, 127.

Beale, J.S., R.P. White, and Shang Po Huang.
EEG and blood pressure effects of TRH in rabbits
Neuropharmacology 1977 16, 499-506.

Beart, P.H., and D. McDonald, 1978.
Neuropharmacological studies of the mesolimbic dopaminergic pathway : somatodendritic mechanisms and gabaetgic neurones in the rat ventral tegmentum
J. Neurochem. 34, 1622-1629.

Beckstead, R.M., V.B. Domesick, W.J.H. Nauta, 1979.
Efferent connections of the substantia nigra and ventral tegmental area in rat
Brain Research, 175 (1979), 191-217.

Belcher, G., and R.W. Ryall, 1977.
Substance P and Renshaw cells : A new concept of inhibitory synaptic interactions
J. Physiol. 272, 105-119.

Ben-Ari, Y., G. Le Gal La Salle, and G. Levesque, 1978.
Effects of substance P on neurones of the medial nucleus of the amygdala Iontophoresis and Transmitter mechanisms in the Mammalian Central Nervous System, 93-95.

Ben-Ari, Y., and J.S. Kelly, 1976.
Dopamine evoked inhibition of single cells of the feline putamen and basolateral amygdala
J. Physiol. 256, 1-21.

Benkert, O., A. Gordon, and D. Maitchke, 1974.
Comparison of TRH LHRH and Placebo in depressive patients using a double blind α over technique
Psychopharmacologia (Berl) 40, 191-198 (1974) 11.

Bergman, J., M. Bienert, H. Niedrich, B. Mehlis, and P. Oehme, 1974.
Uber den Einflussder Kettenlange bei C-terminalen Sequenzen der substanz P
Experentia, 30, 401-403.

Bernardi, G., M.G. Marciani, C. Morocutti, and P. Stanzione, 1978.
The action of GABA and dopamine on rat caudate neurones intracellularly recorded
In: Iontophoresis and Transmitter Mechanism in the Mammalian CNS, 44-46.
Eds_ Ryall and Kelly.

Bevan, P., C.M. Bradshaw, Pun Ryk, N.T. Slater,and E. Szabadi, 1978.
Responses of single cortical neurones to noradrenaline and dopamine
Neuropharmacol. 17, 611-617.

Bjorkman, S., T. Lewander, and T. Zetterstrom, 1980.
MIF (Pro-Leu-Gly-NH_2): failure to effect oxotremorine effects in mice and rats as well as fluphenazine catalepsy or amphetamine hyperactivity in rats
J. Pharm. Pharmacol. 32, 296-297.

Bjorkman, Sven, and H. Sievertson, 1977.
MIF effect on oxotremorine induced tremor, big dose causes no effect, small dose
Arch. of Pharmacol. 298, 79-81.

Bloom, F.E., E. Costa, and G.C. Salmoiraghi, 1965.
Anaesthesia and the responsiveness of individual neurones of the caudate nucleus of the cat to acetylcholine, norepinephrine and dopamine administered by electrophoresis
Journal of Pharmacology and Experimental Therapeutics 150, 244-252.

Blumberg, S., and V.I. Teichberg 1979.
Biological activity and enzymic degradation of substance P analogs - implications for studies of the substance P receptor
Biochem. Biophys. Res. Commun. (Eng) Vol. 90, Issue N1, Year 1979, p. 347, ref. 22.

Blumberg, S., V.I. Teichberg, J.L. Charli, L.B. Hersh, and J.F. McKelvy, 1980.
Cleavage of substance P to an N-terminal tetrapeptide and a C-terminal heptapeptide by a post proline cleaving enzyme from bovine brain
Brain Res. 192, 477-486.

Bradshaw, C.M., M.H.T. Roberts, and E. Szabadi, 1973.
Kinetics of the release of noradrenaline from micropipettes: interaction between ejecting and retaining currents
Bri. J. Pharmac. 49, 667-677.

Briggs, I., 1979.
Excitatory effects of TRH on medullary reticular neurones
Neurosci. lett. 15, 33-36.

Broekkamp, C.L.E., P.A.M. VanDongen, and J.M. VanRossum,
Psychobiology of the striatum, eds. A.R. Cools, A.H.M. Lohman, J.H.L. Vanden Berken
North Holland Amsterdam 1977, 61-72.

Broekkamp, C.L.E., A.G. Phillips, and A.R. Cools,
Stimulant effects of enkephalin microinjection into the dopaminergic A10 area
Nature 278 1979, 560-562.

Brownstein, M.J., E.A. Mroz, J.S. Kizer, M. Palkovits, and S.E. Leeman, 1976.
Regional distribution of substance P in the brain of the rat
Brain Research 116, 299-305.

Bunney, B.S., G.K. Aghajanian, and R.H. Roth.
Comparison of effects of L-Dopa, Amphetamine and Apomorphine on firing rate of rat dopaminergic neuroes
Nature (new biology) 245 (1973), 123-125.

Bunney, B.S., J.R. Walters, R.H. Roth, and G.K. Aghajanian.
Dopaminergic neurones: effect of antipsychotic drugs on single cell activity
The Journal of Pharmacology and Experimental Therapeutics 185 (1973), 560-571.

Bunney, B.S., and G.K. Aghajanian, 1975.
Antipsychotic drugs and central dopaminergic neurones: a model for predicting therapeutic efficacy and incidence of extrapyramidal side effects
Predictability in psychopharmacology: preclinical and clinical correlations.
Publ. Raven Press New York 1975, 225-245.

Bunney, B.S., J.R. Walters, R.H. Roth, G.K. Aghajanian, 1975.
DA neurones effect of Antipsychotic drugs and amphet on single cell activity
J. Pharm. Exp. Ther. 185 1975, 560-571.

Bunney, B.S., and G.K. Aghajanian, 1976.(a)
d-Amphetamine induced inhibition of central dopaminergic neurones: mediation by a striato-nigral feedback pathway
Science 192, 391-393.

Bunney, B.S., and G.K. Aghajanian, 1976 (b)
Dopamine and norepinephrine innervated cells in the rat prefrontal cortex: pharmacological differentiation using microiontophoretic techniques
Life Sci. 19, 1783-1792.

Bunney, B.S., and G.K. Aghajanian.
d-Amphetamine-induced depression of central dopamine neurones: evidence for mediation by both autoreceptors and a striato-nigral feedback pathway
Naunyn-Schmiedebergs Arch. Pharmacol. 304 (1978), 255-261.

Bunney, B.S., and A.A. Grace.
Acute and chronic haloperidol treatment: comparison of effects on nigral dopaminergic cell activity
Life Sciences 23 (1978), 1715-1728.

Bunney, B.S., 1979.
The electrophysiological pharmacology of midbrain dopamine systems
In, The Neurobiology of Dopamine
Eds. A.S. Horn, J. Korf, B.H.C. Westerink
Academic Press London, New York, San Francisco, 417-452.

Burt, D.R., and S.M. Snyder, 1975.
TRH: Apparent receptor binding in rat brain membranes
Brain Res. 93, 309-328.

Bury, R.W., and M.L. Mashford, 1975.
Biological activity of C-terminal partial sequences of substance P
J. Med. Chem. 19, 854-856.

Bustard, T.M., and R.S. Egan, 1971.
The conformation of dopamine hydrochloride
Terrahedron 27, 4457-4469.

Cannon, J.G., 1975.
Chemistry of dopaminergic agonists
Adv. Neurol. 9, 177-183.

Caraceni, T., E.A. Parati, F. Girotti, I. Celano, C. Frigerio, D. Cocchi, and E.E. Muller.
Failure of MIF-1 to affect behavioural responses in patients with Parkinsons Disease under L-dopa therapy
Psychopharmacology (Eng) Vol. 63, Issue N3, Year 1979, 217-221.

Carino, M.A., J.R. Smith, B.G. Weik, and A. Horita.
Effects of TRH microinjected into various brain areas of coreous and peuto-barbital pre-treated rabbits
Life Sci. 19, 1976, 1087-1692.

Carlsson, A.
Antipsychotic drugs, neurotransmitters, and schizophrenia
American J. Psychiatry 135, 1-78, 164-173.

Carlsson, A., T. Magnusson, G.H. Fisher, D. Chang, and K. Folkers, 1977.
Effect of synthetic substance P on control monoaminergic mechanisms
In Substance P, Nobel Symposium 37,
Eds. U.S. von Euler and B. Pernow, 201-207.

Carolei, A., V. Margotta, and G. Palladini, 1977.
MIF: Lack of DA and Ach activity
Neuroend. 23, 129-132.

Carter, R.J., and S. Shuster, 1978.
Melanocyte-stimulating hormone mimetic action of phenothiazines
J. Pharm. Pharmacol. (Eng) Vol. 30, 1978, 233-235.

Celis, M.E., Staleisnik, R. Walter.
Regulation of formation and proposed structure of the factor inhibiting the release of MSH
Proc. Nat. Avad. Sci. 68, No. 7, July 1971, 1428-1433.

Celis, M.E., S. Hase, and R. Walter, 1972.
Structure activity studies of MSH-release inhibiting hormone
Febs letters vol. 27, no. 2, 327-330.

Celis, M.E., A. Vivas, R.C. Orlowski, and R. Walter.
Neurohypophyseal hormone fragment induced release of MSH
Brain Res. (Eng Tecn) Vol. 149, Issue N2, 1978, 516, ref. 9.

Chang, M.M., S.E. Leman, and H.D. Niall.
Amino acid sequence of substance P
Nature 232, 86-89.

Cheramy, A., A. Nieoullon, R. Michelot, and J. Glowinski.
Effects of intranigral application of dopamine and substance P on the vivo release of newly synthesized (^{3}H) Dopamine in the ipsilateral caudate nucleus of the cat
Neuroscience Letters 4 1977, 105-109.

Cheramy, A., R. Michelot, V. Leviel, A. Nieoullo, J. Glowinsk, and B. Kerdelhu.
Effect of immunoneutralization of substance P in cat substantia nigra on release of dopamine from dendrites and terminals of dopaminergic neurons
Brain Res. (Eng, Tecn) Vol. 155, Issue N2, 1978, 404, ref. 21.

Chi Yiu Yim, and G.J. Mogenson, 1980.
Electrophysiological studies of neurones in the ventral tegmental area of Tsia
Brain Research,181, 301-313.

Chipkin, R.E., J.M. Stewart, V.E. Sweeney, K. Harris, and R. Williams, 1979.
Invitro activities of some synthetic substance P analogues
Arch. Int. Pharmacodyn. Ther. 240, 193-202.

Chiodo, L.A., S.M. Antelman, A.R. Caggiula, and C.G. Lineberry, 1980.
Sensory stimuli alter the discharge rate of dopamine (DA) neurones: evidence for two functional types of DA cells in the substantia nigra
Brain Research 189, 544-549.

Chiu, S., and R.K. Mishra.
Antagonism of morphine-induced catalepsy by L-prolyl-L Leucyl-Glycinamide
European Journal of Pharmacology 53 (1979), 119-125.

Chiu, S., Y.W. Wong, and R.K. Mishra.
Direct evidence for L-Prolyl-Leucyl-Glycinamide PLG < receptor in mammalian brain
Fed. Proc., Fed. Am. Soc. Exp. Biol. (Eng. Proc) Vol. 39, Issue N3, 1980, 387.

Christensen, C.W., C.T. Harston, A.J. Kastin, R.M. Kostrews, and M.A. Spirtes.
Investigations on a-MSH and MIF-I effects on cyclic AMP levels in rat brain
Pharmacology Biochemistry and Behaviour, 5, suppl 1, 117-120. (1976).

Collingridge, G.L., and J. Davies, 1980.
Responses of nigral compacta and reticulata neurones to putative inhibitory neurotransmitters and striatal stimulation
J. Physiol. 301, 41-42.

Constantinidis, J., F. Geissbuhler, and J.M. Gaillard.
Enhancement of cerebral NA turnover by TRH evidence by fluoresence histo-chemistry
Tm Houaguimian R T15507 Ex. 30, 1974, 1182-1183.

Cools, A.R., and J.M. VanRossum, 1976.
Excitation-mediating and inhibition-mediating dopamine receptors : a new concept towards a better understanding of electrophysiological, biochemical, pharmacological, functional and clinical data
Psychopharmacol 45, 243-254.

Cooper, B.R., and C.E. Boyer, 1978.
Stimulant action of Thyrotropin Releasing hormone on cat spinal cord
Neuropharmacol 17, 153-156.

Costall, B., S.G. Hui, and R.J. Naylor, 1980.
Differential actions of substituted benzamide on pre- and post synaptic dopamine receptor mechanism in the nucleus accumbens
J. Pharm. Pharmacol. 32, 594-595.

Costall, B., D.H. Fortune, and R.J. Naylor.
The induction of catalepsy and hyperactivity by morphine administered directly into the nucleus accumbens of rats
Eur. J. Pharm. 49, 1978, 49-64.

Costall, B., and R.J. Naylor, 1975.
A comparison of circling models for the detection of anti-Parkinson activity
Psychopharmacology 41, 57-64.

Costall, B., R.J. Naylor, J.G. Cannon, T. Lee, 1977.
Differentiation of the DA mechanism mediating stereotyped behaviour and hyperactivity in the Nacc and caudate putamen
J. Pharm. Pharmac. 29, 337-342.

Costall, B., and R.J. Naylor, 1977.
Further aspects of nomifensine pharmacology
Br. J. Clin. Pharmac. 4, 895-995.

Costall, B., and R.J. Naylor, 1978.
Studies on the dopamine agonist properties of 8- amino-2-methyl-4-(3, 4-dihydroxyphenyl)-1, 2, 3, 4-tetrahydiosoquinoline, a derivative of nomifensine
J. Pharm. Pharmac. 30, 514, 516.

Costall, B., S.G. Hui, G. Metcalf, and R.J. Naylor, 1979.
A study of the changes in motor behaviour caused by TRH on intracerebral injection
Eur. J. Pharm. 53, 143-150.

Cotzias, G.C., M.A. Van Woert, and L.M. Schiffer.
Aromatic amino acids and modification of Parkinsonism
New England Journal of Medicine 276 (1967), 374-379.

Cox, B., A.J. Kastin, and H. Schnieden.
A comparison between a melanocyti-stimul H inh fact (MIF-I) and substances known to activate central dopamine receptors
Eur. J. Pharm. 36, 1976, 141-147.

Crossman, A.R., R.J. Walker and G.N. Woodruff, 1974.
Problems associated with iontophoresis in the substantia nigra and caudate nucleus
Neuropharmacology 13, 547-552.

Crossman, A.R., R.J. Walker and G.N. Woodruff.
Pharmacol studies on single neurones in SN of rat (ionto)
Brit. J. Pharmal. 51, 1974, 137-138.

Crumly, H.J., R.M. Pinder, W.B. Hinshaw, and L.I. Goldeberg, 1976.
Dopamine-like renal and mesenteric vasodilation caused by apomorphine, 6-propylnorapomorphine and 2-amino-6, 7-dihydroxy-1, 2, 3, 4-tetrahydronaphthalene
Nature 259, 584-587.

Cuello, A.C., and J.S. Kelly, 1977.
Electron microscopic autoradiographic localization of (^{3}H)-dopamine in the dendrites of the dopaminergic neurones of the rat substantia nigra in vivo
Brit. J. Pharmacol. 59, 527-528.

Cuello, A.C., P.C. Emson, G. Paxinos, and T. Jessel, 1978.
Substance P containing and cholinergic projections from the habenula
Brain Reasearch 149, 413-429.

Cuello, A.C., and I. Kanazawa.
The distribution of substance P immunoreactive fibres in the rat central nervous system
Journal of comparative Neurology 178 (1978), 129-156.

Cuello, A.C., T.M. Jessel, I. Kanazawa, and L.L. Iversen, 1977.
Substance P : localization in synaptic vesicles in rat central nervous system
Journal of Neurochemistry, 29, 747-751-

Dahlstrom, A., and K. Fuxe, 1965.
Evidence for the existence of monoamine containing neurones in the central nervous system
Acta. Physiol. Scand. 62 (Suppl. 232), 1-55.

Davies, J., and A. Dray, 1978.
Substance P and opiate receptors
Nature Vol. 268 28 July 1977, 351-2.

Davies, J., and A. Dray, 1976.
Substance P in the substantia nigra
Brain Research 107, 623-627.

Davies, J., and P. Tongroach, 1978.
Neuropharmacological studies on the nigro-striatal and raphe-striatal system in the rat
European Journal of Pharmacology 51, 91-100.

Davis, A., J.A. Poat, and G.N. Woodruff, 1980.
The use of R(+)-ADTN in dopamine receptor binding assays
Eur. J. Pharmacol. 63, 237-238.

Davis, A., P.J. Roberts, and G.N. Woodruff, 1978.
The uptake and release of (^{3}H)-2-amino-6, 7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) by striatal nerve terminals
Br. J. Pharmac. 63, 183-190.

De Caro, G., 1966.
Azione scialogoga di 53 polipeptidi sinctetici fisalemino ed eledoisimili nel ratto
Arch. int. Pharmacodyn. 162, 437-446.

Deniau, J.M., A.M. Thierry, and J. Feger, 1980.
Electrophysiological identification of mesencephalic ventromedial tegmental (VMT) neurones projecting to the frontal cortex, septum and nucleus accumbens
Brain Research 189, 315-326.

Diamond, B.I., J.E. Comaty, G.S. Sudakoff, H.S. Havdala, R. Walter, and R.L. Borison, 1979.
Role of Substance P as a transducer for dopamine in model choreas
Adv. Neurol. (Eng, revs) Vol. 23, 505, ref. 27.

Di Chiara, G., M.L. Porceddu, P.F. Spano,and G.L. Gessa, 1977.
Haloperidol increases and apomorphine decreases striatal dopamine metabolism after destruction of striatal dopamine sensitive adenylate cyclase by kainate
Brain Res. 130, 374-382.

Dingledine, R., L.L. Iversen, and E. Breuker, 1978.
Naloxone as a GABA antagonist : evidence from iontophoretic,receptor binding and convulsant studies
E.J.P. 47, 19-27.

Dray, A., 1979.
The striatum and substantia nigra : a commentry on their relationships
Neuroscience 4, 1407-1439.

Dray, A., T.J. Goyne, and N.R. Oakley, 1976.
Effects of a-fluphenthixol on dopamine and 5-hydroxy tryptamine responses of substantia nigra neurones
Neuropharmacology 15, 793-796.

Duffy, M.J., J. Wong, and D. Powell, 1975.
Stimulation of adenylate cyclase activity in different areas of human brain by substance P
Neuropharmacology 14, 615-618.

Duggan, A.W., B.T. Griersmi, P.M. Headley, and J.G. Hill, 1979.
Lack of effect by substance P at sites in the substantia gelatinose where met-enkephalin reduces the transmission of nociceptive impulses
Neurosci. lett. (Eng) 12, 313-317.

Dupont, A., Fhabrie, A. Pelletier, and R. Puviani, 1972.
Organ distribution of TRH
General and comparative Endocrinology 19, 522-526.

Dupont, A., Fhabrie, A. Pelletier, R. Puviani, D.M. Coy, A.V. Schally, and A.J. Kastin, 1975.
Distribution of radioactivity in the organs of the rat and mouse after injection of L (^{3}H) Prolyl Leucyl Glycinamide
J. Endocr. 64, 243-248.

Dyer, R.G., 1974.
Direct effect of LRF and TRF on neurones in hypothalamus
Nature 252, 486-488.

Ehrensing, R.H., and A.J. Kastin, 1978.
Dose-related biphasic effect of Prolyl-Leucyl-Glycinamide (MIF-1) in depression
American Journal of Psychiatry 135:5, 562-566.

Elkhawad, A.O., and G.N. Woodruff, 1975.
Studies on the behavioural pharmacology of a cyclic analogue of dopamine following its injection tnto the brains of conscious rats
Brit. J. Pharmacol. 54, 107-114.

Elliot, P.N.C., P. Jenner, G. Huizing, C.D. Marsden, and R. Miller, 1977.
Substituted benzamides as cerebral dopamine antagonists in rodents
Neuropharmacology 16, 333-342.

Emson, P.C., 1979.
Peptides as neurotransmitter candidates in the mammalian CNS
Prog. Neurobiol. 13, 61-116.

Erspamer, V., and A. Anastasi, 1962.
Structure and pharmacological actions of eledoisin, the active endecapeptide of the posterior salivary glands of eledone
Experentia 15, 58-59.

Erspamer, V., G.F. Erspamer, and G. Linari, 1977.
Occurance of tachykinins (physalemin- or substance P-like peptides) in the amphibian skin and their actions on smooth muscle preparations, 67-74.
In_ Substance P, Nobel Symposium 37
Eds. V.S. von Euler and B. Pernow, Raven Press, New York.

Evered, M.D., J.T. Fitzimons, and G. De Caro, 1977.
Drinking behaviour induced by intracranial injections of eledoisin and substance P in pigeon
Nature 268, 28/7/77, 332-333.

Fallon, J.H., and R.Y. Moore, 1978.
Catecholamine innervation of the basal forebrain
J. Comp. Neur. 180, 545-580.

Feltz, P., 1969.
Dopamine, amino acids and caudate unitary responses to nigral stimulation
J. Physiol. 205, 8-9.

Feltz, P, 1971.
Sensitivity to haloperidol of caudate neurones excited by nigral stimulation
European Journal of Pharmacology 14, 360-364.

Feltz, P., 1971.
-aminobutyric acid and caudato-nigral inhibition
Canadian Journal of Physiology and Pharmacology 49, 1113-1115.

Feltz, P., and D. Albe-Fessard, 1972.
A study of an ascending nigro-caudate pathway
Electroenceph. clin. Neurophysiol. 33, 179-193.

Feltz, P., and J. De Champlain, 1972.
Enhanced sensitivity of caudate neurones to microiontophoretic injections of dopamine in 6-hydroxydopamine treated cats
Brain Research 43, 601-605.

Feltz, P., and J. De Champlain, 1972.
Persistance of caudate unitary responses to nigral stimulation after destruction and functional impairment of the striatal dopaminergic terminals
Brain Research 43, 595-600.

Feltz, P., and J.S. MacKenzie, 1969.
Properties of caudate unitary responses to repetitive nigral stimulation
Brain Research 13, 612-616.

Feltz, P., and M. Rasminsky, 1974.
A model for the mode of action of GABA on primary afferent terminals: depolarizing effects of GABA applied iontophoretically to neurones of mammalian dorsal root ganglia
Neuropharmacolog. 13, 553-563.

Fields, H.L., P.C. Emson, B.K. Leigh, R.F.T. Gilbert, and L.L. Iversen,
Multiple opiate receptor sites on primary afferent fibres
Nature 284, 351-353.

Finnerty, E.P., and S.H.H. Chan, 1979.
Morphine supression of substantia nigra zona reticulata neurones in the rat: implicated role for a novel striatonigral feedback mechanism
European Journal of Pharmacology 59, 307-310.

Fitzpatrick, T.B., M. Seiji, and A.D. McGugan, 1961.
Melanin pigmentation
The New England Journal of Medicine 265, 328-332.

Floor, E., and S.E. Leeman, 1980.
Substance P sulfoxide - separation from substance P by high-pressure liquid chromatography, biological and immunological activities, and chemical reduction
Anal. Biochem. (Eng), Vol. 101, Issue N2, 498-503.

Freedman, S.B., C.P. Wait, and G.N. Woodruff, 1979.
Effect of dopamine receptor agonists and antagonists in the rat nucleus accumbens
Br. J. Pharmacol. 79, 430.

Freedman, S.B., J.A. Poat, and G.N. Woodruff, 1980.
In press.

Freedman, S.B., G.N. Woodruff, and J.A. Poat, 1980.
Neurosci., in press.

Friedman (Eitan), J. Friedman, and S. Gershon, 1973.
Dopamine synthesis, stimulation by a hypothalamic factor
Science 182, 831-832.

Gayton, R.J., L.A. Lambert, and P.B. Bradley, 1978.
Failure of (+)-Naloxone to antagonize responses to opiod peptides
Neuropharmacology 17, 549-551.

German, D.C., D. Dalsass, and R. Sanford Kiser, 1980.
Electrophysiological examination of the ventral tegmental (A10) area in the rat
Brain Research 181, 191-197.

Goldberg, L.I., P.F. Sonneville, and J.L. McNay, 1968.
An investigation of the structural requirements for dopamine-like renal vasodilation: phenylethylamine and apomorphine
J. Pharm. and Exp. Ther. 163, 188-197.

Goldberg, L.I., J.D. Kohli, A.N. Kotake, and P.H. Volkman, 1978.
Characteristics of the vascular dopamine receptor: comparison with other receptors
Fed. Proc. 37, 2396-2402.

Gonce, M., and A. Barbeau, 1978.
Essais physiologiques avec le H.I.F.-1 dans la maladie de parkinson
Rev. Neurol. 134, 141-149.

Gozlan, H., G. Le Gal La Salle, R. Michelot, and Y. Ben-Ari, 1977.
Rapid degradation of substance P and related peptides during microiontophoresis experiment
Neurosci. Lett. 6, 27-33.

Grabowsk, M., and N.E. Anden, 1976.
Apomorphine in the rat nucleus accumbens: effects on the synthesis of 5-hydroxytrptamine and noradrenaline, the motor activity and the body temperature
J. Neural. Trans. 38, 1-8.

Grace, A.A., and B.S. Bunney, 1979.
Paradoxical excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurones
European Journal of Pharmacology 59, 211-218.

Green, A.R. and Heal, D.J. 1978 Release of dopamine in the nucleus accumbens of rats by TRH. Br. J. Pharmacol. 5, 233-241.
Proceedings of the BPS — also in journal.

Growcott, J.W., and N.N. Petter, 1980.
Tachyphylaxis due to the D-phe-7-analogue of substance P
J. Pharm. Pharmacol. 32, 376-377.

Guyenet, P.O., and G.K. Aghajanian, 1977.
Extraction of neurones in the locus coerulueus by substance P and related peptides
Brain Res. 136, 178-184.

Guyenet, P.G., and G.K. Aghajanian, 1978.
Antidromic identification of dopaminergic and other output neurones of the substantia nigra
Brain Research 150, 69-84.

Guyenet, P.G., E.A. Muoz, G.K. Aghajanian, and S.E. Leeman, 1979.
Delayed iontophoretic ejection of substance P from glass micropipettes - correlation with time - course of neuronal excitation invivo
Neuropharmacology (Eng) 18, 553-558.

Guyenet, P.G. and G.K. Aghanjanian, 1979.
Ach, substance P and met-enkephalin in the locus coeruleus: pharmacological evidence for independant sites of action
European Journal of Pharmacology 53, 319-328.

Haase, H.J., L. Floru, and F. Ulrich, 1974.
Klinisch-neuroleptische Untersuchuny des Sulpiride (Dogmatil) an akut erkranlen Schizophrenen
Int. Pharmacopsychiat. 9, 77-94.

Hardy, J.A., J.S. De Belleroche,D. Border, and H.F. Bradford, 1980.
Differential transmitter release from nerve terminals isolated from basal ganglian and substantia nigra
Journal of Neurochemistry 34, 1130-1139.

Harmer, A., J.G. Schofield, and P. Keen, 1980.
Cyclohexamide-sensitive synthesis of substance P by isolated dorsal root ganglia
Nature 284, 267-269.

Heal, D.J., and A.R. Green, 1979.
Administration of Thyrotropin Releasing Hormone (TRH) to rats releases dopamine in N. Accumbens but not N. Caudatus
Neuropharmacology 18, 23-31.

Hefti, F., and W. Lichtensteiger, 1978.
Dendritic dopamine: studies on the release of endogenous dopamine from subcellular particles derived from dendrites of rat nigro-striatal neurones
Neuroscience Letters 10, 65-70.

Hellon, R.F., 1971.
The marking of electrode tip positions in nervous tissue
J. Physiol. (Lond.) 214, 12.

Henry, J.L., 1976.
Effects of substance P on functionally identified units in cat spinal cord
Brain Res. 114, 439-451.

Henry, J.L., K. Krnjevic, and M.E. Morris, 1974.
Substance P and spinal neurones
Can. J. Physiol. Pharmacol. 53, 423-432.

Herrling, P.L., and C.D. Hull, 1980.
Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurones
Brain Res. 192, 441-462.

Hersh, L.B., and J.F. McKelvy, 1979.
Enzymes involved in the degradation of Thyrotropin Releasing Hormone (TRH) and Luteinizing Hormone Releasing Hormone (LHRH) in Bovine brain
Brain Res. 168, 553-564.

Hiler, J.M., and E.J. Simon, 1979.
^{3}H-ethylketocyclazocine binding: lack of evidence for a separate α receptor in rats CNS
Europ. J. Pharmacol. 60, 389-390.

Hockfelt, T., K. Fuxe, O. Johansson, S. Jeffcoate, and N. White, 1975.
Distribution of TRH in the central nervous system as revealed with immunohistochemistry
Eur. J. Pharm. 34, 389-392.

Hockfelt, T., O. Johansson, A. Ljungdahl, J.M. Lundberg, and M. Schultzberg, 1980.
Peptidergic neurones
Nature 284, 515-521.

Hockfelt, T., A. Ljungdahl, L. Terenius, R. Elde, and G. Nilsson, 1977.
Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P
Proc. Natl. Acad. Sci. U.S.A. 74, 3081-3085.

Hoffman, M., G.C. Jommi, O. Montefusco, G.C. Tonon, P.F. Spano, and M. Trabucchi, 1979.
Stereospecific effects of (-)-sulpiride on brain dopamine metabolism and prolactin release
J. Neurochem. 32, 1547-1550.

Honda, F., Y. Satoh, K. Shimomura, H. Satoh, H. Noguchi, S. Uchida, and R. Kato, 1977.
Dopamine receptor blocking activity of sulpiride in the central nervous system
Japan J. Pharmacol. 27, 397-411.

Hong, J.S., H-Y. T. Yang, and E. Costa, 1978.
Substance P content of substantia nigra after chronic treatment with antischizophrenic drugs
Neuropharmacology 17, 83-85.

Horn, A.S., D. De Kaste, D. Dijkstra, H. Rollema, M. Feenstra, B.H.C. Westerink, C. Grol, and A. Westerbrink, 1978.
A new dopaminergic prodrug
Nature 276, 405-407.

Horn, A.S., 1974.
The conformation of dopamine at its uptake site; further studies with rigid analogues
J. Pharm. Pharmac. 26, 735-737.

Hornykiewicz, O., 1979.
Brain dopamine in Parkinsons Disease and other neurological disturbances, p. 633-654
in The Neurobiology of Dopamine,Eds. Horn, A.S., J. Korf, and B.H.C. Westerink. Academic Press, London, New York, San Francisco.

Huidobro-Toro,J.P., A. Scotti de Carolis, and V.G. Longo, 1975.
Intensification of central catecholaminergic and seroteonergic processes by the hypothalamic factors MIF and TRF and by Angiotensin II
Pharmacology Biochemistry and Behaviour 3, 235-242.

Iversen, L.L., 1975.
Dopamine receptors in the brain
Science 188, 1084-1089.

Iversen, L.L., T. Jessel, and I. Kanazawa, 1976.
Release and metabolism of substance P in the rat hypothalamus
Nature 264, 81-83.

Iversen, L.L., T. Jessel, and I. Kanazawa, 1976.
Release and metabolism of substance P in rat hypothalamus
Nature 26, 81-83.

Iversen, S.D., E.M. Jones, A.E. Kelly, and L. Stinus, 1978.
The behavioural consequences of the interaction between morphine, substance P and non striatal dopamine neurones
7th International Congress of Pharmacology, Iuphar Paris 1978, Abstract 559.

Iwatsubo, K., and D.H. Clout, 1977.
Effects of morphine and haloperidol on the electrical activity of rat nigrostriatal neurones
Jnl. of Pharm. and Exptl. Ther. 202, 429-436.

Iwatsubo, K., 1977.
Effect of morphine on dopamine-sensitive adenylate cyclase activity of nucleus accumbens
Japanese Journal of Pharmacology 27, 903-905.

James, T.A., M.S. Starr, 1977.
Behavioural and biochemical effects of substance P injected into SN of the rat
J. Pharmacy and Pharmacology, Vol. 29, No. 3, 181-182.

James, T.A., and M.S. Starr, 1979.
Effects of substance P injected into the substantia nigra
British Journal of Pharmacology 65, 423-429.

Jenner, P., and C.D. Marsden, 1979.
The substituted benzamides - a novel class of dopamine antagonists
Life Sciences 25, 479-486.

Jessell, T.M., and L.L. Iversen, 1977.
Opiate analgesics inhibit substance P release from rat trigeminal nucleus
Nature 268, 549-551.

Johansson, O., T. Hockfelt, R.P. Elde, S.L. Jeffloate, and N. White, 1978.
Thyrotropin releasing hormone (TRH) and somatostatin: immunohistochemical distribution in the central nervous system
Neuroscience Letters suppl., 5220.

Juraska, J.M., C.J. Wilson and P.M. Groves, 1977.
The substantia nigra of the rat: a golgi study
J. Comp. Neurol. 172, 585-600.

Kanazawa, I, E. Bird, R. O'Connel, D. Powell, 1977.
Evidence for a decrease in substance P content of substantia nigra in Huntington's Chorea
Brain Research 120, 387-392.

Kanazawa, I., I.D. Bird, J.S. Gale, L.L. Iversen, T.M. Jessell, O. Huramoto, E.G. Spokes, and D. Sutoo, 1979.
Substance P: decrease in substantia nigra and globus pallidus in Huntington's Disease
Adv. in Neuro. 23, eds. Chase, T.N., et al. Raven Press New York, 495-504.

Kanazawa, I., S. Mogaki, O. Muramoto, and S. Kuzuhara, 1980.
Origin of substance P containing fibres in the entopenduncular nucleus and the substantia nigra of the rat
Brain Res. (Eng, Tecn), Vol. 184, Issue N2, 481-485, ref. 19.

Kastin, A.J., D.H. Coy, A.V. Schally, and L.H. Miller, 1973.
Peripheral administration of hypothalamic peptides results in CNS changes
Pharmacological Research communications 10, no. 4, 293-312.

Kastin, A.J., C.A. Sandman, L.O. Stratton, A.V. Schally, and L.H. Miller, 1975.
Behavioural and electrographic changes in rat and man after MSH
Prog. in Br. Res. 42, 143-150.

Kastin, A.J., M.C.I. Inton Miller, L. Ferrel, and A.V. Schally, 1973.
General activity in intact and hypop rats after MSH, melatonin and MIF admin.
Kastin - see Plotnikoff, Prange, Kostrzewa
Physiology and Behaviour 10, 399-401.

Kastin, A.J., D.H. Coy, and F. Bymaster, 1979.
MIF-I and postsynaptic receptor sites for dopamine
Brain Res. Bulletin 4, 657-662.

Kastin, A.J., A.V. Schally, S. Viosca, and M.C. Miller III, 1969.
MSH activities in plasma and pituitaries of rats after various treatments
Endocrinology 84, 20-28.

Kastin, A.J., and A.V. Schally, 1966.
MSH activity in pituitary glands of rats treated with tranquilizing drugs
Endocrinology 79, 1018-1022.

Kebabian, J.W., and J.M. Saavedra, 1976.
Dopamine-sensitive adenylate cyclase occurs in a region of substantia nigra containing dopaminergic dendrites
Science 193, 683-685.

Kebabian, J.W., and D.B. Calne, 1979.
Multiple receptors for dopamine
Nature 277, 93-96.

Kebabian, J.W., G.L. Petzhold, and P. Greengard, 1972.
Dopamine-sensitive adenylate cyclase in caudate nucleus and its similarity to the dopamine receptor
Proc. Natn. Acad. Sci. U.S.A., 69-2145-2149.

Kelly, P.H., P.W. Seviour, and S.D. Iversen, 1975.
Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum
Brain Res. 94, 507-522.

Kelly, P.H., and K.E. Moore, 1976.
Mesolymbic DA neurones in the rotational model of nigro-striatal function
Nature 263, 695-696.

Kelly, P.H., and K.E. Moore, 1977.
Mesolimbic dopamine neurones: effect of 6-hydroxydopamine-induced destruction and receptor blockade on drug-induced rotation of rats
Psychopharmacol. 55, 35-41.

Kelley, A.E., and S.D. Iversen, 1978.
Behavioural effects of substance P injections into zona reticulata of substantia nigra
Neuroscience letter supplement 1, 5220.

Kelley, A.E., and S.D. Iversen, 1979.
Substance P infusion into substantia nigra of the rat - behavioural analysis and involvement of striatal dopamine
Eur. J. Pharmacol. (Eng) Vol 60, Issue N2-3, 171 – 179

Kelley, A.E., L. Stinus, and S.D. Iversen, 1979.
Behavioural activation induced in the rat by substance P infusion into ventral tegmental area - implication of dopaminergic A10 neurones
Neurosci. Lett. (Eng) Vol. 11, 335-339.

Kelley, A.E., L. Stinus, and S.D. Iversen, 1980.
Interactions between d-ala-met-enkephalin, A10 dopaminergic neurones, and spontaneous behaviour in the rat
Behavioural Brain Research 1, 3-24.

Kerwin, R.W., and C.J. Pycock, 1979.
Thyrotropin releasing hormone stinulates release of (^{3}H)-dopamine from slices of rat nucleus accumbens in vitro
Br. J. Pharmac. 67, 323-325.

Kitai, S.T., and J.D. Koscis, 1978.
An intracellular analysis of the action of the nigro-striatal pathway on the caudate spiny neurone
In: Iontophoresis and transmitter mechanisms in the mammalian CNS, 17-23.
Eds. Ryall and Kelly.

Kohli, J.D.,and L.I. Goldberg, 1980.
Effects of 3'-4'-dihydroxynomifensine on the dopamine vascular receptor
J. Pharm. Pharmacol 32, 225-226.

Koida, M., and R. Walter, 1976.
Post-proline cleaving enzyme
J. Biol. Chem. 251, 7593-7599.

Kondo, Y., and K. Iwatsubo, 1980.
Diminished response of nigral dopaminergic neurones to haloperidol and morphine

Konig,J.F.R. and Klippel,R.A. 1963
The Rat Brain.Waverely Press Inc.

7RH and antidepressants on hypothalamic multiunt activity in the cat

Korany, L., Whitmoyer, D.I. and Sawyer, C.H. 1977 Effect of TRH, LHRH and SRIF on neuronal activity of brain stem reticular formation and hippocampus in the female rat. Expt. Neurol. 57, 807-816.

Noradrenergic neurones: morphine inhibition of spontaneous activity
European Journal of Pharmacology 25, 165-169.

Koscis, J.D., and S.T. Kitai, 1977.
Dual excitatory inputs to caudate spiny neurones from substantia nigra stimulus
Brain Res. 138, 271-283.

Kostrzewa, R.M., A.J. Kastin, and M.A. Spirtes, 1975.
a-MSH and MIF-I effects on catecholamine levels and synthesis in various rat brain areas
Pharmacology Biochemistry and Behaviour 3, 1017-1023.

Kostrzewa, R.M., M.A. Spirtes, J.W. Klara, C.W. Christensen, A.J. Kastin and T.H. Joh, 1976.
Effects of MIF on DA nerves
Pharmacology Biochem. and Behaviour Vol. 5, suppl. 1, 125-127.

Kostrzewa, R.M., J.C. Hardin, R.L. Snell, A.J. Kastin, D.H. Coy, and F. Bymaster, 1979.
MIF-I and postsynaptic receptor sites for dopamine
Brain Research Bulletin 4, 657-662.

Krivoy, W.A., J.R. Couch, J.L. Henry, and J.M. Stewart, 1979.
Synaptic modulation by substance P
Fed. Proc. Fed. Am. Soc. Exp. Biol. (Eng) Vol. 38, Issue N9, 2344-2347, ref. 22.

Krnjevic, K., and M.E. Morris, 1974.
An excitatory action of substance P on cuneate nuerones
Canadian J. of Physiol. and Pharmacol. 52, 736-744.

Krueger, B.K., J. Forn, J.R. Watters, R.H. Roth, and P. Greengard, 1976.
Stimulation by dopamine of adenosine cyclic 3', 5'-monophosphate formation in rat caudate nucleus: effect of lesions of the nigro-neostriatal pathway
Molecular pharmacology 12, 639-648.

Kubicki, J., 1977.
Some behavioural effects of SP_{6-11} hexapeptide C-terminal of substance P injected into the cerebral ventricles in rats
Acta Physiology Polish 28, 489-492.

Labella, F.S., C. Pinsky, and V. Havlicek, 1979.
Morphine derivatives with diminished opiate receptor potency show enhanced central excitatory activity
Brain Research 174, 263-271.

Le Gal La Salle, G., and Y. Ben-Ari, 1977.
Microiontophoretic effects of substance P on neurones of medial amygdala and putamen of rats
Brain Res. 135, 174.

Lee, C.M., A. Arregui, and L.L. Iversen, 1979.
Substance P degradation by rat brain peptidases:inhibition by SQ 20881
Biochemical Pharmacology 28, 553-556.

Lee, T., P. Seeman, A. Rajput, I.J. Farley, and O. Hornykiewicz, 1978.
Receptor basis for dopaminergic supersensitivity in Parkinsons Disease
Nature 273, 59-61.

Lembeck, F., and G. Zetler, 1971.
Substance P
Int. Eccycl. Pharmacol. Ther. Sects. 72 1, 29-71.

Lichtensteiger, W., and R. Lienhart, 1977.
Response of mesencephalic and hypothalamic DA neurones to α-MSH mediated by area postrema
Nature 266, 635-637.

Lord, J.A.H., A.A. Waterfield, J. Hughes, and H.W. Kosterlitz, 1977.
Endogenous opiod peptides: multiple agonists and receptors
Nature 267, 495-499.

Malthe-Sørenssen, D., P.L. Wood, D.L. Cheney, and E. Costa, 1978.
Modulation of the turnover rate of acetyl choline in rat brain by intraventricular injections of Thyrotropin Releasing Hormone, Somatostatin, Neurotensin and Angiotensin II
Journal of Neurochemistry 31, 685-691.

Marshall, J.F., and U. Ungerstedt, 1977.
Striatal efferent fibres play a role in maintaining rotational behaviour in the rat
Science 198, no. 4312, 62-64.

Martin, W.R., C.G. Eades, J.A. Thompson, R.E. Huppler, and P.E. Gilbert, 1976.
The effects of morphine and nalorphine like drugs in the non dependant and morphine dependant chronic spinal dog
J. Pharmacol. Exp. Ther. 197, 517-532.

Mehlis, B., S. Bohm, M. Becker, and M. Bienert, 1975.
Circular dichroism and infrared studies of substance P and C-terminal analogues
Biochem. Biophys. Ros. Commun. 66, 1447.

Mehlis, B., M. Rueger, H. Becker, M. Bienert, H. Niecrich, and P. Oehme, 1980.
Circular dichroism studies of substance P and its C-terminal sequences - CD spectra in aqueous-solution and effects of hydrogen -ion concentration
Int. J. Pept. Protein Res. 15, 20-25.

Melzack, R., and P.D. Wall, 1965.
Pain mechanisms: a new theory
Science 150, 971-979.

Metcalf, G., 1974.
TRH: a possible mediator of thermoregulation
Nature 252, 22/11/74, 310-311.

Miller, R.J., 1978.
Enkephalin: a peptide with morphine-like properties
Tins, August 1978, 29-31.

Miller, R.J., and J. McDermed, 1979.
Dopamine-sensitive adenylate cyclase
In: The Neurobiology of Dopamine, eds. A.S. Hora, J. Korf and B.H.C. Westerink, p. 159-177, Chapter 13.

Miyamoto (Masaomi), and Y. Nagawa, 1977.
Mesolimbic involvement in the locomotor stimulant action of TRH in rats
Eur. J. Pharm. 44, 143-152.

Miyamoto, M., S. Narumi, Y. Nagai, T. Shima, and Y. Nagawa, 1979.
Thyrotropin releasing hormone hyperactivity and mesolimbic dopamine system in rats
JPN. J. Pharmacol. (Eng) Vol. 29, Issue N3, 335. ref. 35.

Moore, R.Y., and F.E. Bloom, 1978.
Central catecholamine neurone systems: anatomy and physiology of the dopamine systems
Ann. Rev. Neurosci. 1, 129-169.

Moss, R.L., C.A. Dudley, and M.J. Kelly, 1978.
Hypothalamic polypeptide releasing hormones: modifiers of neuronal activity
Neuropharm. 17, 87-93.

Moss, R.L., and C.A. Dudley, 1978.
Changes in responsiveness of medial preoptic neurones to the microelectrophoresis of releasing hormones as a function of ovarian hormones
Brain Research 149, 511-515.

Mroz, E.A., and S.E. Leeman, 1977.
Substance P, vitamine, hormones
(N.Y.) (Eng) 35, 209-281.

Mudge, A.W., S.E. Leeman, and G.D. Fischbach, 1980.
Enkephalin inhibits release of substance P from sensory neurones in culture and decreases action potential duration
Proc. Natl. Acad. Sci. USA. 76, 526-530.

Munday, K.A., J.A. Poat, and G.N. Woodruff, 1974.
Increase in the cyclic AMP content of rat striatum produced by a cyclic analogue of dopamine
J. Physiol. Lond. 241, 119-120.

Munday, K.A., J.A. Poat, and G.N. Woodruff, 1976.
Structure activity studies on dopamine receptors: a comparison between rat striatal adenylate cyclase and Helix Aspersa neurones
Br. J. Pharmacol. 57, 452.

Munday, K.A., J.A. Poat, K.J. Watling and G.N. Woodruff, 1977.
On the structural requirements for dopamine-like activity in homogenates of rat nucleus accumbens
Br. J. Pharmacol. 61, 150.

McCarthy, P.S., R.J. Walker, and G.N. Woodruff, 1977.
Depressant actions of enkephalins on neurones in the nucleus accumbens
J. Physiol. 267, 40.

McGeer, E.G., and E.A. Singh, 1979.
Inhibition of Angiotensin converting enzyme by substance P
Neurosci. lett. (Eng) Vol. 14, Issue N1, 105, ref. 12.

Nair, R.M.G., A.J. Kastin,and A.V. Schally, 1971.
Isolation and structure of hypothalamic MSH release inhibiting hormone
Biochem. and Biophys. Res. Comm. 43, 1376-1381.

Nakamura, S., K. Iwatsubo, C.T. Tsai, and K. Iwama, 1979.
Neuronal activity of the substantia nigra (pars compacta) after injection of kainic acid into the caudate nucleus
Experimental Neurology 66, 682-691.

Nakazawa, Y., T. Kotorii, S. Horikawa, M. Kotorii, M. Ohshima, and H. Hasuzawa, 1979.
Individual variations in the effects of flurazepam, clorazepate, L-dopa and thyrotropin releasing hormone on REM sleep in man
Psychopharmacology 60, 203-206.

Narumi, S., and Y. Maki, 1978.
Stimulatory effects of substance P on neurite extension and cAMP levels in cultured neuroblastoma cells
Journal of Neurochemistry 30, 1321-1326.

Nicoll, R.A., 1977.
Excitatory action of TRH on spinal motoneurones
Nature 265, 242-243.

Nicoll, R.A., G.R. Siggins, N. Ling, F.E. Bloom and Guilemin, 1977.
Neuronal actions of endorphins and enkephalins among brain regions: a comparative microiontophoretic study
Proc. Nat. Acad. Sci. 74, 2584-2588.

North, R.A., 1979.
Opiates, opiod peptides and single neurones
Life Sci. 24, 1527-1546.

Ogata, N., 1979.
Substance P causes direct depolarization of neurones of guinea pig interpeduncular nucleus in vitro
Nature 277

Olpe, H.R., and W.P. Koella, 1977.
Rotatory behaviour in rats by intranigral application of substance P and an eledoisin fragment
Br. Res. 126, 576-579.

Oparil, S., 1977.
Angiotensin I converting enzyme and inhibitors, 157-167
In: Hypertension, eds. Jacques Genest, Erich Koiw, Otto Kuchil.

Olpe, H.R., W.P. Koella, P. Wolf, and H.L. Haas, 1977.
The action of baclofen on neurones of the substantia nigra and of the ventral tegmental area
Brain Research 134, 577-580.

Otsuka, A., S. Konishi, and T. Takahashi, 1975.
Hypothalamic substance P as a trasmitter candidate for primary afferent neurons
Federation Proceedings 34, 1922-1928.

Otsuka, M., S. Konishi, 1974.
Excitatory action of substance P on spinal motoneurones
Nature 252, 734-735.

Otsuka, M., and S. Konishi, 1976.
Release of substance P like immunoreactivity from isolated spinal cord of newborn rat
Nature 264, 83-84.

Pelletier, G., F. Labrie, A.J. Kastin, D. Coy, and A.V. Schally, 1975.
Radioautographic localization of radioactivity in rat brain after intraventricular or intracarotid injection of ^{3}H-L-Prolyl-L-Leucyl Glycinamide
Pharmacology Biochemistry and Behaviour 3, 675-679.

Pert, A., and C. Sivit, 1977.
Neuroanatomical focus for morphine and enkephalin-induced hypermobility
Nature 265, 645-647.

Piercey, M.F., F.J. Einspahr, P.J. Dobry, L.A. Schroede, and R.P. Ho Lliste, 1980.
Morphine does not antagonize the substance P mediated excitation of dorsal horn neurons
Brain Res. 186, 421.

Piercey, M.F., and F.J. Einspahr, 1980.
Use of substance P partial fragments to characterize substance P receptors of cat dorsal horn neurones
Brain Research 187, 481-486.

Pijnenberg, A.J.J., G.N. Woodruff, and J.M. Van Rossum, 1973.
Ergometrine induced locomotor activity following intracerebral injection into the nucleus accumbens
Brain Res. 59, 289-302.

Pijnenberg, A.J.J., and J.M. Van Rossum, 1973.
Stimulation of locomotor activity following injection of DA into the nucleus accumbens
J. Pharm. Pharmacol. 25, 1003-1005.

Pijnenberg, A.J.J., W.M.M. Honig, J.A.M. Van Den Heyden, and J.M. Van Rossum, 1976.
Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity
European Journal of Pharmacology 35, 45-58.

Pinder, R.M., W.B. Hinshaw, and L.I. Goldberg, 1976.
Dopamine-like renal mesenteric vasodilation caused by apomorphine 6-propylnorapomorphine and 2-amino-6, 7-dihydroxy-1, 2, 3, 4-tetrahydronaphthalene
Nature 259, 584-586.

Plotnikoff, N.P., M.S. Anderson, A.J. Prange, G.R. Breese, and I.C. Wilson, 1972.
TRH enhancement of DOPA activity
Science 178, 417-418.

Plotnikoff, N.P., A.J. Kastin, M.S. Anderson, and A.V. Schally, 1973.
DOPA potentiation by MIF
Life Sci. 10, 1279-1283.

Plotnikoff, N.P., A.J. Kastin, M.S. Anderson, and A.V. Schally, 1973.
Deserpidine antagonism by MIF
Neuroendocrinol. 11, 67-71.

Plotnikoff, N.P., F.N. Minard, and A.J. Kastin, 1974.
DOPA potentiation in ablated animals and normal animals after MIF
Neuroendocrinol 14, 271-279.

Plotnikoff, N.P., A.J. Prang, G.R. Breese, and I.C. Wilson, 1974.
TRH: Enhancement of DOPA activity in thyroidutomzeel rats
Life Sci. 14, 1271-1278.

Poat, J.A., G.N. Woodruff, and K.J. Watling, 1978.
Direct effect of nomifensine derivative on dopamine receptor
J. Pharm. Pharmac. 30, 495-497.

Pollard, H., C. Llorens, J.C. Schwartz, C. Gros, and F. Dray, 1978.
Localization of opiate receptors and enkephalins in the rat striatum in relationship with the nigrostriatal dopaminergic system: Lesion studies
Brain Research 151, 392-398.

Pollard, H., C. Llorens-Cortes, and J.C. Schwartz, 1977.
Enkephalin receptors on dopaminergic neurones in the rat striatum
Nature 268, 745-747.

Prange, A.J. Jr., G.R. Breese, J.M. Cott, B.R. Martin, B.R. Cooper, I.C. Wilson, and N.P. Plotnikoff, 1974.
TRH antagonism of pentobarbital in rodents
Life Sci. 14, 447-455.

Prange, A.J., I.C. Wilson, A. Knox, T.K. McLane, and M.A. Lipton, 1970.
Am. J. Psychiatry 127, 191-194.

Prange, A.J., I.C. Wilson, P.P. Lara, L.B. Alltop, and G.R. Breese, 1972.
Lancet 2, 999-1002.

Prasad, C., T. Matsul, and Peterkofsky, 1977.
Antagonism of ethanol narcosis by histidyl-proline diketopiperazine
Nature 268, 122-144.

Pugsley, T.A., and W. Lippmann, 1977.
Synthetic melanocyte stimulating hormone releasing inhibiting factor
Arzniem. Forch/Drug Res. 27, 2293-2296.

Pycock, C.J. and Marsden, C.D. 1978 The rotating rodent a two component system. Eur. J. Pharmacol. 47, 167-175.

Raja, S.N., and Guyenet, P.G., 1980.
Effects of phencyclidine on the spontaneous activity of monoaminergic neurons
European Journal of Pharmacology 63, 229-233.

Randie, M., and V. Miletic, 1978.
Depressant action of methionine enkephalin and somatostatin in cat dorsal horn neurones activated by noxious stimuli
Brain Research 152, 196-202.

Redding, T.W., A.J. Kastin, R.M.G. Nair, and A.V. Schally, 1973.
Distribution, half life and excretion of 14 C- and ^{3}H- labelled L-Prolyl-L-Leucyl-Glycinamide in the rat
Neuroendocrinology 11, 92-100.

Rekker, R.F., D.J.C. Engel, and G.G. Nys, 1972.
Apomorphine and its dopamine like action
J. Pharm. Pharmac. 24, 589-591.

Renaud, L.P., J.B. Martin, and P. Brazeau, 1976.
Hypothalamic releasing factors: physiological evidence for a regularory action on central neurones and pathways for their distribution in brain
Pharmacology Biochem. and Behaviour 5, Suppl 1, 171-178.

Renaud, L.P., H.W. Blume, Q.J. Pittman, Y. Lamour, and A.T. Tan, 1979.
Thyrotropin releasing hormone selectively depresses glutamate excitation of cerebral cortical neurones
Science 205, 1275-1277.

Renaud, L.P., and J.B. Martin, 1975,
TRH: depressant action on central neuronal activity
Brain Res. 86, 150-154.

Renaud, L.P., J.B. Martin, and P. Brazeau, 1975.
TRH, LHRH and SRIF, depressant action on CNS neuron activity
Nature 255, 233-235.

Rethy, C.R., C.B. Smith, and J.E. Villarreal, 1971.
Effects of narcotic analgesics upon the locomotor activity and brain catecholamine content of the mouse
Journal of Pharmacology and Experimental Therapeutics 176, 472-479.

Rondeau, D.B., F.B. Jolicoeu, F. Belanger, and A. Barbeau, 1978.
Motor activity induced by substance P in rats
Pharmacol. Biochem. Behav. 9, 769-775.

Roques, B.P., C. Garbay-Jaureguiberry, R. Oberlin, M. Anteunis, and A.K. Lala, 1976.
Conformation of Met^5-Enk determined by high field PMR spectrosopy
Nature 262, 778-779.

Rupnow, J.H., W.L. Taylor, and J.E. Dixon, 1979.
Purification and characterization of a thyrotropin releasing hormone deamidase from rat brain
Biochemistry 18, 1206-1212.

Ruffieux, A., and W. Schultz,1980.
Dopaminergic activation of reticulata neurones in the substantia nigra
Nature 285, 240-241.

Rusterholz, B.D., J.P. Long, J.R. Flynn, J.R. Glyn, C.F. Barnfnecht, R.W. Lind, and A.K. Johnson, 1978.
Inhibition of apomorphine induced behaviours by derivatives of 2-amino-1, 2, 3, 4-tetrahydronaphthalene
Arch. int. Pharmacodyn. 232, 246-260.

Samanin, R., S. Benasconi, and S. Grattini, 1975.
The effect of nomifensine on the depletion of brain serotonin and catecholamines induced respectively by fenfluramine and 6-hydroxy dopamine in rats
Eur. J. Pharmacol. 34, 377-380.

Sandman, C.A., and A.J. Kastin, 1978.
Interaction of α-MSH and MIF-I with d-amphetamine on open-field behaviour of rats
Pharmacology Biochemistry and Behaviour 9, 759-762.

Sastry, B.R., 1978.
Effects of substance P, acetylcholine and stinulation of habenula on rat interpeduncular neuronal activity
Br. Res. 144, 404-410.

Schaeffer, J.M., J. Axelrod, and M.J. Brownstein, 1977.
Regional diffs in DA mediated release of TRH material from synaptosomes
Brain Res. 138, 571-574.

Schally, A.V., A. Arimura, and A.J. Kastin, 1973.
Hypothalamic regulatory hormones
Science 179, 341-350.

Schenker, C., E.A. Mroz, and S.E. Leeman, 1976.
Release of substance P from isolated nerve endings
Nature 264, 790-791.

Schenkel-Hulliger, L., W.P. Koella, and A. Hartmann, 1974.
Tremorigenic effect of TRH in rats
L Maitre Experatia 30, 1168-1170.

Schneider Von, E., P.A. Fischer, P. Jacobi, and W. Reh, 1978.
Der Einflub von MIF (Melanozyteninhibierender Faktor) auf Psychomotorik und Stimmungsverhalten von Parkinsonkranken
Arzneim Forsch/Drug Res. 28 II, 1296.

Schulz, H., G.L. Kovacs, and G. Telegdy, 1978.
Action of posterior pituitary neuropeptides on the nigrostriatal dopaminergic system
European Journal of Pharmacology 57, 185-190.

Schwarz, R., I. Creese, J.T. Coyle, and S.H. Snyder, 1978.
Dopamine receptors localised on cerebral cortical afferents to rat corpus striatum
Nature 271, 766-769.

Seeman, P., M. Wong, and T. Lee, 1974.
Dopamine receptor - block and nigral fiber impulse - blockade by major tranquillizers
Fed. Proc. 33, 246.

Seeman, P., 1977.
Anti-schizophrenic drugs - membrane receptor sites of action
Biochem. Pharmacol. 26, 1741-1748.

Shuster, S., A.J. Thody, S.K. Goolamat, J.L. Burton, N. Plummer and D. Bates, 1973.
MSH and Parkinsonism
Lancet 1, 463-464.

Silbergeld, E.K., and J.R. Walters, 1979.
Synaptosomal uptake and release of dopamine in substantia nigra : effects of -aminobutyric acid and substance P
Neuroscience Letters 12, 119-126.

Simon, H., M. LeMcal, and A. Calas, 1979.
Efferents and afferents of the ventral tegmental-A10 region studied after local injection of ^{3}(H)leucine and horse radish peroxidase
Brain Res. 178, 17-40.

Skirboll, L.R., and B.S. Bunney, 1979.
The effects of acute and chronic haloperidol on spontaneously firing neurones in the caudate nucleus of the rat
Life Sciences 25, 1419-1434.

Skirboll, L.R., A.A. Grace, and B.S. Bunney, 1979.
Dopamine auto and postsynaptic receptors: electrophysiological evidence for differential sensitivity to dopamine agonists
Science 206, 80-82.

Smith, G.D., and J.F. Griffin, 1978.
Conformation of (Leu^{5}) enkephalin from X-ray diffraction: features important for recognition at opiate receptor
Sci. 199, 1214-1216.

Snyder, S.H., S.P. Banerjee, H.I. Yamamura, and D. Greenberg, 1974.
Drugs, neurotransmitters and schizophrenia
Science 184, 1243-1253.

Spano, P.F., M. Trabucchi, and G. Di Chiara, 1977.
Localization of nigral dopamine-sensitive adenylate cyclase in neurons originating from the corpus striatum
Science 196, 1343-1345.

Spano, P.F., S. Govoni, and M. Trabucchi, 1978.
Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system
Adv. Biochem. Psychopharmacol. 19, 155-165.

Spirtes, H.A., N.P. Plotnikoff, C.T. Kostrzewa, C.T. Harston, A.J. Kastin, and C.W. Christensen, 1976.
Possible association of increased rat behavioural effects and increased striatal dopamine and norepinephrine levels during the dopa potentiation test
Pharmacol. Biochem. and Behav. 5, 121-124.

Spirtes, M.A., C.W. Christen, C.T. Harston, and A.J. Kastin, 1978.
Alpha-MSH and MIF-I effects on CGMP levels in various rat brain regions
Brain Res. 144, 189-193.

Stanton, T.L., A. Winokur, and A.L. Beckman, 1980.
Reversal of natural CNS depression by TRH action in the hippocampus
Brain Res. 181, 470-475.

Stefanini, E., A.M. Marchisio, P. Devoto, F. Vernaleone, R. Collu, and P.F. Spano, 1980.
Sodium-dependent interaction of benzamides with dopamine receptors
Brain Res. 198, 229-233.

Steinacker, A., 1976.
Effect of substance P on synaptic transmission at the frog neuromuscular junction
Biol. Bull. (Woods Hole, Mass.) 151, 432.

Stinus, L., G.F. Koob, N. Ling, F.E. Bloom, and M. LeMoal, 1980.
Locomotor activation induced by infusion of endorphins into the ventral tegmental area: evidence for opiate-dopamine interactions
Proc. Natl. Acad. Sci. USA 77, 2323-2327.

Szeli, J., E. Molina, L. Zappia, and G. Bertacinni, 1977.
Action of some natural polypeptides on the longitudinal muscle of the guinea pig ileum
Eur. J. Pharm. 43, 285-287.

Tache, Y., P. du Ruisseau, J.R. Duchamre, and R. Collu, 1977.
Antagonism of pentobarbital-induced hormonal changes by TRH in rat
Eur. J. Pharm. 45, 369-376.

Theriault, E., M. Otsuka, and T. Jessell, 1979.
Capsaicin-evoked release of substance P from primary sensory neurons
Brain Res. 170, 209-213.

Theodorou, A., M. Crockett, P. Jenner, and C.D. Marsden, 1979.
Specific binding of ^{3}H-sulpiride to rat striatal preparations
J. Pharm. Pharmacol. 31, 424-426.

Titeler, M., and P. Seeman, 1979.
In-vitro measurement of brain receptors for dopamine and neuroleptics
In: Neurobiology of Dopamine, Academic Press, 179-195,
Eds. Horn, A.S., J. Korf, and B.H.C. Westerink.

Titeler, M., P. Weinreich, D. Sinclair, and P. Seeman, 1978.
Multiple receptors for brain dopamine
Proc. Natl. Acad. Sci. USA 75, 1153-1156.

Trabucchi, M., R. Longoni, P. Fresia, and P.F. Spano, 1975.
Sulpiride a study on the effects on dopamine receptors in rat neostriatum and limbic forebrain
Life Sci. 17, 1551-1556.

Ungerstedt, U., 1978.
The role of dopamine as a nueotransmitter in the central and the autonomic nervous system
Acta. Endocrinologica Suppl. 216 88, 13-26.

Ungerstedt, U., 1971.
Stereotaxic mapping of the monoamine pathways in the rat brain
Acta. Physiol. Scand. 82, Suppl. 367, 1-48.

Van Ree, J.M., D. De Weid, 1977.
Modulation of heroin self administration by neurohypophysed principles
Eur. J. Pharm. 43, 199-202.

Voight, K.H., H.L. Fehm. R.E. Lang, and R. Walter,
Effect of somatostatin and MIF on ACTH release in dispersed pituitary cells
Life Sci. 21, 732-748.

Voith, K., 1977.
Synthetic MIF analogues
Anzniem-Forch./Drug Res. 27, 2290-2293.

Von Euler, U.S., and J.H. Gaddum, 1931.
An unidentified depressor substance in certain tissue extracts
J. Physiol. 72, 74-87.

Walker, R.J., Kemp, J.A., Yajima, H. and Woodruff, G.N. 1976
The action of substance P on mesencephalic reticular and substantia nigra neurones of the rat. Experentia 32, 214-215.

Dopaminergic neurones: an invivo system for measuring drug interactions with presynaptic receptors
Naunyn-Schmeidegerg's Arch. Pharmacol. 296, 5-14.

Wang, R.Y., and G.K. Aghajanian, 1977.
Recording of single unit activity during electrical stimulation and micro-iontophoresis : a method of minimizing stinulus
Elec. and Clin. Neuro. 43, 434-437.

Waterfield, A., J.A.H. Lord, J. Hughes, and H.W. Kosterlitz, 1978.
Diffs. in the inhibitory effects of normorphine and opiod peptides on the responses of the vasa diferentia of two strains of mice
Eur. J. Pharm. 47, 249-250.

Watling, K.J., G.N. Woodruff, and J.A. Poat, 1979.
Dopamine sensitive adenylate cyclase in homogenates of rat nucleus accumbens: structure-activity studies and effects of agonists and antagonists
Europ. J. Pharmacol. 56, 45-49.

Westerink, B.H.C., D. Dijkstra, M.G.P. Feenstra, C.J. Grol, A.S. Horn, H. Rollema, and E. Wirix, 1980.
Dopaminergic prodrugs: brain concentrations and neurochemical effects of 5-6 and 6-7 ADTN after administration as dibenzoyl esters
Eur. J. Pharmacol. 61, 7-15.

Wilson, I.C., and P.L. Lara, 1973.
TRH in Schizophrenia
Lancet 11 7/8/73, 43-44.

Wilson, C.J., S.J. Young, and P.M. Groves, 1977.
Statistical properties of neuronal spike trains in the substantia nigra: cell types and their interactions
Brain Res. 136, 243-260.

Wilson, C.J., P.M. Groves, and E. Fifkova, 1977.
Monoaminergic synapses, including dendro-dendritic synapses in the rat substantia nigra
Exp. Brain Res. 30, 161-174.

Wilson, C.J., G.A. Fenster, S.J. Young, and P.M. Groves, 1979.
Haloperidol-induced alteration of post-firing inhibition in dopaminergic neurones of rat substantia nigra
Brain Res. 179, 165-170.

Winokur, A., M.S. Kreider, J. Dugan, and R.D. Utiger, 1978.
The effects of 6-hydroxydopamine on thyrotropin-releasing hormone in rat brain
Br. Res. 152, 203-208.

Winokur, A., and A.L. Beckman, 1978.
Effects of thyrotropin releasing hormone, norepinephrine and acetylcholine on the activity of neurones in the hypothalamus, septum and cerebral cortex of the rat
Brain Research 150, 205-209.

Winokur, A., M.S. Kreider, J. Dugan, and R.D. Utiger, 1978.
The effects of 6-hydroxydopamine on thyrotropin-releasing hormone in rat brain
Brain Research 152, 203-208.

Wirz-Justice, A., and M. Lichtsteiner, 1977.
Differential effect of TRH on MAO activity in the median eminence of male and pro-oestrous female rats
Neuropharmacology 16, 419-423.

Woodruff, G.N., and R.J. Walker, 1969.
The effect of dopamine and other compounds on the activity of neurones of Helix Aspersa, structure activity relationships
Int. J. Neuropharmacol. 8, 279-289.

Woodruff, G.N., 197[illegible].
Dopamine receptors: a review
Comp. Gen. Pharmacol. 2, 439-455.

Woodruff, G.N., A.O. El-Khawad, A.R. Crossman, and R.J. Walker, 1974.
Further evidence for the stimulation of rat brain dopamine receptors by a cyclic analogue of dopamine
J. Pharm. Pharmac. 26, 740-741.

Woodruff, G.N., A.O. Elkhawad, and R.M. Pinder, 1974.
Long lasting stimulation of locomotor activity produced by the intraventricular injection of a cyclic analogue of dopamine into conscious mice
Eur. J. Pharmac. 25, 80-86.

Woodruff, G.N., P.S. McCarthy, and R.J. Walker, 1976.
Studies on the pharmacol of neurones in the N Acc of the rat
Br. Res. 115, 233-242.

Woodruff, G.N., 1977.
In discussion on nomifensine and epilepsy
Brit. J. Clin. Pharmacol. 4, 1055.

Woodruff, G.N., S.B. Freedman, and J.A. Poat, 1980.
Why does sulpiride not block the effect of dopamine on the dopamine sensitive adenylate cyclase
J. Pharm. Pharmacol. 32, 802-803.

Woodruff, G.N., K.J. Watling, C.D. Andrews, J.A.Poat, J.D. McDermed, 1977.
DA receptors in rat striatum and N Acc: conformational studies using rigid analogues of DA
J. Pharm. Pharmac. 29, 422-427.

Woodruff, G.N., and C.D. Andrews, 1979.
Some effects of sulpiride on dopaminergic systems: comparison with other neuroleptics
In: Sulpiride and other benzamides, 33-46.
Eds. Spano, P.F., M. Trabucchi, G.U. Corsini, and G.L. Gessa, Italian Brain Research Foundation, Milan.

Woodruff, G.N., A. Davis, C.D. Andrews, and J.A. Poat, 1979.
Dopamine receptors in the mammalian brain
Recent Adv. in Receptor Chem.

Woodruff, G.N., 1979.
Dopamine receptors in invertebrates
In: The Neurobiology of Dopamine, 523-539.
Eds. Horn, A.S., J. Koif, and B.H.C. Westerink, Academic Press, London, New York, San Francisco.

Wright, D.M., and M.H.T. Roberts, 1978.
Supersensitivity to a substance P analogue following dorsal root section
Life Sci. 22, 19-24.

Yarbrough, G.G., J.C. McGuffin, and C. Clineschmidt, 1979.
MK-771-induced electromyographic (EMG) activity in the rat: comparison with thyrotropin releasing hormone (TRH) and antagonism by neurotensin
European Journal of Pharmacology 60, 41-46.

Yarbrough, G.G., 1978.
Studies on the neuropharmacology of thyrotropin releasing hormone (TRH) and a new TRH analog
Eur. J. Pharm. 48, 19-27.

Yarbrough, G.G., D.R. Haubrich, R.W. Ryall, and J.S. Kelly, 1978.
Iontophresis and transmitter mechanisms in the mammalian CNS, 136-138.

Yim, C.Y., and G.J. Mogenson, 1980.
Electrophysiological studies of neurones in the ventral tegmental area of tsai
Brain Res. 181, 301-313.

Youngblood, W.W., M.A. Lipton and J.S. Kizer, 1978.
TRH-like immunoreactivity in urine, serum and extrahypothalamic brain: non indentity with synthetic pyroglu-hist-pro-NH_2 (TRH)
Brain Research 151, 99-116.

Zieglgansberger, W., and I.F. Tulloch, 1979.
Effects of substance P on neurones in the dorsal horn of the spinal cord of the cat
Brain Research 166, 273-282.