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Interaction Between Nigro-Striatal Lesions and Drugs Affecting Dopamine Receptors

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INTERACTION BETWEEN NIGRO-STRIATAL LESIONS AND DRUGS
AFFECTING DOPAMINE RECEPTORS

BY

MARTIN DENNIS HYNES III

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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1975

NIGRO-STRIATAL LESIONS AND DRUGS

ABSTRACT

Lesioning the nigro-striatal dopamine neuron system produces aphagia and adipsia with an intensity proportional to the size of the lesion. Those rats which received small lesions produced by a 1 mA current for a 15 second duration of the nigro-striatal system initially lost weight and then spontaneously recovered from that loss. Pretreatment with alpha-methyl-para-tyrosine (50 mg/kg) or haloperidol (0.4 mg/kg) given twice a day for a three-day period prior to lesioning facilitated the recovery. When large lesions (2mA for 30 sec) were used to destroy this dopaminergic neuron system, a severe aphagia and adipsia resulting in death occurred in all saline treated animals. Haloperidol (2 mg/kg) or morphine sulfate (30 mg/kg) injected twice a day for six days preceding extensive nigro-striatal destruction promoted survival. The pharmacological denervation of dopaminergic receptors produced by haloperidol, morphine sulfate, or alpha-methyl-para-tyrosine prior to surgical destruction of the nigro-striatal pathway is felt to facilitate recovery.

Symptoms of morphine withdrawal such as, wet shakes, ptosis, weight loss and hypothermia were enhanced when lesions were made in the nigro-striatal tract prior to and following the production of morphine dependence. This exacerbation of the primary abstinence syndrome was seen at either of two different terminal doses of morphine sulfate.

Apomorphine, a dopamine receptor stimulant, was effective in reducing the withdrawal wet shakes in these lesioned animals. The administration of apomorphine to intact withdrawn rats also resulted in a significant decrease in wet shakes. The intensity of morphine withdrawal aggression observed in response to social grouping of the animals seventy-two hours after the termination of morphine administration was decreased by nigro-striatal lesioning. Destruction of the medial forebrain bundle in morphine dependent rats results in increased withdrawal ptosis, temperature and weight loss. These results suggest a role for brain noradrenergic and dopaminergic neuronal systems in morphine withdrawal. In particular, withdrawal wet shakes seem to be related to dopaminergic mechanisms.

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TABLE OF CONTENTS

	PAGE
ABSTRACT.	ii
ACKNOWLEDGEMENTS.	iv
TABLE OF CONTENTS	v
LIST OF TABLES.	ix
LIST OF FIGURES	x
 CHAPTER	
I INTRODUCTION	1
II LITERATURE SURVEY.	8
A. Central Nervous System Dopaminergic Pathways	8
1) Mesolimbic Dopamine Neurons.	8
2) Tubero-infundibular Dopamine Neurons	9
3) Nigro-neostriatal Dopamine Neurons . .	11
a. Electrical stimulation of the nigro-neostriatal pathway.	12
b. Motor functions.	14
c. Mental functions	16
d. Autonomic functions.	16
e. Sensory neglect following removal of the nigrostriatal dopamine system	16
B. Adipsia and Aphagia After Degeneration of the Nigro-Striatal Dopamine System	18
C. Cortical Dopaminergic Terminals	24
D. Recovery Mechanism Within the Central Nervous System	25

	PAGE
1) Catecholamine Turnover.	26
2) Postjunctional Supersensitivity	28
3) Evidence for Regeneration Axon Sprout- ing of Central Catecholamine Neurons. .	32
E. Morphine and Brain Dopamine	35
1) Acute Effects of Morphine	35
2) Effects of Chronic Morphine	41
3) Effects of Morphine Withdrawal.	42
F. Apomorphine and Brain Dopamine.	44
G. Neuroanatomical Pathways Related to Morphine Dependence and Abstinence.	47
H. Influence of Chemical Denervation on Mor- phine Physical Dependence and Tolerance . .	54
1) 5,6-Dihydroxytryptamine	54
2) 6-Hydroxydopamine	54
III GENERAL METHODS AND MATERIALS	57
A. Animals	57
B. Drugs	57
C. Brain Lesions	57
D. Drug Administration	58
E. Measurement of Withdrawal Symptoms	59
F. Histology	60
1) Physical Characteristics of the Brain Lesion.	61
2) Neurochemical Effects of the Lesion . .	62
G. STATISTICAL ANALYSIS.	65
IV EFFECT OF HALOPERIDOL, METHYL-PARA-TYROSINE AND MORPHINE ON WEIGHT LOSS AND LETHALITY DUE TO NIGRO-STRIATAL LESIONS	70
A. Introduction.	70

	PAGE
B. Method.	73
C. Results	74
D. Discussion.	80
V MODIFICATION OF NARCOTIC WITHDRAWAL SYMPTOMS BY NIGRO-STRIATAL AND MEDIAL FORE BRAIN BUNDLE LESIONS.	85
A. Introduction.	85
B. Method.	87
C. Results	88
1) Modification of Narcotic Withdrawal Symptoms by Nigro-Striatal Lesioning.	88
a. Lesions made prior to the produc- tion of dependence.	88
b. Lesions made after the production of morphine dependence.	93
1. Effect of small nigro-striatal lesions	93
2. Effect of large nigro-striatal lesions	97
c. Lesions made during the withdrawal period.	100
1. Effect of small nigro-striatal lesions	100
2. Effect of large nigro-striatal lesions	100
d. Modification of narcotic withdrawal symptoms by apomorphine	100
1. Effect of apomorphine on nar- cotic withdrawal symptoms in the intact dependent rat.	100
2. Effect of apomorphine on nar- cotic withdrawal symptoms in rats with nigro-striatal le- sions made after the produc- tion of dependence.	102

	PAGE
2) Modification of Narcotic Withdrawal Symptoms by Medial Fore Brain Bundle Lesions.	102
a. Lesions made after the production of morphine dependence	102
1. Effect of small medial fore brain bundle lesions	105
2. Effect of large medial fore brain bundle lesions	105
b. Small lesions made during the withdrawal period.	108
D. Discussion	108
VI DISCUSSION	116
VII SUMMARY AND CONCLUSIONS.	119
VIII BIBLIOGRAPHY	121

LIST OF TABLES

Table	Page
1 Weight Changes Following Small Lesions of the Nigro-Striatal Bundle With Pretreatment by Haloperidol or Alpha-Methyl -Para-Tyrosine. . . .	75
2 Weight Changes Following Large Lesions of the Nigro-Striatal Bundle With Pretreatment by Morphine or Haloperidol	78
3 Modification of the Narcotic Withdrawal Syndrome by Nigro-Striatal Lesioning Prior to the Production of Morphine Dependence	90
4 Seventy-two Hour Morphine Withdrawal Aggression in Rats Receiving Brain Lesions	92
5 Narcotic Withdrawal Symptoms in Rats with Small Nigro-Striatal Lesions Made After the Terminal Dose of Morphine Sulfate.	95
6 Narcotic Withdrawal Symptoms After Large Nigro-Striatal Lesions Following the Terminal Dose of Morphine Sulfate.	98
7 Nigro-Striatal Lesioning at Forty-Eight Hours After the Last Morphine Sulfate Injection: Effect on Seventy-Two Hour Withdrawal Symptoms. .	101
8 The Effect of Apomorphine on Narcotic Withdrawal Symptoms in the Intact Dependent Rat.	103
9 The Effect of Apomorphine on Narcotic Withdrawal Symptoms in Rats With Nigro-Striatal Lesions Made After the Terminal Dose of Morphine.	104
10 The Effect of Medial Fore Brain Bundle Lesions on the Narcotic Withdrawal Syndrome	106
11 Narcotic Withdrawal Symptoms at Seventy-Two Hours After Small Lesions of the Medial Fore Brain Bundle at Forty-Eight Hours	109

LIST OF FIGURES

Number		Page
1	Typical Cross Section of Rat Brain Showing Nigro-Striatal Destruction.	66
2	Representative Small Nigro-Striatal Lesion Superimposed on a Figure from the König and Klippel Atlas (1963).	67
3	Representative Large Nigro-Striatal Lesion Superimposed on a Figure from the König and Klippel Atlas (1963).	68
4	Striatal Dopamine Levels at Various Time Intervals Following Bilateral Lesions of the Nigro-Striatal Pathway.	69
5	Percent Weight Changes Following Small Lesions of the Nigro-Striatal Tract with Pretreatment by Haloperidol or Alpha-Methyl-Para-Tyrosine	76
6	Grams of Weight Lost Following Small Lesions of the Nigro-Striatal Bundle with Pretreatment by Haloperidol or Alpha-Methyl-Para-Tyrosine.	77
7	Percent Weight Changes Following Large Lesions of the Nigro-Striatal Bundle Lesions With Pretreatment by Morphine Sulfate or Haloperidol	79
8	Grams of Weight Lost Following Large Lesions of the Nigro-Striatal Bundle with Pretreatment by Morphine or Haloperidol	81
9	Percent Surviving as a Function of Days Following Large Lesions of the Nigro-Striatal Bundle with Pretreatment by Morphine Sulfate or Haloperidol.	82

I. INTRODUCTION

The existence of the nigro-striatal pathway arising in the substantia nigra and terminating in the neostriatum is well established by histological (Anden et al., 1964; Anden et al., 1965), biochemical (Anden et al., 1964; Poirier and Sourkes, 1965) and electromicroscopic (Hokfelt and Ungerstedt, 1969) determinations. The nigro-striatal neuron system is known to regulate the dopamine content of the neostriatum (Anden et al., 1964; Anden et al., 1965). The physiological importance of this system is evident from its involvement in motor function, mental processes, thermoregulation and normal feeding behavior (Anden et al., 1966b; Fuxe et al., 1970). The function of this dopamine system is particularly interesting in connection with the actions of a number of psychopharmacological agents such as apomorphine (Anden et al., 1967; Ernst, 1967), morphine (Puri et al., 1973) and neuroleptics (Anden et al., 1971) which are known to influence dopaminergic function. The objective of this study is to investigate the interaction between the effects of lesioning the nigro-striatal neuron system and pharmacological agents affecting dopamine receptors.

The ascending fibers of the nigro-striatal system originate mainly from the dopamine cell bodies situated in the zona compacta of the substantia nigra. The fibers be-

come aggregated in a bundle situated just medial and ventromedial to the lemniscus medialis in the area of the nucleus ventralis tegmenti. This bundle lies dorso medial to the ventral part of the crus cerebri in the H₂ area of Forel before it enters the rostral part of the crus cerebri. After entering the crus cerebri the tract diverges into the internal capsule to innervate the neostriatum (Anden et al., 1964; Anden et al., 1965; Poirier and Sourkes, 1965; Faull and Lavery, 1969). This system is of great importance for normal movements and postures. The symptoms of rigidity, hypokinesia and tremor found in parkinsonism are probably due to the degeneration of the nigro-striatal system (Hornykiewicz, 1966). The pathophysiology of schizophrenia may be in part due to the activity of dopamine at its receptor sites in the striatum (Klawans et al., 1972). A role for dopamine neurons in thermoregulation has been suggested since apomorphine decreases body temperature via a central action (Fuxe and Sjogrist, 1972). The nigro-striatal neurons appear to be necessary for normal eating and drinking behavior (Ungerstedt, 1970, 1971; Oltsman and Harvey, 1972).

Unilateral lesions of the corpus striatum or the nigro-striatal dopamine system are known to produce asymmetric movements and postures (Poirier et al., 1965; Anden et al., 1966). Lesioned animals show a pronounced rotational behavior which is linked to differences in dopamine receptor activity on two sides of the brain (Anden et al., 1966a).

Three to four days after unilateral removal of the nigro-striatal system there is a lack of orienting response to all sensory stimuli on the side contralateral to the lesion (Ungerstedt, 1973). The period from five days to two months after the operation is characterized by partial recovery of sensory function (Ungerstedt, 1973). Degeneration of the nigro-striatal system, bilaterally, in the rat has been shown to result in the development of adipsia and aphagia (Ungerstedt et al., 1970, 1971). Zigmond and Stricker (1972, 1973) have also found that depletion of brain dopamine was critical for the production of adipsia and aphagia. The adipsic and aphagic syndrome seen after nigro-striatal destruction is severe and if not force-fed animals die several days after lesioning (Ungerstedt, 1970, 1971; Oltsman and Harvey, 1972). Force-fed animals recover from aphagia and adipsia, in a predictable sequence of stages culminating in the ability to maintain their body weight on food pellets and water (Marshall and Teitelbaum, 1973). Recovery of feeding behavior has been suggested to involve a denervation supersensitivity mechanism (Glick et al., 1972). Supersensitivity may be defined as the phenomenon in which the amount of a neurotransmitter required to produce a given biological response is less than normal. Thus, the one consistent sign of supersensitivity is a shift to the left of the dose response curve (Fleming et al., 1973).

Supersensitivity in addition to being a useful model in explaining recovery of feeding behavior has also been

forwarded to explaining dependence on narcotics (Jaffe and Sharpless, 1968; Collier, 1969). Based upon results from morphine withdrawal aggression it has been suggested that central dopamine receptors are supersensitized during chronic morphine administration. Morphine withdrawal aggression in dependent animals can be selectively potentiated by dopaminergic stimulating agents and blocked by drugs which block dopamine receptors (Puri et al., 1971; Lal and Puri, 1972; Puri and Lal, 1973). Further evidence for the interaction between brain dopamine and morphine comes from biochemical studies. Acute morphine elevates brain homovanillic acid and 3-4 dihydroxyphenylacetic acid (Fukui and Tagaki, 1972; Kuschinsky and Hornykiewicz, 1972), increases the synthesis of labeled dopamine from labeled tyrosine in brain (Clouet and Ratner, 1970; Fukui et al., 1972; Smith et al., 1970, 1972) and accelerates the disappearance of dopamine after the administration of alpha-methyl-para-tyrosine (Gunne et al., 1969; Puri et al., 1973). Based upon these studies it was proposed that acute administration of morphine blocks dopamine receptors. Chronic administration of morphine therefore would produce a persistent blockade of dopamine receptors and cause the development of latent supersensitivity of central dopamine receptors. This supersensitivity may therefore contribute to the morphine withdrawal syndrome.

Treatment with haloperidol (Gianutsos et al., 1974a, 1975) and methyl-para-tyrosine (Tarsy and Baldessarini, 1973)

has also been shown to produce a supersensitivity of dopamine receptors in the central nervous system. Haloperidol is a potent neuroleptic that is known to block dopamine receptors (Janssen, 1967; Anden et al., 1971). The dopamine receptor supersensitivity produced by chronic administration of haloperidol is manifested by an enhanced stereotypy and aggression in response to small otherwise ineffective doses of apomorphine (Gianutsos et al., 1974a). Apomorphine has been shown to effectively decrease turnover of dopamine in haloperidol-treated rats at doses which were without effect in drug-naive rats (Gianutsos et al., 1975). A shift in the dose response curve in the direction of increased sensitivity was found in response to apomorphine, a dopaminergic stimulant, after withdrawal of chronically administered alpha-methyl-para-tyrosine (Tarsy and Baldessarini, 1973). Alpha-methyl-para-tyrosine is a well known inhibitor of catecholamine synthesis (Nagatsu et al., 1964). Apomorphine, which has been used in these studies to show increased sensitivity, is believed to stimulate the dopaminergic receptors in the central nervous system (Anden et al., 1967; Ernst, 1967). Biochemical studies have shown that apomorphine decreases the turnover rate of striatal dopamine; this effect is completely blocked by haloperidol (Anden et al., 1967; Persson, 1970; Anden and Becard, 1971; Lahti et al., 1972; Puri, 1973).

This investigation is focused on the nigro-striatal neuron system and dopaminergic supersensitivity. These factors

were studied in relation to aphagia, adipsia and the morphine withdrawal syndrome. Nigro-striatal lesions will be made and body weight changes recorded. Prior to the production of the lesion, animals will be treated with either saline, alpha-methyl-para-tyrosine, a blocker of catecholamine synthesis (Nagatsu et al., 1964), haloperidol, a dopaminergic blocking agent (Janssen, 1967; Van Rossum, 1967; Anden et al., 1970), or morphine which may also block dopaminergic receptors (Puri et al., 1973). Drug treated and control groups will be compared with respect to lesion induced weight changes and lethality.

Nigro-striatal lesions will be produced both prior to and subsequent to the production of morphine dependence. After the abrupt termination of morphine injections subjects will be observed for the symptoms of morphine withdrawal. Narcotic withdrawal symptoms will be compared between lesioned and non-lesioned groups. The dopaminergic stimulating agent apomorphine (Anden et al., 1967; Ernst, 1967) will be administered to both intact and nigro-striatal lesioned morphine withdrawn subjects to further assess the role of dopamine receptor activity in the abstinence phenomena. In order to determine if the results are specific for the dopaminergic system the medial fore brain bundle, a nor-adrenergic and serotonergic neuron system, will also be lesioned after the production of dependence and abstinence signs observed.

This research has the following significance. First-

ly, evidence for the role of the nigro-striatal dopamine neurons in aphagia and adipsia will be gathered. Secondly, it will clarify the role of receptor supersensitivity in recovery from nigro-striatal lesioning and in the morphine withdrawal syndrome. Thirdly, it will give insight into the role of this dopamine system in narcotic dependence.

II. LITERATURE SURVEY

A. Central Nervous System Dopaminergic Pathways

The dopamine neuron system discovered so far are two large ascending fiber systems and one small dopamine neuron system. The existence of these dopaminergic pathways has been demonstrated through studies employing lesions, stimulating electrodes, biochemical and histochemical techniques. Based upon these studies the following dopaminergic pathways have been described:

- 1) Mesolimbic Dopamine Neurons
- 2) Tubero-infundibular Dopamine Neurons
- 3) Nigro-Neostriatal Dopamine Neurons

1) Mesolimbic Dopamine Neurons

The mesolimbic dopamine neurons have their cell bodies in the area surrounding the nucleus interpeduncularis and fibers from these cell bodies terminate in the tuberculum olfactorium, nucleus accumbens, the dorsolateral part of the nucleus interstitialis striae terminalis and in the nucleus amygdaloideus centralis (Anden et al., 1966a). Dopaminergic fibers of this system run medially to the nigro-neostriatal dopamine fibers whereas cranially they mainly lie ventral to the nigro-neostriatal dopamine fibers. The mesolimbic dopamine neurons probably contain less dopamine than does nigro-neostriatal dopamine neurons. This is probably due

to the fact that the terminal network of each mesolimbic dopamine neurons is much smaller than that of the nigro-neostriatal dopamine neurons (Anden et al., 1966b; Fuxe et al., 1970).

Lesions placed at the level of the rostral hypothalamus which specifically interrupt the pathway to the mesolimbic area are found to modify neuroleptic catalepsy. These lesions caused an initial potentiation followed by a reduction in the cataleptic effect of neuroleptic agents but cause a potentiation of cholinergic catalepsy at all times of testing (Costall and Naylor, 1974). The mesolimbic area thus appears to be involved with the mediation of neuroleptic catalepsy and the cholinergic dopaminergic balance controlling cataleptic behavior would also appear to involve the mesolimbic dopamine neurons (Costall and Naylor, 1974a). Lesions of the dopaminergic mesolimbic innervation partially reduce morphine catatonia (Costall and Naylor, 1974b). Although stereotypy has been considered by many purely in terms of extrapyramidal actions, studies employing apomorphine and ET 495 have shown that mesolimbic functions are important for the initiation of stereotyped responses (Costall and Naylor, 1973c). Ablation of the mesolimbic innervation reduce the weaker components of methylphenidate stereotypy (Costall and Naylor, 1974c).

2) Tubero-infundibular Dopamine Neurons

The tubero-infundibular dopamine neurons have their

cell bodies mainly localized to the anterior part of the nucleus annuatus and anterior periventricular nuclei. The axons of this system run ventrally toward the lateral border of the median eminence (Fuxe, 1963; Fuxe and Hokfelt, 1966, 1969). In the external layer of the medial eminence, the axons give rise to a densely packed plexus of dopaminergic nerve terminals, which exerts an axo-axonic influence in the layer (Hokfelt, 1967).

This very short dopaminergic intrahypothalamic system regulates the discharge of the releasing and inhibiting factors from the median eminence (Fuxe et al., 1970). The release of dopamine in the median eminence acts locally on terminals storing luteinizing hormone releasing factor (LHRF) to inhibit the release of LHRF from the medial eminence. This system also participates in mediating the negative feedback action of estrogen and testosterone on gonadotropic secretion, because estrogen and testosterone markedly increase the turnover of the tubero-infundibular dopamine neurons of castrated rats, resulting in increased release of dopamine in this area (Fuxe et al., 1967, 1969). The blockade of ovulation by synthetic estrogen and its derivatives may at least partly be mediated via activation of the neuron system (Fuxe and Hokfelt, 1969). This system is also highly sensitive to prolactin, which markedly increases the turnover of dopamine in the tubero-infundibular dopamine neurons (Fuxe and Hokfelt, 1969, 1970).

3) Nigro-neostriatal Dopamine Neurons

The nigro-neostriatal system seems to originate mainly in the pars compacta of the substantia nigra (Anden, 1964). Support for this comes from fluorescence microscopy and the high dopamine content observed in this area (Hornykiewicz, 1963). Unilateral pars compacta lesions result in a sixty per cent lowering of the dopamine level in the corpus striatum of the operated side when compared with the unoperated side (Faull and Lavery, 1969). The caudate nucleus and putamen shows a fairly strong green to yellow fluorescence due to the high dopamine content. This fluorescence was reduced in animals with lesions of the substantia nigra. A clear correlation was found between the fluorescence reduction and the extent of destruction of the pars compacta (Anden, 1964). Some cell bodies are also found in the zona reticulata and the pars lateralis of the substantia nigra, which also belong to the nigro-neostriatal dopamine neurons (Fuxe et al., 1970). Recently, studies after removal of the nucleus caudatus putamen suggest that the cell bodies in the ventrolateral part of the midbrain tegmentum belong to this large uncrossed neuron system, inasmuch as they show marked reduction in fluorescence intensity and signs of atrophy after such operations (Fuxe et al., 1970). There is now a virtually complete picture of the distribution of these dopamine fibers. Upon leaving the pars compacta of the substantia nigra, most of the nigro-neostriatal dopamine

fibers become aggregated in a bundle which ascends just medial and dorso medial to the ventral part of the crus cerebri. At the level of the posterior part of the medial eminence, the bundle turns to the ventralrostral part of the crus cerebri and enters and diverges into the lentolenticular part of the internal capsule. Running rostrally and dorsally in the internal capsule, the fibers then ascend in the fibers of the internal capsule to innervate the neostriatum (Anden et al., 1965).

Biochemical and histochemical investigation have yielded some quantitative data on the unilateral nigro-neostriatal dopamine system in the rat. The number of neurons making up this system is 3500 on the average. There are about 1.2×10^{-10} grams of dopamine per neuron with terminals containing fifty times more dopamine than the cell bodies. There are approximately 500,000 varicosities in the terminals, each with an average diameter of 0.4μ , containing 2.5×10^{-16} grams of dopamine and has a dopamine concentration of around 8,000 $\mu\text{g}/\text{grwet weight}$. One dopamine cell body of the substantia nigra, with a mean diameter of 30μ , contains 2.5×10^{-18} grams and has a dopamine concentration of from 60 to 200 $\mu\text{g}/\text{gr}$ (Anden et al., 1966c).

a. Electric stimulation of the nigro-neostriatal pathway

The direct electrical stimulation of either the caudate nucleus or the pars compacta of the substantia nigra causes a frequency and intensity related release of H-3

dopamine into the cerebrospinal fluid (Von Voightlander and Moore, 1971, 1972) and also into ventricles (Von Voightlander and Moore, 1971). These results imply that dopamine is a neurotransmitter of the nigro-neostriatal pathway. There is evidence to indicate that dopamine may function as an inhibitory neurotransmitter at the terminals of the nigro-neostriatal pathway. Bloom et al., (1965), demonstrated a primarily inhibitory action of dopamine when it was applied micro-iontophoretically to caudate neurons. Most of the neurons that respond to dopamine are inhibited by exogenous dopamine applied in the caudate nucleus of anesthetized and decerebrated cats (McLellan and York, 1967; York 1970). Following the application of dopamine iontophoretically from multibarrel micropipette assemblies near caudate cells, the rate of discharge of fifty to sixty per cent of these neurons is depressed, while the spike rate of approximately ten per cent of the cells is facilitated. Electrical stimulation of substantia nigra evokes depressant and facilitatory responses from individually recorded nucleus neurons (Connor, 1968, 1970).

Catecholamine involvement in the phenomenon of intracranial self stimulation is well known. Until recently, the major role has been assigned to noradrenergic nerves. This has been challenged by neuroanatomical and histochemical evidence of a possible dopaminergic involvement in intracranial self-stimulation (Phillips and Fibiger, 1973). When

the facilitatory effects of d and l isomers of amphetamine on self-stimulation were assessed, it was found that the two isomers were equipotent (Phillips and Fibiger, 1973). These data would seem to indicate that the dopaminergic systems in part subserves positive reinforcement.

b. Motor functions

The nigro-neostriatal neurons play an important role in normal movement and postures. Degeneration of the system is believed to be the cause of symptoms such as rigidity, hypokinesia and tremors found in parkinsonism (Hornykiewicz, 1966). In agreement with this it has been found possible to restore normal motor functions in Parkinsonian patients by treatment with L-dopa (Cotzias et al., 1967).

The drug-induced stimulation of the nigro-neostriatal pathway causes an induction of locomotion, stereotyped behaviors and finally compulsive gnawing (Randrup and Munkvad, 1968; Fuxe and Ungerstedt, 1970). Thus the changes in these behaviors involve the stimulation of dopaminergic receptors. Similar results were obtained when animals were treated with L-dopa, the precursor of dopamine, following dopa decarboxylase inhibition. Locomotor activity was markedly increased (Bartholini et al., 1969; Butcher et al., 1970). The increase in locomotor activity is mainly composed of stereotyped movements (Butcher et al., 1970). The systemic administration of apomorphine produces stereotyped behavior in rats which is characterized by continuous and compulsive sniffing, licking and gnawing (Ernst, 1965).

The results of several studies have supported the hypothesis that stereotyped behaviors induced by apomorphine or by amphetamine is due to increased dopamine receptors activity in the neostriatum (Ernst, 1969; Fuxe and Ungerstedt, 1970).

Unilateral lesions of the corpus striatum or the nigrostriatal dopamine system are known to produce asymmetries in movement and posture (Poirier et al., 1965; Anden et al., 1966a). The difference between the two sides of the brain may be further aggravated by treatment with drugs that release dopamine from the non-lesioned side. Such animals show a pronounced rotational behavior (Anden et al., 1966a). The rotational behavior is further linked to the differences in dopamine levels on the two sides of the brain by finding that unilateral striatal injections of dopamine cause the rats to turn or slowly rotate away from the side where dopamine was injected (Ungerstedt et al., 1969). Spontaneous rotations toward the intact side is seen twenty-four to thirty-four hours after a lesion of the nigrostriatal dopamine system. The direction of the rotation as well as the time point of its occurrence is indicative of a degeneration release of dopamine from the lesioned side (Ungerstedt, 1973). In a chronically lesioned animal there is a striking difference between the effects of dopamine-releasing drugs and dopamine receptor-stimulating drugs. Amphetamine causes the animal to rotate toward its lesioned side, apomorphine causes it to rotate toward its intact side (Ungerstedt, 1973). These results indicate that amphetamine preferenti-

ally influences the non-lesioned side, whereas apomorphine exerts its strongest effect on the denervated side.

c. Mental functions

It is now accepted that stereotyped behavior induced by amphetamine or apomorphine is principally due to the increased dopaminergic neurotransmission in the neostriatum. Stereotyped behavior is also often seen in patients with schizophrenia (Snyder, 1971) and it has, therefore, been assumed to be partly due to an abnormal increased activity of nigro-neostriatal dopamine neurons (Fuxe et al., 1970). This view is substantiated by the fact that neuroleptics which are potent antipsychotic drugs block dopaminergic neurotransmission (Corrodi et al., 1971).

d. Autonomic functions

The observation that dopamine increases cardiac output and increases systemic blood pressure (Noyer et al., 1971) suggest that dopaminergic neurons may play a role in central vasomotor mechanism. Since the administration of dopaminergic blocking agents such as spiroperdal and pimozide decrease blood pressure (Fuxe et al., 1970).

e. Sensory neglect following removal of the nigrostriatal dopamine system

Three to four days after a unilateral removal of the nigrostriatal system there is an almost complete lack of orienting response to all sensory stimuli on the side contralateral to the lesion, while the animal reacts in an es-

sentially normal way to stimuli to the side ipsilateral to the lesion (Ungerstedt, 1973). Simple reflexes like the withdrawal reaction or the corneal reflex, are normal on both sides. The period from five days to two months after the operation is characterized by partial recovery of sensory functions. Even two months after the operation none of the animals show normal responses on the side contralateral to the lesion. The sense of smell seems to recover first, then vision and yet none of the animals regained a normal reaction to touch (Ungerstedt, 1973).

B. Adipsia and Aphagia After Degeneration of the Nigro-Striatal Dopamine System

Detailed mapping of the central monoamine pathways (Ungerstedt, 1971) show the dopamine axons to be located in a dense bundle in the lateral hypothalamus before entering into the crus cerebri. There is a vast literature in the field of physiological psychology and especially in connection with consumatory behavior where the effects of lesions and stimulations in this area are carefully studied. However, the dopamine system has received little or no attention, probably because detailed information of its anatomy has been lacking.

A great deal has been learned concerning the changes in food and water regulation following lateral hypothalamic lesions; little is known concerning the specific anatomical structures or systems involved. Several investigations have suggested that the critical areas for producing the lateral hypothalamic syndrome may be outside or include only a lateral segment of the lateral hypothalamus (Morgane, 1961; Gold, 1967; Grossman and Grossman, 1971; Wampler, 1971). Gold (1967) outlined a critical forebrain area for producing aphagia and adipsia. This area included a portion of the globus pallidus, the medial portion of the internal capsule. It is known that several fiber systems pass through these three areas which regulate the telencephalic content of norepinephrine, dopamine and serotonin. The nigro-striatal bundle, a fiber system that regulates the

dopamine content of the neostriatum, passes through each of the three areas described by Gold (1967).

Electrocoagulation in the lateral hypothalamus interrupting the axons of the nigro-striatal dopamine pathway cause the dopamine terminals in the corpus striatum to degenerate thus resulting in a decrease in the dopamine content of the neostriatum (Poirier et al., 1967; Faull and Laverty, 1969; Moore et al., 1971; Ungerstedt, 1971). Symptoms of adipsia and aphagia appear after the lesions; since other than dopamine neurons may have been destroyed the results may not be due to interruption of dopamine fibers alone. The technique of introcerebral injections of 6-hydroxydopamine permits a more selective degeneration of dopamine and noradrenergic pathways (Ungerstedt, 1971). The development of adipsia and aphagia was correlated to the histochemical effects of the 6-hydroxydopamine lesions. Adipsia and aphagia always followed a complete bilateral degeneration of the nigro-striatal dopamine system regardless if the 6-hydroxydopamine was injected into the substantia nigra, the area ventralis tegmenti or the lateral hypothalamus (Ungerstedt, 1970, 1971). However, where the ascending noradrenergic pathways were lesioned no adipsia and aphagia developed in spite of the fact that most of the hypothalamus degenerated (Ungerstedt, 1971). In agreement with previous reports, intraventricular administration of 6-hydroxydopamine in monoamine oxidase inhibited animals or bilateral injections of 6-hydroxydopamine into substantia nigra

produced aphagia and adipsia (Fibiger et al., 1973). Oltsman and Harvey (1972) found that electrolytic lesions of the lateral hypothalamus which destroyed the dopaminergic nigro-striatal tract produced severe aphagia and adipsia.

The adipsic and aphagic syndrome seen after nigro-striatal destruction is severe. Ungerstedt (1970, 1971) found that if not supported, the animals died four to five days after lesions. Their condition was generally worse than that which is seen where a normal animal is deprived of food and water (Ungerstedt, 1971). Oltsman and Harvey (1972) also reported a severe aphagia and adipsia after nigro-striatal lesioning. Recovery of food and water intake is reported to have occurred within ten days of intraventricular 6-hydroxydopamine (Zigmond and Stricker, 1972; Fibiger et al., 1973). Nigral injections of 6-hydroxydopamine produce a more severe effect with recovery failing to occur during a three month period (Fibiger et al., 1973). Animals with nigro-striatal lesions show deficits in water regulation and food intake as have been found in lateral hypothalamic lesioned animals (Oltsman and Harvey, 1972; Fibiger et al., 1973; Marshall and Teitelbaum, 1973). Rats with nigro-striatal destructions progress through the same sequence of stages in the recovery of feeding as do rats with lateral hypothalamic lesions (Marshall and Teitelbaum, 1973).

Ungerstedt (1970, 1971) has shown that adipsia and aphagia is the result of a loss in striatal dopamine. Zigmond and Stricker (1972, 1973) have also found that depletion of

brain dopamine was more critical for producing symptoms of the lateral hypothalamic syndrome than was depletion of brain norepinephrine. Glick et al., (1974) have found lesion-induced weight loss to be highly correlated with depletion of striatal dopamine but not telencephalic norepinephrine. In rats with severe dopamine depletions, the degree of weight loss was related more to the striatum with the highest remaining level of dopamine suggesting that a critical level of dopamine in one striatum may be essential for lateral hypothalamic recovery. Zigmond and Stricker (1973) have also reported that residual brain catecholamines appear to make a significant contribution to the recovery of ingestive behavior in rats with either 6-hydroxydopamine or electrolytic lesions. Rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions decrease food and water intake markedly after the administration of methyl-para-tyrosine at doses that did not affect the ingestive behaviors of controlled rats (Zigmond and Stricker, 1973). These results suggest that recovery from aphagia and adipsia is dependent upon compensatory processes occurring with the damaged systems. Several mechanisms have been proposed which might account for this compensation, such as an increase in catecholamine turnover in terminals of remaining fibers (Bloom et al., 1969; Uretsky et al., 1971), an increased sensitivity of postsynaptic receptors (Ungerstedt, 1971; Uretsky and Schoenfeld, 1971; Schoenfeld and Uretsky, 1972), and sprouting of new terminals from transected axons (Katzman et al.,

1971; Nygren et al., 1971).

Recently, various kinds of chemical treatments have been found to attenuate the severity of the aphagia and adipisia following bilateral lesions of the lateral hypothalamus. Recovery of feeding behavior has been facilitated by systemic injections of either methyl-p-tyrosine or insulin for a few days prior to surgery. Rats with bilateral hypothalamic lesions die of starvation within seven days of surgery. But when these rats are pretreated with methyl-p-tyrosine they spontaneously eat, drink and gain weight after surgery (Glick et al., 1972). These data suggest that recovery of functions after lateral hypothalamic damage involves denervation supersensitivity since, methyl-p-tyrosine should have pharmacologically produced a partial denervation of neurons subserving recovery. Glick and Greenstein (1972) have also suggested that recovery from lateral hypothalamic lesions may involve the sprouting of intact inputs to the remaining lateral hypothalamic noradrenergic neurons. They found that if frontal cortical lesions were produced thirty days prior to lateral hypothalamic lesioning recovery was facilitated. The period of recovery after bilateral electrolytic lesions of the lateral hypothalamus is shortened if insulin is given for five days before surgery (Balagura et al., 1973). Recovery of feeding has also been facilitated when rats are reduced by food deprivation to seventy-five per cent of their normal body weight. This food deprivation facilitated recovery has been shown for lateral hypothalamic lesions

(Powley-Keeseey, 1970) and 6-hydroxydopamine treatment (Myers and Martin, 1973).

Enhanced recovery of feeding after hypothalamic damage has been reported in response to an intraventricular injection of nerve growth factor (Berger et al., 1973). These authors speculate that nerve growth factor may facilitate behavioral recovery by promoting the development of supersensitivity to norepinephrine and possibly also by stimulating the growth of regenerating noradrenergic neurons in the brain. Postoperative alpha-methyl-para-tyrosine treatment has been shown to facilitate survival but the dose of alpha-methyl-para-tyrosine which will produce this effect is critical (Glick and Greenstein, 1974). Initially, alpha-methyl-para-tyrosine improved feeding mechanisms involving catecholamine and thereby promoted the development of denervation supersensitivity which became behaviorally manifested after the effect of alpha-methyl-para-tyrosine on catecholamine synthesis had subsided (Glick and Greenstein, 1974). Electrical stimulation through the same electrodes that induced aphagia by means of a mechanical lesion has been shown to shorten the post lesion recovery period (Harrell et al., 1974). Alteration of the esculent property of the diet given following lateral hypothalamic lesioning has also been shown to effect recovery from the syndrome (Myers and Martin, 1973). Recovery of a postoperative weight loss following bilateral ablations of frontal cortex in rats is quicker when food pellets are scattered on the cage floor

than when pellets were available only in attached food hoppers (Glick and Greenstein, 1973). The period of recovery after bilateral electrolytic lesions of the lateral hypothalamus in rats is lengthened if glucagon is given during the preoperative period (Balagura et al., 1973). Bilateral lesions made in the habenular nuclei had little effect on recovery of feeding following lateral hypothalamic lesions (Mok et al., 1973), thus suggesting that the habenular nucleus is not a crucial part of the feeding system which mediates the recovery from the lateral hypothalamic syndrome.

C. Cortical Dopaminergic Terminals

For a number of years it had been generally assumed that all catecholaminergic cortical nerve terminals were nor-adrenergic. However, high concentrations of dopamine have been found in the cortex of various species (Bertler and Rosengren, 1959; Valzelli and Garattini, 1968). It appears that most of the dopamine found in the cortex of the rat is not localized in nor-adrenergic terminals. Lesions of the dorsal nor-adrenergic system or a combined lesion of the dorsal and the ventral nor-adrenergic systems, which both significantly decrease the cortical levels of norepinephrine, did not induce a parallel reduction in cortical dopamine content (Thierry et al., 1973b). These findings strongly suggest the existence of dopaminergic neurons in the cortex. Further evidence for the existence of dopaminergic terminals in the rat cortex is taken from the fact

that cortical synaptosomes have the ability to synthesize ^3H -dopamine from ^3H -tyrosine (Thierry et al., 1973a).

The destruction of ascending noradrenergic pathways which abolishes the in vitro synthesis of ^3H -norepinephrine did not abolish the synthesis of ^3H -dopamine (Thierry et al., 1973a). Also, a specific dopamine reuptake process has been demonstrated in the cerebral cortex of normal rats and rats whose ascending noradrenergic pathways have been selectively destroyed (Tassin et al., 1974). Visualization of these dopaminergic terminals has been achieved by combined pharmacological and histochemical methods (Lidbrink et al., 1974). The occurrence of dopaminergic nerve endings was further supported by the demonstration of a dopaminergic receptor in the cortex. Specifically, dopamine was found to activate an adenylate cyclase system and this effect was inhibited by the dopamine receptors blocker haloperidol (von Hungen and Roberts, 1973).

D. Recovery Mechanism Within the Central Nervous System

Recovery from central nervous system lesions appears to be dependent on compensatory processes occurring within the damaged system. Several mechanisms have been proposed which might account for this compensation, such as an increase in catecholamine turnover in terminals of remaining fibers, an increase in sensitivity of postsynaptic receptors and sprouting of new terminals from transected axons.

1) Catecholamine Turnover

Reduced accumulation of intraventricularly administered ^3H -1-norepinephrine was seen in the brain of rats who were treated with intraventricular injections of 6-hydroxydopamine. The loss of catecholamine uptake sites produced by the 6-hydroxydopamine pretreatment was probably responsible for the reduction in accumulation (Uretsky *et al.*, 1971). The rate constant of disappearance of ^3H -norepinephrine was used to estimate the fraction of the endogenous norepinephrine pool turning over per unit of time. An increase in the rate constant in the pons-medulla region was seen in 6-hydroxydopamine treated rats. An increase in the rate constant may indicate that norepinephrine containing neurons which survive the degeneration effects of 6-hydroxydopamine show an increase in their physiological state of activity to compensate for the loss of neuronal function after degeneration (Uretsky *et al.*, 1971). Alternatively, the noradrenergic neurons which have a higher turnover than average may survive the effects of 6-hydroxydopamine. Since 6-hydroxydopamine causes a profound and long-lasting depletion of norepinephrine in all brain regions (Uretsky and Iversen, 1970), the turnover rate of norepinephrine in 6-hydroxydopamine pretreated animals was much lower than in control brains despite the increase in rate constant

observed in the pons-medulla (Uretsky et al., 1971). These results are consistent with those of Bloom et al. (1969), who found a decrease in the turnover of norepinephrine in whole brain of 6-hydroxydopamine treated rats. In these experiments there was an increase in the rate constant in the striatum; this reflects the disappearance of ^3H -norepinephrine from mainly dopaminergic neurons. This increase in the rate constant may nevertheless reflect some compensatory change in the level of activity of the surviving dopamine-containing neurons.

A marked increase in dopamine specific activity has been found after intravenous administration of ^3H -tyrosine in animals following injections of 6-hydroxydopamine into the substantia nigra. Furthermore, these lesioned animals showed enhancement of the $^3\text{H}\text{-H}_2\text{O}/\text{DA}$ ratio on the lesioned side following injections of the radioactive precursor. An increase in the ratio of homovanillic acid to the dopamine content in the striatum on the side lesioned with 6-hydroxydopamine has also been found (Agid et al., 1974). These results indicate a hyperactivity of the remaining dopaminergic neurons following partial degeneration of the nigrostriatal pathway.

2) Postjunctional Supersensitivity

Supersensitivity may be defined as the phenomenon in which the amount of a substance required to produce a given biological response is less than normal. Thus, the one consistent sign of supersensitivity is a shift in the maximum response to a drug (Fleming et al., 1973). An apparent change in sensitivity of the postjunctional element to nerve impulses could result from any one of several changes in the transmission apparatus. There could be a change in the postjunctional element so that it actually became more sensitive to the transmitter (Thesleff, 1960). The mechanism inactivating the transmitter could change so that it is removed from its site of action more slowly (Trendelenburg, 1963). Prejunctional elements could increase their capacity to deliver transmitter or change their recovery process to repetitive stimulation (Sharpless, 1969). Many of these changes are known to occur in disused or denervated peripheral structures.

There is evidence that spinal motor neurons become more reactive to a variety of stimuli following cord section (Cannon and Rosenblueth, 1949; Stavraky, 1961), and destruction of sensory nerve fibers (Cannon and Rosenblueth, 1949; Stavraky, 1961; Loeser and Ward, 1967). Several weeks after a tenotomy, which relieves tension on muscle spindles and thus reduces the activity of Group Ia sensory fibers, monosynaptic discharge elicited by stimulating Group Ia fibers had greatly increased in strength (Beranek and Hnik, 1959;

Kozak and Westerman, 1961).

Supersensitivity of the temperature regulating center of the hypothalamus has been shown to develop in response to chronic administration of scopolamine (Friedman and Jaffe, 1969; Friedman et al., 1969). When scopolamine was administered to mice for various periods ranging from five days to four weeks and then withdrawn, an exaggerated hypothermic response to pilocarpine and other centrally acting cholinergic drugs was seen. Sharpless and Halpern (1962) found that in the isolated cerebral cortex of the cat supersensitivity emerged in two to three weeks after surgical denervation. Supersensitivity to metamphetamine has been shown to occur after treatment with alpha-methyl-para-tyrosine (Poschel and Nintemon, 1966).

Following intraventricular administration of 6-hydroxy-dopamine which destroys catecholamine containing nerve terminals, L-dihydroxy-phenylalanine (dopa) produces a marked increase in the locomotor activity of treated rats, while it has little effect on the activity of untreated rats (Uretsky and Schoenfeld, 1971). These results suggest the enhanced effects of L-dopa may be due to a central supersensitivity to catecholamine. The behavioral response to apomorphine is altered by 6-hydroxydopamine pretreatment while lowering the ED_{50} for apomorphine (Schoenfeld and Uretsky, 1972). A modified response to apomorphine, administered directly into the striatum has also been reported in reserpine-treated rats (Fuxe and Ungerstedt, 1970).

Jalfre and Haefely (1971) have reported that 6-hydroxydopamine treated rats showed increased motor activity after low doses of apomorphine which were ineffective in normal animals. A shift in the dose-response curve in the direction of increased sensitivity was found in response to apomorphine after withdrawal of chronically administered reserpine, alpha-methyl-para-tyrosine or chlorpromazine (Tarsy and Baldessarini, 1973). Chronic administration of haloperidol has been shown to result in a supersensitivity of dopamine receptors. This supersensitivity is manifested by an enhanced stereotypy and aggression in response to small, otherwise ineffective doses of apomorphine (Gianutsos et al., 1974). Apomorphine has been shown to effectively decrease turnover of dopamine in haloperidol treated rats at doses which were without effect in drug-naive rats (Gianutsos et al., 1974).

Chronic denervation of the rat pineal gland leads to an increase in the cyclic AMP response to norepinephrine within three weeks (Weiss and Costa, 1967). Pineal denervation has been shown to also induce supersensitivity in the postsynaptic beta adrenergic receptor site on the pineal cell to catecholamines. Elevation of adenosine cyclic 3', 5' monophosphate was also seen in response to denervation and this resulted in the superinduction of N-acetyl-transferase in the pineal gland (Deguchi and Axelrod, 1973). The responsiveness of the pineal beta adrenergic receptor has been found to change sensitivity, in response to diurnal

changes (Romero and Axelrod, 1974). An increase in the cyclic AMP response to norepinephrine has been found to occur in the hypothalamus, cerebrum and brainstem of rats treated seven days beforehand with 6-hydroxy-dopamine (Palmer, 1972). Prior to destruction of the dopaminergic innervation in the striatum by 6-hydroxydopamine or chronic inhibition of dopamine synthesis by alpha-methyl-para-tyrosine fails to alter dopamine stimulated cyclic AMP formation. These results indicate that dopamine sensitive adenylyl cyclase does not appear to increase during dopaminergic denervation supersensitivity (Von Voigtlander et al., 1973).

A model proposed for physical dependence and associated tolerance is what Emmelin (1961) called the supersensitivity of pharmacological denervation. According to the disuse theory of physical dependence, presence of the drug entity is only indirectly responsible for the development of dependence; the direct or proximal cause is depression of nervous activity for long periods of time (Sharpless, 1969). Withdrawal phenomena generally seem to represent rebound effects, opposite in character to those produced by the drug itself, as if depressed pathways become hyperexcitable during withdrawal and stimulated pathways become depressed (Sharpless, 1969). Sharpless and Halpern (1962) suggest that supersensitivity of receptors might underly the convulsions resulting from withdrawal of barbituates after the induction of physical dependence. It has also been suggested to account for physical dependence and tolerance towards

morphine (Jaffee, 1965; Collier, 1966). The effectiveness of apomorphine and amphetamine in enhancing morphine withdrawal aggression when given in otherwise ineffective doses has led Lal and co-workers to suggest receptor supersensitivity during narcotic dependence (Lal et al., 1971; Lal and Puri, 1972; Puri and Lal, 1973).

3) Evidence for Regeneration Axon Sprouting of Central Catecholamine Neurons

For many years a general conclusion of neurohistological investigators was that adult mammal cerebral nerve fibers show only feeble and abortive regenerative growth when severed (Cajal, 1928; Clark, 1943). Thus, many have reported sprouting from cut central axons, but this process soon terminated and the newly formed axons sprouts were described as degenerating within a few weeks. There is now data to suggest the brain may be capable of some plastic modifications in response to deafferenting lesions; there is now evidence for more persistent and functional regeneration.

It is well known that upon severing or traumatizing a monoamine nerve axon or an axon collateral, the transmitter accumulates within the axon itself (Dahlstrom and Fuxe, 1964; Dahlstrom, 1965). In fact, it is this phenomenon that has permitted the mapping out of catecholamine-containing fibers tracts in the central nervous system. The intraaxonal catecholamines accumulate very rapidly after the injury and remain approximately twelve days after which

they gradually disappear (Dahlstrom and Fuxe, 1965). During the seventh to nineteenth days after electrolytic lesions in the mesencephalon of the rat a type of densely packed, delicate, fluorescent, varicose fiber become visible in the vicinity of the axonal accumulations (Katzman et al., 1971). This increase is ascribed to regeneration or sprouting of catecholamine fibers at the border of the lesion. Although the catecholamine fluorescence in this fiber on the border of the lesion had decreased at seven weeks, the normal catecholamine concentration in the preterminal part of the axon is too low to be visualized with the histochemical method (Katzman et al., 1971). These newly formed fibers could either represent the growth of the central catecholamine neurons, whose axons have been cut by the lesion, or sprouting from the intact axons passing near the lesion. Studies employing lesions in the rat spinal cord show the development and growth of newly formed fiber sprouts from severed axons (Katzman et al., 1971; Bjorklund et al., 1971). Reinnervation of the distal part of the spinal cord by new noradrenergic fibers following 6-hydroxydopamine denervation has been shown (Nygren et al., 1971). Reinnervation was attributed to result from outgrowth of axotomized fibers, but growth in the form of collaterals sprouting from a few possibly surviving fibers in the distal region may be involved. A normal pattern of innervation was seen within one to two months after denervation (Nygren et al., 1971).

After a unilateral entorhinal lesion (a major extrin-

sic afferent to the hippocampal formation), a new fiber projection from the remaining contralateral entorhinal cortex grows to reinnervate the dentate gyrus. These new fibers establish electrophysiologically functional synaptic connections with the denervated dentate granule cells (Steward et al., 1974). These results indicate that reinnervation has functional significance and that the reinnervating fibers may be functionally homologous to those which were destroyed by the lesions. The manner of reinnervation by contralateral entorhinal fibers also suggest that the growing fibers are guided to the denervated site (Steward et al., 1974). Collateral reinnervation has been shown after partial deafferentation of the septal nuclei. It is a predictable phenomenon, which follows a rigid time course and results in a characteristic pattern of synapse formation (Raisman and Field, 1973). Abundant growth of nerve fibers into and across thin laminar lesions of the rabbit cortex have been reported by Rose et al. (1960). Another case of significant neuronal regeneration has been reported in the hypothalamo-hypophysial tract after pituitary stalk transection in the ferret (1968). Lesioned central catecholamine neurons in the rostral mesencephalon show a considerable capacity for growth into smooth muscle transplants and into and along the walls of cerebral blood vessels (Bjorklund and Stenevi, 1971).

E. Morphine and Brain Dopamine

The relationship between neurotransmitters in the brain and the pharmacological activity of narcotic analgesics has received considerable attention in the last several years. Serotonin, acetylcholine, norepinephrine and dopamine are among the neurotransmitters that have been implicated in the effect of morphine.

1) Acute Effects of Morphine

Morphine in a dose of 20 mg/kg has been shown to cause a thirty-two per cent decrease in mouse brain dopamine and twenty-three per cent decrease in norepinephrine when assayed fluorometrically. The time course of this effect on dopamine is seen to correspond to analgesia produced by morphine (Takagi et al., 1966). The acute administration of morphine has been shown by others to cause a similar decrease of brain dopamine as well as norepinephrine in mice (Reis et al., 1969; Rethy et al., 1971.). In rats, there was no difference in the brain dopamine levels one hour after an acute dose of morphine (Gunne et al., 1969; Wantanabe et al., 1969; Johnson and Clouet, 1973; Puri et al., 1973). However, a transient increase in dopamine levels at two and four hours after morphine has been reported (Johnson and Clouet, 1973).

The conversion of ^{14}C -tyrosine in vivo into ^{14}C -catecholamines has been shown to increase after the acute administration of either morphine or levorphenol (Smith et al., 1970, 1972). The increased incorporation of ^{14}C -tyrosine

into ^{14}C -catecholamine was seen in the whole brain of mice after 30 mg/kg or 100 mg/kg of morphine (Smith et al., 1970, 1972). Morphine was found to increase the synthesis of catecholamines in the cerebral cortex, diencephalon, striatum, brainstem and cerebellum. Naloxone, a narcotic antagonist, blocked the effects of morphine upon the incorporation of ^{14}C -tyrosine into ^{14}C -catecholamines. Tolerance has been shown to occur to this effect of morphine (Smith et al., 1972). Clouet and Ratner (1970) found that acute morphine administration increased the accumulation of ^{14}C -dopamine, when ^{14}C -tyrosine was injected cisternally. A maximum increase was reached in the striatum and hypothalamus one hour after morphine administration. Enhanced accumulation of ^3H -dopamine was seen after the i.v. administration of C-3,5- H -tyrosine in rats treated with morphine. Increased accumulation of ^3H -dopamine and $^3\text{H}\text{-H}_2\text{O}$ was seen in tissue and medium of striatal slices incubated with ^3H -tyrosine in response to morphine treatment (Gauchy et al., 1973). These results indicate that morphine stimulated dopamine synthesis. Increased release of newly synthesized ^3H -dopamine was also seen which was evidenced by a greater accumulation of ^3H -dopamine in incubating medium of slices of morphine pre-treated rats (Gauchy et al., 1973). Similar results of increase in the synthesis of dopamine and reversal by naloxone were also observed by Loh et al. (1973).

Another pharmacological approach to study catecholamine turnover is the use of a catecholamine synthesis in-

hibitor, alpha-methyl-para-tyrosine. Acute administration of morphine has been found to cause an accelerated depletion of brain dopamine after catecholamine synthesis inhibition. This effect is interpreted as an increased activity within the ascending dopamine neuron system (Gunne et al., 1969). Similarly, Puri et al. (1973) have shown a faster depletion of striatal dopamine by morphine in methyl-para-tyrosine treated rats, suggesting an increased dopamine turnover. Kuschinsky (1973) has also shown morphine to significantly increase the depleting effect of methyl-para-tyrosine.

In rats, the catalepsy induced by analgesic doses of morphine has been shown to parallel a dose-dependent increase in the concentrations of homovanillic acid, a dopamine metabolite, in the striatum (Kuschinsky et al., 1972, 1974 and Ahtee et al., 1973). In the whole mouse brain Fukui and Tukagi (1972) have found a significant rise in the dopamine metabolites, homovanillic acid and 3,4-dihydroxyphenyl acetic acid, with analgesic doses of morphine. This increase in homovanillic acid is explained by an increased dopamine turnover, thus a rise in dopamine utilization in the striata (Kuschinsky, 1973 and Kuschinsky et al., 1974). The catalepsy and the rise in striatal homovanillic acid concentrations produced by morphine were inhibited by naloxone (Kuschinsky et al., 1972) a morphine antagonist, whereas the corresponding effects of chlorpromazine were not influenced by the naloxone. Morphine had an additional effect on striatal homovanillic acid increase when combined

with a maximally effective dose of chlorpromazine (Kuschinsky et al., 1974). These results are interpreted to suggest that it is unlikely that morphine excited its effects on homovanillic acid in a manner like chlorpromazine (Kuschinsky et al. 1974). They conclude that the main site of morphine's action may be presynaptic, where the drug might interfere directly or indirectly with the metabolism of dopamine at the corresponding terminals.

On the basis of their studies with methadone, Sasame et al. (1972) have postulated that the cataleptic effect and the increase of dopamine utilization, induced by narcotic analgesics, are due to a postsynaptic dopamine receptor blocking effect. Puri et al. (1973) have also argued for a postsynaptic blocking effect of morphine. They have shown that morphine treatment blocked stereotyped behavior in rats induced by amphetamine or apomorphine which are due to stimulation of dopamine receptors. Further evidence that morphine acts as a dopaminergic receptor blocker comes from the study of asymmetries and turning produced in rats after unilateral removal of the caudate nucleus or lesioning of the nigro-striatal dopamine neurons. Morphine in a dose of 2 mg/kg caused asymmetry to the operated side, as did haloperidol (5 mg/kg) a known dopaminergic blocking agent (Fuxe and Ungerstedt, 1970). This data along with the blockade by morphine of amphetamine and apomorphine induced stereotyped behavior (Fuxe and Ungerstedt, 1970; Puri et al., 1973) support the view that morphine acutely acts as a dopamine

receptor blocker. Such a blockade can explain the increased turnover found in the ascending dopamine neurons after morphine (Gunne et al., 1969; Puri et al., 1973). The blockade of dopamine receptors could induce a compensatory nervous feedback onto the presynaptic dopamine cell bodies in the mesencephalon. This will result in an increased nervous impulse flow in dopamine neurons, which would result in increased dopamine turnover (Fuxe and Ungerstedt, 1970).

The intensity of the catecholamine fluorescence measured in nerve cells of substantia nigra, ventromedial tegmental area, and midbrain reticular formation by a modification of the histochemical method of Falck and Hillarp showed increased intensity in the male mouse in response to 40 mg/kg of morphine (Heinrich et al., 1971). In female rats only the cells of the substantia nigra showed a rise in fluorescence intensity after 20 mg/kg of morphine (Heinrich et al., 1971). Gunne et al. (1969) have shown that acute morphine plus methyl-para-tyrosine produced an increased depletion of fluorescence from the dopamine terminals in the nucleus caudatus putamen, nucleus accumbens and tubererculum olfactorium. No change in the degree of depletion in various noradrenergic terminals systems was seen after methyl-para-tyrosine (Gunne et al., 1969). This change in fluorescence appears to be due to an enhancement of amine synthesis.

Subanalgetic levels of morphine have been shown to produce mixed inhibitions of dopamine transport into slices.

from the mouse brain cortex and uncompetitive inhibition in the diencephalon. Increased concentrations of morphine produced a greater effect on dopamine affinity rather than reaction velocity which was observed in the cortex although inhibition remained mixed. In the diencephalon the type of inhibition changed from uncompetitive to mixed kinetics (Hitzemann et al., 1973). The uptake of ^{14}C -dopamine into rat striatum synaptosomes and of ^{14}C -norepinephrine into hypothalamic synaptosomes was inhibited by morphine in concentrations of 10^{-5} and 10^{-6}M , respectively. Kinetic analyses of dopamine uptake in the presence of morphine suggest that low affinity uptake was inhibited rather than high affinity uptake (Clouet et al., 1974).

The in vitro and in vivo activity of tyrosine hydroxylase after the administration of morphine has been studied by measuring the conversion of ^{14}C -tyrosine to DOPA and catecholamines (Fuxe et al., 1972; Lok et al., 1973).

Morphine did not modify the activity of tyrosine hydroxylase in brain homogenates. In vivo experiments show a significant increase in brain tyrosine hydroxylase activity after morphine. The authors suggest that an acceleration of dopamine biosynthesis may be due to the activation of feedback mechanism in vivo. Furthermore, a significant increase in the specific activity of brain tyrosine was observed after morphine, by other investigators and they also found this effect was blocked by the morphine antagonist, naloxone (Loh et al., 1973).

2) Effects of Chronic Morphine

Gunne (1963) noted no change in dopamine content in the telencephalon of dogs treated daily for seventy to ninety days with increasing doses of morphine up to 120 mg/kg. Dopamine was also found unchanged in various brain regions of the morphine dependent monkey (Segal et al., 1962; Segal et al., 1972). During chronic administration of morphine its effect on brain dopamine disappeared and the impulse flow became normal within ascending dopamine neurons system (Gunne et al., 1969). In rats, there was a slight increase in the brain levels of dopamine after chronic administration of morphine (Sloan et al., 1963; Johnson and Clouet, 1973). The increase in brain dopamine concentrations was suggested to be attributed to the increase in the synthesis of dopamine after chronic administration of morphine (Clouet and Ratner, 1970; Johnson and Clouet, 1973). The increase in dopamine synthesis was suggested to be associated with the increase in tyrosine hydroxylase activity after chronic morphine (Reis et al., 1970). Contrary to these findings Smith et al. (1972) have shown that after repeated administration of either morphine or levorphenol tolerance and cross tolerance developed to the effect of these drugs upon the synthesis of ^{14}C -dopamine and ^{14}C -norepinephrine. It is interesting to note that the excretion of dopamine was reported to be increased in rats (Sloan and Eisenmen, 1968) and male human volunteers (Weil-Malberbe et al., 1965) during the addiction phase.

3) Effect of Morphine Withdrawal

Gunne (1963) reported that brain dopamine was decreased seventy-two hours after morphine withdrawal. At this period dogs exhibited moderate to severe abstinence. Maynert and Klingman (1962) observed similar findings during withdrawal in dogs and rabbits but observed no change in brain dopamine on rats during withdrawal from morphine. Abrupt withdrawal has been shown to reduce activity in the brain dopamine neurons. Histochemical results showed that methyl-para-tyrosine did not cause any effect within the dopamine or noradrenergic neurons that could be distinguished from the effect of synthesis inhibition alone. Nalorphine-induced abstinence caused increased activity within the noradrenaline neuron system in practically all parts of the brain (Gunne et al., 1969). Contrary to this study a transient increase in dopamine levels was reported in rats and mice after naloxone induced withdrawal (Iwamoto et al., 1973), which is suggested to be responsible for the stereotyped jumping in mice and rats during withdrawal. In rats, brain dopamine and norepinephrine levels decreased during withdrawal from morphine when compared with chronic morphine levels (Sloan et al., 1973). The striatal dopamine turnover in morphine dependent animals was shown not to differ from the turnover in non-dependent animals when measured one, twenty-four and seventy-two hours after the last morphine injection (Puri, 1973). Also, dopamine levels were

replenished at a more rapid rate during morphine withdrawal after the administration of reserpine (Gunne et al., 1970).

The urinary excretion of dopamine in rats was increased during withdrawal with peaks occurring on the third and eighth day (Sloan and Eiseman, 1968). In humans, however, the urinary excretion of dopamine was found to be decreased during the withdrawal phase (Weil-Malherbe et al., 1965).

Upon withdrawal from morphine, aggregation of the dependent rats elicited intense aggression. Pretreatment of the withdrawn rats with L-dopa, amphetamine or apomorphine before aggregation enhanced the aggressive responses several-fold. Haloperidol, a dopaminergic blocking agent, blocked the aggressive behavior (Lal et al., 1971; Puri et al., 1971; Lal and Puri, 1972; Puri and Lal, 1973; Gianutsos et al., 1974). These data are interpreted to suggest a dopaminergic basis of morphine withdrawal aggression and the development of a latent supersensitivity of dopaminergic neuropathways during morphine dependence.

F. Apomorphine and Brain Dopamine

In both the central nervous system and in the periphery, apomorphine is believed to stimulate the dopaminergic receptors (Anden et al., 1967; Ernst, 1967). Biochemical studies have shown that apomorphine decreases the turnover rate of striatal dopamine. The incorporation of C-14 tyrosine into dopamine was found to be decreased after the administration of apomorphine (Persson, 1970; Nyback et al., 1970). Apomorphine also decreases the disappearance of dopamine after alpha-methyl-para-tyrosine (Anden et al., 1967; Anden and Bedard, 1971; Puri et al., 1973). Similarly, there was a decrease in the rate of formation of homovanillic acid (Roos, 1965, 1969; Lahti et al., 1972). The administration of apomorphine can also result in the decreased accumulation of dopa after the administration of NSD 1025 (Koe, 1973). The biochemical changes of brain dopamine produced by apomorphine were completely blocked by haloperidol (Anden et al., 1967; Persson, 1970; Anden and Bedard, 1971; Lahti et al., 1972; Puri et al., 1973). Apomorphine has also been shown to inhibit tyrosine hydroxylase activity directly, but only in higher doses than those necessary to induce the functional changes (Goldstein et al., 1970).

Single unit recording from dopaminergic areas has made it possible to test further and investigate the effect of apomorphine. Apomorphine given intravenously in a dose of 0.1 mg/kg has been shown to inhibit dopamine unit activity (Bunney et al., 1973). This effect is not influenced by

alpha-methyl-para-tyrosine but is blocked by haloperidol. It is therefore possible to explain a decrease in synthesis and turnover of dopamine by the concept of a compensatory neuronal feedback mechanism (Anden et al., 1969; Carlsson and Linquist, 1973). The interruption of impulse flow in dopamine neurons cause a marked increase in striatal dopamine (Walters et al., 1973) an effect that can be blocked by apomorphine (Anden et al., 1973). This finding suggests that dopamine neurons are at least partly autoregulatory. Released or extraneuronal dopamine seems to act on presynaptic inhibitory dopamine receptors. Stimulation of the inhibitory dopamine receptors on dopamine neurons, either by dopamine or apomorphine, may inhibit both nerve activity and dopamine synthesis (Christiansen and Squires, 1974).

The systemic administration of apomorphine produces stereotyped behavior in rats which is characterized by continuous and compulsive sniffing, licking and gnawing (Ernst, 1965). The results of several studies have supported the hypothesis that stereotyped behavior induced by apomorphine is due to increased dopamine receptor activity (Ernst, 1969; Fuxe and Ungerstedt, 1969). Apomorphine has been shown to produce hypothermia in mice. This hypothermic effect is antagonized by haloperidol and pimozide, indicating that dopaminergic mechanisms are involved in temperature control (Fuxe and Sjoqrist, 1972). In a number of rats apomorphine consistently facilitated self-stimulation but inhibited this behavior in others (Broekkamp and van Rossum, 1974).

These results indicate that apomorphine is able to replace the reinforcing action of intracranial rewarding stimulation.

G. Neuroanatomical Pathways Related to Morphine Dependence and Abstinence

Physical dependence on morphine is characterized by the appearance of abstinence signs when morphine intake is abruptly terminated or when an opioid antagonist is administered. The specific action of morphine and its surrogates on the central nervous system suggest the presence of neuroanatomical pathways which are selectively sensitive to opioid compounds.

The neuroanatomical sites of morphine action have been the subject of many investigations. Lotti et al. (1965) demonstrated that the hypothermic effect of morphine could be induced when morphine was applied locally to the anterior hypothalamus. Injection of 50 ug of morphine sulfate into the region of the preoptic anterior hypothalamic nuclei led to a fall in core temperature while injections into other regions of the hypothalamus were ineffective. Introduction of the drug into the area of the mammillary nuclei caused hyperactivity and hyperthermia. It was suggested that the hypothermic effect of morphine is due to a depression of the set point of the hypothalamic thermostat, possibly by rendering the cells insensitive to the stimulus of the input from the cold receptors in the skin. Tsou and Jang (1964) also using the technique of intracerebral microinjections, localized the site of the analgesic action of morphine in the rabbit to the periventricular gray of the third ventricle. Analgesia has also been reported

by Herz et al. (1970) after application onto the surface of the fourth ventricle and Buxbaum et al. (1970) after application into the anterior nuclei of the rat thalamus. Naloxone has been found to reverse morphine analgesia in the medial thalamic nuclei and in medial portions of the mid-brain (Collins et al., 1974). These results tend to indicate that medial mesodiencephalic areas of the brain are involved in morphine analgesia. The caudate nuclei has also been implicated as important in mediating the altered reaction to pain induced by morphine (Glick, 1974). Bilateral lesions of the caudate nuclei were shown to produce a persistent potentiation of the effect of morphine on escape latencies (Glick, 1974). These studies indicate that although the opioid molecule may act on a common biochemical element in different tissues, the amount and the coupling of the biochemical element of specific tissue functions determine the specificity of opioid actions.

The central sites related to physical dependence have been studied by localized manipulations of brain tissue. Wikler (1948, 1952) reported that removal of the cortex in dogs did not attenuate the abstinence syndrome but that spinal cord sections interfered with some withdrawal signs. This work has been confirmed by the demonstration that morphine dependence has a supraspinal and spinal component (Martin and Eades, 1964). It has been reported that bilateral rostral cingulotomy markedly attenuates abstinence phenomena after withdrawal of morphine in monkeys and a variety

of opioid analgesis in patients with intractable pain. Foltz et al., 1957; Wilker (1972) has reported that the salutary effects of cingulotomy on the morphine withdrawal syndrome consist largely of attenuation of non-specific (emotional) reaction to the unmodified specific morphine withdrawal syndrome. Bilateral lesions in the cingulum, the dorsomedial thalamic nucleus, the anterior temporal lobe, or the septum do not alter the specific signs of the primary morphine withdrawal syndrome in the rat (Wilker, 1972). Nor do cingulotomy or lesion in the dorsomedial thalamic nucleus, the anterior temporal lobe or the septum prevent relapse (Wilker, 1972). Stereotaxic lesions in the ventromedial nucleus suppressed or diminished the autonomic signs of withdrawal and produced a marked intolerance to morphine (Kerr and Pozuelo, 1971). Subsequent studies carried out in monkeys showed that lesions of the hypothalamus, amygdala and septal nuclei, even if the tolerance for morphine and severity of the withdrawal had been modified the lesion had little or no effect on the craving for morphine, as the animals continued self-injected approximately the same amount of morphine sulfate as they did before receiving their lesion (Kerr and Pozuelo, 1971). Lesions placed stereotaxically in monkeys in the organs of the nigro-striatal system and the nucleus tegementi ventralis, known to be mainly dopaminergic pathways, abolish craving for morphine and the phenomena of withdrawal, as evidenced, respectively, by lack of bar pressing and by absence of the

manifestations of withdrawal (Pozuelo and Kerr, 1972). Further evidence for the involvement of dopamine pathways in morphine abstinence has been shown by Gianutsos et al. (1973, 1974b). They have found that electrolytic lesioning abolished the morphine withdrawal aggression in thirty day abstinent rats while lesioning of the medial forebrain bundle was ineffective in blocking the aggression (Gianutsos et al., 1973, 1974b).

A central component to morphine dependence has been suggested by the studies by Eidelberg and Barstow (1971) in the monkey and by Wantanabe (1971) in the rat showing that dependence on morphine can be induced by chronic intracranial applications of chronic intracranial applications of morphine. These investigations also demonstrated that withdrawal could be precipitated by administration of opioid antagonist into the ventricular fluids. Herz et al. (1972) reported that withdrawal signs were elicited in the morphine dependent rabbits after administration of nalorphine into the fourth ventricle. Wei et al. (1972, 1973) have found that medial thalamus and areas in the diencephalic-mesencephalic junction are more sensitive than neocortical, hippocampal, striatal, hypothalamic and mesencephalic structures to naloxone precipitated withdrawal. Their investigation indicates that the medial thalamus and rostral mesencephalic structures are involved in precipitated abstinence behaviors - neocortical, hippocampal, hypothalamic, striatal and tegmental areas of the brain are relatively insensitive

to naloxone precipitated withdrawal. The regional application of naloxone to the brain to precipitate abstinence signs indicates that the site of adaptation to morphine has neuro-anatomical specificity. Wei concluded from these results that medial thalamic nuclei and closely adjacent structures may be the primary sites for the development of opioid dependence. The medial thalamus is also believed to play a role in tolerance. Morphine sulfate administration results in drastic alterations in brain bioelectrical activity. After repeated drug administrations EEG effects disappear (Teitelbaum et al., 1974). An intense morphine response was seen after the administration of a single dose of morphine to tolerant rats with lesions of the medial thalamus. Despite extensive damage to the medial and lateral habenular nuclei, the fasciculus retroflexus, and dentate gyri, naloxone still reversed the effect of morphine (Teitelbaum et al., 1974). It appears that medial thalamic lesions have little effect on precipitated withdrawal while they have a drastic effect on sensitivity to morphine in tolerant rats.

Recently the occurrence of opioid receptor binding has been reported - its localization in the nervous tissue (Pert and Snyder, 1973). Their studies of the tissue distribution provide evidence for the locus of the pharmacological actions of opioids. The greatest amount of binding occurred in the brain. Within the brain the opioid receptor binding revealed the greatest amount of binding in the

corpus striatum where binding exceeded that of the cerebral cortex more than fourfold. Of the known neurotransmitters only dopamine and acetylcholine are found in high concentration in the corpus striatum. Other areas to show binding were the midbrain cortex, brainstem, and the cerebellum, in that order.

Several studies have taken the approach of lesioning discrete brain areas and then administering chronic morphine; upon the termination of morphine withdrawal symptoms are recorded. It has been found that rats with bilateral lesions in the anterior cingulate cortex show less opiate directed behavior following passive morphine injections (Trafton and Marques 1971, 1974). Less withdrawal induced weight loss is seen in rats with posterior medial forebrain bundle lesions. These lesioned rats consume morphine solution much more readily than controls (Glicks and Charap, 1973). Glick suggests from this data that the addictive and dependence properties may have separate mechanisms.

A characteristic behavior of rats undergoing withdrawal from morphine is the appearance of repetitive shaking movements of the body. Several investigations have been undertaken to study the neuroanatomical pathways related to morphine abstinence, in particular the effects of brain lesions on the wet shake behavior of morphine abstinence. Wei et al. has reported that naloxone precipitated shakes are generated in neural elements rostral to the inferior colliculus and caudal to the fasciculus retroflexus (Wei et

al., 1973). The areas in the rat brain where knife cuts attenuate the wet shakes response to ice water and where naloxone precipitated the shaking behavior, bears considerable resemblance to the primary motor area for shivering in the cat described by Hemingway as "... in the dorsomedial caudal hypothalamus near the third ventricle just ventral to the caudal border of the massa intermedia of the thalamus...within the reticular substance dorsal to the mammillary bodies, dorsomedial to the cerebral peduncles, substantia nigra and subthalamus...". This evidence of Wei's would indicate that the acute and chronic effects of morphine on wet shakes are mediated in the mesodiencephalon of the brain.

H. The Influence of Chemical Denervation on Morphine Physical Dependence and Tolerance

1) 5,6-Dihydroxytryptamine

Recently it was found that a strongly reducing congener of serotonin, synthesized by Schlossburger and Kuck (1960), 5,6-dihydroxytryptamine (5,6-DHT) induces a selective chemical destruction of brain 5-HT nerve terminal (Baumgarten et al., 1971) thus reducing brain serotonin contents. The antinociceptive effect of morphine is not significantly changed by 5,6-DHT pretreatment (Blasing, 1973). The intracerebral administration of 5,6-DHT in the mouse inhibited the development of tolerance to and physical dependence on morphine induced by morphine pellet implantation (Ho et al., 1972).

2) 6-Hydroxydopamine

Intraventricular administration of 6-hydroxydopamine (6-OHDA) markedly reduces brain catecholamines (Bloom et al., 1969). Morphine administration one week following intraventricular administration of 6-OHDA has been found to increase morphine induced analgesia when measured by the tail-flick response. The bilateral administration of 6-OHDA into the medial hypothalamic areas at the level of the ventricular or the dorsomedial hypothalamic nuclei also markedly augmented morphine's effect on the tail-flick latency. In addition to these neuroanatomical structures, when 6-OHDA was injected into the medial forebrain bundle morphine effect on the tail-flick latency was enhanced.

Bilateral local administration of 6-OHDA into nucleus caudate-putamen reduced morphine analgesia (Nakamura et al., 1973). This data would seem to indicate that 6-OHDA induced depletion of norepinephrine in the hypothalamus potentiates morphine analgesia whereas depletion of dopamine in the caudate nucleus decreases morphine analgesia.

The involvement of brain dopamine in the morphine antinociceptive effect has also been shown in other studies. Rats treated intracisternally at two weeks of age with 6-OHDA which depleted both brain norepinephrine and dopamine showed antagonism of morphine antinociception six weeks later when measured by the hot-plate and tail-flick tests. Preferential depletion of brain dopamine by desmethyldipramine and 6-OHDA in these rats produced greater antagonism of morphine antinociception in the tail-flick test and complete antagonism in the hot-plate test (Elchisak et al., 1973). A reduced analgesic response to morphine has been found in both tolerant and nontolerant mice after the intracerebral administration of 6-OHDA without modifying the brain uptake of morphine (Friedler et al., 1972). A decrease in sensitivity to morphine has also been reported for tolerant and nontolerant rats following pretreatment by 6-OHDA (Bhargava et al., 1973).

The morphine dependent state has also been found to be altered by 6-OHDA. Precipitated abstinence, measured by naloxone induced withdrawal jumping in mice was enhanced by 6-OHDA pretreatment; weight loss after abrupt withdrawal was

also increased by 6-OHDA (Friedler et al., 1972). Intraventricular injection of 6-OHDA has been found to exacerbate morphine withdrawal in the rat (Bhargava et al., 1973).

The intraventricular administration of 6-OHDA caused a marked depletion of brain-norepinephrine in saline treated rats and in rats treated either chronically or with a single dose of morphine. The increase in brain dopamine seen twenty-four hours after the administration of 6-OHDA to control rats was not observed when 6-OHDA was administered to rats previously treated with morphine - a decrease in brain dopamine was observed in these rats. One week after treatment with 6-OHDA both brain noradrenaline and morphine concentrations markedly decreased. While chronic morphine treatment with morphine caused no significant effect on the depletion of brain norepinephrine after 6-OHDA, chronic treatment with morphine did inhibit the depletion of brain dopamine (Nakamura et al., 1972). These results are interpreted to suggest that chronic treatment with morphine may induce changes in the uptake process of the nigrostriatal system.

III. GENERAL METHODS AND MATERIALS

A. Animals

Male hooded rats of the Long-Evans strain, random-bred, weighing 250-400 grams were obtained from Charles River Breeding Farms, Wilmington, Massachusetts. Prior to their use in this investigation the rats were housed in individual cages and allowed free access to food and water at all times.

B. Drugs

Haloperidol (Haldol) was obtained through the courtesy of McNeil Laboratories, Inc., Fort Washington, Pennsylvania and Janssen Pharmaceutica, Beerse, Belgium. Morphine sulfate and apomorphine hydrochloride were obtained from the Mallinckrodt Chemical Works, St. Louis, Missouri. The dl-alpha-methyl-para-tyrosine was obtained from Regis Chemical Company, Chicago, Illinois.

Haloperidol was dissolved in 0.3% tartaric acid. Morphine sulfate and apomorphine hydrochloride were dissolved in distilled water. Alpha-methyl-para-tyrosine was suspended in 0.5% carboxymethylcellulose.

C. Brain Lesions

Under ether anesthesia, bilateral electrolytic lesions were made with a monopolar stainless steel electrode with a 0.5 mm non-insulated tip. A current of either 2mA for 30 seconds duration or 1mA for 15 seconds duration generated

by a Lesion Producing Device (Stoelting Co. Model 58040, Chicago, Ill.) was passed through the electrode placed into the brain with a David Kopf Stereotaxic instrument. With the dorsal cranium horizontal, the coordinates for the nigro-striatal bundle were 1.5 mm posterior to bregma, 2.0 mm lateral to the midline suture and 8.5 mm below the surface of the cranium. Lesions of the medial forebrain bundle were placed 2.0 mm posterior to bregma, 1.5 mm lateral to the midline suture and 8.5 mm below the surface of the cranium in a horizontal position.

D. Drug Administration

Rats were made dependent upon morphine by injecting them intraperitoneally with systematically increasing doses of morphine sulfate three times a day. The starting dose of 15 mg/kg/injection was increased by 15 mg/kg every day until a dose of 405 mg/kg/day was reached. In those rats who received a terminal dose of 200 mg/kg only two injections a day were given 12 hours apart. The starting dose of 10 mg/kg/injection was increased by 10 mg/kg every day until a dose of 200 mg/kg was reached. They were maintained at this dose for at least five days before using them in withdrawal studies.

In the investigation of recovery from nigro-striatal lesions, haloperidol, morphine sulfate and alpha-methyl-para-tyrosine were administered intraperitoneally twice a day for a three or six day period with the last injection being given 24 hours prior to surgery.

E. Measurement of Withdrawal Symptoms

Abstinence symptoms were measured after the abrupt termination of chronic morphine administration and the lesioning of various brain regions. Symptoms were measured once every 24 hours for the first 72 hours after the withdrawal of morphine and/or brain lesioning. In addition to these initial observations the occurrence of withdrawal symptoms in brain lesioned subjects was measured three weeks following the production of the lesion. The rats were removed from their home cages and placed individually into a novel cage. Symptoms were measured for 30 minutes. Prior to these observations the rats were weighed and their rectal temperature taken (Martin et al., 1963). The abnormal behaviors noted during the observation period consisted of wet shakes (Martin et al., 1963), writhing (Buckett, 1964), ptosis and piloerection (Wikler, 1960). Aggression (Lal et al., 1971) was measured seventy-two hours after the last morphine injection. Violent shaking movements of the head and/or body of the rat which resemble the action of an animal which has been drenched with water are defined as wet shakes and the rate of occurrence for the observation period is recorded. Writhing consists of dragging of the abdomen along the floor of the cage and drawing in of the abdominal wall (Buckett, 1964) or arching of the back, neither of which is accompanied by yawning. The number of wriths occurring during the 30 minute observation period was noted. The presence or absence of piloerection, the condition in which the animal's fur stands

on end, more than a 45° angle, is noted during the period of observation. Ptosis is defined as the condition where the rat's eyelids are drooping, the eye thus appears as slit-like. The length of time during the observation period that the rat was exhibiting ptosis is measured. The reduction of body temperature during morphine abstinence, hypothermia, was measured using a digital thermister thermometer (Digitec Model 8500-3 United Systems, Corporation). The rectal probe was inserted 5cm and maintained in place for one minute before recording the temperature. The extent of hypothermia was calculated from the body temperature one hour after the last morphine injection or the period just prior to production of the lesion. The change in body weight during abstinence and/or after brain lesions is calculated from the body weight after the last injection of morphine and/or the production of the brain lesion. To measure aggressive responses the rats were aggregated in groups of four 72 hours after the last morphine sulfate injection. The aggression was measured as duration of rearing (in seconds), number of vocalizations and the number of attacks and bites. Attacks, including bites and rearing, were recorded by the experimenter. Vocalizations were recorded automatically through an Audio Threshold relay.

F. Histology

Representative animals from the various drug-treated and saline control groups were used to evaluate the site of lesioning. Those subjects chosen for evaluation were per-

fused with Dietrick's Fixative at the conclusion of the experiment. Dietrick's Fixative was prepared by combining 1800 ml of water, 90 ml of 95% ethyl alcohol; 300 ml of 40% formaldehyde and 60 ml of glacial acetic acid. The brain was then removed from the cranium and allowed to harden in Dietrick's Fixative. After the brain tissue had hardened for at least 24 hours, it was then washed in water for several hours. Increasing concentrations of ethanol were used to dehydrate the tissue prior to cleansing with chloroform. When each brain was embedded in paraffin, 10 micron sections were taken from the area containing the lesion site. The sections were then mounted on slides and stained with Harris' hematoxylin and eosin according to the method of Luna (1968).

1) Physical Characteristics of the Brain Lesion

The typical localization of the nigro-striatal lesion employed in these experiments based on examination of histological material from a representative animal is shown in Figure 1. Examination of these sections showed the lesion to be localized in the area of the lateral hypothalamus, with destruction of the nigro-striatal pathway and some portions of the medial forebrain bundle.

When a current of 1mA for 15 seconds was employed microscopic examination showed the lesion to have destroyed an area of 0.30 ± 0.03 mm in diameter; this is a mean of four representative subjects. The area destroyed by this lesion is shown in Figure 2. Increasing the current and

duration to 2mA for 30 seconds produced a greater destruction of brain tissues. The mean lesion diameter was increased to 0.47 ± 0.01 mm when measured in four animals. The area destroyed by this lesion is shown in Figure 3.

In those experiments where the lesion was directed at the medial forebrain bundle microscopic examination showed the damage to be localized to this area. In some cases some slight damage to the immediately adjacent nigro-striatal fibers cannot be ruled out.

2) Neurochemical Effects of the Lesion

In order to further confirm the site of the lesion to the nigro-striatal dopamine neuron system the dopamine concentration in the corpus striatum was determined spectrofluorometrically following either a 1mA 15 second or 2mA 30 second brain lesion.

To evaluate dopamine levels after nigro-striatal lesioning the animals were starved for 18-24 hours prior to being sacrificed by decapitation. The brains were rapidly removed from the cranium and the two cerebral hemispheres separated. The lateral ventricle was opened to expose the corpus striatum which was then removed. All of these manipulations were carried out on ice and with ice-cold surgical equipment. Striata obtained from lesioned and non-lesioned subjects were separately weighed and then homogenized in 3 ml of 0.4 N perchloric acid containing 0.05% sodium metabisulfite. The homogenate was then transferred into a

centrifuge tube. The homogenizer was rinsed with 1 ml perchloric acid solution which was added to the homogenate. The homogenate samples were then centrifuged at 10,000 RPM for 10 minutes in a Servall RC 2B refrigerated centrifuge.

The supernatant from each centrifuge tube was transferred into a large centrifuge tube to which 10 ml of 0.5 M tris buffer (pH 9.5) was added. The resultant pH of the sample was 8.5 ± 0.1 . The samples were then poured onto alumina columns and allowed to pass through at a rate of 30 drops per minute. After the solution had run through the alumina column 5 ml of deionized glass distilled water was added to the large centrifuge tube which was then passed through the column. The eluate collected was discarded. The dopamine from the alumina column was then extracted by 4 ml of 0.2 N acetic acid. The acetic acid eluate was collected in a graduated centrifuging tube containing 1 ml of EDTA-reagent. A two and a half ml sample from each test tube was used for the dopamine determination. The two and a half ml sample of 0.2 N acetic acid eluate was transferred into another test tube and to it 0.2 ml of iodine solution was added. Each test tube was shaken in a Vortex Gemie test tube shaken for 15 seconds and after exactly 3 minutes, 0.2 ml of alkaline sulfite was added. The tissue blanks were obtained by the residual 0.2 N acetic acid eluate. The tissue blanks were prepared by reversing the addition of iodine and alkaline sulfite solution. Exactly 3 minutes after the addition of alkaline sulfite solution 0.2 ml of 5 N acetic acid solution

was added to each test tube. Again each test tube was shaken for 15 seconds, placed into a bath of boiling water for 5 minutes and then cooled to room temperature. The concentration of dopamine was estimated by reading the samples at 370 NM while activated at 320 NM on the Amico-Bowman spectrofluorometer. The reagent blank consisted of all the reagents but dopamine. The concentration of dopamine in the experimental samples was determined by comparing the fluorescence with the known concentration of a standard dopamine solution. The calculated concentration of dopamine was then expressed as micrograms of dopamine per gram of wet tissue.

Twenty-four hours following the 1mA 15 second lesion there was a non-significant increase in the corpus striatal dopamine levels. The average dopamine concentration of the corpus striatum in non-lesioned rats was 8.63 ± 0.49 ug/g. At both four and seven days following this lesion, corpus striatal dopamine levels were significantly decreased, Student's 't' test ($p < 0.05$). The decrease in dopamine levels was greatest on the seventh post-lesion day. These results are summarized in Figure 4.

Striatal dopamine levels were seen to significantly increase, Student's 't' test ($p < 0.05$), when more extensive nigro-striatal lesions, 2mA for 30 seconds, were made twenty-four hours prior to measurement. The corpus striatal dopamine concentration was found to be 6.31 ± 0.40 ug/g in non-lesioned controls. Four days after lesioning there was a dramatic and significant Student's 't' test ($p < 0.05$), de-

crease in the concentration of striatal dopamine. The levels of striatal dopamine seven days following this large lesion were not measured due to the death of all experimental animals. These changes in dopamine levels are shown in Figure 4. The results achieved here correspond well with those of other investigators (Faull et al., 1969; Anden, et al., 1972; Agid et al., 1974).

G. Statistical Analysis

An analysis of variance for repeated measures was used to test for significant differences between treated and control conditions in the nigro-striatal lesion induced weight loss experiments.

The two-tailed Student's 't' test for independent means was used to test for the differences between the means of lesioned and non-lesioned groups in the morphine withdrawal experiments. The level of significance was chosen to be $p < 0.05$ for rejection of the null hypothesis.

The results obtained from the effect of drugs pre-treatment on survival after extensive nigro-striatal lesions was analyzed by the Chi-Square test.

FIGURE 1. Typical Cross Section of Rat Brain Showing
Nigro-Striatal Destruction.

FIGURE 2. Representative Small Nigro-Striatal Lesion
Superimposed on a Figure From the König
and Klippel Atlas (1963).

FIGURE 3. Representative Large Nigro-Striatal Lesions
Superimposed on a Figure From the König and
Klippel Atlas (1963).

FIGURE 4. Striatal Dopamine Levels at Various Time Intervals Following Bilateral Lesions of the Nigro-Striatal Pathway Expressed as Percent of Non-Lesioned Controls. Dopamine Levels Were Measured at 1 (N=9), 4 (N=11) and 7 (N=8) Days Following 15 Sec 1mA Current Lesions and at 1 (N=8) and 4 (N=8) Days After 30 sec 2mA Current Lesions. Control Dopamine Levels are Given in the Text.

IV. EFFECT OF HALOPERIDOL, METHYL-PARA-TYROSINE AND MORPHINE ON WEIGHT LOSS AND LETHALITY DUE TO NIGRO-STRIATAL LESIONS

A. Introduction

Bilateral lesions of the lateral hypothalamus produce a complete cessation of eating and drinking (aphagia and adipisia) eventually leading to death in spite of ample food and water availability (Anand and Brobeck, 1951). Although extensive information has been accumulated on the changes in food and water regulation following LH lesions, knowledge about anatomical structures is still incomplete. Several newer experiments have questioned the role of the LH as a "feeding center," and several studies have suggested that the critical areas for producing aphagia and adipsia may be outside or include only a far lateral segment of the lateral hypothalamus (Morgane, 1961; Gold, 1961; Grossman and Grossman, 1971; Wampler, 1971). These critical areas include portions of the globus pallidus, the medial portion of the internal capsule, and a small portion of the lateral hypothalamus area adjacent to the internal capsule. Several fiber systems pass through these areas and are known to regulate the content of norepinephrine, dopamine and serotonin in the telencephalon.

One such fiber system, the nigro-striatal bundle (NSB),

regulates the dopamine content of the neostriatum (the caudate and putamen). Nigrofugal fibers pass through areas described as critical for producing the LH syndrome. Rats sustaining a complete electrolytic (Oltzman and Harvey, 1972) lesion of the NSB or chemical lesion (Ungerstedt, 1970, 1971) of the NSB show a severe aphagia and adipsia resulting in death, unless the animals are force-fed.

When animals that have received lateral hypothalamus lesions are force-fed they gradually recover from their feeding deficits (Teitelbaum and Epstein, 1962). The basis of recovery after lateral hypothalamic lesioning is not known despite much investigation. A denervation supersensitivity model may be useful in explaining recovery (Sharpless, 1964). If a supersensitivity phenomenon were the factor responsible for the recovery of food regulating behavior, then certain results could be predicted; namely, neurons which were made supersensitive at some time prior to destruction of the NSB could then be expected to facilitate recovery.

Administration of alpha-methyl-para-tyrosine (MPT), a known blocker of catecholamine synthesis (Nagatsu et al., 1964) will produce a partial denervation of catecholamine pathways (Tarsy and Baldessarini, 1973). Recent research has shown that pretreatment with MPT before lesioning of the lateral hypothalamus facilitates recovery from the syndrome (Glick et al., 1972). However, MPT blocks the synthesis of all the catecholamines, and further, the lesion used by Glick et al. (1972) was located in the lateral hypothalamus;

thus, their results do not distinguish between noradrenergic and dopaminergic effects either pharmacologically or neuro-anatomically. The issue is especially significant since the suggestion has been made that recovery of nutritive regulation after lateral hypothalamus lesioning is dependent upon recovery within a noradrenergic reward system (Berger et al., 1971, 1973).

This experiment was undertaken to establish the role of dopaminergic neurons in the regulation of body weight. Both partial (1mA 15 sec) and extensive (2mA 30 sec) nigro-striatal lesions will be produced and post lesion recovery studied. Rats were injected with either saline, haloperidol or alpha-methyl-para-tyrosine for three days prior to the production of partial (1mA 15 sec) nigro-striatal lesions. Alpha-methyl-para-tyrosine pretreatment were employed since previous reports indicated that it facilitated recovery from lateral hypothalamic lesions. Haloperidol served to distinguish between dopaminergic and adrenergic mechanisms in recovery. Following lesioning animals were observed daily for changes in body weight. Drug treated groups were compared with saline controls to determine if drug pre-treatment effected weight changes following nigro-striatal lesioning. Prior to extensive (2mA 30 sec) lesioning of the nigro-striatal tract either saline, haloperidol or morphine were administered for a six day period. Since previous evidence suggested that acute morphine blocks dopamine receptors it was thus incorporated to test the role of

dopaminergic supersensitivity in post lesion recovery. These drug treated animals were compared to saline treated controls with respect to lesion induced weight changes and lethality. It was hypothesized that lesioning of the nigro-striatal tract would result in aphagia and adipsia as indicated by a loss of body weight. Drugs which reduce dopaminergic receptor activity are hypothesized to facilitate recovery from lesion induced weight loss, thus establishing a role for brain dopaminergic systems in the regulation of body weight.

B. Method

Haloperidol (0.4 mg/kg), methyl-para-tyrosine (50 mg/kg) or saline were administered intraperitoneally (i.p.) twice a day (8 a.m. and 8 p.m.) for three consecutive days with the last injection given 24 hours prior to the production of a partial (1mA 15 sec) bilateral nigro-striatal lesion. A six-day pretreatment period of morphine (30 mg/kg), haloperidol (2 mg/kg) or saline, injected twice daily was given prior to the production of an extensive (2mA 30 sec) bilateral nigro-striatal lesion. The last injection was given 24 hours before surgery.

Throughout the experiment the rats were housed individually and given free access to dry food and water. Force feeding was not attempted. Body weights were taken daily during both pre- and post- lesion periods. The occurrence of a death in either the experimental or control groups was recorded. A representative group of surviving rats were sacrificed 16 days after the production of the lesion and

the lesion site determined by histological procedures as described in Chapter III.

C. Results

Those animals receiving partial (1mA 15 sec) nigro-striatal lesions continued to lose body weight until the fifth post-operative day as can be seen from Table 1. Analysis of variance for repeated measures (Winer, 1962) showed that those groups pretreated with alpha-methyl-para-tyrosine or haloperidol lost significantly ($p < 0.05$) less body weight than did saline controls. The extent of total weight loss was diminished in these drug treated animals as evidenced by their higher percentage body weights as compared to saline treated controls. These results are shown in Figure 4. The saline treated controls showed a greater loss in gram of body weight indicating that the drug treated group began to recover earlier. These results are summarized in Figure 5.

When a current of 2mA for a 30 second duration was employed to make a more extensive lesion of the nigro-striatal tract the resulting aphagia and adipsia produced death in all saline treated controls. These results are shown in Table 2. All of the rats in the control group died within two weeks of the lesion, nearly half of them in less than eight days. Percentage body weight changes were similar for both treated and control groups for the first six post-lesion days as can be seen in Figure 6. From the 9th to the 14th post-operative day, the saline treated rats showed a greater loss in grams of body weight than did the groups

Table 1. Weight Changes¹ Following Small Lesions² of the Nigro-Striatal Bundle with Pretreatment by Haloperidol³ or Alpha-Methyl-Para-Tyrosine⁴⁻⁶

Postoperative Day	Treatment		
	Saline (N=8)	Haloperidol N=8)	Methyl-p-N=5)
1	89.8 $\pm$ 0.4	93.4 $\pm$ 1.4	91.6 $\pm$ 1.6
2	85.3 $\pm$ 0.9	92.0 $\pm$ 1.6	88.2 $\pm$ 2.8
3	83.7 $\pm$ 1.1	90.0 $\pm$ 1.3	87.6 $\pm$ 3.6
4	83.6 $\pm$ 2.0	88.8 $\pm$ 2.2	86.4 $\pm$ 3.9
5	84.1 $\pm$ 2.7	90.7 $\pm$ 1.5	89.2 $\pm$ 5.1
6	86.5 $\pm$ 2.6	91.8 $\pm$ 1.3	91.0 $\pm$ 4.9
7	86.4 $\pm$ 2.9	93.3 $\pm$ 1.4	90.2 $\pm$ 4.9

1. Weights are expressed as means and standard errors of percent weight relative to preoperative weight. Number of subjects per condition is indicated in parentheses.
2. Each subject was lesioned bilaterally using 1 mA for 15 sec.
3. 0.8 mg/kg/day was administered i.p. for 3 days.
4. 100 mg/kg/day was administered i.p. for 3 days.
5. An analysis of variance for repeated measures¹⁴ comparing the haloperidol and control conditions on days 2 through 7 indicated a significance ($F=7.6$, $p < .05$ for $df = 1, 14$).
6. Preoperative weights were 367.8 ± 6.5 , 318.4 ± 19.1 , 371.5 ± 9.2 grams respectively for saline, haloperidol and methyl-p-tyrosine treated groups.

TABLE 1
PERCENTAGE OF THE TOTAL POPULATION OF THE UNITED STATES
IN THE VARIOUS RACES AND ETHNIC GROUPS, 1900-1950
Source: U. S. Bureau of Census, "The Negro Population, 1900-1950,"
U. S. Census Bureau, Washington, D. C., 1955.

Table 2. Weight Changes¹ Following Large Lesions² of the Nigro-Striatal Bundle With Pretreatment by Morphine³ or Haloperidol⁴⁻⁵

Postoperative Day	Treatment		
	Saline	Morphine	Haloperidol
1	87.1 $\pm$ 1.3 (11)	88.4 $\pm$ 0.6 (15)	89.3 $\pm$ 1.2 (28)
2	80.4 $\pm$ 1.1 (11)	81.7 $\pm$ 0.6 (15)	83.0 $\pm$ 1.1 (28)
3	74.7 $\pm$ 1.1 (11)	76.8 $\pm$ 0.7 (15)	77.6 $\pm$ 1.1 (27)
4	70.9 $\pm$ 1.0 (11)	73.0 $\pm$ 0.8 (15)	74.1 $\pm$ 1.2 (25)
5	67.0 $\pm$ 1.1 (11)	69.4 $\pm$ 0.9 (15)	70.0 $\pm$ 1.4 (25)
6	63.0 $\pm$ 1.4 (10)	66.2 $\pm$ 0.7 (15)	68.7 $\pm$ 1.6 (24)
7	60.7 $\pm$ 1.8 (7)	63.9 $\pm$ 1.4 (13)	67.1 $\pm$ 2.0 (21)
8	60.6 $\pm$ 2.3 (4)	65.3 $\pm$ 2.8 (10)	67.2 $\pm$ 2.7 (16)
9	54.5 $\pm$ 3.5 (2)	69.7 $\pm$ 4.2 (7)	70.9 $\pm$ 3.5 (12)
10	51.0 $\pm$ 2.8 (2)	75.0 $\pm$ 4.5 (6)	70.5 $\pm$ 4.2 (11)
11	48.0 $\pm$ 1.4 (2)	75.8 $\pm$ 5.1 (6)	76.0 $\pm$ 5.6 (8)
12	46.0 $\pm$ 0.0 (1)	76.1 $\pm$ 5.6 (6)	77.8 $\pm$ 7.1 (7)
13	44.0 $\pm$ 0.0 (1)	76.8 $\pm$ 6.4 (6)	90.3 $\pm$ 7.8 (6)
14	-- -- (0)	78.4 $\pm$ 6.8 (6)	91.4 $\pm$ 4.4 (5)

1. Weights are expressed as means and standard errors of percent weight relative to preoperative weight. Number of subjects per condition is indicated in parentheses.
2. Each subject was lesioned bilaterally using 2 mA for 30 sec.
3. 60 mg/kg/day of morphine sulfate was given i.p. for 6 days.
4. 4 mg/kg/day of haloperidol was given i.p. for 6 days.
5. Preoperative weights were 316.8 $\pm$ 23.3, 365.4 $\pm$ 10.7, 330.7 $\pm$ 7.4 grams respectively for saline, morphine and haloperidol treated groups.

FIGURE 7. Vertical depth changes following storm passage
and for the first 24 hours after passage of storm.
with respect to the storm center at
the respective locations and time and place.
in Figure 7.

pretreated with haloperidol (4 mg/kg/day) or morphine sulfate (60 mg/kg/day). These results are summarized in Figure 7. A significantly greater number of rats pretreated with morphine or haloperidol were still alive on the 15th post-operative day when compared to saline treated controls (morphine vs saline $p < 0.001$ and haloperidol vs saline $p < 0.01$ by the Chi-Square test). These data are shown in Figure 8.

D. Discussion

The extent of weight loss in the lesioned animals was dependent upon the amount of nigro-striatal damage. These results support previous suggestions (Oltzman and Harvey, 1972; Ungerstedt, 1970, 1971) that the destruction of the nigro-striatal pathway is critical for the production of the lateral hypothalamic syndrome. Facilitations of recovery from the syndrome produced by large lateral hypothalamic lesions has previously been demonstrated with methyl-para-tyrosine treatment (Glick *et al.*, 1972), and the present results show an analogous effect of methyl-para-tyrosine on recovery from nigro-striatal damage of a less extensive area. Recovery was also facilitated by pretreatment with haloperidol. Lethality from massive nigro-striatal damage was reduced if subjects were treated with haloperidol or morphine for a six-day period prior to surgery.

A definite mechanism underlying recovery from the destruction of the nigro-striatal pathway cannot as yet be proposed. Several suggestions are worthy of consideration, first an increase in sensitivity of postsynaptic receptors

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(Cannon and Rosenbleuth, 1949; Stovraky, 1961; Glick et al., 1972); secondly the regenerative sprouting from transected axons (Katzmon et al., 1971; Nygren et al., 1971); third and lastly is an increase in catecholamine turnover in surviving neurons (Bloom et al., 1969; Uretsky et al., 1971; Agid et al., 1973). Any or all of these changes are likely to promote recovery. All the drugs found active in promoting recovery have one property in common, that is, they produce dopamine deficiency at receptor sites. Alpha-methyl-para-tyrosine is a well-known inhibitor of catecholamine synthesis (Nagatsu et al., 1964) haloperidol blocks dopamine receptors directly (Janssen, 1967; Van Rossum, 1967; Anden et al., 1970) and morphine blocks dopamine receptors indirectly, possibly through an effect on non-dopaminergic neurons (Puri et al., 1973). The other pharmacological effects of the drugs employed in this study are not common to all three drugs.

A drug induced deficiency of receptor activity can cause both an increase in receptor sensitivity as a consequence of pharmacological denervation (Sharpless, 1964) and stimulate neuronal feedback mechanism (Fuxe and Ungerstedt, 1970). Chronic treatment with haloperidol (Gianutsos et al., 1974a, 1975), methyl-para-tyrosine (Tarsy and Baldessareni, 1973), and morphine sulfate (Puri and Lal, 1973) is known to cause supersensitivity of dopamine receptors in the central nervous system. Both

haloperidol and morphine sulfate increase dopamine synthesis in the nigro-striatal system (Janssen, 1967; Gunne et al., 1969; Puri et al., 1973). The pretreatment with these drugs, that increase neurotransmitter synthesis, could promote the development of a compensatory change in the level of activity of the surviving dopamine containing neurons (Bloom et al., 1969; Uretsky et al., 1971; Agid et al., 1973). The stimulation of neurotransmitter synthesis could possibly accompany stimulation of several other processes which play a part in the regenerative process, which, of the suggested effects of these drugs under consideration might actually account for the observed promotion of recovery after nerve injury needs further investigation.

V. MODIFICATION OF NARCOTIC WITHDRAWAL SYMPTOMS
BY NIGRO-STRIATAL AND MEDIAL FORE
BRAIN BUNDLE LESIONS

A. Introduction

The participation of dopaminergic neuronal systems in narcotic dependence and withdrawal has been suggested in recent years by several lines of evidence. Morphine withdrawal aggression in dependent animals can be selectively potentiated by dopaminergic stimulating agents and blocked by drugs which block dopamine receptors (Puri et al., 1971; Lal and Puri, 1972; Puri and Lal, 1973). Haloperidol, a drug with some specificity for blocking dopamine receptors (Janssen 1967; Von Rossum, 1967; Anden et al., 1970) has been reported to reduce morphine withdrawal syndrome in animals and humans (Lal et al., 1971; Karkalas and Lal, 1972). Haloperidol also reduces the self administration of morphine in addicted rats (Hanson and Cimino-Venema, 1972) and monkeys (Pozuelo and Kerr, 1972). Lesions placed stereotaxically in monkeys in the origin of the nigro-striatal system and the nucleus tegmenti ventralis, known to be mainly dopaminergic pathways, abolish craving for morphine and the phenomena of withdrawal, as evidenced, respectively by lack of bar pressing and by lack of withdrawal manifestations (Pozuelo and Kerr, 1972). Further evidence for the involvement of dopamine

pathways in morphine abstinence has been shown by Gianutsos et al. (1973,1974). They have found that electrolytic lesioning of the nigro-striatal bundle abolished morphine withdrawal aggression in thirty-day abstinent rats while lesioning of the medial forebrain bundle was ineffective in blocking the aggression (Gianutsos et al., 1973, 1974b).

Further evidence for the interaction between brain dopamine and morphine comes from biochemical studies. Acute morphine elevates brain homovanillic and 3-4 dihydrophenylacetic acid (Fukui and Tugaki, 1972; Kuschinsky and Hornykiewicz, 1972), increase the synthesis of labeled dopamine from labeled tyrosine in the brain (Clouet and Ratner, 1970; Fukui et al., 1972; Smith et al., 1970, 1972) and accelerates the disappearance of dopamine after the administration of alpha-methyl-para-tyrosine (Gunne et al., 1969; Puri et al., 1973). These observations strongly suggest the involvement of dopaminergic pathways in morphine action and dependence.

In order to understand the role of the nigro-striatal dopaminergic pathways in morphine dependence, this study investigated the effect of nigro-striatal lesions produced prior to and following the production of dependence on withdrawal symptoms. Morphine dependence was produced in rats with partial (1mA 15 sec) nigro-striatal lesions. After the abrupt termination of morphine injections narcotic withdrawal symptoms were compared between lesioned and non-lesioned groups. Both partial (1mA 15 sec) and extensive

(2mA 30 sec) nigro-striatal lesions were produced during the withdrawal period. These lesions were made at both 0 and 48 hours after the terminal dose of morphine. Withdrawal symptoms were measured in lesioned and non-lesioned groups and compared. The aforementioned experiments were conducted at two different levels of morphine dependence, at terminal doses of 200 and 405 mg/kg/day of morphine sulfate. The dopaminergic stimulating agent, apomorphine (Anden et al., 1967; Ernst, 1967) was administered to both intact and nigro-striatal lesioned morphine withdrawn subjects to further assess the role of dopaminergic receptor activity in the abstinence phenomena. In order to determine if the results were specific for the dopaminergic system the medial fore brain bundle, a noradrenergic and serotonergic neuron system, was also lesioned after the production of dependence and abstinence signs observed. It was hypothesized that nigro-striatal lesions would modify the withdrawal symptoms that involved dopaminergic mechanisms.

B. Method

Male hooded rats were housed in individual cages during the addiction and withdrawal phases of these experiments. Brain lesions were made under anesthesia either prior to or following the production of dependence. At the completion of each experiment a representative group of animals were sacrificed for histological localization of the lesion site.

Rats were made dependent upon morphine by injecting them intraperitoneally with systematically increasing doses

of morphine sulfate three times a day. The starting dose of 15 mg/kg/injection was increased by 15 mg/kg every day until a dose of 405 mg/kg was reached.

In those rats who received a terminal dose of 200 mg/kg only two injections a day were given twelve hours apart. The starting dose of 10 mg/kg/injection was increased by 10 mg/kg every day until a dose of 200 mg/kg was reached. They were maintained at this dose for at least five days after which no morphine was injected.

Abstinence symptoms were measured after the abrupt termination of chronic morphine administration. Symptoms were measured once every 24 hours for the first 72 hours after morphine withdrawal. The rats were removed from their home cages and placed individually into novel cages. Symptoms were measured for 30 minutes; prior to observation rats were weighed and then rectal temperature taken. The abnormal behavior noted during the observation period consisted of wet shakes, writhing, ptosis and piloerection. Aggression in response to social grouping was measured seventy-two hours after the last morphine sulfate injection. A more detailed description of these procedures can be found in Chapter III.

C. Results

1) Modification of Narcotic Withdrawal Symptoms by Nigro-Striatal Lesioning

a. Lesions made prior to the production of dependence

Partial destruction of the nigro-striatal path-

way prior to the production of morphine dependence modified the intensity of the narcotic withdrawal syndrome. Wet shakes and ptosis were seen to significantly ($p < 0.02$) increase when compared to non-lesioned addicted controls by the Student's 't' test at 24 and 48 hours of withdrawal respectively. The Student's 't' test indicated a significant ($p < 0.05$) lesion effect on weight loss at 24 and 48 hours of withdrawal. Hypothermia was found to be significantly ($p < 0.001$) increased over the non-lesioned groups by the Student's 't' test at 48 hours of withdrawal. Table 3 provides a summary of these results. There was a significant reduction in 72 hour morphine withdrawal aggression in the animals receiving nigro-striatal lesions prior to the production of dependence. These results are shown in Table 4.

Replication of this experiment in which the terminal dose of morphine was increased to 405 mg/kg/day yielded similar results. Analysis of the data with the Student's 't' test showed that lesioned subjects showed significantly ($p < 0.001$) more wet shakes at 48 and 72 hours of withdrawal than did non-lesioned addicted controls. Writhing was found to be significantly ($p < 0.01$) decreased at 72 hours of withdrawal. At 48 hours of withdrawal lesioned rats showed a significant ($p < 0.01$) increase in ptosis time and weight loss when compared to non-lesion addicted controls by the Student's 't' test. Using this same statistical test a significant ($p < 0.001$) decrease in 24 hours withdrawal hypothermia was found. These results are summarized in Table 3. Morphine withdrawal aggression measured at 72 hours of

Table 3. Modification of the Narcotic Withdrawal Syndrome by Nigro-Striatal Lesioning¹
Prior to the Production of Morphine Dependence

Symptoms ¹⁰ (Hours of Withdrawal)	Non Addicted		Addicted			
			200 mg/kg/day		405 mg/kg/day	
	Non- Lesioned	Lesioned	Non- Lesioned	Lesioned	Non- Lesioned	Lesioned
Wet Shakes						
24	0.2±0.1	1.5±1.1	2.0±0.3	5.4±1.1 ³	9.1±1.3 ⁹	10.6±2.0 ⁵
48	0.2±0.1	0.5±0.2	5.6±0.7	7.8±1.4	6.9±0.9	13.6±1.7 ⁵
72	0.2±0.1	1.0±0.4	6.1±0.7	7.3±1.6	6.3±0.6	13.6±1.7 ⁵
Writhing						
24	0.0±0.0	0.5±0.5	0.5±0.1 ⁴	0.4±0.0	1.9±0.3 ⁷	2.1±0.8
48	0.0±0.0	0.2±0.2	1.0±0.4	1.4±0.5	2.5±0.4 ⁷	2.0±0.8 ⁴
72	0.0±0.0	0.0±0.0	0.3±0.1	1.0±0.3	3.2±0.9 ⁸	0.5±0.2 ⁴
Piloerection (No positive/No observed)						
24	0/12	4/4	15/15	13/13	27/27	14/16
48	0/12	2/4	15/15	12/13	27/27	16/16
72	0/12	2/4	15/15	13/13	16/16	14/14
Ptosis Time						
24	0.0±0.0	0.0±0.0	28±21	206±116 ³	101±28 ⁶	92±43 ⁴
48	0.0±0.0	0.0±0.0	0.0±0.0	438±158 ³	87±30 ⁷	536±134 ⁴
72	0.0±0.0	0.0±0.0	0.0±0.0	74±41	201±60 ⁸	227±92
Weight Loss						
24	-	-	2.6±0.7	10.3±3.0 ²	12.8±1.5 ⁹	11.0±1.9 ⁴
48	-	-	25.3±2.7	35.9±3.0 ³	26.5±1.5	34.5±2.5 ⁴
72	-	-	30.9±2.1	33.4±4.4	32.8±3.0	37.5±4.3

Table 3 - Continued

Hypothermia						
24	-	0.1±0.01	1.87±0.06	1.80±0.09 ₅	2.05±0.07	1.59±0.08 ⁵
48		0.2±0.40	1.75±0.07	2.79±0.18 ⁵	1.88±0.06	1.90±0.10
72		0.0±0.00	1.70±0.08	1.98±0.11	1.35±0.10	1.64±0.11

- 1mA for 15 seconds.
- Significantly different from non-lesioned addicted controls based on Student's 't' test ($p < 0.05$).
- Significantly different from non-lesioned addicted controls based on Student's 't' test ($p < 0.02$).
- Significantly different from non-lesioned addicted controls based on Student's 't' test ($p < 0.01$).
- Significantly different from non-lesioned addicted controls based on Student's 't' test ($p < 0.001$).
- Significantly different from 200 mg/kg/day morphine withdrawal, based on Student's 't' test ($p < 0.05$).
- Significantly different from 200 mg/kg/day morphine withdrawal, based on Student's 't' test ($p < 0.02$).
- Significantly different from 200 mg/kg/day morphine withdrawal, based on Student's 't' test ($p < 0.01$).
- Significantly different from 200 mg/kg/day morphine withdrawal, based on Student's 't' test ($p < 0.001$).
- All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fraction used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

Table 4. Seventy-Two Hours Withdrawal Aggression in Rats Receiving Brain Lesions

<u>Treatments</u>	<u>N</u>	Mean $\pm$ S.E.		
		<u>Attacks</u>	<u>Rearing</u>	<u>Vocalization</u>
		<u>Addicted to 405 mg/kg/day</u>		
Intact Controls	5	32 $\pm$ 2	3014 $\pm$ 193	1809 $\pm$ 361
Small NSB Lesion Prior to Addiction ¹	1	30	1261	527
Small NSB Lesion After Addiction ¹	1	3	286	80
Large NSB Lesion After Addiction ²	1	21	309	319
Small NSB Lesion After Addiction ¹	1	14	997	293
Large NSB Lesion After Addiction ²	1	5	56	69
<u>Addicted to 200 mg/kg/day</u>				
Intact Controls	3	9 $\pm$ 6	1811 $\pm$ 868	929 $\pm$ 454
Small NSB Lesion Prior to Addiction ¹	2	0 $\pm$ 0	35 $\pm$ 19	13 $\pm$ 24
Small NSB Lesion After Addiction ²	2	58 $\pm$ 4	2639 $\pm$ 165	827 $\pm$ 44
Large NSB Lesion After Addiction ²	2	14 $\pm$ 7	292 $\pm$ 16	209 $\pm$ 110

1. 1 mA 15 sec

2. 2 mA 30 sec

withdrawal showed a reduction in rearing and vocalization in the lesion addicted groups. These results are shown in Table 4.

The withdrawal symptoms exhibited by rats addicted to 200 and 405 mg/kg/day of morphine were compared by the Student's 't' test. Wet shakes were increased in those rats withdrawing from 405 mg/kg/day at 24, 48 and 72 hours but significance ($p < 0.001$) was achieved only at 24 hours. Writhing in these rats was significantly ($p < 0.02$) increased at 24, 48 and 72 hours. Ptosis was significantly increased at 24 ($p < 0.05$), 48 ($p < 0.02$) and 72 ($p < 0.01$) hours of withdrawal in rats withdrawn from 405 mg/kg/day of morphine as compared to those withdrawn from 200 mg/kg/day. Weight loss was found to significantly ($p < 0.001$) increase at 24 hours. Increased hypothermia was observed in rats withdrawn from 405 mg/kg/day but this increase failed to achieve significance. These results are summarized in Table 3.

b. Lesions made after the production of morphine dependence.

1. Effect of small nigro-striatal lesions

Rats made dependent, 200 mg/kg/day terminal dose, and lesioned (1mA for 15 sec) following the last injection of morphine showed a general increase in withdrawal intensity. Withdrawal wet shakes in the lesioned rats were significantly ($p < 0.001$) increased at 24 and 48 hours of withdrawal when compared to their non-lesioned counterparts by the Student's 't' test. At 72 hours of withdrawal a significantly ($p < 0.02$) increased amount of writhing was observed in lesioned subjects.

Ptosis time was found to significantly ($p < 0.05$) increase in nigro-striatal lesioned subjects by the Student's 't' test at 24, 48, and 72 hours of withdrawal. When the same statistical test was employed for the effect of the lesion on weight loss it showed the lesion groups to have lost a significantly ($p < 0.001$) greater amount of body weight at 24, 48 and 72 hours of withdrawal than non-lesioned controls. The Student's 't' test showed a significant ($p < 0.05$) increase in hypothermia at 72 hours. A significant ($p < 0.05$) lesion effect was observed for wet shakes and writhing in non-addicted rats. Weight loss was also seen in non-addicted lesioned rats. These results are summarized in Table 5. Seventy-two hour morphine withdrawal aggression was apparently increased by the lesion, as shown in Table 4.

When the terminal dose of morphine was increased to 405 mg/kg/day a similar lesion effect was noted. The Student's 't' test showed significant increases in wet shakes at 24 ($p < 0.02$), 48 ($p < 0.05$) and 72 ($p < 0.001$) hours in the lesioned groups as compared to the non-lesioned addicted controls. Writhing was found to be significantly ($p < 0.05$) decreased at both 24 and 72 hours of withdrawal in the nigro-striatal lesioned groups. Significant ($p < 0.001$) increases in ptosis time and weight loss were seen in the lesion groups when compared to the non-lesioned controls by the Student's 't' test. Application of this statistical test to withdrawal hypothermia showed a significant ($p < 0.001$) decrease at 24 hours and a significant ($p < 0.01$) increase at

Table 5. Narcotic Withdrawal Symptoms in Rats With Small Nigro-Striatal Lesions¹ Made After the Terminal Dose of Morphine Sulfate

Symptoms ⁶ (Hours of Withdrawal)	Non-Addicted		Addicted			
			200 mg/kg/day		405 mg/kg/day	
	<u>Non- Lesioned</u>	<u>Lesioned</u>	<u>Non- Lesioned</u>	<u>Lesioned</u>	<u>Non- Lesioned</u>	<u>Lesioned</u>
Wet Shakes						
24	0.2±0.1	3.0±0.6 ⁵	2.0±0.3	13.8±4.7 ⁵	9.1±1.3	15.1±2.7 ³
48	0.2±0.1	5.4±1.9 ³	5.6±0.7	12.3±3.3 ⁵	6.9±0.9	17.3±5.0 ²
72	0.2±0.1	2.6±0.8 ⁴	6.1±0.7	7.4±2.3	6.3±0.6	11.3±3.5 ⁵
Writhing						
24	0.0±0.0	1.6±0.6 ³	0.5±0.4	0.7±0.7	1.9±0.3	0.5±0.1 ²
48	0.0±0.0	1.5±0.5 ⁴	1.0±0.4	1.6±0.5 ³	2.5±0.4	1.5±0.0 ⁷
72	0.0±0.0	0.5±0.2 ²	0.3±0.1	1.6±0.9 ³	5.4±1.7	0.8±0.4 ³
Piloerection (No positive/No observed)						
24	0/12	12/21	15/15	9/9	27/27	12/16
48	0/12	6/9	15/15	9/9	27/27	15/16
72	0/12	5/9	15/15	9/9	16/16	16/16
Ptoxis Time						
24	0.0±0.0	193±101	28±21	763±219 ²	101±28	1768±27 ⁵
48	0.0±0.0	119±119	0.0±0.0	670±290 ²	87±30	1178±175 ⁵
72	0.0±0.0	33±33	0.0±0.0	411±205 ²	384±92	1179±193 ⁵
Weight Loss						
24		31.2±1.7	2.6±0.7	45.8±0.9 ⁵	12.8±1.5	36.4±1.7 ⁵
48	-	36.9±7.4	25.3±2.7	68.4±2.5 ⁵	26.5±1.5	56.0±2.8 ⁵
72		46.8±1.4	30.9±2.1	82.0±3.0 ⁵	31.8±3.0	72.9±2.6 ⁵

Table 5 - Continued

Hypothermia					
24	+0.5±0.1	1.87±0.06	1.78±0.13	2.05±0.07	1.50±0.03 ⁵
48	+0.1±0.1	1.75±0.07	2.40±0.25	1.88±0.06	2.58±0.51
72	+0.2±0.1	1.70±0.08	3.05±0.40 ²	1.61±0.15	4.02±0.75 ⁴

1. 1mA for 15 seconds.
2. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.05$).
3. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.02$).
4. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.01$).
5. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.001$).
6. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fraction used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

72 hours. These data are shown in Table 5. The lesioned groups showed significantly less 72 hour morphine withdrawal aggression. These data are given in Table 4.

2. Effect of large nigro-striatal lesions

Large nigro-striatal lesions made after the terminal dose of morphine in rats dependent upon 200 mg/kg/day resulted in increased ptosis time and weight loss. Analysis of these data by the Student's 't' test showed a significant ($p < 0.001$) increase in ptosis time and weight loss at 24, 48 and 72 hours of withdrawal. Hypothermia in the nigro-striatal lesioned rats showed a significant ($p < 0.001$) increase at 72 hours. A significant ($p < 0.05$) lesion effect was observed for wet shakes and writhing in non-addicted rats. Weight loss was also seen in non-addicted lesioned rats. These results are summarized in Table 6. Morphine withdrawal aggression was decreased by the lesion as shown in Table 4.

A similar lesion effect was seen in rats dependent upon 405 mg/kg/day of morphine. Writhing was decreased significantly ($p < 0.05$) at 24, 48 and 72 hours in those rats receiving extensive nigro-striatal lesions after the production of dependence. The Student's 't' showed significant ($p < 0.001$) lesion induced increases in ptosis time and weight loss at 24, 48 and 72 hours as compared to non-lesioned addicted controls. Withdrawal hypothermia significantly increased at 48 ($p < 0.01$) and 72 ($p < 0.001$) hours as compared to non-lesioned counterparts. These results are

Table 6. Narcotic Withdrawal Symptoms After Large Nigro-Striatal Lesions¹ Following the Terminal Dose of Morphine Sulfate

Symptoms ⁶ (Hours of Withdrawal)	Non Addicted		Addicted			
			200 mg/kg/day		405 mg/kg/day	
	<u>Non Lesioned</u>	<u>Lesioned</u>	<u>Non Lesioned</u>	<u>Lesioned</u>	<u>Non Lesioned</u>	<u>Lesioned</u>
Wet Shakes						
24	0.2+0.1	2.2+0.4 ⁵	2.0+0.3	5.2+1.9	9.1+1.3	6.1+1.9
48	0.2+0.1	2.6+0.4 ⁵	5.6+0.7	13.3+4.4	6.9+0.9	10.5+1.7
72	0.2+0.1	2.4+0.7 ³	6.1+0.7	8.5+2.4	6.3+0.6	9.1+2.3
Writhing						
24	0.0+0.0	1.3+0.4 ⁴	0.5+0.4	1.8+1.3	1.9+0.3	0.0+0.0 ²
48	0.0+0.0	2.3+0.6 ⁴	1.0+0.4	0.5+0.2	2.5+0.4	0.7+0.7 ²
72	0.0+0.0	1.3+0.6 ²	0.3+0.1	2.4+1.3	3.2+0.9	0.0+0.0 ²
Piloerection (No positive/No observed)						
24	0/12	24/25	15/15	9/9	27/27	8/8
48	0/12	10/10	15/15	9/9	27/27	7/7
72	0/12	10/10	15/15	7/7	16/16	6/6
Ptosis Time						
24	0.0+0.0	153+88	28+21	1253+166 ⁵	101+28	1800+00 ⁵
48	0.0+0.0	231+92 ²	0+0	1288+178 ⁵	87+30	1740+60 ⁵
72	0.0+0.0	355+213	0+0	1511+176 ⁵	201+60	1800+00 ⁵

Table 6 - Continued

Weight Loss						
24		30.0+2.1	2.6+0.7	37.8+3.5 ⁵	12.8+1.5	36.8+2.2 ⁵
48	-	55.3+5.0	25.3+2.7	68.7+5.1 ⁵	26.0+1.5	54.7+2.2 ⁵
72		70.2+6.3	30.9+2.1	89.1+5.0 ^r	32.8+3.0	69.8+2.2 ⁵
Hypothermia						
24	-	0.00+0.16	1.87+0.06	1.55+0.17	2.05+0.07	2.38+0.40 ⁴
48		0.63+0.43	1.75+0.07	3.00+0.70	1.88+0.06	4.88+1.03 ⁵
72		0.27+0.42	1.70+0.08	3.45+0.41 ⁵	1.35+0.10	6.98+1.81 ⁵

1. 2 mA for 30 seconds.
2. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.05$).
3. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.02$).
4. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.01$).
5. Significantly different from non-lesioned controls by the Student's 't' test ($p < 0.001$).
6. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fraction used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

summarized in Table 6. The nigro-striatal lesioned groups exhibited reduced morphine withdrawal aggression. These data are represented in Table 4.

c. Lesions made during the withdrawal period.

1. Effect of small nigro-striatal lesions

The production of small, 1mA 15 sec , lesions of the nigro-striatal pathway made at 48 hours after the last morphine injection produced an increased occurrence of 72 hour withdrawal symptoms as shown in Table 7. Wet shakes and writhing were significantly ($p < 0.01$) increased as compared to non-lesioned controls by the Student's 't' test. This same test showed a significant ($p < 0.001$) lesion effect on ptosis time and weight loss.

2. Effect of large nigro-striatal lesions

Large, 2mA 30 sec , lesion of the nigro-striatal tract produced increases in some withdrawal symptoms. These data were analyzed by the Student's 't' test. Significant ($p < 0.01$) increases in ptosis time and weight loss were seen in lesioned subjects as compared to their non-lesioned counterparts. These results are summarized in Table 7.

d. Modification of narcotic withdrawal symptoms by apomorphine

1. Effect of apomorphine on narcotic withdrawal symptoms in the intact dependent rat

Dependent rats, 405 mg/kg/day of morphine, were observed for the occurrence of withdrawal symptoms and then

Table 7. Nigro-Striatal Lesioning at Forty-Eight Hours After the Last Morphine Sulfate Injection: Effect on Seventy-Two Hour Withdrawal Symptoms

Symptoms ⁷	Non Addicted			Addicted		
	Non Lesioned	Small Lesion ²	Large Lesion ³	Non Lesioned	Small Lesion ²	Large Lesion ³
Wet Shakes	0.2+0.1	3.0+0.6 ⁶	2.2+0.4	6.3+0.6	17.5+3.7 ⁵	13.4+4.3
Writhing	0.0+0.0	1.6+0.64	1.3+0.4	3.2+0.9	0.3+0.1 ⁵	0.8+0.8
Piloerection (No positive/No observed)	0/12	9/17	24/25	16/16	20/20	7/7
Ptosis Time	0.0+0.0	126+76	153+88	201+60	1310+123 ⁶	1414+255 ⁶
Weight Loss	-	31.2+1.7	30.0+2.1	32.9+3.0	54.4+2.4 ⁶	51.7+3.4 ⁶

1. Rats were dependent upon 405 mg/kg/day of morphine sulfate.
2. 1mA 15 sec nigro-striatal lesions.
3. 2mA 30 sec nigro-striatal lesions.
4. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.02$)
5. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.01$)
6. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.001$)
7. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fraction used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

after being injected with apomorphine, re-observed. The results for this experiment are given in Table 8. The paired 't' test was used to determine if apomorphine had a significant effect on the symptoms of narcotic withdrawal. Wet shakes were significantly ($p < 0.05$) reduced by apomorphine (1.25 mg/kg) at 24, 48 and 72 hours after the terminal dose of morphine. The other symptoms of morphine withdrawal were not significantly affected by this treatment.

2. Effect of apomorphine on narcotic withdrawal symptoms in rats with nigro-striatal lesions made after the production of dependence.

Small (1mA 15 sec) nigro-striatal lesions produced after the terminal dose of morphine were observed to increase the severity of the withdrawal syndrome. (See Table 5.) The effect of an injection of apomorphine (1.25 mg/kg) on the withdrawal syndrome in rats with nigro-striatal lesion made after the terminal dose of morphine is shown in Table 9. Wet shakes were decreased at 24, 48 and 72 hours with significant ($p < 0.05$) reductions occurring at both 48 and 72 hours when compared by the paired 't' test to pre-drug levels. Apomorphine failed to significantly affect the other symptoms of withdrawal in these lesioned rats.

2) Modification of Narcotic Withdrawal Symptoms by Medial Fore Brain Bundle Lesions

a. Lesions made after the production of morphine dependence

Table 8. The Effect of Apomorphine on Narcotic Withdrawal Symptoms in the Intact Dependent Rat

Symptoms ⁵	Time Since the Terminal Dose of Morphine					
	Twenty-four hours		Forty-eight hours		Seventy-two hours	
	Pre-Drug	Post Apomorphine ¹	Pre-Drug	Post Apomorphine ¹	Pre-Drug	Post Apomorphine ¹
Wet Shakes	6.2+1.5	2.0+1.1 ³	5.1+1.9	2.8+1.1 ⁴	5.6+0.9	3.0+0.8 ³
Writhing	1.1+0.3	0.4+0.1	1.4+0.5	1.3+0.4	1.3+0.4	0.6+0.4
Piloerection	21/21	21/21	10/10	10/10	10/10	10/10
(No positive/no observed)						
Ptosis Time	434+112	867+134	125+51	379+114	95+63	157+58
Hypothermia	2.08+0.34	2.48+0.30	1.95+0.35	1.92+0.35	1.24+0.39	1.10+0.38
Weight Loss	25.0+1.8	25.4+2.0	31.4+3.5	33.3+3.9	27.6+3.0	31.1+3.1

1. Apomorphine hydrochloride 1.25 mg/kg administered fifteen minutes prior to observation.
2. Rats were dependent upon 405 mg/kg/day of morphine sulfate, injections were given three times a day.
3. Significantly different from pre-drug based on Paired 't' test ($p < 0.05$).
4. Significantly different from pre-drug based on Paired 't' test ($p < 0.01$).
5. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fractions used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

Table 9. The Effect of Apomorphine¹ on Narcotic Withdrawal Symptoms in Rats With Nigro-Striatal Lesions² Made After the Terminal Dose of Morphine³

Symptoms ⁵	Time Since the Terminal Dose of Morphine Sulfate					
	Twenty-four hours		Forty-eight hours		Seventy-two hours	
	Pre-Drug	Post Apomorphine ¹	Pre-Drug	Post Apomorphine ¹	Pre-Drug	Post Apomorphine ¹
Wet Shakes	15.1+4.1	7.8+1.5	15.0+3.1	6.0+1.4 ⁴	9.1+3.8	2.5+1.1 ⁴
Writhing	0.7+0.4	0.0+0.0	0.4+0.4	0.0+0.0	0.0+0.0	0.0+0.0
Piloerection (No positive/No observed)	9/9	9/9	9/9	9/9	8/8	8/8
Ptosis Time	1800+0	1800+0	1800+0	1785+14	1800+0	1740+35
Hypothermia	2.3+0.4	2.8+0.2	3.8+0.5	4.3+0.4	6.4+0.9	6.6+0.9
Weight Loss	30.7+2.2	36.2+2.0	60.1+2.3	63.0+2.0	81.0+3.0	81.6+2.4

1. Apomorphine hydrochloride 1.25 mg/kg administered fifteen minutes prior to observation.
2. lma 15 sec nigro-striatal lesions.
3. Rats were dependent upon 405 mg/kg/day of morphine sulfate, injections were given twice a day.
4. Significantly different from pre-drug based on Paired 't' test ($p < 0.05$).
5. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fractions used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

1. Effect of small medial fore brain bundle lesions

Withdrawal symptoms were found to be modified by small (1mA 15 sec) medial fore brain bundle lesion when they were made after the terminal dose of morphine; these results are shown in Table 10. The Student's 't' test was used to analyze the difference between the mean of the lesioned groups and that of the non-lesioned addicted controls. Wet shakes were found to be significantly ($p < 0.05$) increased at 48 hours of withdrawal while writhing was significantly ($p < 0.02$) decreased at both 24 and 48 hours after the terminal dose of morphine. Significant ($p < 0.001$) increases were seen in ptosis time and weight loss at 24, 48 and 72 hours of morphine withdrawal. Hypothermia was found to significantly ($p < 0.01$) increase at 72 hours of morphine withdrawal. The lesion by itself produced ptosis and weight loss in non-addicted rats. Medial fore brain bundle lesions were found to decrease social aggression seen at 72 hours of withdrawal; these results are shown in Table 4.

2. Effect of large medial fore brain bundle lesions

The effect of large (2mA 30 sec) medial fore brain bundle lesion on morphine withdrawal are shown in Table 10. At 48 hours of withdrawal a significant ($p < 0.001$) increase in wet shakes was observed; the Student's 't' test was used to determine significance. Writhing was significantly ($p < 0.05$) decreased at both 48 and 72 hours of withdrawal. The Student's 't' test showed ptosis time and weight loss to be significantly ($p < 0.001$) increased in the

Table 10. The Effect of Medial Fore Brain Bundle Lesions on the Narcotic Withdrawl Syndrome

Symptoms ⁵ (Hours of Withdrawal)	Non Addicted			Addicted		
	Non Lesioned	Lesioned		Non Lesioned	Lesioned	
		1mA 15 sec	2mA 30 sec		1mA 15 sec	2mA 30 sec
Wet Shakes						
24	0.2±0.3	1.25±0.7	0.7±0.4	9.1±1.3	10.1±3.4	10.2±4.8 ⁴
48	0.2±0.3	0.75±0.3	2.0±0.8	6.9±0.8	14.5±3.0	16.5±2.1 ⁴
72	0.2±0.3	1.25±0.9	1.1±0.9	6.3±0.9	8.8±2.7	20.0±8.6
Writhing						
24	0.0±0.0	0.75±0.3	0.0±0.0	1.9±0.3	0.3±0.2 ⁴	0.2±0.2
48	0.0±0.0	0.62±0.2	0.3±0.3	2.5±0.4	4.5±2.6	0.1±0.1 ¹
72	0.0±0.0	0.62±0.3	0.0±0.0	3.2±0.9	0.50±0.3 ²	0.0±0.0 ¹
Piloerection (No positive/No observed)						
24	0/12	5/8	2/4	27/27	6/6	8/8
48	0/12	5/8	5/6	27/27	6/6	7/7
72	0/12	8/8	4/6	16/16	6/6	4/4
Ptosis Time						
24	0.0±0.0	562±215 ²	570±396	101±27	1533±178 ⁴	1800±000 ⁴
48	0.0±0.0	615±284 ¹	1600±126 ⁴	87±30	1033±260 ⁴	1611±144 ⁴
72	0.0±0.0	576±258	1058±339 ³	201±60	1270±255 ⁴	1800±000 ⁴

Table 10 - Continued

Hypothermia						
24	-	0.01±0.08	+0.1±0.1	2.04±0.07	1.53±0.39	2.50±1.9 ²
48		+0.08±0.16	0.0±0.1	1.8±0.03	1.90±0.36	4.62±0.9 ²
72		0.31±0.11	0.4±0.2	1.35±0.1	3.30±0.57 ³	4.67±2.5 ²
Weight Loss						
24		43.2±2.0	45.4±4.3	12.4±1.4	36.8±1.7 ⁴	39.6±2.2 ⁴
48	-	61.7±2.5	62.3±4.9	25.5±1.6	59.6±1.6 ⁴	59.4±3.6 ⁴
72		67.5±6.5	72.8±6.4	32.8±3.0	73.8±1.7 ⁴	62.7±7.1 ⁴

1. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.05$).
2. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.02$).
3. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.01$).
4. Significantly different from non-lesioned controls based on Student's 't' test ($p < 0.01$).
5. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fractions used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

medial fore brain lesioned withdrawn rats. Hypothermia was significantly ($p < 0.02$) increased in the lesion groups. Ptosis and weight loss resulted from lesioning non-addicted rats. Morphine withdrawal aggression was decreased by these large medial fore brain bundle. These results are summarized in Table 4.

b. Small lesions made during the withdrawal period

The effects of small (1mA 15 sec) medial fore brain bundle lesions made at 48 hours on narcotic withdrawal symptoms at 72 hours are summarized in Table 11. Statistical significance was determined by the Student's 't' test. Ptosis time, weight loss and hypothermia were found to significantly ($p < 0.01$) increase in medial fore brain bundle lesioned rats as compared to non-lesioned addicted controls.

D. Discussion

The results from these experiments suggest a role for dopaminergic nigro-striatal fibers in morphine withdrawal. Nigro-striatal lesioning prior to the production of morphine dependence resulted in increased wet shakes, ptosis and weight loss at various intervals after the terminal doses of morphine. Similar increases in withdrawal symptoms were observed when small (1mA 15 sec) lesions of the nigro-striatal pathway were made after the terminal dose of morphine. Ptosis time, weight loss and hypothermia were increased when large (2mA 30 sec) nigro-striatal pathway lesions were produced after the production of morphine dependence. Lesions of the nigro-striatal bundle during the

Table 11. Narcotic Withdrawal Symptoms at Seventy-Two Hours After Small Lesions of the Medial Fore Brain Bundle¹ at Forty-Eight Hours

<u>Symptoms</u> ⁷	<u>Non Addicted</u>		<u>Addicted</u>	
	<u>Non Lesioned</u>	<u>Lesioned</u> ²	<u>Non Lesioned</u> ³	<u>Lesioned</u> ⁴
Wet Shakes	0.2+0.3	2.6+0.7	6.3+0.6	6.1+0.9
Writhing	0.0+0.0	0.8+0.3	3.2+0.9	1.5+1.2
Piloerection (No positive/No observed)	0/12	0/12	16/16	16/16
Ptosis Time	0.0+0.0	383+208	201+60	1043+164 ⁵ ₆
Weight Loss	-	31.9+.08	32.9+3.0	52.6+3.4 ⁶ ₆
Hypothermia	-	0.01+.08	1.35+0.1	2.97+0.4 ⁶ ₆

1. 1mA 15 sec medial fore brain bundle lesions.
2. Twenty-four hours after medial fore brain bundle lesioning.
3. Seventy-two hours after the last injection of morphine sulfate, 135 mg/kg.
4. Seventy-two hours after the last injection of morphine sulfate, 135 mg/kg, and twenty-four hours after the production of the lesion.
5. Significantly different from non-lesioned addicted controls by the Student's 't' test ($p < 0.02$).
6. Significantly different from non-lesioned addicted controls by the Student's 't' test ($p < 0.01$).
7. All data are expressed in terms of means and standard errors unless otherwise indicated. The number of animals employed in each group is indicated as the denominator of the fractions used to express the occurrence of piloerection. Ptosis time is expressed in seconds, weight loss in grams and hypothermia in degrees centigrade.

withdrawal period, 48 hours after the terminal dose of morphine, resulted in increased wet shakes, ptosis time and weight loss. Apomorphine, a dopamine receptor agonist (Anden et al., 1967; Ernst, 1967), reduced wet shakes in both intact and nigro-striatal lesioned dependent rats.

Several mechanisms may possibly underly the enhanced withdrawal symptoms in nigro-striatal lesioned subjects. An increased denervation supersensitivity, changes in receptor activity and alterations in the balance between several putative neurotransmitters are mechanisms worthy of consideration.

One possible explanation for the enhanced withdrawal symptoms induced by nigro-striatal lesioning can be offered in terms of a denervation supersensitivity. It is a well recognized phenomenon that after pharmacological or surgical denervation in the peripheral nervous system, the response of the target organ to a transmitter can be greatly augmented. Analogously, it has been suggested that physical dependence might be a manifestation of a central denervation supersensitivity and as a result the withdrawal phenomena would reflect a state of rebound hyperexcitability (Jaffee and Sharpless, 1968). If the withdrawal symptoms that were seen to increase involve dopaminergic pathways, then nigro-striatal lesioning could induce an additional degree of supersensitivity in this pathway and thus enhance the withdrawal symptoms. The production of a biochemical lesion of catecholamine nerve terminal by 6-hydroxy-dopamine has been

reported to exacerbate the signs of morphine withdrawal in mice (Friedler et al., 1972) and in rats (Bhargava et al., 1973). These investigators have suggested that the enhanced withdrawal phenomena observed in their experiments may be due to an increased denervation supersensitivity produced by 6-hydroxydopamine treatment.

Receptor activation may be in part responsible for the expression of several withdrawal symptoms. Nigro-striatal lesions lead to increased wet shakes and a decreased activation of striatal dopamine receptors, whereas apomorphine, a dopaminergic receptor stimulant, decreases the occurrence of wet shakes. These data point to a major role for dopamine receptor activity in the expression of narcotic withdrawal wet shakes. An increase in dopamine receptor activity resulting in decreased symptomology while on the hand decreased receptor stimulation results in the increased occurrence of wet shakes. The apomorphine induced decrease of withdrawal wet shakes is in accordance with the results of Hoffmeister and Schichting (1973). These investigators found a decrease in withdrawal wet shakes of the dog after stimulation of central catecholamine mechanisms with amphetamine and cocaine. The effect of these drugs was interpreted to indicate an antagonism of withdrawal. Herz et al., (1974) have shown that the administration of d-amphetamine, cocaine, L-dopa increased levallorphan precipitated withdrawal jumping and decreased wet shakes. The effect of these drugs was interpreted as a potentiation of withdrawal since

similar changes in withdrawal occurred when withdrawal was precipitated in highly dependent rats (Herz et al., 1974). Following their logic it would be concluded that nigro-striatal lesions reduce narcotic withdrawal. This conclusion does not seem to be justified in view of the fact that rats receiving a terminal dose of 405 mg/kg/day of morphine show a higher occurrence of wet shakes than those dependent upon 200 mg/kg/day of morphine. It has been reported that rats dependent upon 30 or 120 mg/kg/day of morphine show no wet shakes upon withdrawal of the drug (Aker and Brady, 1968). The increase in wet shakes seen in those rats receiving a higher terminal dose of morphine tends to parallel a general increase in other symptoms.

Additional support for the role of denervation supersensitivity and receptor activity in the expression of withdrawal symptoms comes from the effect of the lesion by itself. Nigro-striatal lesioning produced some symptoms similar to those of narcotic withdrawal. Lesioned animals showed wet shakes, writhing, piloerection, ptosis and weight loss. The appearance of these withdrawal like symptoms is thought to be due to a decrease impulse flow in the nigro-striatal system and a resultant denervation supersensitivity. It should be pointed out that the increase in withdrawal symptoms in the animals lesioned after the production of dependence cannot totally be explained in terms of the lesion effect alone. Lesioning the nigro-striatal pathway prior to the production of dependence results in

increased withdrawal signs when the effect of the lesion alone is virtually non-existent.

Alterations in the balance between several putative neurotransmitters have been reported to occur during morphine withdrawal (Merali et al., 1974). These investigators have shown alterations in the cholinergic and dopaminergic mechanism of the striatum during chronic morphine treatment and its subsequent withdrawal. Dopaminergic nigro-striatal fibers are known to influence the metabolism of acetylcholine in the neostriatum. Destruction of this tract results in an exaggerated response in neostriatal cholinergic neurons to apomorphine (Fibiger and Grewaal, 1974). A complex interaction between morphine induced changes in striatal dopamine and acetylcholine and those changes induced by nigro-striatal lesioning may be in part responsible for the enhanced withdrawal symptoms observed in nigro-striatal lesioned subjects. Serotonin has also been implicated in the pharmacological actions of morphine and in the phenomena of narcotic withdrawal (Shen et al., 1970). Recently it has been shown that nigro-striatal fibers are functionally connected with a serotonergic system (Cools et al., 1974). It is thus possible that the nigro-striatal lesion induced increases in withdrawal symptoms are the result of alterations in serotonergic and dopaminergic neuronal systems.

The intense morphine withdrawal aggression observed in response to social grouping 72 hours after the termination of morphine injections was reduced by the production of

partial nigro-striatal lesion prior to the production of dependence. Similarly, when lesions of the nigro-striatal tract were made after the terminal dose of morphine there was a general decrease in the aggressive response of these animals. One notable exception occurred when a small (1mA 15 sec.) lesion was placed in rats dependent upon 200 mg/kg/day morphine after the last injection. It should be noted that these results are of a preliminary nature and further investigation is needed. These results are thus in agreement with those of Gianutsos et al. (1973, 1974b), who have shown that nigro-striatal lesioning blocked morphine withdrawal aggression in thirty-day abstinent rats. It has been previously hypothesized that social aggression during acute withdrawal was caused by the hyperactivity of dopaminergic receptors which have become supersensitized during chronic morphine administration (Lal et al., 1971; Lal and Puri, 1972; Puri and Lal, 1973). The decrease in aggression appears to result from the degeneration of dopamine neurons produced by the lesion, thus leaving little or no transmitter to be released onto supersensitized receptors.

Destruction of the medial fore brain bundle in morphine dependent rats resulted in increased ptosis, temperature and weight loss. Although wet shakes were seen to increase at both 48 hours and 72 hours, the increase was significant only at 48 hours. Seventy-two hour withdrawal ptosis, weight and temperature loss were significantly increased when lesions were produced during the withdrawal period. Wet shakes

were not affected by these 48 hour lesions. These experiments with the medial fore brain bundle suggest a role for nor-adrenergic and serotonergic mechanisms in narcotic withdrawal, especially ptosis, weight and temperature loss. The involvement of the medial fore brain bundle in narcotic withdrawal has been previously suggested (Glick et al., 1973; Herz et al., 1974). It appears that wet shakes are more dependent upon dopaminergic mechanisms, since medial fore brain bundle lesions were effected in significantly increasing wet shakes at only one time interval. A role for this noradren-
ergic neuronal system in wet shakes can not be totally ruled out.

VI. DISCUSSION

Electrolytic lesions of the nigro-striatal dopamine pathway resulted in decreased striatal dopamine, loss of body weight and increased some symptoms of morphine withdrawal.

Increased levels of striatal dopamine were seen 24 hours after both small (1mA 15 sec) and large (2mA 30 sec) lesions of the nigro-striatal tract. At four and seven days after small (1mA 15 sec) lesions the level of striatal dopamine was significantly reduced. A similar reduction was observed four days following a large (2mA 30 sec) nigro-striatal lesion. These results correspond well with those of other investigators (Faull et al., 1969; Anden et al., 1972; Agid et al., 1974).

Lesioning of the nigro-striatal pathway resulted in aphagia and adipsia, indicated by a loss of body weight. The histological examination of the lesion site and the decrease in corpus striatal dopamine produced by the lesion indicates that the extent of weight loss was dependent upon the amount of nigro-striatal damage. Administration of haloperidol or alpha-methyl-para-tyrosine prior to the production of small (1mA 15 sec) nigro-striatal lesions lessened the loss in body weight produced by the lesion. Lethality and the loss of body weight were reduced in those animals who were pretreated with haloperidol and morphine

prior to receiving a large (2mA 30 sec) nigro-striatal lesion. An increase in receptor sensitivity, regenerative sprouting from transected axons and an increase in catecholamine turnover are several possible mechanisms which may be involved in the recovery of body weight following nigro-striatal lesioning.

Morphine withdrawal wet shakes, ptosis, weight loss and hypothermia were increased when nigro-striatal lesions were made either prior to or following the production of morphine dependence. Similar changes were observed when the lesion was made during the withdrawal period. Apomorphine effectively induced withdrawal wet shakes in both intact and nigro-striatal lesioned subjects. Several mechanisms which may be responsible for the enhanced withdrawal symptoms in these lesioned subjects are an increased denervation supersensitivity, changes in receptor activity and alterations in the balance between several putative neurotransmitters. Noradrenergic mechanism also appears to be involved in the narcotic withdrawal syndrome since destruction of the medial fore brain bundle in morphine dependent rats resulted in increased ptosis, weight loss and hypothermia.

This study suggests a role for nigro-striatal fibers in the regulation of body weight and some of the symptomology of morphine withdrawal. An interaction between the nigro-striatal system and drugs effect dopamine receptors is indicated by the pharmacological modification of lesion induced weight loss. The increased morphine withdrawal

wet shakes, ptosis, hypothermia, weight loss and decreased aggression further suggest such an interaction. The supersensitivity of dopamine receptors may be a useful concept in explaining such interactions.

VII. SUMMARY AND CONCLUSIONS

Lesions of the nigro-striatal dopamine neurons system result in aphagia and adipsia. The extent of weight loss in the lesioned animals was dependent upon the amount of nigro-striatal damage. A denervation supersensitivity of striatal dopamine receptors is viewed as being in part responsible for the recovery of nutritive function following nigro-striatal destruction. Administration of drugs capable of producing supersensitivity at central dopamine receptors, alpha-methyl-para-tyrosine, haloperidol and morphine, have been found to facilitate recovery from the lesion effects.

The symptoms of morphine withdrawal were intensified when the nigro-striatal pathway was destroyed prior to or following the production of dependence. Withdrawal wet shakes, ptosis, weight loss and temperature loss were seen to significantly increase. Apomorphine produced a significant decrease in wet shakes in both lesioned and non-lesioned dependent rats. These results suggest that withdrawal wet shakes are dependent upon dopaminergic mechanism. A denervation supersensitivity mechanism, changes in receptor activity and alterations in the balance between several putative neurotransmitters are mechanisms which may be useful in explaining the increased withdrawal phenomena.

Nigro-striatal destruction was found to have decreased the intensity of seventy-two hour withdrawal aggression.

Noradrenergic mechanisms also appear to be involved in the narcotic withdrawal syndrome. The symptoms of ptosis, weight and temperature loss appear to be at least in part dependent upon the activity and functional integrity of the medial fore brain bundle.

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