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## MECHANISM AND CHARACTERISTICS OF DRUG-INDUCED AGGRESSION

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MECHANISM AND CHARACTERISTICS OF  
DRUG-INDUCED AGGRESSION

BY

GERALD GIANUTSOS

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE  
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DOCTOR OF PHILOSOPHY DISSERTATION

OF

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1975

**MECHANISM OF DRUG INDUCED AGGRESSION**

#### ABSTRACT

Gerald Gianutsos, Ph.D.: Mechanism and Characteristics of Drug-Induced Aggression.

This research investigated the pharmacological alteration of drug-induced aggression in naive, morphine-dependent and chronically haloperidol treated rats. Naive rats were treated with apomorphine (1.25 - 20 mg/Kg) and aggregated in groups of four for aggression. It was determined that 20 mg/Kg of apomorphine produced maximal aggression, as measured by number of attacks, duration of aggressive posturing and number of vocalizations. Doses less than 5 mg/Kg were without any effect. The neuroleptics, haloperidol and oxyperomide, the narcotic, morphine, and the cholinergic agonist, pilocarpine all reduced apomorphine-induced aggression in a dose-dependent manner. Centrally acting anti-cholinergic drugs partially reversed the blockade of aggression produced by all the drugs except morphine which was preferentially antagonized by naloxone, a narcotic antagonist. Aggression was elicited by sub-threshold doses of apomorphine when combined with clonidine; large doses of dexetimide, an anti-cholinergic; or when administered three days after an injection of p-chloroamphetamine (12 mg/Kg). None of these drugs were capable of eliciting aggression by themselves. Amphetamine potentiated the apomorphine-induced aggression, but

failed to elicit aggression with sub-threshold doses of apomorphine unless combined with cyproheptadine.

In morphine-dependent rats, aggression was observed during withdrawal produced by withholding of injections, but not in withdrawal induced by naloxone. The aggression - which was observed at 72 hours and at 30 days of withdrawal - was reduced by morphine, haloperidol or by lesions of the dopaminergic nigro-striatal bundle, and enhanced by small doses of apomorphine. Apomorphine was also capable of eliciting aggression when administered at four hours of withdrawal, with or without naloxone. The 72 hour withdrawal aggression was similar to apomorphine-induced aggression in naive rats since it was dose-dependently increased by dexetimide or clonidine and decreased by pilocarpine. However, in contrast to the effect in naive rats, anticholinergic drugs failed to reverse the blockade of aggression produced by haloperidol in morphine dependent rats.

Following chronic treatment with haloperidol, rats demonstrated signs of dopaminergic supersensitivity as shown by enhanced apomorphine- or amphetamine-induced stereotypy, increased spontaneous hyperactivity and a shift to the left of the dose-response curve for amphetamine stimulation and for apomorphine-induced reduction in striatal dopamine turnover. In addition, the rats exhibited an increased sensitivity to the stimulation of activity produced by dexetimide and a decrease in the depression of activity produced by pilocarpine. Although there was no spontaneous aggression after discontinuation of chronic halperidol, the threshold

dose of apomorphine required to elicit aggression was dramatically reduced. Amphetamine failed to produce aggression after chronic haloperidol, in contrast to the effect normally seen after chronic morphine.

The results of this study demonstrated the requirement of central dopaminergic stimulation for drug-induced aggression, and suggested that the aggression was antagonized by the activity of acetylcholine and serotonin and possibly facilitated by norepinephrine. In addition, it suggested that morphine and haloperidol produce an anti-aggression action by different mechanisms, possibly involving a cholinergic component in the case of haloperidol. Finally, the research provided evidence that the dopaminergic supersensitivity following chronic treatment with morphine may be qualitatively or quantitatively different from the supersensitivity following chronic treatment with haloperidol, since spontaneous and amphetamine-stimulated aggression are noted only in the former case. It was proposed that morphine interferes with cholinergic and/or serotonergic compensatory mechanisms and that these contribute to the aggression.

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## I. INTRODUCTION

Drugs are capable of producing a variety of behavioral changes in laboratory animals. Under the appropriate conditions, drugs can elicit aggressive behavior. Over the past several years, aggression has been reported after the administration of several different types of drugs, most notably, apomorphine (Senault, 1970) and amphetamine (Chance, 1948). In addition, aggression has been reported during narcotic withdrawal (Boshka et al., 1966; Lal et al., 1971), which is intensified by treatment with either apomorphine or amphetamine (Puri and Lal, 1973a).

The mechanism underlying drug-induced aggression is not well established. Apomorphine is believed to directly stimulate dopamine receptors in the brain (Ernst, 1967; Anden et al., 1967). Amphetamine is believed to release both norepinephrine (Carlsson et al., 1966; Carr and Moore, 1969) and dopamine (Besson et al., 1971; Chiueh and Moore, 1973), but its behavioral effects are generally regarded as the result of dopaminergic stimulation (Van Rossum and Hurkmans, 1964; Costa et al., 1972; Thornburg and Moore, 1973a). Therefore, a working hypothesis may be formulated which states

that a hyperstimulation of dopamine neuronal systems may be responsible for aggression. A study of the types of drugs which both increase and decrease the degree of aggression may provide some insight into the mechanisms involved in this type of behavior and a method for comparing different types of drugs.

Haloperidol and morphine share a number of behavioral effects (see Lal et al., 1975a), including an anti-aggressive action in fighting elicited by shock (Lal et al., 1975a), DOPA + amphetamine (Lal et al., 1972b) or morphine withdrawal (Puri and Lal, 1973a). The two drugs also both produce an acceleration of dopamine turnover (Puri et al., 1973). Yet, there are obvious differences between the two drugs.

Dopamine's behavioral effects are modulated by activity of other neurotransmitters. For instance, amphetamine induced stereotypy is decreased by cholinergic (Arnfred and Randrup, 1968) or serotonergic (Weiner et al., 1973) activity. These types of interactions are rarely studied in drug-induced aggression.

The objectives of this research, therefore, are three-fold. First, the research will examine the role of brain dopamine in drug-induced aggression. Secondly, it will investigate the interaction between dopamine and acetylcholine and serotonin in this behavior, and thirdly, it will compare and contrast haloperidol and morphine after both acute and chronic treatment.

The significance of this research is manifold. The primary significance will be the determination of the role of brain dopamine in the expression of drug-induced aggression.

Secondly, this research will provide information relevant to the interaction between dopamine and acetylcholine and serotonin in the CNS, especially in the control of drug-induced aggression.

Thirdly, this research will examine similarities and differences between morphine and haloperidol after both acute and chronic treatment. Since only the former produces physical dependence (though both share many behavioral actions, see above), this research may provide information relevant to drug addiction.

Lastly, this research may have practical significance by serving as a potential model for schizophrenia research. It has been suggested that schizophrenia may be due to a hyperactivity of dopamine neuronal systems in the CNS (Klawans et al., 1972a; Matthysse, 1973). It is proposed here, that a similar hyperactivity may be the cause underlying the drug-induced aggression in rodents. If so, the drug-induced aggression may serve as a useful tool for studying brain changes which may be related to schizophrenia and serve as a test for screening new potential therapeutic agents.



## II. LITERATURE SURVEY

### A. AGGRESSION

#### 1) General Aspects

Violence is a well recognized component of human society. However, fighting is not restricted to humans, but is found in a wide variety of animals. Because of the ubiquity of this behavior, it has long attracted the attention of psychologists, psychiatrists, ethologists, sociologists and other investigators in the behavioral sciences. In recent years, it has also been studied by pharmacologists who have used aggression to provide information on the complex workings of the brain, to gain insight into the actions of centrally acting drugs and to attempt to rationally develop drugs for the treatment of psychopathological disorders.

Aggression has been defined by Lorenz (1966) as "the fighting instinct in beast and man which is directed against members of the same species." This behavior, in the view of Valzelli (1967), is directed towards removing or overcoming what is menacing the physical or psychologi-

cal integrity of the organism. Aggression is such a widespread phenomenon, that Scott (1958) concluded that it is not an accident of evolution but rather a necessary and useful part of an animal's life. Aggression is necessary, Lorenz (1966) concluded, to establish territoriality, for the selection of the strongest member of a species, and for the defense of the young.

Collias (1944) investigated the role of aggression in the ecological pattern of living systems and observed that a great deal of fighting in vertebrates is associated with territoriality. In addition, he observed that fighting in animal groups tends to become organized into social dominance hierarchies which has the net effect of greatly reducing the amount of fighting and that one of the major physiological factors connected with fighting in vertebrates is the male sex hormone.

Considerable attention has been focused on further delineating the variables underlying aggressive behavior. The physiological mechanisms responsible for aggression are very complex and involve interactions between neural, hormonal and sensory factors, as well as equally complex psychological factors. For example, it is believed that aggression is strongly stimulus bound. As an illustration, it is known that rats will kill mice, but not rat pups; male mice will attack other males, but will not attack females; attacks precipitated by stimulation of the

hypothalamus of the cat will be directed at a live rat, but not at a stuffed rat (see Moyer, 1968). Furthermore, it is well recognized that animals respond to postures of their opponents which results in inhibition of aggression. In addition, other psychological factors such as success at fighting influence aggression. Successful, experienced animals are much more likely to fight than inexperienced ones or animals which have lost a battle (Scott, 1962). Thus it is clear that aggression is an extremely complex behavior and any hypothesis which purports to explain aggressive behavior would have to be equally complex.

Moyer (1968) has classified aggression into seven categories. These are predatory, inter-male, fear-induced, irritable, territorial defense, maternal and instrumental. These types of aggression were differentiated on the basis of stimulus situations which elicit the behavior. Moyer pointed out that different neuroanatomical loci, neural mechanisms and topographical patterns may accompany the different types of aggression, so that a global explanation encompassing all types of aggression may be an insurmountable task. Therefore, it is not surprising that a drug may affect different types of aggression in different ways. Some of these methods used to study the effects of drugs on aggression are presented in the next section.

## 2) Models of Aggression

There is a startling variety of methods by which aggression may be studied in the laboratory. The most widely used procedures are aggression produced by a painful stimulation (Tedeschi et al., 1959; Ulrich and Azrin, 1962), prolonged social isolation of mice (Yen et al., 1959) or the administration of drugs. Some other frequently utilized models for the study of aggression include mouse killing (muricide) by rats (Karli, 1956); frog killing (rana-cide) by rats (Bernstein and Moyer, 1970); hyperemotionality induced by lesions of the septum (Brady and Nauta, 1953); rage responses following intracerebral injections of chemicals (see Myers, 1975); aggression during extinction of an operant task (Azrin et al., 1966); and the introduction of a strange rat into the residence of another (Miczek, 1974). It is probable that the models each have a different physiological basis. The characteristics of the most popular models will be briefly described.

In the isolation paradigm, male mice are housed singly for a period of at least three weeks (Yen et al., 1959). The aggression occurs spontaneously when the isolated mouse is exposed to another mouse. The mice become progressively more aggressive with increasing duration of isolation and progressively less aggressive as the size of the group in which they live increases (Welch and Welch, 1970). The isolated mice are more sensitive to the effects of CNS

stimulants and less sensitive to the effects of CNS depressants (Lal et al., 1972a). This type of aggression has been extensively utilized for the testing of drugs and is blocked by a wide variety of pharmacological substances (Janssen et al., 1960; Valzelli, 1967) apparently with little selectivity. The isolation reportedly produces a decrease in the turnover of brain serotonin and norepinephrine and an increase in the turnover of brain dopamine (Valzelli, 1970).

In pain-induced aggression, two animals are enclosed in an area from which they cannot escape and a painful stimulus, usually an electric shock, is delivered to the animals. The aggression occurs in females as well as males, but is not directed towards inanimate objects in the case of rats (Ulrich and Azrin, 1962). This procedure has also been used for screening drugs (Tedeschi et al., 1959). Pain-induced aggression appears to be primarily related to the activity of brain catecholamines, especially norepinephrine (Eichelman, 1973).

Pharmacological investigation of aggression may also be carried out in fighting that occurs as a result of the prior administration of a drug. Several types of drugs are capable of producing aggression in laboratory animals, especially apomorphine (Senault, 1970) and amphetamines (Lal et al., 1970; Hasselager et al., 1972). Aggression also occurs during withdrawal following chronic treatment with narcotics (Boshka et al., 1966; Lal et al., 1971). The apomorphine-induced and morphine withdrawal aggression

will be treated more fully below. The drug-induced aggression paradigm appears to be related to dopaminergic activity in the CNS

a) Apomorphine-Induced Aggression- Large doses of apomorphine, a dopamine receptor agonist (Anden et al., 1967), produce aggression in rats (Senault, 1970; McKenzie, 1971). The aggression is directed only towards a similarly treated rat and is not observed when a non-treated rat or a mouse is put into contact with an apomorphine treated rat (McKenzie, 1971). The aggression occurs with increased frequency in older (84 day old) rats and is absent in rats less than 49 days of age (McKenzie, 1971).

This effect is seen only in male rats (McKenzie, 1971). Castration or hypophysectomy decreases, while large doses of testosterone increase the degree of aggression (Senault, 1972).

When lesions are made in the amygdala or the substantia nigra of rats, apomorphine-induced aggression is reduced. In contrast, lesions which destroy the septum or olfactory bulb enhance the degree of aggression but fail to produce aggression in normally non-aggressive rats (Senault, 1973).

The effect of various drugs on apomorphine-induced aggression have been studied. Neuroleptics and anxiolytics reduce the degree of aggression (Senault, 1970) as do the alpha-adrenergic blocker, phenoxybenzamine (Senault, 1974); the beta-adrenergic blocker, propranolol (Butter-

worth et al., 1975); and the anticholinergic, atropine (Senault, 1970; Dlabac, 1973). A slight decrease in the aggression is produced by 5-hydroxytryptophan (Senault, 1974). The aggression is increased by L-DOPA, MAO inhibitors (Senault, 1974), reserpine (McKenzie, 1971) and to some extent by clonidine (Senault, 1974; Butterworth et al., 1975). A sensitization to the effect of apomorphine has been shown following withdrawal from narcotics (Puri and Lal, 1973a) or treatment with 6-hydroxydopamine (Eichelman and Thoa, 1973). It has been reported that little if any effect on apomorphine-induced aggression is produced by antidepressants (Dlabac, 1973), PCPA or alpha-m-dopa (Senault, 1974).

Lastly, it has been noted that ET-495, another drug believed to stimulate dopamine receptors (Corrodi et al., 1972) also produces aggression (Senault, 1974).

b) Morphine Withdrawal Aggression - Rats can be made dependent on narcotics and will exhibit a number of behavioral changes during withdrawal from these drugs (Martin et al., 1963). Dependent rats undergoing withdrawal from morphine are hyperirritable and, when placed together, will show vigorous fighting (Boshka et al., 1966). The aggression is blocked by continued treatment with morphine, but emerges when drug administration is discontinued. The aggression reaches its maximum approximately three days after withdrawal has been initiated (Thor et al., 1970; Puri and Lal, 1973a). The fighting consists of vocaliza-

tions, aggressive postures, biting and other forms of attack and often leads to severe injury or death of some of the rats (Lal, 1975). Abstinence from other narcotics, such as etonitazene (Florea and Thor, 1968), methadone (Singh, 1973) or fentanyl (Lal et al., 1975b) also results in withdrawal aggression, but spontaneous aggression is not noted after chronic treatment with amphetamine, alcohol or barbiturates (Puri and Lal, 1974b). Morphine withdrawal aggression has also been reported in mice (Weissman, 1973) and guinea pigs (Goldstein and Schulz, 1973). Rage and difficulty in handling has also been reported during narcotic withdrawal in monkeys (Seevers and Deneau, 1963).

The aggression differs from natural aggression in that posturing by the subordinate animal which usually leads to cessation of aggression, does not inhibit fighting during withdrawal, and a withdrawn rat will attack a dead rat (Crabtree and Moyer, 1973). Some aggression, although to a lesser degree, is shown by females in morphine withdrawal (Crabtree and Moyer, 1973). Older rats are more responsive than younger ones (Davis and Khalsa, 1971).

The aggression occurs spontaneously during withdrawal but can be markedly increased by directly (Puri and Lal, 1973a) or indirectly (Thor, 1969; Lal et al., 1971) acting dopamine receptor stimulants. These observations first suggested that dopamine supersensitivity develops during narcotic addiction (Puri and Lal, 1973a). Small doses of d- or l-amphetamine potentiate the aggression and are near-



ly equipotent in this regard (Lal et al., 1971). Hydroxyamphetamine, which has only a peripheral action, does not enhance the aggression (Lal et al., 1971). Similarly, the aggression is markedly enhanced by small doses of apomorphine or L-DOPA and is decreased by dopamine receptor blockade by haloperidol or by inhibition of dopamine synthesis by alpha-methyl-p-tyrosine (Puri and Lal, 1973a). Apomorphine also induces aggression in morphine dependent mice (Iorio et al., 1975). These results strongly suggest the involvement of brain dopamine in morphine addiction and withdrawal. In addition, morphine withdrawal aggression is also enhanced by extracts of Cannabis sativa (Carlini and Gonzalez, 1972).

## B. DOPAMINE IN THE CENTRAL NERVOUS SYSTEM

### 1) Dopamine Pathways

Dopamine, like other central neurotransmitters, has an uneven distribution in the CNS. The discovery of high levels of dopamine in the basal ganglia by Bertler and Rosengren (1959) gave rise to the idea that dopamine has a function of its own, independent of being a precursor of norepinephrine. Dopamine is distributed in the basal ganglia, the limbic system, the hypothalamus and the cortex. The existence of dopamine fiber pathways in the CNS has been demonstrated through studies employing lesions, stimulating electrodes, biochemical and histochemical techniques. Based upon these studies, the following dopaminergic path-

ways have been described:

- a) Nigro-neostriatal Dopamine neurons
- b) Mesolimbic Dopamine neurons
- c) Tubero-infundibular Dopamine neurons

a) Nigro-Neostriatal Dopamine Neurons - The nigro-neostriatal system seems to originate mainly in the pars compacta of the substantia nigra (Anden et al., 1964). Support for this comes from fluorescence microscopy and the high dopamine content observed in this area (Hornykiewicz, 1966). Unilateral pars compacta lesions result in a 60 per cent lowering of the dopamine level in the corpus striatum of the operated side when compared with the unoperated side (Faull and Laverty, 1969). The caudate nucleus and putamen show 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 et al., 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 system (Fuxe et al., 1970). Recently, studies after the removal of the nucleus caudatus putamen suggest that the cell bodies of the ventrolateral part of the midbrain tegmentum belong to this uncrossed neuron system, in as much 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 medially and dorso-medial to the ventral part of the crus cerebri. At the level of the posterior part of the median eminence, the bundle turns to the ventrolateral part of the crus cerebri and enters and diverges into the retrolenticular 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 investigations 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  $1.2 \times 10^{-10}$  grams of dopamine per neuron with terminals containing 50 times more dopamine than the cell bodies. There are approximately 500,000 varicosities in the terminals, each with an average diameter of 0.4u, containing  $2.5 \times 10^{-16}$  grams of dopamine and has a dopamine concentration of around 8000 ug/gr wet weight. One dopamine cell body of the substantia nigra, with a mean diameter of 30u, contains  $2.5 \times 10^{-18}$  grams and has a dopamine concentration of from 60 to 200 ug/gr (Anden et al., 1966d).

These data 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-striatal pathway. Bloom and coworkers (1965) demonstrated that dopamine has primarily an inhibitory action when applied microiontophoretically to caudate neurons. Most of the neurons that respond to dopamine are inhibited by exogenous dopamine applied to the caudate nucleus of anesthetized and decerebrated cats (McLellan and York, 1967; York, 1970). Following the application of dopamine iontophoretically from multibarrel micropipettes near caudate cells, the rate of discharge of 50 to 60 per cent of these neurons is depressed, while the spike rate of approximately 10 per cent of the cells is facilitated. Electrical stimulation of the substantia nigra evokes depressant and facilitatory responses from individually recorded caudate neurons (Connor, 1968; 1970).

b) Meso Limbic 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 interstitiata striae terminalis and in the nucleus amygdaloideus centralis (Anden et al., 1966b). These areas have been shown to contain large quantities of dopamine which is

not associated with norepinephrine synthesis (Koslow et al., 1974). A dopamine sensitive adenylate cyclase has also been found in the tuberculum olfactorium, nucleus accumbens and amygdala (Clement-Cormier et al., 1974). These cyclase systems are considered to resemble the "dopamine receptor" (Keibabian et al., 1972). Dopaminergic fibers of this system run medially to the nigro-striatal dopamine fibers, whereas cranially they lie mainly ventral to the nigro-striatal system (Anden et al., 1966b).

The mesolimbic dopamine neurons probably contain less dopamine than do nigro-striatal neurons. This is probably because the terminal network of each mesolimbic neuron is much smaller than that of the nigro-neostriatal dopamine neurons (Anden et al., 1966c; Fuxe et al., 1970).

c) Tubero-Infundibular Dopamine Neurons - The tubero-infundibular dopamine neurons have their cell bodies mainly localized in 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 median 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 median 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 tubero-infundibular dopamine in neurons of castrated rats, resulting in increased release of dopamine in the area (Fuxe et al., 1967; 1969). The blockade of ovulation by synthetic estrogens and their derivatives may at least be partly mediated via activation of this neuron system (Fuxe and Hokfelt, 1969). This system is also highly sensitive to prolactin, which markedly increases the turnover of dopamine in tubero-infundibular neurons (Fuxe and Hokfelt, 1969).

d) Cortical Dopamine Neurons - In addition to the well defined tracts described above, large quantities of dopamine have also been found in the cortex of various species (Bertler and Rosengren, 1959). It appears that most of the dopamine found in the cortex of the rat is not localized in noradrenergic terminals. Lesions of the dorsal noradrenergic system or combined lesions of the dorsal and

ventral noradrenergic systems, which both significantly decrease cortical levels of norepinephrine, did not induce a parallel reduction of 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 provided by the fact that cortical synaptosomes have the ability to synthesize <sup>3</sup>H-dopamine from <sup>3</sup>H tyrosine (Thierry et al., 1973a). The destruction of ascending noradrenergic pathways which abolishes the in vitro synthesis of <sup>3</sup>H-norepinephrine did not affect the synthesis of <sup>3</sup>H-dopamine (Thierry et al., 1973a). In addition, a specific dopamine reuptake process has been demonstrated in the cerebral cortex of normal rats and in rats whose ascending noradrenergic pathways have been selectively destroyed (Tassin et al., 1974). The dopamine cells in the cortex have recently been visualized by histofluorescence techniques (Hokfelt et al., 1974). The dopamine was shown to be distributed throughout the limbic cortex, but was not present in the neocortex (Hokfelt et al., 1974). Part of the dopamine terminals in the cortex originate in the A9 and A10 areas of the substantia nigra (Lindvall and Bjorklund, 1974). A dopamine-sensitive adenylate cyclase has also been found in the cortex (Von Hun- gen and Roberts, 1973).

## 2) Dopamine Release and Turnover, in the CNS

Using a push-pull cannula, McLennan (1964) demonstrated the resting release of dopamine from the caudate nucleus, which was estimated to occur at the rate of 1ng/min. Electrical stimulation of the substantia nigra produced an increase in dopamine output in the putamen (McLennan, 1965) and an increase in dopamine and its metabolite, homovanillic acid (HVA) in the lateral ventricle of the cat (Portig and Vogt, 1969). A frequency and intensity related release of labelled dopamine from the caudate nucleus was also demonstrated after stimulation of the nigro-striatal bundle or the caudate (Von Voightlander and Moore, 1971a; 1971b). Similar results were shown when caudate dopamine stores were specifically labelled by radioactive tyrosine (Chiueh and Moore, 1973). Electrode placements more medial or lateral in the hypothalamus failed to induce dopamine release (Chiueh and Moore, 1973). These results clearly implicate dopamine as the neurotransmitter in the nigro-striatal pathway.

The release of dopamine can be further enhanced by potassium or by drugs such as amphetamine (Besson et al., 1971). The amphetamine induced release is prevented by lesions of the nigro-striatal bundle (Von Voightlander and Moore, 1973a).

Lesioning of the nigro-striatal bundle produces a gradual degeneration of dopamine terminals and a marked reduction of dopamine levels in the striatum (Poirer and



Sourkes, 1965; Moore et al., 1971; Oltsman and Harvey, 1972). However, inhibition of the firing of the nigro-striatal bundle by lesions or administration of gamma-hydroxybutyrate causes an immediate increase in the levels of caudate dopamine (Walters et al., 1973).

The effect of drugs on the release and utilization of brain dopamine is usually indirectly determined after assessing the drug's effect on dopamine turnover. The turnover of dopamine refers to the overall rate at which the dopamine stores are replaced in a given tissue. The turnover of rat brain dopamine has been estimated to occur at the rate of 0.21 ug/g/hr, with a turnover time of 3.6 hours (Neff and Costa, 1966). The turnover rate can be influenced by a variety of physiological situations and can also be affected by drugs (see section B.4). The overall rate of catecholamine formation is governed by the activity of tyrosine hydroxylase (Levitt et al., 1965), the first enzyme in the synthetic pathway. The synthesis of new dopamine is regulated by a complex process involving end-product inhibition (Udenfriend et al., 1959); activity of post-synaptic receptors (Carlsson and Lindqvist, 1963); activity of pre-synaptic autoreceptors (Roth et al., 1975); and brain tyrosine concentrations (Wurtman et al., 1974). Drugs may interact at any point, but drug effects are generally considered to occur by increasing or decreasing dopamine receptor stimulation. In general, a drug which increases dopamine receptor activity would decrease dopa-

mine turnover, while a drug that decreases dopamine receptor activity would increase dopamine turnover.

### 3) Behavioral Effects of Dopamine

Activity of dopaminergic neurons in the CNS is responsible for the regulation of numerous behaviors. These include motor effects (catalepsy, Parkinsonism, locomotor activity); reward mechanisms (self-administration, self-stimulation); consummatory behaviors; and bizarre behaviors (stereotypy, aggression, schizophrenia). These effects will be briefly described.

a) Motor Functions - The degeneration of the nigro-striatal pathway causes the motor dysfunctions associated with Parkinson's Disease (Hornykiewicz, 1966). This degeneration leads to akinesia, rigidity and tremors in human patients. It is possible to restore normal motor function in Parkinsonian patients by treatment with L-DOPA (Cotzias et al., 1967) which markedly elevates the level of brain dopamine (Everett and Borcharding, 1971). In animals, the deficiency of striatal dopamine leads to an immobility termed catalepsy. This can be accomplished by large lesions of the mesencephalic-diencephalic areas of the brain which disrupt dopamine activity in the neostriatum (Anden et al., 1966e) or by the administration of neuroleptic drugs either systemically (Janssen et al., 1965) or directly into the caudate-putamen or globus pallidus (Costall et al., 1972).

Unilateral lesions of the nigro-striatal pathway or the corpus striatum are known to produce asymmetries in movement and posture (Poirer and Sourkes, 1965; Anden et al., 1966b). 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., 1966b). The rotational behavior is further linked to the differences in dopamine levels on the two sides of the brain by the finding that unilateral striatal injections of dopamine causes the rats to turn or slowly rotate away from the side where dopamine was injected (Ungerstedt, et al., 1969). Spontaneous rotations towards the intact side are seen 24 to 34 hours after a lesion of the nigro-striatal 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, 1971a). 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 towards the lesioned side, apomorphine causes it to rotate towards the intact side (Ungerstedt, 1971a).

Intrastriatal injections of dopamine produce motor hyperactivity in rats (Benkert and Kohler, 1972). Similarly, large doses of L-DOPA combined with a peripheral aromatic acid decarboxylase inhibitor produce an increase

in spontaneous locomotor activity which is associated with a seven-fold increase in brain dopamine levels, while norepinephrine remains unchanged (Butcher et al., 1972).

In addition, the locomotor stimulation produced by amphetamine is believed to be due to release of dopamine (Van Rossum and Hurkmans, 1964; Thornburg and Moore, 1973a; Hollister et al., 1974). Depletion of brain norepinephrine by inhibition of dopamine-beta-hydroxylase (Thornburg and Moore, 1973a) or treatment with 6-hydroxydopamine (Hollister et al., 1974) failed to alter the amphetamine-induced stimulation, while selective dopamine depletion did reduce the amphetamine effect.

b) Reward Mechanisms - Reward systems are usually thought of as being noradrenergic (Stein, 1968), but recent evidence has suggested a major role for dopamine as well. In addition to the traditional sites of electrode placement, self-stimulation behavior may also be maintained from electrodes placed in the substantia nigra (Arbuthnott et al., 1970) or the nucleus accumbens (Phillips et al., 1975), both of which are important dopaminergic sites. Self-stimulation from the lateral hypothalamus is blocked by the dopamine receptor blockers, haloperidol and pimozide (Wauquier and Niemegeers, 1972; Lippa et al., 1973), but not by phentolamine, a noradrenergic receptor blocker, or by the inhibition of norepinephrine synthesis with FLA-63 (Lippa et al., 1973). Furthermore, depletion of dopamine

by 6-hydroxydopamine depressed brain self-stimulation, while similar treatments which deplete norepinephrine were ineffective (Cooper et al., 1974). The action of amphetamine in enhancing self-stimulation was similarly affected by 6-hydroxydopamine treatment (Cooper et al., 1974). Lastly, it was shown that d- and l- amphetamine were equipotent in enhancing self-stimulation from dopaminergic sites while d-amphetamine was more potent in noradrenergic sites (Phillips and Fibiger, 1973; Phillips et al., 1975). These differences in potencies closely paralleled the in vitro effect of amphetamine in releasing norepinephrine or dopamine (Azzaro and Rutledge, 1973).

In addition, the self-administration of amphetamine is apparently dependent on dopaminergic mechanisms since low doses of dopamine receptor blockers selectively increase lever pressing for amphetamine (analogous to the effect seen after decreasing the dose of amphetamine in the self-administered solution), while higher doses produce extinction (Yokel and Wise, 1975). Lastly, apomorphine, a direct dopamine agonist, is also self-administered by rats (Baxter et al., 1974).

c) Consummatory Behavior - Aphagia and adipisia result from the destruction of dopamine fibers coursing through the lateral hypothalamus by electrolytic lesions or 6-hydroxydopamine treatment (Ungerstedt, 1971b). This behavior is correlated with biochemical and histochemical evidence of

decreased dopaminergic input to the striatum (Ungerstedt, 1971b; Zigmond and Stricker, 1972; Glick et al., 1974; Hynes et al., 1975). Similar destruction of noradrenergic fibers failed to produce these deficits in eating and drinking (Ungerstedt, 1971b).

d) Bizarre Behaviors - Injections of drugs which increase brain dopaminergic activity, such as apomorphine, DOPA or amphetamine, result in a compulsive gnawing behavior (Harnack, 1874; Randrup and Udsen, 1963; Ernst, 1965) termed stereotypy. This behavior consists of repetitive sniffing, licking and chewing of the cage. This behavior is also noted after direct injection of apomorphine into the striatum (Smelik and Ernst, 1966) or the olfactory tubercles (McKenzie, 1972).

Various dopaminergic structures have been reported to be the site responsible for stereotypy on the basis of studies involving lesions and local injections. These include the striatum (Smelik and Ernst, 1966; Fog et al., 1970; Ungerstedt et al., 1969); the olfactory tubercles (McKenzie, 1972); and various other portions of the meso- limbic dopamine system (Costall and Naylor, 1973).

It is interesting to note that recent theories concerning the etiology of schizophrenia have suggested that it is the result of dopaminergic hyperactivity in the striatum (Klawans et al., 1972a); the limbic system (Matthysse, 1973); or the cortex (Hokfelt et al., 1974).

The blockade of stereotypy has been used as a screening procedure for detecting anti-psychotic activity of drugs (Janssen et al., 1965).

The hypothesis put forward in this thesis is that drug-induced aggression is also the result of dopaminergic hyperactivity. Apomorphine and amphetamine generally produce aggression in rats and mice, respectively (see Section II. A.2). Apomorphine is believed to directly stimulate dopamine receptors (Anden et al., 1967), while amphetamine releases dopamine (Besson et al., 1971). ET-495, another dopamine receptor stimulant (Corrodi et al., 1972a), also produces aggression in rats (Senault, 1974). Similarly, DOPA administration results in a substantial increase in brain dopamine and elicits fighting, while similar treatments which raise norepinephrine are ineffective in producing fighting (Benkert et al., 1973a). A more detailed discussion of the relevant data is found in Section V.

#### 4) Effects of Drugs on Dopamine Levels and Turnover

This research is primarily concerned with the actions of apomorphine, haloperidol and morphine. All of these drugs have profound effects on brain dopamine, which are important in the determination of their mechanisms of action. These effects will be examined in this section

a) Apomorphine - Apomorphine is believed to directly stimulate dopamine receptors. This conclusion is based on be-

havioral experiments in which apomorphine-induced stereotypy was unaffected by depletion of dopamine by alpha-methyl-p-tyrosine (MPT) (Ernst, 1967) or by 6-hydroxydopamine lesions of the substantia nigra (Price and Fibiger, 1974). These treatments abolished the stereotypy produced by amphetamine (Ernst, 1967; Price and Fibiger, 1974), which is believed to release dopamine from pre-synaptic terminals (Besson et al., 1971; Chiueh and Moore, 1973).

Neurochemically, apomorphine decreases the turnover of striatal dopamine. The drug decreases the incorporation of C<sup>14</sup> tyrosine into C<sup>14</sup> dopamine (Persson, 1970; Nyback etal., 1970), decreases the disappearance of dopamine after MPT administration (Anden et al., 1967; Puri et al., 1973), decreases the rate of formation of homovanillic acid (Roos, 1969) and decreases the accumulation of DOPA after decarboxylase inhibition by NSD 1025 (Koe, 1974). These biochemical changes are blocked by prior administration of neuroleptics (Anden et al., 1967; Anden and Bedard, 1971; Lahti et al., 1972). Recently, apomorphine was also shown to decrease the turnover of dopamine in cortical areas (Scatton et al., 1975).

In experiments where histofluorescence was measured, apomorphine retarded the reduction in dopamine fluorescence which normally occurs after MPT (Anden et al., 1967). Small doses of apomorphine also depress the firing rate of dopamine cells (Bunney et al., 1973a), presumably by a feedback mechanism. These effects are likewise blocked



by haloperidol while the effects on firing rate are unaffected by MPT.

These results have been interpreted as due to a decrease in activity of dopamine neurons as a compensatory mechanism for receptor stimulation by the apomorphine (Anden et al., 1967; Lahti et al., 1972; Puri et al., 1973). Although apomorphine can inhibit tyrosine hydroxylase (Goldstein et al., 1970), the doses required in vitro are substantially higher than necessary to produce functional changes.

b) Haloperidol - Haloperidol, and related anti-psychotic drugs, are believed to block dopamine receptors. The behavioral consequences of this effect are the induction of catalepsy, ptosis and inhibition of operant avoidance and brain self-stimulation (Fielding and Lal, 1974).

Neurochemically, haloperidol increases the turnover of dopamine in the striatum (Anden et al., 1970a), the meso-limbic areas (Anden et al., 1970a) and the cortex (Scatton et al., 1975). This is accomplished without altering the levels of dopamine in the brain (Anden et al., 1966a). There is also no change in brain dopamine levels after chronic haloperidol treatment (Gyorgy et al., 1969; Puri and Lal, 1974a; Hyttel, 1974). Tolerance to the dopamine turnover enhancing effect of haloperidol has been reported after chronic treatment (Asper et al., 1973), but Puri and Lal (1974a) failed to observe this phenomenon, although they did find that morphine dependent rats

were tolerant to the effects of haloperidol. Haloperidol increases the synthesis of labelled dopamine from radioactive tyrosine (Nyback et al., 1970), increases the depletion of brain dopamine after the administration of MPT (Neff and Costa, 1966; Corrodi et al., 1967), increases the rate of accumulation of the dopamine metabolites, homovanillic acid and 3,4-dihydroxyphenylacetic acid (Anden et al., 1966a; Roos, 1965) and 3-methoxytyramine and normetanephrine (after MAO blockade) (Carlsson and Lindqvist, 1963) and increases the accumulation of DOPA after NSD 1025 (Cegrell et al., 1970). The haloperidol induced increase in dopamine depletion following MPT requires intact nigro-striatal fibers for the effect to occur (Anden et al., 1971). In addition, haloperidol reduces the stimulation of adenylyl cyclase caused by dopamine (Clement-Cormier et al., 1974) and increases the firing rate of dopamine cells (Bunney et al., 1973b).

The interpretation of these results has been that haloperidol blocks dopamine receptors and this causes a compensatory activation of dopamine neurons to increase the concentration of dopamine in the synaptic cleft (Carlsson and Lindqvist, 1963). However, haloperidol failed to alter the in vivo efflux of labelled dopamine into the ventricle of the cat (Von Voightlander and Moore, 1973b). It has also been proposed that haloperidol may block the pre-synaptic coupling between

nerve impulses and transmitter release (Seeman and Lee, 1975). Recent evidence suggests that the increased firing of the dopamine neuron produced by the neuroleptics enhances the affinity of tyrosine hydroxylase for its pteridine cofactor (Zivkovic et al., 1974).

c) Morphine - Morphine is also believed to decrease the effect of dopamine in the CNS. Morphine does not alter the steady state level of dopamine in the rat (Gunne et al., 1969; Puri et al., 1973) or cat (Moore et al., 1965), although a transient increase in dopamine levels occurring at two and four hours after drug administration has been reported in the rat (Clouet and Ratner, 1970). Morphine causes a decrease in dopamine levels in the mouse (Takagi and Nakama, 1966; Rethy et al., 1971) and an increase in levels in the monkey (Segal et al., 1972).

The exact relationship between dopamine and narcotic drugs may not be defined by the measurements of dopamine content of nervous tissue since these reflect only gross changes and may not reveal local changes or alterations in the turnover of dopamine.

Morphine increases the conversion of C<sup>14</sup> tyrosine to labelled dopamine in the rat (Clouet and Ratner, 1970; Costa et al., 1973). The C<sup>14</sup> dopamine reaches maximum concentrations in the striatum one hour after morphine administration (Clouet and Ratner, 1970). Morphine also increases the turnover of dopamine in the mouse (Smith

et al., 1970; Loh et al., 1973), an effect which is blocked by the narcotic antagonist naloxone (Smith et al., 1972).

Morphine also causes an increase in the depletion of dopamine in the rat brain after inhibition of catecholamine synthesis by MPT (Gunne et al., 1969; Puri et al., 1973). This depletion was determined by histofluorescence to occur in the dopamine terminals of the striatum, the nucleus accumbens and the olfactory tubercles (Gunne et al., 1969). In addition, morphine increases the concentration of the dopamine metabolites, homovanillic acid and 3,4-dihydroxyphenylacetic acid (Kuschinsky and Hornykiewicz, 1972; Fukui and Takagi, 1972; Kuschinsky, 1973). This effect is also blocked by naloxone, while the corresponding increase in homovanillic acid produced by chlorpromazine is unaffected by naloxone (Kuschinsky and Hornykiewicz, 1972). Lastly, morphine increases cyclic AMP formation by a dopamine sensitive adenylate cyclase (Puri et al., 1975).

The mechanism by which morphine enhances dopamine turnover is not well established. Morphine does not alter either in vitro (Clouet et al., 1973) or in vivo (Cicero et al., 1973) tyrosine hydroxylase, nor does it alter the affinity of tyrosine hydroxylase for its cofactor, substrate or end product (Cicero et al., 1973). Puri and coworkers (1973) proposed that morphine blocks dopamine receptors and the increased turnover reflects a compensatory increase in neuronal activity analagous to the effect of neuroleptics. Consistent with this hypothesis is the

observation that the increased synthesis of new dopamine produced by morphine is associated with an increased release of dopamine into an incubation medium from striatal slices (Gauchy et al., 1973). Methadone has also been proposed to act via post synaptic dopamine receptor blockade (Sasame et al., 1972). Alternatively, it has been suggested that morphine produces a functional deficiency of dopamine by diverting newly synthesized dopamine from storage sites to sites of catabolism (Kuschinsky and Hornykiewicz, 1972).

After chronic treatment with morphine, there is a slight increase in the dopamine content of the brain in rats (Sloan et al., 1963; Johnson et al., 1974), but dopamine levels remain unchanged after prolonged treatment in dogs (Gunne, 1963) or monkeys (Segal et al., 1972). Although most researchers report that a tolerance develops to the effect of morphine on brain dopamine turnover (Gunne et al., 1969; Smith et al., 1972; Costa et al., 1973; Puri and Lal, 1974a), it has also been reported that morphine continues to increase the synthesis of dopamine after chronic treatment (Johnson et al., 1974). This effect may be associated with the increase in tyrosine hydroxylase activity which reportedly occurs during chronic morphine treatment (Reis et al., 1970). However, decreased tyrosine hydroxylase activity has also been reported in rats implanted with morphine pellets (Branchey et al., 1974).

During morphine withdrawal, brain dopamine was decreased in dogs, 72 hours after withdrawal was initiated (Gunne, 1963). In rats, brain dopamine levels appear to remain unchanged during withdrawal (Maynert and Klingman, 1962; Puri and Lal, 1974a), although a decrease in levels, relative to inflated levels during addiction, has been reported (Sloan et al., 1963). In contrast to these results, which were obtained in animals which were withdrawn by withholding of morphine injections, abstinence precipitated by naloxone administration reportedly increases the level of brain dopamine in rats and mice (Iwamoto et al., 1973). The turnover of brain dopamine also appears to remain unchanged during morphine withdrawal (Puri and Lal, 1974a) although decreased dopamine turnover has also been reported in rats (Gunne et al., 1969) and mice (Rosenman and Smith, 1972). In addition, caudate tyrosine hydroxylase activity is reportedly elevated at 48 hours of withdrawal in rats (Reis et al., 1970) and decreased at 36 hours of withdrawal in mice (Marshall and Smith, 1974).

### C. DOPAMINE INTERACTIONS IN THE CNS

The effects of dopamine in the CNS are modulated by activity of other brain neurotransmitters. The complexity of the brain demands that activity in one area will have consequences in another area, so that it is foolhardy to investigate the actions of one transmitter as if it existed

independently. These delicate equilibria are important aspects of the normal functioning of the brain and drug interference with the normal balance of transmitter function may be an essential component of the drug's action. The most widely studied of dopamine's interactions are with acetylcholine and serotonin.

#### 1) Acetylcholine

The concept of an interaction between dopamine (DA) and acetylcholine (ACh) developed from the clinical observations that Parkinsonism could be relieved either by increasing dopamine activity or by decreasing cholinergic activity (Klawans, 1968). Subsequently, considerable behavioral and neurochemical evidence has been gathered to support the idea of a reciprocal balance between ACh and DA.

Catalepsy, which is produced by drugs which decrease dopaminergic activity in the brain, is also produced by cholinergic drugs (Costall et al., 1972), while the neuroleptic-induced catalepsy is reversed by anticholinergics (Morpurgo and Theobald, 1964). Similarly, the anticholinergics enhance, while cholinergics block, the stereotypy produced by dopamine receptor stimulation (Arnfred and Randrup, 1968; Scheel-Kruger, 1970; Klawans et al., 1972b). In addition, anticholinergic drugs are reported to reverse the neuroleptic-induced blockade of methylphenidate stereotypy (Fjalland and Moller-Nielsen, 1974a).

Likewise, the amphetamine-induced locomotor stimulation is enhanced by scopolamine (Fibiger et al., 1970) and inhibited by tremorine (Mennear, 1965)., while the locomotor stimulation produced by anticholinergics is blocked by reducing the synthesis of dopamine (Thornburg and Moore, 1973b).

Neurochemically, dopaminergic stimulation appears to reduce the release of ACh while cholinergic stimulation appears to increase the turnover of DA. Some of the experiments which have demonstrated these effects are summarized here. Dopamine released via stimulation of the nigro-striatal bundle blocks the release of ACh (Stadler et al., 1974). Similarly, apomorphine, amphetamine or L-DOPA increases the level of ACh in the striatum (Sethy and Van Woert, 1974). The increase in ACh levels produced by apomorphine (Guyenet et al., 1975) or ET-495 (Ladinsky et al., is blocked by neuroleptics but is not blocked by alpha-methyl-p-tyrosine (Ladinsky et al., 1974) or lesions of the nigro-striatal bundle (Guyenet et al., 1975), suggesting that the effect is mediated via dopaminergic receptor stimulation. Similarly, neuroleptic drugs increase the release of ACh with a resultant decrease in levels in the striatum (Stadler et al., 1973; Guyenet et al., 1975), and increase ACh turnover (Trabucchi et al., 1974). Promethazine, a phenothiazine related to chlorpromazine which has little or no DA receptor blocking activity, does not alter ACh levels (Stadler et al., 1973). This effect is noted in



the striatum, but not in other dopaminergic areas (Lloyd et al., 1973), nor is it noted in the hippocampus, which receives a rich cholinergic input but does not contain dopamine terminals (Rommelspacher and Kuhar, 1975). These results are poorly correlated with the direct anticholinergic activity of the neuroleptics (Rommelspacher and Kuhar, 1975) again suggesting that the effect is mediated by dopamine. These results have been interpreted to mean that striatal ACh is under an inhibitory influence of DA. However, lesions of the nigro-striatal pathway did not affect striatal ACh levels (Butcher and Butcher, 1974; Guyenet et al., 1975).

In an analagous manner, cholinergic drugs increase striatal dopamine turnover (Corrodi et al., 1967a) and the rate of formation of homovanillic acid (HVA) (Ahtee and Kaariainen, 1974). Similarly, anticholinergics reduce the turnover of striatal dopamine (Bowers and Roth, 1972; Corrodi et al., 1972a) and reduce the concentration of HVA (O'Keefe et al., 1970). However, intraventricular infusion of atropine increases striatal HVA (Bartholini and Pletscher, 1971).

Of considerable interest is the additional interaction between ACh and DA in which anticholinergics have been reported to inhibit the increase in DA turnover produced by the neuroleptics (O'Keefe et al., 1970; Anden, 1972; Bowers and Roth, 1972). It appears that this effect is limited to the neuroleptic induced increase in striatal dopamine turnover, since the increase in turnover in limbic areas is un-

affected by anticholinergics (Anden and Bedard, 1971; Anden, 1972).

## 2) Serotonin

Behavioral evidence supporting an interaction between serotonin and DA has grown in recent years, although the evidence is not as strong as in the case of ACh (previous section). In general, increasing serotonergic activity opposes the effect of dopaminergic stimulation.

Lycke and coworkers (1969) found that depressing the level of serotonin by parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis (Koe and Weissman, 1966), increased the excitation produced by DOPA and resulted in aggression in their mice. Similarly, PCPA enhances the stimulation produced by amphetamine (Mabry and Campbell, 1973), while blocking serotonin receptors enhanced the stimulation produced by apomorphine (Grabowska and Michaluk, 1974). Breese and coworkers (1974) further investigated this relationship and observed that pargyline reduced the stimulant effect of amphetamine and that this was correlated with an increase in serotonin levels. PCPA reversed the inhibition. Similarly, destruction of serotonin neurons with 5,6-dihydroxytryptamine likewise potentiated the amphetamine response. PCPA was unable to alter the inhibition of amphetamine-induced stimulation produced by 6-hydroxydopamine (Breese *et al.*, 1974), suggesting that the serotonin effect is indirect.

Further evidence for an interaction between DA and serotonin has been provided by studies which measured stereotypy produced after DA agonists, but these results have been contradictory. Decreasing serotonergic activity has been reported to increase the stereotypy produced by either amphetamine or apomorphine (Weiner et al., 1973; 1975). However, it has also been reported that decreasing serotonergic stimulation has no effect (Breese et al., 1974) and even decreases the stereotypy (Costall and Naylor, 1975) induced by these DA agonists.

It has also been reported that the catalepsy induced by neuroleptics is antagonized by PCPA or by the destruction of raphe neurons (Kostowski et al., 1972), lending further support to an interaction.

The evidence thus suggests that activation of dopaminergic neurons leads to a compensatory increase in the activity of serotonergic neurons which results in a reduction of the effect of the initial dopamine stimulation (Grabowska and Michaluk, 1974; Cools, 1974).

#### D. DOPAMINERGIC SUPERSENSITIVITY

Supersensitivity refers to the phenomenon in which the amount of a substance required to produce a given biological response is less than normal. This implies a shift in the dose response curve for an agonist to lower agonist concentrations. Such a shift in the dose response curve is considered the major criterion for supersensitivity (Trendelenburg, 1966). Evidence for the concept of supersensitivity was provided by experiments in the peripheral nervous system. In early experiments, it was shown that spinal motor neurons became more reactive to a variety of stimuli following transection of the spinal cord or destruction of sensory nerve fibers (Cannon and Rosenblueth, 1949). Supersensitivity in the periphery is considered to be of two types. In the first type, termed denervation supersensitivity, physical removal of the pre-junctional nerve terminals leads to an enhanced effect of the neurotransmitter due to loss of the re-uptake function of the nerve terminal (Langer and Trendelenburg, 1966). In decentralization supersensitivity, the neurotransmitter fails to reach the target organ and leads to an actual increase in the sensitivity of the receptor (Langer et al., 1967). This type of supersensitivity was demonstrated by the enhanced response of the cat nictitating membrane to norepinephrine following removal of the afferent nerves to the superior cervical ganglion (Langer et al., 1967). The time course of each type of supersensi-

tivity is different, with denervation supersensitivity requiring a shorter period of time to develop. The actual molecular mechanism by which supersensitivity occurs is unknown, but several suggestions have been made. There could be a change in the post-junctional element so that it actually becomes 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); or pre-junctional elements could increase their capacity to deliver transmitter or change their recovery process to repetitive stimulation (Sharpless and Jaffe, 1969). Many of these changes have been demonstrated in disused or denervated peripheral structures.

In the central nervous system, the term supersensitivity is loosely applied to any procedure which results in an exaggerated response to an agonist. This has been studied, in dopaminergic brain areas, after either the destruction of neurons which supposedly synapse with dopamine receptors or after the administration of drugs which decrease the availability of dopamine for its receptors. In both cases, an enhanced response to stimulation by dopamine and related agonists is presumed to occur as a compensatory adaptation to the lack of stimulation as a result of the effect of the lesion or the drug. It should be noted that supersensitivity has been used to explain the development of toler-

ance to and physical dependence on drugs (e. g. Jaffe and Sharpless, 1968; Collier, 1966; Sharpless and Jaffe, 1969; Puri and Lal, 1973a), since withdrawal phenomena seem to represent rebound effects which are opposite in character to those produced by the drug itself. The characteristics of the two methods of producing supersensitivity in the CNS are described below.

#### 1) Lesion Induced Supersensitivity

Intracerebral injections of 6-hydroxydopamine produce a degeneration of catecholamine containing nerve terminals in the CNS (Breese and Traylor, 1970; Uretsky and Iversen, 1970). Following this treatment, rats exhibit a marked increase in locomotor activity in response to L-DOPA (Uretsky and Schoenfeld, 1971) or apomorphine (Schoenfeld and Uretsky, 1972) as well as enhanced apomorphine-induced stereotypy (Iversen and Creese, 1975) and ET-495 induced hypothermia (Reid, 1975). Similarly, destruction of the substantia nigra results in enhancement of apomorphine-induced stereotypy, while the stereotypy produced by amphetamine is blocked (Iversen and Creese, 1975).

Unilateral destruction of dopaminergic nigro-striatal fibers or terminals provides an interesting model for the study of dopaminergic supersensitivity (Ungerstedt, 1971a; Von Voightlander and Moore, 1973c). After such lesions, animals circle away from the side of the lesion in response to apomorphine and towards the side of the lesion in response

to amphetamine, presumably because apomorphine has more of an effect on the lesioned (supersensitive) side. An injection of 6-hydroxydopamine into the striatum, which induces this behavior, markedly decreases the concentration of dopamine but has little effect on norepinephrine or serotonin (Von Voightlander and Moore, 1973c). In addition, the dose response curve for eliciting the turning by apomorphine or L-DOPA gradually shifts to the left over the course of 30 days (Thornburg and Moore, 1975). Similarly, the rate of apomorphine-induced circling is well correlated with the reduction of forebrain dopamine (Thornburg and Moore, 1975).

Following lesions of the substantia nigra, an enhancement of striatal dopamine-stimulated adenylate cyclase has been reported (Mishra et al., 1974) which would provide neurochemical substantiation for the supersensitivity. Furthermore, caffeine and theophylline enhanced the turning response to apomorphine in unilaterally lesioned rats, but failed to alter turning by themselves or that produced by amphetamine (Fuxe and Ungerstedt, 1974) which was interpreted as due to a preferential effect of these compounds on cyclic AMP formed in the lesioned (supersensitive) side. However, it has also been reported that there is no difference in caudate dopamine-stimulated adenylate cyclase after destruction of pre-synaptic nerve terminals by 6-hydroxydopamine (Von Voightlander et al., 1973). Further

evidence for denervation supersensitivity is provided by Fibiger and Grewaal (1974) who found that in rats with unilateral substantia nigra lesions, the effect of apomorphine in increasing striatal acetylcholine levels was greater on the lesioned side compared with the non-lesioned side, although the response to haloperidol was unaffected by the lesion. In addition, it is interesting to note that the dopamine cells which do not degenerate after 6-hydroxydopamine treatment have a higher rate of dopamine turnover than cells on the non-lesioned side (Agid et al., 1973), which may represent another type of compensation for reduced dopamine stimulation on the lesioned side.

## 2) Drug Induced Supersensitivity

Changes in behavior during and after chronic administration of drugs has long been recognized. Boyd (1960) noted a tolerance to the depressant effect of chlorpromazine after 30 weeks of treatment without a cross tolerance to pentobarbital. Furthermore, he noted an increase in locomotor activity during "withdrawal" of the drug. Similarly, Moore (1968) reported a tolerance to the depressant effect of alpha-methyl-p-tyrosine (MPT) during chronic feeding of mice, despite the fact that catecholamine levels remained depressed. Similarly, daily treatment with reserpine led to enhanced locomotor activity when the drug was discontinued which was correlated with an increase in norepinephrine stimulated adenylyl cyclase (Williams and Pirch, 1974).



Subsequently, it was noted that exaggerated responses to dopamine agonist drugs could be elicited following chronic treatment with drugs which would normally block their effect in naive rats. The stereotypy produced by amphetamine, apomorphine or methylphenidate was augmented following the cessation of repeated treatment with neuroleptics in rats (Schelkunov, 1967; Tarsy and Baldessarini, 1973), mice (Fjalland and Moller-Nielsen, 1974b) and guinea-pigs (Klawans and Rubovits, 1972). This effect was also noted following chronic MPT, but was not seen following chronic treatment with the non-neuroleptics, pentobarbital or diazepam (Tarsy and Baldessarini, 1973), which do not affect CNS dopamine. Interestingly, it has been reported that this effect also fails to develop following chronic treatment with the novel neuroleptic, clozapine (Sayers et al., 1975), a drug which does not block apomorphine stereotypy in naive rats (Stille et al., 1971).

Similarly, amphetamine stimulation of locomotor activity was enhanced following chronic MPT, but the stimulation produced by methylphenidate or pipradol, which is not blocked by MPT in naive animals, was unaffected following chronic MPT (Dominic and Moore, 1969). Concomitant administration of L-DOPA along with MPT, which would bypass the inhibition of catecholamine synthesis and prevents the depletion of dopamine produced by the MPT, blocked the development of supersensitivity (Gudelsky et al., 1975).

Recently, it was shown that supersensitivity following chronic haloperidol could also be demonstrated neurophysiologically (Yarbrough, 1975), such that a larger proportion of caudate cells were inhibited by dopamine following chronic haloperidol treatment. However, there is apparently no change in striatal dopamine-sensitive adenylate cyclase activity in mice chronically treated with neuroleptics, although behavioral evidence of supersensitivity was present (Von Voightlander et al., 1975).

Puri and Lal (1973a) proposed that a dopaminergic supersensitivity also develops during chronic treatment with narcotics. This suggestion was based on experiments involving aggression during morphine withdrawal in which normally ineffective doses of apomorphine or amphetamine stimulated the aggression (Lal et al., 1971; Puri and Lal, 1973a). Subsequently, it was also shown that the reduction in striatal dopamine turnover produced by apomorphine could be elicited with ordinarily ineffective doses in morphine withdrawn rats (Puri and Lal, 1973b).

### III. EXPERIMENTAL

#### A. Animals

Male, hooded rats of the Long-Evans strain were obtained from Charles River Breeding Farms, Wilmington, Massachusetts and were used in this study. The rats weighed 275-350 g at the start of experiments involving chronic drug treatment. Rats used for apomorphine-induced aggression weighed 375-475 g. The animals were housed in a room with thermostatically controlled constant temperature (70°F or 21°C  $\pm$  1°C) and an alternating period (12 hours) of light and dark (7 AM to 7 PM, light). They were allowed free access to food and water at all times unless otherwise stated. Rats used for chronic morphine treatment were housed in individual cages; all others were housed in standard colony cages.

#### B. Drugs

Drugs used in this study were: apomorphine hydrochloride (Mallinckrodt Chemical Works), morphine sulfate (Merck & Co.), haloperidol (Janssen Pharmaceutica and McNeil Laboratories), amphetamine sulfate (Smith, Kline & French), pilocarpine hydrochloride (Mallinckrodt Chemical Works), dexetimide (R16470) (Janssen Pharmaceutica), isopropamide (ZR79) (Janssen Pharmaceutica), naloxone hydrochloride (Endo Lab-

oratories), atropine sulfate (Mallinckrodt Chemical Works), pentobarbital sodium (Abbott Laboratories), cyproheptadine (Merck, Sharpe & Dohme), clonidine hydrochloride (Boeringer-Ingelheim) and alpha-methyl-p-tyrosine (MPT) methyl ester hydrochloride (Aldrich Biochemicals). All drugs were freshly prepared and administered by the intraperitoneal route, except in those experiments in which more than two drugs were used. In these, the first drug was administered subcutaneously. All drugs were dissolved in distilled water except haloperidol and oxyperomide which were dissolved in 0.3% tartaric acid and cyproheptadine which was solubilized with the aid of two drops of Tween 20. The doses and pretreatment times for the drugs are shown in Table 1.

### C. Chronic Drug Treatment

#### 1) Morphine Addiction

Rats were made dependent on morphine by injecting them with systematically increasing doses of morphine sulfate three times a day. The starting dose of 15 mg/kg/injection (45 mg/kg/day) was increased by 15 mg/kg/injection daily for nine days until a maintenance dose of 135 mg/kg/injection (405 mg/kg/day) was reached. The rats were maintained at this dose for at least five days. On the last morning, a final injection of 135 mg/kg was administered, after which no further morphine was injected. This was considered zero time of withdrawal. This is the usual addiction schedule

TABLE 1  
DOSE RANGES AND PRE TREATMENT TIMES  
FOR DRUGS USED

Drug	Dose Range (mg/Kg)	Time Waited Before Testing (minutes)
d-amphetamine	0.1 - 16	60
l-amphetamine	2 - 32	60
apomorphine	0.5 - 20	10
atropine	2.5 - 10	30
clonidine	0.06- 0.50	30
cyproheptadine	2 - 8	45
dexetimide	0.32- 20	40
haloperidol	0.16- 10	120
isopropamide	2.5 - 5	30
morphine	5 - 135	40
MPT	100 - 300	240
naloxone	1 - 16	10
oxyperomide	1.25- 5	40
pentabarbital	5 - 20	15
pilocarpine	1.25- 10	15

utilized in this study. In early pilot experiments, other schedules were also employed. In these procedures, injections were given every 12 hours and the terminal dose was 200 mg/kg/day. In the first schedule, a starting dose of 10 mg/kg/injection was increased by 10 mg/kg/injection each day for 10 days. This is referred to as the short, low dose schedule. In the other, the starting dose of 5 mg/kg/injection was increased by 5 mg/kg/injection daily for 20 days. This is referred to as the long, low dose schedule. These treatment schedules are illustrated in Table 2.

## 2) Chronic Haloperidol

Rats were injected with haloperidol twice daily beginning with a dose of 2.5 mg/kg/injection. This dose was increased by 2.5 mg/kg/injection every fifth day until the terminal dose of 10 mg/kg/injection (20 mg/kg/day) was reached. This dose was maintained for four days. On the following morning, a final injection of 10 mg/kg was administered after which no further haloperidol was injected and this was considered as zero time of withdrawal. This schedule is also illustrated in Table 2.

## D. Brain Lesions

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

TABLE 2  
SCHEDULES OF CHRONIC DRUG TREATMENTS

Doseage of mg/Kg/injection (per day)

<u>Day</u>	<u>Morphine Addiction</u>			<u>Haloperidol</u>
	<u>Regular</u>	<u>Short, Low</u>	<u>Long, Low</u>	
1	15 (45)	10 (20)	5 (10)	2.5 (5)
2	30 (90)	20 (40)	10 (20)	2.5 (5)
3	45 (135)	30 (60)	15 (30)	2.5 (5)
4	60 (180)	40 (80)	20 (40)	2.5 (5)
5	75 (225)	50 (100)	25 (50)	5 (10)
6	90 (270)	60 (120)	30 (60)	5 (10)
7	105 (315)	70 (140)	35 (70)	5 (10)
8	120 (360)	80 (160)	40 (80)	5 (10)
9	135 (405)	90 (180)	45 (90)	7.5 (15)
10	135 (405)	100 (200)	50 (100)	7.5 (15)
11	135 (405)	100 (200)	55 (110)	7.5 (15)
12	135 (405)	100 (200)	60 (120)	7.5 (15)
13	135 (405)	100 (100) <sup>a</sup>	65 (130)	10 (20)
14	135 (135) <sup>a</sup>		70 (140)	10 (20)
15			75 (150)	10 (20)
16			80 (160)	10 (20)
17			85 (170)	10 (10) <sup>a</sup>
18			90 (180)	
19			95 (190)	
20-22			100 (200)	
23			100 (100) <sup>a</sup>	
Doses Per Day	3	2	2	2

<sup>a</sup> Last injection of sequence (one morning injection).

ated by a Lesion Producing Device (Stoelting Co. Model 58040) was passed through the electrode placed into the brain with a David Kopf Stereotaxic Instrument. With the dorsal cranium horizontal, the coordinates for the nigrostriatal 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 8.0 mm below the surface of the cranium.

#### E. Measurement of Withdrawal Signs

At selected times after discontinuation of drug treatment, rats were removed from their home cages and placed individually into a novel cage. Signs were measured for 30 minutes. The signs which were noted consisted of "wet shakes," defined as the violent shaking movement of the head or body which resembles movements made by an animal which has been drenched with water; writhing, defined as the dragging of the abdomen along the floor of the cage and drawing in of the abdominal wall (Buckett, 1964) unaccompanied by yawning; ptosis, defined as the drooping of the eyelids to form a slit-like appearance, unaccompanied by sleep; piloerection; diarrhea and teeth chattering. The frequency of "wet shakes" and writhing was noted during the observation session along with the duration of ptosis. The presence or absence of the other signs was noted as present or not present.



In addition, the rat was weighed and his colonic temperature was measured one hour after receiving the final injection of morphine. The temperature was measured using a digital thermistor thermometer (Digitec Model 581C, United Systems Corp.). The rectal probe was inserted 5 cm and maintained in place for one minute before recording the temperature. Immediately prior to the observation session, the rats were again weighed and their temperatures taken. These values were compared with the initial measurement in order to establish the magnitude of change in weight and temperature. The descriptions and measurements of these signs may be found in Gianutsos et al. (1975).

#### F. Aggression

Aggression was measured by placing similarly treated rats in groups of four into a chamber (23 cm X 20 cm X 19 cm) made of Plexiglas with a wire floor. The walls of the chamber were covered with a sound-absorbing rubber material, with the front left uncovered to allow for observations.

The rats were observed for one hour during which time, three parameters of aggression were measured as described in Lal (1975). These were: attacks (including bites), rearing time and vocalizations. An attack was recorded each time one rat bit, violently shoved or leaped upon another animal. Rearing time consisted of the total elapsed time, in seconds, in which two or more rats assumed an aggressive posture in which they stood on their hind paws facing each

other in close proximity with their mouths open and vocalizing or when one rat stood over another while vocalizing. These typical postures are illustrated in Figures 1A and 1B. Vocalizations were monitored via an audio detection relay (Scientific Prototype Model 761-G) which automatically sent a pulse to a counter when a vocalization was made. The relay was modified to detect sounds in the range of 2400-4800 Hz. All three parameters were recorded for six consecutive 10 minute intervals and were summed to arrive at the figures for responses per hour. Each group of rats was treated statistically as one subject.

#### G. Locomotor Activity

At various time intervals after drug treatment (see Table 1), rats were placed individually in clear Plexiglas bins (42 cm X 27 cm X 16 cm) on top of a Selective Activity Meter (Columbus Industries, Model S) for the measurement of locomotor activity. Each meter was enclosed within a separate wooden cabinet equipped with a fan and a house light, both of which were constantly on during the activity measurements.

Each time the rat crossed one of the six electromagnetic sensors on the meter, a count was recorded. The counts were accumulated in a print-counter (Data Pac, LVE/BRS Model No. 421-09) and printed out at 10 minute intervals. The counts for one hour (six consecutive 10 minute intervals) were summed and activity was expressed as counts

FIG 1A--Aggressive posture of tern during  
displayed agonistic reaction. Note that the  
birds are standing on their hind feet in close pro-  
ximity with open mouths.







per hour. All activity measurements were made during the morning.

#### H. Stereotypy

Stereotypy was measured by placing rats into individual cages (24 cm X 18 cm X 17.5 cm) with a wire grid floor and front. The rats were observed during five consecutive five minute observation periods. Each observation period was separated from the next observation by a five minute recess. The rats were observed at 0, 10, 20, 30 and 40 minutes after the session began.

During the observation period, the rats were rated for the intensity of stereotypy according to the five point scale devised by Ernst (1967). The ratings were as follows:

- 0 - No stereotyped movements.
- 1 - Rats walking around cage, sniffing over grid, occasionally licking the wires and putting their nose in the grids.
- 2 - Rats moving around, occasionally biting and gnawing at the wires.
- 3 - Rats restricting their locomotion to a small area and gnawing intensely at the bottom.
- 4 - Rats remaining on the same spot for at least 5 minutes while jerkily gnawing and clinging their teeth around the wires convulsively for longer periods, sometimes interrupted by short intermissions.

### I. Dopamine Turnover

The rate of dopamine turnover was evaluated by measuring the rate of disappearance of dopamine from the corpus striatum after the administration of alpha-methyl-p-tyrosine (MPT) methyl ester hydrochloride (300 mg/Kg), which inhibits the synthesis of the catecholamines (Spector et al., 1965). The animals were deprived of food for 18-24 hours prior to being sacrificed by decapitation. The brains were rapidly removed from the cranium and placed on ice. The lateral ventricles were opened to expose the corpus striatum which was removed from each side and pooled. All manipulations were carried out on ice using ice-chilled equipment. The striata were weighed and homogenized in 3 ml of 0.4 N perchloric acid containing 0.05% sodium metabisulfite. The homogenate was transferred to a centrifuge tube. The homogenizer was rinsed with 1 ml perchloric acid solution which was added to the homogenate and centrifuged at 12,000 X g for 10 minutes in a Servall RC 2B refrigerated centrifuge.

The supernatant from each centrifuge tube was transferred into a large centrifuge tube containing 10 ml of 0.5 M tris buffer (pH 9.5). The resultant pH of the sample was  $8.5 \pm 0.1$ . The samples were allowed to pass through a 9 mm glass column packed with 500 mg of alumina at a rate of 30 drops a minute. The eluate as well as a 5 ml distilled water wash were discarded. The dopamine on the column was then extracted by 4 ml of 0.2 N acetic acid and collected in a graduated centrifuge tube containing 1 ml of



EDTA reagent. A two and a half ml sample was transferred to a test tube and assayed spectrofluorometrically (Carlsson and Waldeck, 1958). To the test tube was added 0.2 ml of iodine solution and shaken. Exactly three minutes later, 0.2 ml alkaline sulfite was added. Tissue blanks were prepared from the residual acetic acid eluate with the addition of alkaline sulfite and iodine reversed. Exactly three minutes after the addition of alkaline sulfite, 0.5 ml of 5N acetic acid was added to each test tube and placed into a bath of boiling water for five minutes. Once they had cooled to room temperature, the concentration of dopamine in each sample was estimated by reading the samples at 370 nm while activating at 320 nm in an Aminco-Bowman Spectrofluorometer. 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 was expressed as micrograms of dopamine per gram of wet tissue. Recovery was found to be 88%. The samples were not corrected for the recovery loss.

The turnover of dopamine was calculated from the rate of striatal dopamine depletion observed two hours after the injection of MPT according to the method of Costa and Neff (1970). The quantity of dopamine in the sample obtained after the MPT was compared with the quantity in the striatum immediately after the MPT injection. The calculation of dopamine turnover was carried out using the URI IBM Systems 370 computer and consisted of solving the equation:

$$\text{Log } DA_t = \text{Log } DA_0 - 0.434 kt$$

where  $DA_0$  is the initial steady state concentration of dopamine,  $DA_t$  is the concentration of dopamine at any time,  $t$ , after the administration of MPT and  $k$  is the fractional rate constant. Multiplication of  $k$  by the initial steady state level of dopamine yields the apparent turnover rate.

### J. Statistics

The following statistical procedures were used in this study: When differences were obvious, no statistical procedure was used. Student's "t" test was employed for comparisons involving two groups. Multiple comparisons were made using the Tukey (b) test except in those cases where all comparisons were made with control, in which case Dunnett's test was used. In one experiment, where the comparison was planned a priori, the Test of Individual Comparisons was utilized. All procedures are described in Winer (1971).

Where data followed a binomial distribution, the Chi Square Test was used. In those instances in which the data was on an ordinal scale, or seriously violated certain assumptions of parametric statistics, the Mann Whitney Test or the Kruskal-Wallis Analysis of Variance was used, depending on the number of groups being tested. These procedures are described in Conover (1971). Multiple comparisons were made in these cases by Dunn's Procedure (Hollander and Wolfe, 1973).

## IV. RESULTS

The results of this investigation are divided into three sections: aggression induced by apomorphine in naive rats; morphine withdrawal aggression; and behaviors following chronic haloperidol.

### A. Apomorphine Induced Aggression in Naive Rats

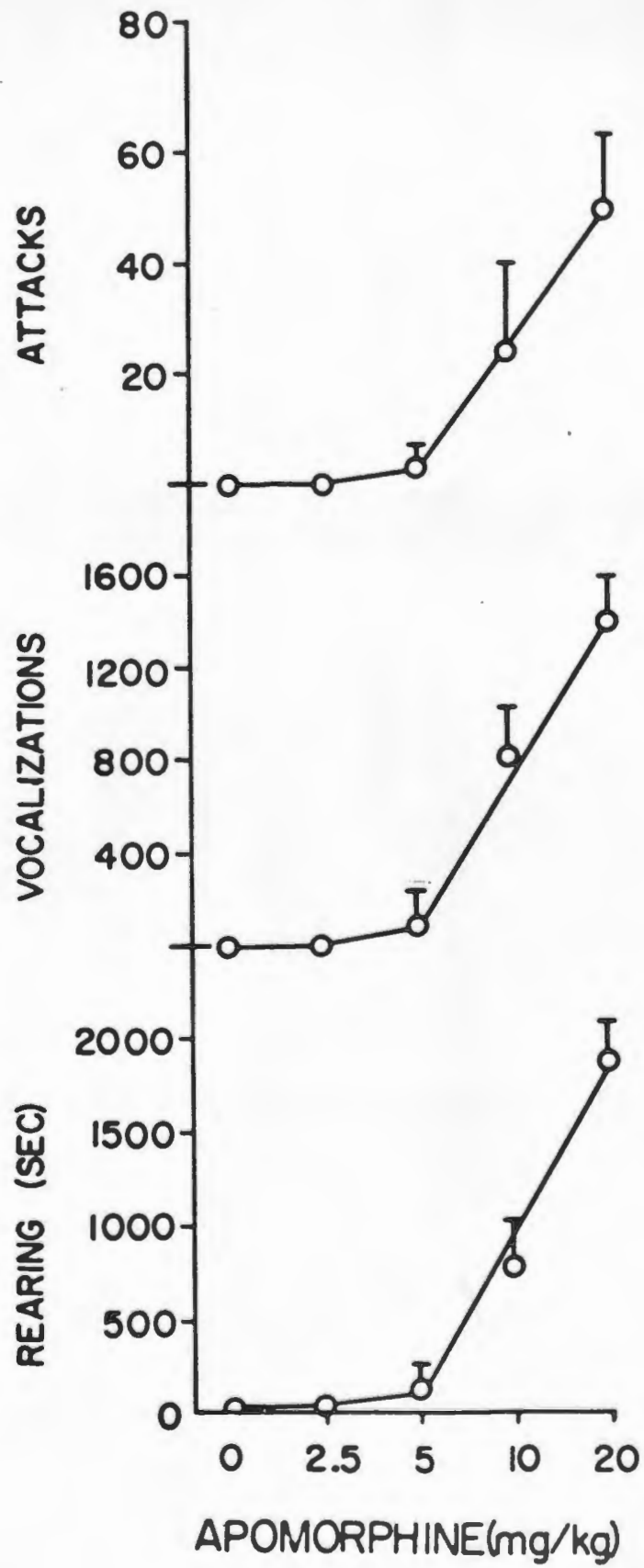
Apomorphine produced aggression in a dose-dependent manner as illustrated in Figure 2. This was determined for use in subsequent experiments. The aggression was characterized by vocalizations, aggressive postures and attacks and biting. At the higher doses, this frequently led to severe injury and bleeding. Doses less than 2.5 mg/kg failed to produce any aggression, while the 5 mg/kg dose produced aggression in approximately 20 per cent of the groups tested. The 20 mg/kg dose reliably produced a substantial aggression and was utilized in further studies. Doses of 5 mg/kg or less were used in studies where it was predicted that the treatment would increase the aggression.

#### 1) Effect of Anti-Dopaminergic Drugs

If the aggression is due to dopaminergic hyperactivity, it would be expected that drugs which reduce dopaminergic activity would also reduce the aggression. Drugs from two

Fig. 2--Dose related elicitation of aggression by apomorphine in naive rats. Apomorphine was injected into groups of four rats, five minutes prior to observations. Points are mean  $\pm$  S. E. for five different groups. Attacks were counted by the experimenter. Rearing refers to the time, in seconds, spent in aggressive postures (see Figures 1a, 1b). Vocalizations were counted automatically. The rats were observed for one hour. The dose of the drug was well correlated with the intensity of aggression on all three measures ( attacks:  $r= 0.78$ ; rearing:  $r= 0.82$ ; vocalizations:  $r= 0.80$ ).

RESPONSES/h, MEAN  $\pm$  S.E.



different classes which decrease dopamine activity were used, neuroleptics and narcotics (see Section II.B).

The neuroleptics, haloperidol and oxyperomide, produced a dose-dependent reduction of the aggression induced by the 20 mg/kg dose of apomorphine (Table 3). This effect was noted on all three parameters of aggression measured. Haloperidol was maximally effective at a dose of 2.5 mg/kg, while the 5 mg/kg dose of oxyperomide abolished the aggression. Similarly, morphine produced a dose-dependent blockade of the apomorphine-induced aggression, with a dose of 10 mg/kg being maximally effective (Table 4). The morphine-induced blockade of aggression was prevented by the narcotic antagonist, naloxone, which had no effect of its own. The specificity of this effect is evident from the fact that naloxone had no effect on the reduction in aggression produced by haloperidol (Table 4). These results confirmed the suspected anti-aggression action of anti-dopaminergic drugs.

In contrast to the effect of naloxone, an anticholinergic drug, dexetimide, partially reversed the anti-aggression action of haloperidol or oxyperomide as illustrated in Table 5. This effect was produced by doses of dexetimide which apparently had little effect on apomorphine-induced aggression of their own (c.f. Table 9). However, dexetimide was unable to alter the blockade of aggression produced by morphine (Table 5), suggesting that morphine and the neuroleptics produce their effects by different mechanisms. If

TABLE 3  
EFFECTS OF NEUROLEPTICS ON  
APOMORPHINE-INDUCED AGGRESSION

<u>Drug</u>	<u>Dose (Mg/Kg)</u>	<u>N<sup>3</sup></u>	<u>Aggressive Response/Hour (mean ± S.E.)</u>		
			<u>Attacks</u>	<u>Rearing</u>	<u>Vocalizations</u>
Saline	-	5	61 ± 9	2750 ± 251	2219 ± 204
Haloperidol	0.63	2	59 ± 5	2358 ± 756	1351 ± 97
	1.25	5	28 ± 8	2358 ± 544	1190 ± 487
	2.5	5	2 ± 2	89 ± 89	17 ± 15
Oxyperomide	1.25	2	52 ± 2	2621 ± 220	889 ± 95
	2.5	5	15 ± 6	1163 ± 510	386 ± 148
	5.0	5	2 ± 2	33 ± 33	25 ± 25

1 Apomorphine (20 mg/Kg) was injected into all groups

2 Haloperidol was injected two hours before testing while oxyperomide was injected 40 minutes before testing.

3 Number of groups (four rats per group) tested.

Values for haloperidol (2.5 mg/Kg) and oxyperomide (5 mg/Kg) are significantly different ( $P < 0.05$ ) from saline control (Dunn's Procedure).

TABLE 4

EFFECT OF MORPHINE ON APOMORPHINE-INDUCED AGGRESSION<sup>1</sup>

Drugs <sup>2</sup>	Dose (mg/Kg)	N <sup>3</sup>	Aggressive Response/Hour (mean $\pm$ S.E.)		
			Attacks	Rearing (secs)	Vocalizations
Saline	-	5	58 $\pm$ 10	1787 $\pm$ 301	1188 $\pm$ 252
Morphine	5	5	28 $\pm$ 11	1150 $\pm$ 285	702 $\pm$ 221
	10	5	1 $\pm$ 1	30 $\pm$ 30	10 $\pm$ 10
Naloxone + Morphine	4	5	42 $\pm$ 5	1654 $\pm$ 384	1163 $\pm$ 196
	10				
Haloperidol + Naloxone	2.5	3	0	0	0
	4				
Naloxone	4	3	46 $\pm$ 5	2189 $\pm$ 263	1283 $\pm$ 107

<sup>1</sup>Apomorphine (20 mg/Kg) was injected into all groups

<sup>2</sup>Morphine was injected 40 minutes before testing, haloperidol was injected 2 hours before testing and naloxone was injected 10 minutes before testing.

<sup>3</sup>Number of groups (4 rats/group) tested.



TABLE 5  
 REVERSAL OF NEUROOPTIC-INDUCED BLOCKADE OF APOMORPHINE-INDUCED AGGRESSION<sup>1</sup>  
 BY DEXETIMIDE

<u>Pre-Treatment Drug</u>	<u>Dose</u>	<u>Post-Treatment Drug</u>	<u>Dose</u>	<u>N</u>	<u>Aggressive Responses/Hour</u> (mean ± S.E.)		
					<u>Attacks</u>	<u>Rearing</u>	<u>Vocalizations</u>
Saline	-	Saline	-	5	47 ± 10	1633 ± 315	1119 ± 306
Haloperidol	2.5	Saline	-	3	3 ± 3	71 ± 71	22 ± 23
		Dexetimide	1.25	2	18 ± 4	986 ± 152	583 ± 120
			2.5	6	14 ± 3	571 ± 191	403 ± 109
			5.0	3	45 ± 4	1392 ± 209	1214 ± 342
Oxyperomide	5	Saline	-	3	0	0	0
		Dexetimide	1.25	4	19 ± 9	615 ± 278	433 ± 207
			2.5	4	20 ± 4	562 ± 118	388 ± 110
			5.0	3	37 ± 13	1087 ± 389	998 ± 303
Morphine	10	Saline	-	3	0	0	0
		Dexetimide	1.25	3	1 ± 0	6 ± 5	12 ± 9
			2.5	3	0	0	0
			5.0	6	4 ± 2	76 ± 52	101 ± 51

<sup>1</sup>All groups were injected with apomorphine (20 mg/Kg) following treatment with other drugs

an anticholinergic drug reverses the neuroleptic effect, a cholinergic drug should synergize with the neuroleptic. As can be seen in Table 6, the combination of an approximately ED<sub>50</sub> dose of oxyperomide with an ED<sub>50</sub> dose of the cholinergic drug, pilocarpine, resulted in a reduction of aggression which was greater than either drug alone. These results suggest a reciprocal interaction between dopamine and acetylcholine. If so, cholinergic stimulation should decrease apomorphine-induced aggression.

## 2) Effect of Drugs Affecting Acetylcholine

Data summarized in Table 7 shows that pilocarpine reduced the aggression induced by the 20 mg/kg dose of apomorphine in a dose-dependent manner, with the dose of 5 mg/kg of pilocarpine being maximally effective. The experiments summarized in Table 8 were designed to test the effect of various anticholinergics on the pilocarpine-induced blockade of aggression. The data is divided into two consecutive 30 minute segments, rather than a one hour observation period as usually presented. This is because it was noted that the centrally active anticholinergics, dexetimide and atropine, when combined with the high dose of apomorphine used in this experiment, resulted in convulsive-like toxic effects towards the middle of the observation period. These convulsions became apparent after the rats had begun to fight. Therefore, the measurements recorded in the first period were analyzed separately from

TABLE 6

EFFECT OF PILOCARPINE PLUS OXYPEROMIDE ON APOMORPHINE-<sup>1</sup>  
INDUCED AGGRESSION

Drug	N <sup>2</sup>	Aggressive Responses/Hour (mean $\pm$ S.E.)		
		Attacks	Rearing (secs)	Vocalizations
Saline	6	53 $\pm$ 4	2702 $\pm$ 122	1693 $\pm$ 221
Pilocarpine <sup>3</sup>	6	30 $\pm$ 6	1621 $\pm$ 334	451 $\pm$ 88
Oxyperomide <sup>4</sup>	6	28 $\pm$ 6	1410 $\pm$ 434	745 $\pm$ 245
Pilocarpine <sup>3</sup> + Oxyperomide <sup>4</sup>	6	4 $\pm$ 3*	465 $\pm$ 307*	125 $\pm$ 94

\* Significantly different ( $p < 0.05$ ) from pilocarpine or oxyperomide alone by Test of Individual Comparisons (Winer, 1971.)

<sup>1</sup> 20 mg/Kg of apomorphine injected into all groups.

<sup>2</sup> Number of groups (4 rats per group) tested.

<sup>3</sup> 2.5 mg/Kg 10 minutes before testing.

<sup>4</sup> 2.5 mg/Kg 40 minutes before testing.

TABLE 7

BLOCKADE OF APOMORPHINE<sup>1</sup>-INDUCED AGGRESSION BY PILOCARPINEAggressive Responses/Hour (Mean  $\pm$  S.E.)

<u>Dose of Pilocarpine<sup>2</sup> (mg/Kg)</u>	<u>N</u>	<u>Attacks/Bites</u>	<u>Rearing (seconds)</u>	<u>Vocalizations</u>
0	7	51 $\pm$ 9	1929 $\pm$ 261	1222 $\pm$ 204
2.5	5	33 $\pm$ 12	1363 $\pm$ 572	291 $\pm$ 114
5	5	1 $\pm$ 1*	81 $\pm$ 81*	16 $\pm$ 14*
10	3	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

\* Significantly different ( $P < 0.05$ ) from control (Dunn's Procedure)

<sup>1</sup> Apomorphine (20 mg/Kg) was injected into all rats.

<sup>2</sup> Pilocarpine (or saline) was injected 15 minutes before testing.

TABLE 8

EFFECT OF ANTICHOLINERGICS ON PILOCARPINE-INDUCED BLOCKADE OF  
APOMORPHINE-INDUCED AGGRESSION<sup>1</sup>Responses/30 Min (Mean  $\pm$  S.E.)<sup>3</sup>

Drug	Dose (mg/Kg)	N <sup>2</sup>	First 30 Minutes			Second 30 Minutes		
			Attacks	Rearing (secs)	Vocalizations	Attacks	Rearing (secs)	Vocalizations
None <sup>4</sup>	-	5	19 $\pm$ 8	729 $\pm$ 271	493 $\pm$ 219	32 $\pm$ 9	1200 $\pm$ 268	729 $\pm$ 201
Saline <sup>5</sup>	-	5	0	0	0	0	0	0
Isopropamide <sup>6</sup>	2.5	5	0	0	0	0	0	0
Dexetimide	0.63	3	0	0	55 $\pm$ 35	0	1 $\pm$ 1	3 $\pm$ 3
	1.25	3	3 $\pm$ 2	170 $\pm$ 158	103 $\pm$ 54	9 $\pm$ 3	183 $\pm$ 99	159 $\pm$ 101
	2.5	8	9 $\pm$ 4	448 $\pm$ 157	223 $\pm$ 68	4 $\pm$ 2	209 $\pm$ 126	134 $\pm$ 54
	5	5	16 $\pm$ 6	458 $\pm$ 135	331 $\pm$ 107	4 $\pm$ 2	71 $\pm$ 35	105 $\pm$ 38
Atropine	5	3	5 $\pm$ 5	171 $\pm$ 171	139 $\pm$ 113	5 $\pm$ 5	338 $\pm$ 338	201 $\pm$ 201
	10	3	8 $\pm$ 4	164 $\pm$ 101	132 $\pm$ 72	8 $\pm$ 4	231 $\pm$ 153	265 $\pm$ 157

<sup>1</sup> Apomorphine 20 mg/Kg was injected into all groups.<sup>2</sup> Number of groups tested (4 rats/group)

Table 8 Continued

- 3 Groups were observed for two consecutive 30 minute periods (see text).
- 4 Apomorphine alone
- 5 This group and all subsequent groups received apomorphine + pilocarpine (5 mg/Kg) and the appropriate drug.
- 6 This dose of isopropamide blocked pilocarpine-induced (5 mg/Kg) diarrhea.

the second segment in order to take the convulsions into account for proper interpretation of the data. Dexetimide and atropine partially reversed the blockade of aggression by pilocarpine in the first period. During the second half of the observation, these drugs were ineffective in reinstating the aggression, but this effect is probably an artifact attributable to the hyperstimulation and convulsions produced by the drug combinations. The third anticholinergic drug used, isopropamide, does not penetrate into the CNS (Janssen and Niemegeers, 1967). Isopropamide effectively blocked the diarrhea produced by the pilocarpine at the dose used (2.5 mg/kg) but had no effect on the aggression-blocking property of pilocarpine, indicating that the effect of cholinergic stimulation in inhibiting aggression is centrally mediated.

Since the cholinergic drug, pilocarpine, blocked the aggression produced by apomorphine, it would be expected that an anticholinergic may increase the aggression. Large doses of dexetimide were combined with a threshold dose (5 mg/kg) and a sub-threshold dose (2.5 mg/kg) of apomorphine (see Figure 2). Dexetimide alone, in any dose including one as high as 20 mg/kg, was unable to elicit aggression in the rats (Table 9), although signs resembling amphetamine agitation were present at this dose. However, dexetimide in a dose of 10 mg/kg elicited aggression when combined with a sub-threshold dose of apomorphine and significantly increased the aggression produced by threshold doses of apomor-

TABLE 9

## EFFECT OF DEXETIMIDE ON APOMORPHINE INDUCED AGGRESSION

Dose (mg/Kg)		N <sup>2</sup>	Aggressive Responses/Hour (Mean $\pm$ S.E.)		
Apomorphine	Dexetimide <sup>1</sup>		Attacks/ Bites	Rearing (secs)	Vocalizations
0	20	3	0	0	0
2.5	0	5	0	0	3 $\pm$ 1
	5	5	0	0	110 $\pm$ 72
	10	5	11 $\pm$ 6*	244 $\pm$ 148*	427 $\pm$ 171*
5.0	0	5	4 $\pm$ 4	257 $\pm$ 257	148 $\pm$ 148
	2.5	3	5 $\pm$ 5	261 $\pm$ 261	177 $\pm$ 177
	5	6	21 $\pm$ 6*	876 $\pm$ 147*	651 $\pm$ 183*

\* Significantly different from corresponding no dexetimide group ( $p < .05$ ) by student's test.

<sup>1</sup> Dexetimide was injected 30 minutes before apomorphine. Tests were made 5 minutes later.

<sup>2</sup> Number of groups (4 rats/group) tested.



phine at a dose of 5 mg/kg (Table 9).

### 3) Effect of Amphetamines

It was predicted that amphetamine, which releases dopamine, would intensify the apomorphine-induced aggression. Amphetamine, at a dose of 8 mg/kg, did exacerbate the aggression produced by threshold doses of apomorphine (5 mg/kg), but failed to elicit aggression when combined with sub-threshold doses of apomorphine (Table 10). Doses higher than those reported produced severe toxic reactions when the rats were placed in the small confinement used for testing. Since amphetamine releases norepinephrine and serotonin (McLean and McCartney, 1961) as well as dopamine, an investigation into the possible role of these other transmitters as antagonists of an amphetamine effect on aggression was undertaken.

Clonidine, which stimulates alpha noradrenergic receptors (Anden et al., 1970b) was used to explore the role of norepinephrine. As is illustrated in Table 11, combinations of clonidine and sub-threshold doses of apomorphine resulted in a marked and dose-dependent increase in the aggression. Doses of clonidine as small as 0.13 mg/kg produced a violent behavior characterized by an excessively large proportion of biting when combined with normally ineffective doses of apomorphine.

The serotonergic influence was measured in two ways. Some preliminary data is presented in Table 12 in which

TABLE 10

## EFFECT OF AMPHETAMINE ON APOMORPHINE-INDUCED AGGRESSION

Apomorphine <sup>1</sup> Dose	Amphetamine <sup>2</sup> Dose	N <sup>3</sup>	Aggressive Responses/Hour (mean ± S.E.)		
			Attacks	Rearing Secs)	Vocalizations
2.5	0	4	0	0	0
	8	4	0	0	31 ± 31
	12	2	0	0	27 ± 27
5	0	5	4 ± 4	101 ± 101	81 ± 66
	8	5	26 ± 9*	1058 ± 359*	747 ± 242*

\* Significantly different ( $p < 0.05$ ) from no amphetamine group.  
(Student's t test)

<sup>1</sup> Injected five minutes before grouping.

<sup>2</sup> Injected 60 minutes before testing.

<sup>3</sup> Number of groups (four rats per group) tested.

TABLE 11

## EFFECT OF CLONIDINE ON APOMORPHINE-INDUCED AGGRESSION

<u>Drug</u>	<u>Dose</u> (mg/Kg)	<u>N</u> <sup>1</sup>	<u>Aggressive Responses/Hour</u> (mean $\pm$ S.E.)		
			<u>Attack</u>	<u>Rearing</u>	<u>Vocalizations</u>
Saline <sup>2</sup>	-	4	0	0	0
Clonidine <sup>2</sup> (+ apomorphine)	0.07	4	3 $\pm$ 3	73 $\pm$ 73	16 $\pm$ 16
	0.13	4	27 $\pm$ 9	1163 $\pm$ 409	404 $\pm$ 162
	0.25	4	51 $\pm$ 3	1364 $\pm$ 209	605 $\pm$ 78
Clonidine <sup>3</sup> (alone)	0.25	3	0	0	0
	0.50	2	0	0	0

<sup>1</sup>Number of groups (four rats/group) tested.

<sup>2</sup>The drug was administered 25 minutes prior to apomorphine (2.5 mg/Kg). Observations were made five minutes later.

<sup>3</sup>Clonidine was injected 30 minutes before observation. No other drug was administered.

TABLE 12

EFFECT OF AMPHETAMINE PLUS CYPROHEPTADINE ON APOMORPHINE  
INDUCED AGGRESSION

<u>Drug<sup>1</sup></u>	<u>Apomorphine Dose (mg/Kg)</u>	<u>N<sup>2</sup></u>	<u>Aggressive Responses/Hour (mean ± S.E.)</u>		
			<u>Attacks</u>	<u>Rearing (secs)</u>	<u>Vocalizations</u>
Saline	1.25	3	0	0	0
Amphetamine <sup>3</sup>	1.25	3	0	0	18 ± 18
Amphetamine <sup>3</sup> + Cyproheptadine <sup>4</sup>	1.25	3	6 ± 6	186 ± 186	103 ± 87
Saline	2.5	4	0	0	0
Amphetamine <sup>5</sup>	2.5	4	0	0	30 ± 30
Cyproheptadine <sup>6</sup>	2.5	2	0	0	0
Amphetamine <sup>5</sup> + Cyproheptadine <sup>6</sup>	2.5	4	20 + 11*	966 + 583*	495 + 167*

\* Significantly different ( $p < 0.05$ ) from saline control (Mann Whitney Test)

<sup>1</sup> Drugs were injected followed by apomorphine at the indicated doses.

TABLE 12 continued

<sup>2</sup> Number of groups tested (4 rats per group).

<sup>3</sup> 8 mg/Kg. 60 minutes before testing.

<sup>4</sup> 8 mg/Kg. 45 minutes before testing.

<sup>5</sup> 4 mg/Kg. 60 minutes before testing.

<sup>6</sup> 4 mg/Kg. 45 minutes before testing.

cyproheptadine, a blocker of serotonin receptors (Stone et al., 1961), was used. When combined with amphetamine, cyproheptadine appeared to produce a weak elicitation of aggression by sub-threshold doses of apomorphine. Cyproheptadine alone, at the doses used, did not influence the apomorphine-induced aggression. Secondly, parachloroamphetamine (PCA), a related phenethylamine which produces a long-lasting depletion of brain serotonin (Sanders-Bush et al., 1972) markedly lowered the effective dose of apomorphine. When PCA was given in a dose of 12 mg/kg, three days before testing, there was no spontaneous aggression in the rats, but there was considerable aggression after small (0.63- 2.5 mg/kg), normally ineffective doses of apomorphine (Figure 3). These results would suggest that serotonin opposes dopamine-induced aggression.

#### B. Morphine Withdrawal Aggression

##### 1) 72 Hour Withdrawal Aggression

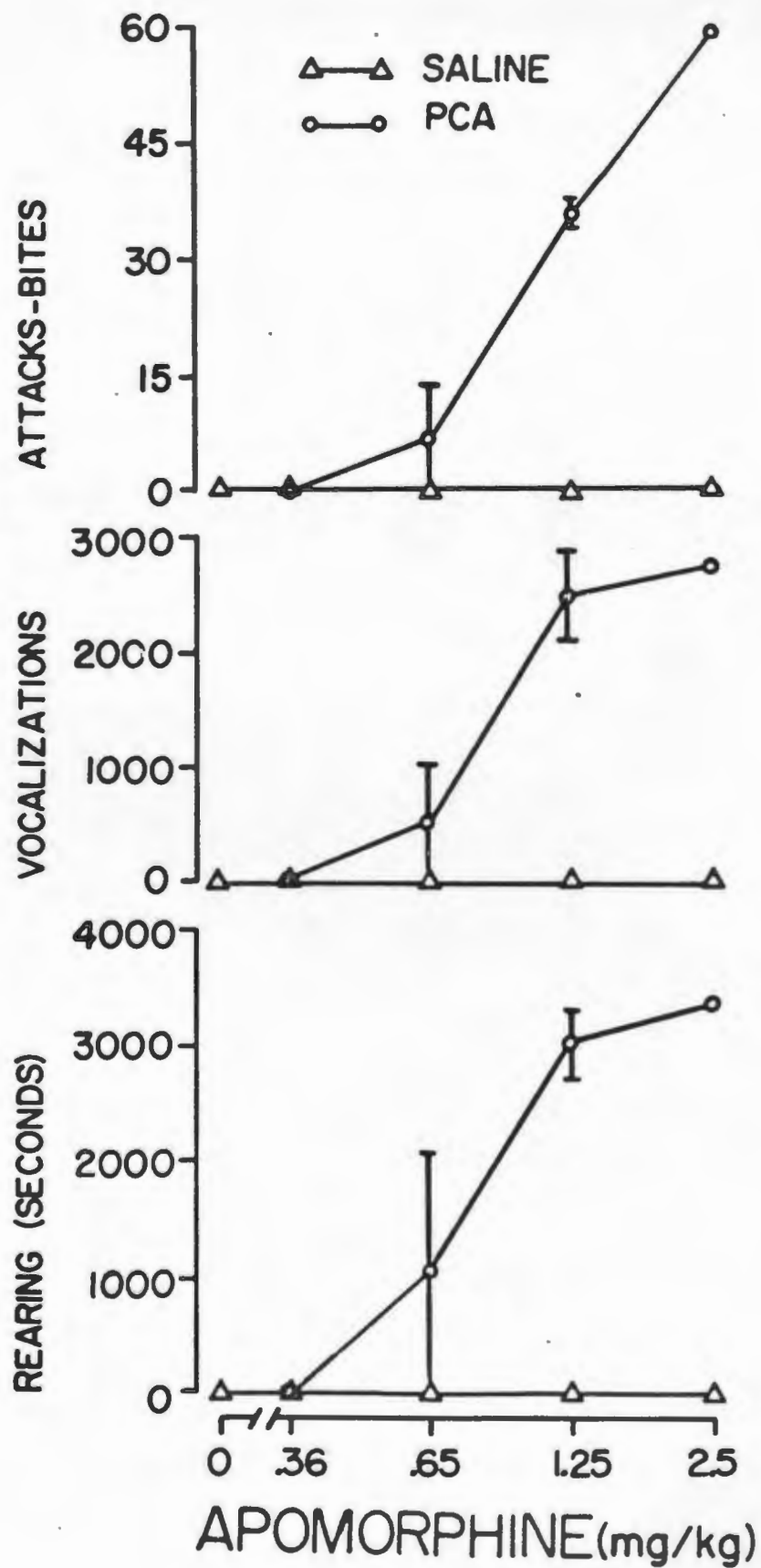
Morphine withdrawal aggression was studied because it also may have a dopaminergic basis (Puri and Lal, 1973a). Any similarities between morphine withdrawal aggression and apomorphine-induced aggression would provide further support for the mechanisms proposed.

##### a) Effect of Dopamine

Rats were made dependent on morphine by the three methods described in Section III.c.1. As expected, the

Fig. 3--Dose related elicitation of aggression by apomorphine in p-chloroamphetamine (PCA) treated rats. Apomorphine was injected into groups of four rats, three days after PCA (12 mg/kg in a single injection). Points are mean  $\pm$  S. E. for four groups, except for the 2.5 mg/kg dose of apomorphine which represents data for one group. Apomorphine or saline was injected five minutes before observations.

AGGRESSIVE RESPONSES/HOUR (mean  $\pm$  S.E.)





rats addicted to a terminal dose of 405 mg/kg/day demonstrated considerable aggression 72 hours after their last morphine injection (Table 13). Decreasing the amount of morphine injected during addiction to a terminal dose of 200 mg/kg resulted in significantly less aggression during withdrawal on all three parameters tested, while increasing the length of time that the rats received morphine injections from 15 to 25 days, while maintaining the terminal dose at 200 mg/kg, produced aggression which was indistinguishable from the usual high dose schedule (Table 13). This indicates that the degree of aggression is dependent on the amount of morphine administered and the duration of addiction. The high dose schedule was utilized for all further experiments.

It is interesting to note that the aggression peaks at 72 hours of withdrawal, while other signs of withdrawal (e. g. "wet shakes", ptosis, hypothermia), peak at 24-48 hours of withdrawal (Martin et al., 1963; Gianutsos et al., 1975). Spontaneous locomotor activity was also measured during withdrawal to determine if any pattern was evident. It can be seen from the data summarized in Table 14 that locomotor activity was significantly lower than in normal rats at 48 hours of withdrawal, when most signs of withdrawal are at their most intense. The activity was significantly higher than control at 72 hours of withdrawal, when aggression was at its maximum. The activity was normal at 24 hours of withdrawal.

TABLE 13

EFFECT OF DIFFERENT SCHEDULES OF ADDICTION ON 72 HOUR  
MORPHINE WITHDRAWAL AGGRESSION

Terminal Dose <sup>1</sup> (mg/Kg)	Length of <sup>2</sup> Addiction (days)	N <sup>3</sup>	Aggressive Responses/Hour (mean $\pm$ S.E.)		
			Attacks	Rearing (secs)	Vocalizations
405	14	13	32 $\pm$ 2	3216 $\pm$ 118	1864 $\pm$ 150
200	15	6	13 $\pm$ 4*	1877 $\pm$ 532*	904 $\pm$ 323
200	25	3	29 $\pm$ 5	3399 $\pm$ 161	2524 $\pm$ 103

\* Significantly different ( $p < 0.05$ ) from 405 group (Student's t test).

<sup>1</sup> Final daily dose of morphine at which rats were maintained (see methods).

<sup>2</sup> Total number of days during which morphine injections were given.

<sup>3</sup> Number of groups (4 rats per group).

TABLE 14

## LOCOMOTOR ACTIVITY OF RATS DURING MORPHINE WITHDRAWAL

<u>Period of Withdrawal<sup>1</sup></u>	<u>N</u>	<u>Locomotor Activity Counts (mean <math>\pm</math> S.E.)</u>
Control <sup>2</sup>	10	1138 $\pm$ 106
24 hours	10	1101 $\pm$ 131
48 hours	10	810 $\pm$ 84*
72 hours	10	1429 $\pm$ 101*

---

\* Significantly different ( $p < 0.05$ ) from control (Dunnet's Test)

<sup>1</sup> Hours since last morphine injection (135 mg/Kg)

<sup>2</sup> Naive, non-addicted rats

As expected from the data of Puri and Lal (1973a), a small dose of apomorphine (1.25 mg/kg) increased, while haloperidol (0.64 mg/kg) decreased the 72 hour morphine withdrawal aggression (Table 15). Lesions of the dopaminergic nigro-striatal bundle (NSB) reduced the intensity of the aggression (Table 16). Small lesions (1 mA, 15 sec) of the bundle decreased aggression by about 50 per cent, while more extensive lesions (2 mA, 30 sec) produced a virtually complete blockade of the aggression. These lesions have been shown to produce the characteristic rise in dopamine levels in the striatum 24 hours after the lesion, followed by a gradual depletion of striatal dopamine, with the larger lesion producing a more complete depletion (Hynes et al., 1975). These results provide further evidence for the dopaminergic basis of morphine withdrawal aggression.

In contrast to the effects in normal rats (c.f. Table 5), dexetimide did not affect the blockade of aggression by haloperidol in morphine withdrawn rats (Table 17). It was determined in pilot experiments that the 0.64 mg/kg dose of haloperidol which was used in this study was the minimum effective dose in blocking withdrawal aggression. Dexetimide by itself did not reduce the aggression, while the combination of haloperidol and dexetimide produced the usual blockade of aggression (Table 17). These results suggest an alteration of the normal dopaminergic/cholinergic balance during morphine withdrawal and point up the first difference between morphine withdrawal aggression and apomorphine-induced

TABLE 15

EFFECT OF APOMORPHINE OR HALOPERIDOL ON 72 HOUR  
MORPHINE WITHDRAWAL AGGRESSION

Drug	Dose (mg/Kg)	N <sup>1</sup>	Aggressive Responses/Hour (mean $\pm$ S.E.)		
			Attacks	Rearing (secs)	Vocalizations
Saline	-	5	30 $\pm$ 3	3060 $\pm$ 315	1771 $\pm$ 181
Apomorphine <sup>2</sup>	1.25	7	82 $\pm$ 5	3324 $\pm$ 210	1995 $\pm$ 377
Haloperidol <sup>3</sup>	0.64	4	0 $\pm$ 0	3 $\pm$ 3	15 $\pm$ 11

<sup>1</sup> Number of groups (4 rats/group) tested.

<sup>2</sup> Apomorphine or saline was injected 5 minutes before testing.

<sup>3</sup> Haloperidol was injected 2 hours before testing.

TABLE 16

EFFECT OF BRAIN LESIONS ON 72 HOUR MORPHINE WITHDRAWAL  
AGGRESSION

Lesion	N <sup>1</sup>	Aggressive Response/Hour (mean $\pm$ S.E.)		
		Attacks	Rearing (secs)	Vocalizations
None	5	29 $\pm$ 3	3013 $\pm$ 241	2091 $\pm$ 128
NSB Small	5	24 $\pm$ 8	771 $\pm$ 201*	477 $\pm$ 116*
NSB Large	5	1 $\pm$ 0*	18 $\pm$ 6*	26 $\pm$ 8*
MFB Small	5	22 $\pm$ 12	1524 $\pm$ 621	588 $\pm$ 229*
NSB Large + Apomorphine	2	47 $\pm$ 5*	1209 $\pm$ 200	702 $\pm$ 29

All lesions were made at 48 hours of withdrawal. Tests were performed 24 hours later. NSB designates nigro-striatal bundle, MFB designates medial forebrain bundle. Small means 1 mA of current was passed through electrode for 15 seconds, large means 2 mA for 30 seconds.

\* Significantly different ( $p < 0.05$ ) from normal rats (Tukey Test).

<sup>1</sup> Number of groups tested (4 rats per group).

TABLE 17

EFFECT OF DEXETIMIDE ON HALOPERIDOL INDUCED BLOCKADE  
OF MORPHINE WITHDRAWAL AGGRESSION

<u>Drug<sup>1</sup></u>	<u>Dose</u> <u>mg/Kg</u>	<u>N<sup>2</sup></u>	<u>Aggressive Responses/Hour</u> ( <u>mean</u> $\pm$ <u>S.E.</u> )		
			<u>Attacks</u>	<u>Rearing</u> ( <u>secs</u> )	<u>Vocalizations</u>
72 hour withdrawal <sup>3</sup>					
Saline	-	5	30 $\pm$ 2	3114 $\pm$ 121	1507 $\pm$ 158
Haloperidol	0.64	5	0	2 $\pm$ 2	4 $\pm$ 4
Haloperidol + Dexetimide	0.64 5	9	4 $\pm$ 4	334 $\pm$ 296	284 $\pm$ 246
7 day withdrawal <sup>3</sup>					
Apomorphine	1.25	6	30 $\pm$ 6	3391 $\pm$ 89	2387 $\pm$ 464
Apomorphine + Haloperidol	1.25 0.64	3	0	0	0
Apomorphine + Dexetimide	1.25 5	3	42 $\pm$ 5	2792 $\pm$ 533	1948 $\pm$ 525
Apomorphine + Dexetimide + Haloperidol	1.25 5 0.64	4	1	4 $\pm$ 4	8 $\pm$ 6

<sup>1</sup> Haloperidol was injected two hours before testing while dexetimide and apomorphine were injected 40 minutes and 10 minutes, respectively, before testing.

<sup>2</sup> Number of groups (four rats per group) tested.

<sup>3</sup> Time since last morphine injection.

aggression.

#### b) Effect of Acetylcholine

In order to further test the similarities between morphine withdrawal aggression and apomorphine-induced aggression, the effect of cholinergic stimulation and blockade was tested. The cholinergic agonist, pilocarpine, reduced the 72 hour morphine withdrawal aggression in a dose-dependent manner (Figure 4), with the 10 mg/kg dose being maximally effective in reducing all three measures of aggression. The anticholinergic, dexetimide, produced a dose-dependent increase in the aggression (Table 18) with the 10 mg/kg dose being effective. Similar results were noted in apomorphine-induced aggression.

#### c) Effect of Clonidine

Clonidine, at a dose of 0.25 mg/kg, intensified the aggression normally seen at 72 hours of withdrawal (Table 19). Clonidine produced a similar enhancement of apomorphine-induced aggression, suggesting that noradrenergic activity may be important in the aggression.

## 2) ELICITED WITHDRAWAL AGGRESSION

Naloxone, a narcotic- antagonist, is capable of precipitating a withdrawal syndrome if administered to dependent rats within a few hours of a narcotic (Wei et al., 1973), but certain characteristics of the withdrawal differ from spontaneous withdrawal (see Gianutsos et al., 1975). It



Fig. 4--Dose related reduction of 72 hour morphine withdrawal aggression by pilocarpine. Pilocarpine or saline was injected into groups of four morphine withdrawn rats 10 minutes prior to observations.

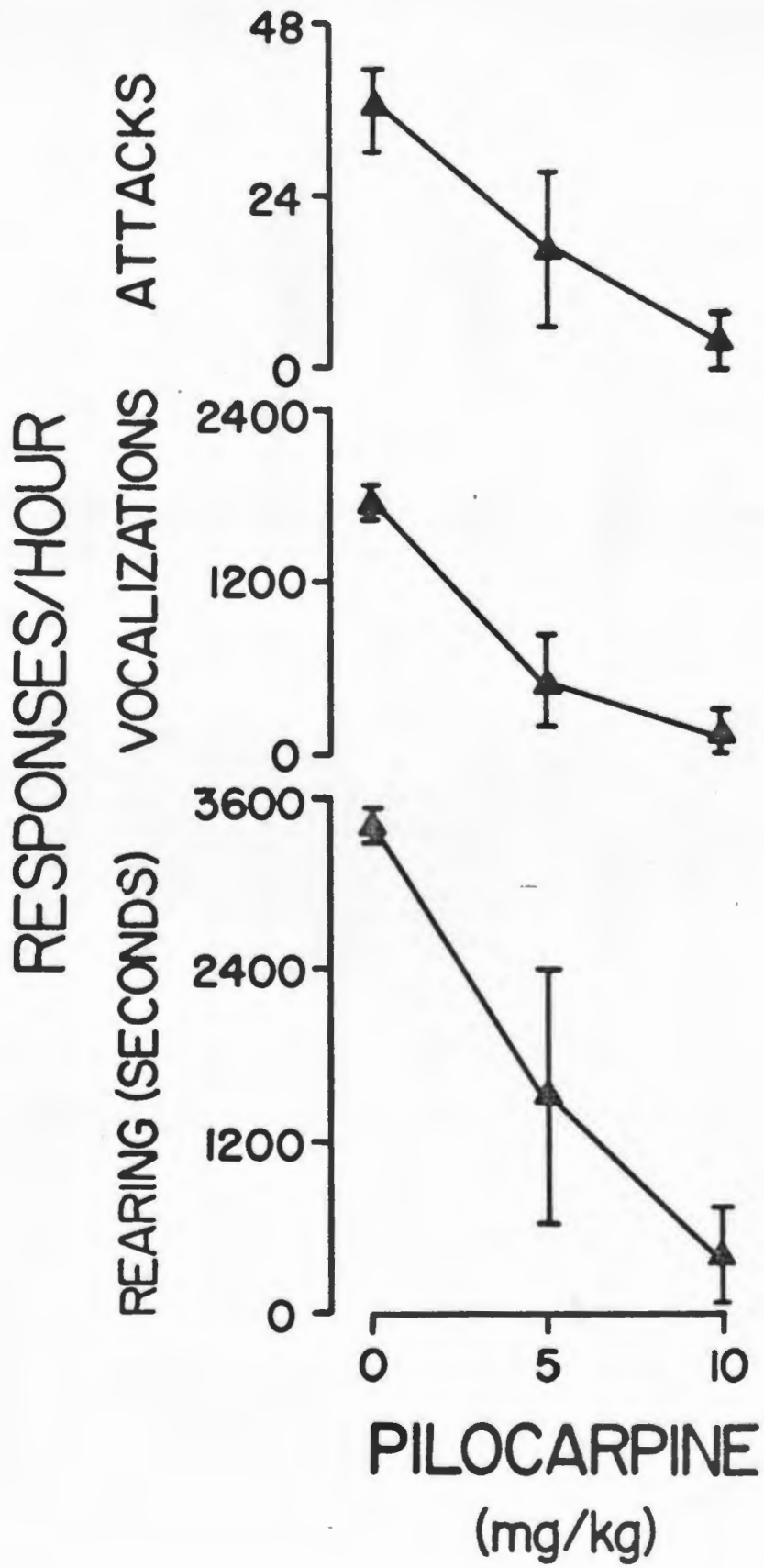


TABLE 18

EFFECT OF DEXETIMIDE ON 72 HOUR MORPHINE  
WITHDRAWAL AGGRESSION

<u>Dose of Dexetimide<sup>1</sup> (mg/Kg)</u>	<u>N<sup>2</sup></u>	<u>Aggressive Responses/Hour (mean <math>\pm</math> S.E.)</u>		
		<u>Attacks</u>	<u>Rearing (secs)</u>	<u>Vocalizations</u>
0	5	22 $\pm$ 7	1515 $\pm$ 875	1117 $\pm$ 406
5	3	22 $\pm$ 9	1870 $\pm$ 1009	1221 $\pm$ 570
10	4	57 $\pm$ 8*	2891 $\pm$ 641	2098 $\pm$ 308*

\* Significantly different ( $p < .05$ ) from saline control (0mg/Kg) by (Tukey (b) Test).

<sup>1</sup> Dexetimide was injected 35 minutes before grouping.

<sup>3</sup> Number of groups (4 rats/group) tested.

TABLE 19

EFFECT OF CLONIDINE ON MORPHINE-WITHDRAWAL AGGRESSION<sup>1</sup>

<u>Drug</u>	<u>N</u> <sup>2</sup>	<u>Aggressive Responses/Hour</u> (mean $\pm$ S.E.)		
		<u>Attacks</u>	<u>Rearing</u> (secs)	<u>Vocalizations</u>
Saline	4	13 $\pm$ 4	861 $\pm$ 311	352 $\pm$ 205
Clonidine <sup>3</sup>	6	37 $\pm$ 4*	1808 $\pm$ 253*	723 $\pm$ 67

\* Significantly different ( $p < 0.05$ ) from saline (Student's t test).

<sup>1</sup> 72 hours after last morphine injection.

<sup>2</sup> Number of groups (four rats/group) tested.

<sup>3</sup> Clonidine was injected at a dose of 0.25mg/Kg 30 minutes before observations.

was decided to investigate whether naloxone could likewise precipitate aggression in dependent rats. When administered to morphine-dependent rats four hours after their terminal dose of morphine, 4 mg/kg of naloxone abruptly precipitated signs of withdrawal as described in Table 20. Most notable were diarrhea, a loss in body weight and a profound hypothermia. Dependent rats given saline or apomorphine at this time showed no signs of withdrawal except for a mild hypothermia following apomorphine (Table 20). When rats treated with naloxone were grouped, they failed to consistently show aggression even when a higher dose of naloxone (8 mg/kg) was used as illustrated in Table 21. In contrast, when these rats were injected with a small dose of apomorphine (1.25 mg/kg) either alone or in combination with naloxone four hours after their last morphine injection, they exhibited considerable aggression (Table 21). Amphetamine likewise produced some aggression, either alone or when combined with naloxone (Table 21). In order to control for a possible direct anti-aggression action of naloxone, the drug was administered to 72 hour morphine-withdrawn rats. As can be seen from the data in Table 22, naloxone failed to affect either the spontaneous 72 hour withdrawal aggression or the potentiation of aggression produced by apomorphine. These data provide further evidence for a latent supersensitivity of dopamine receptors as a result of narcotic dependence.

TABLE 20

PRECIPITATED NARCOTIC WITHDRAWAL FOUR HOURS  
AFTER TERMINAL MORPHINE INJECTION

Symptom	Occurrence <sup>1</sup>					
	No Drug <sup>2</sup> (12)		Naloxone <sup>2</sup> (16)		Apomorphine <sup>2</sup> (7)	
Diarrhea	1/12		15/16**		0/7	
Teeth Chattering	0/12		11/16**		0/7	
Weight Change (g) <sup>3</sup>	+8.0	± 1.6	-4.9	± 1.6	+7.4	± 1.2
Temperature Reduction (deg) <sup>4</sup>	1.26	± 0.11	4.95	± 0.49*	2.41	± 0.23*
Wet Shakes	0.2	± 0.1	3.0	± 0.6*	0.3	± 0.2
Ptosis	5.0	± 5.0	550.0	± 138.0*	45.0	± 36.0
Writhes	0.1	± 0.1	0.3	± 0.2	0	± 0

\* Significantly different from no drug (Student's t Test).

\*\* Significantly different from no drugs (Chi Square)

<sup>1</sup> Mean ± S.E. or frequency of occurrence out of total number observed.

<sup>2</sup> Saline, naloxone (4mg/Kg) or apomorphine (1.25mg/Kg) injected 4 hours after terminal dose of morphine (135mg/Kg). Observations made for 30 min., 10 minutes after injection. Number in ( ) denotes number of animals observed.

<sup>3</sup> Change in body weight from weight immediately prior to last morphine injection.

<sup>4</sup> Fall in body temperature from hyperthermia seen one hour after last morphine injection.

TABLE 21

## ELICITATION OF WITHDRAWAL AGGRESSION IN MORPHINE DEPENDENT RATS FOUR HOURS AFTER MORPHINE INJECTION

<u>Drug</u> <sup>1</sup>	<u>Dose</u> (mg/Kg)	<u>N</u> <sup>2</sup>	<u>Responses/Hour</u> (mean $\pm$ S.E.)		
			<u>Attacks</u>	<u>Rearing</u> (secs)	<u>Vocalizations</u>
None	-	3	0	2 $\pm$ 2	0
Naloxone	4	8	0	3 $\pm$ 2	130 $\pm$ 48
	8	9	4 $\pm$ 3	77 $\pm$ 50	237 $\pm$ 69
Apomorphine	1.25	4	46 $\pm$ 12	2472 $\pm$ 558	1233 $\pm$ 189
Amphetamine	2	8	7 $\pm$ 3	1347 $\pm$ 496	423 $\pm$ 187
	4	4	0	3 $\pm$ 3	42 $\pm$ 29
Naloxone +	4	4	60 $\pm$ 14	2896 $\pm$ 167	2162 $\pm$ 109
Apomorphine	1.25				
Naloxone +	8	4	40 $\pm$ 9	2326 $\pm$ 365	1793 $\pm$ 236
Apomorphine	1.25				
Naloxone +	4	4	16 $\pm$ 4	978 $\pm$ 306	1359 $\pm$ 460
Amphetamine	2				

<sup>1</sup> Drugs injected 4 hours after last morphine injection (135mg/Kg). Apomorphine was injected 5 minutes, while naloxone was injected 10 minutes before observation. Amphetamine was injected 60 minutes prior to testing.

<sup>2</sup> Number of groups tested (4 rats/group).

TABLE 22

EFFECT OF APOMORPHINE OR NALOXONE ON 72 HOUR  
MORPHINE WITHDRAWAL AGGRESSION

Drug <sup>2</sup>	Dose (mg/Kg)	N <sup>1</sup>	Response/Hour (mean $\pm$ S.E.)		
			Attacks	Rearing (secs)	Vocalizations
None	-	6	30 $\pm$ 4	3296 $\pm$ 185	1869 $\pm$ 195
Apomorphine	1.25	7	87 $\pm$ 5	3324 $\pm$ 210	1995 $\pm$ 377
Naloxone	4	5	42 $\pm$ 5	3474 $\pm$ 94	2145 $\pm$ 115
	8	6	28 $\pm$ 3	2294 $\pm$ 248	1562 $\pm$ 222
Apomorphine + Naloxone	1.25 4	2	75 $\pm$ 2	3409 $\pm$ 90	1838 $\pm$ 355

<sup>1</sup> Number of group tested (4 rats/group).

<sup>2</sup> Apomorphine was injected 5 minutes before testing while naloxone was injected 10 minutes before testing. Injections were made 72 hours after last morphine injection.



### 3) Protracted Aggression

It is known that some of the signs of narcotic abstinence can be detected even weeks or months after withdrawal. In rats (Martin and Sloan, 1971) and humans (Himmelsbach, 1941), a phenomenon known as protracted abstinence. Therefore, it was of interest to determine if aggression would likewise be present during protracted abstinence. Rats were made dependent on morphine and allowed to undergo continuous withdrawal for 30 days. When grouped, these rats exhibited aggression as shown in Table 23. The aggression was markedly enhanced by apomorphine (1.25 mg/kg), a normally ineffective dose, and blocked by a small dose of morphine (10 mg/kg) (Table 23). A large (2 mA, 30 sec) lesion of the nigro-striatal bundle produced 24 hours before testing completely blocked the aggression. When the effect of the lesion was bypassed by stimulating the post-synaptic receptors with apomorphine, the aggression was reinstated (Table 23). Lesions of the adjacent medial fore-brain bundle, which carries noradrenergic and serotonergic fibers, did not significantly affect the aggression. These results suggest that the dopaminergic supersensitivity which develops during morphine dependence is a long lasting phenomenon.

TABLE 23

EFFECT OF APOMORPHINE AND BRAIN LESIONS ON  
MORPHINE-WITHDRAWAL AGGRESSION.

Treatment	N <sup>1</sup>	Aggressive Responses/Hour (mean $\pm$ S.E.)		
		Attacks/Bites	Rearing (secs)	Vocalizations
ISOLATED CONTROLS <sup>2</sup>				
No Drug	6	1 $\pm$ 0.3	52 $\pm$ 48	4 $\pm$ 2
Apomorphine <sup>3</sup>	4	0	9 $\pm$ 9	14 $\pm$ 3
72 HOUR MORPHINE WITHDRAWAL				
No Drug	3	26 $\pm$ 6	3599 $\pm$ 1	2191 $\pm$ 271
30 DAY MORPHINE WITHDRAWAL				
No Drug	6	23 $\pm$ 9	1513 $\pm$ 280	828 $\pm$ 260
Morphine <sup>4</sup>	2	0	0	0
Apomorphine <sup>3</sup>	5	56 $\pm$ 10	3017 $\pm$ 138	1961 $\pm$ 150
MFB Lesion <sup>5</sup>	3	33 $\pm$ 5	1423 $\pm$ 298	753 $\pm$ 59
NSB Lesion	7	1 $\pm$ 1	39 $\pm$ 39	18 $\pm$ 6
NSB Lesion <sup>5</sup> + Apomorphine <sup>3</sup>	4	42 $\pm$ 22	625 $\pm$ 267	528 $\pm$ 222

<sup>1</sup> Number of groups (4 rats/group) tested.

<sup>2</sup> Rats housed one per cage for 30 days.

<sup>3</sup> 1.25 mg/Kg

<sup>4</sup> 10 mg/Kg, 30 minutes prior to testing.

<sup>5</sup> Lesions were produced 24 hours before testing. MFB denotes medial forebrain bundle and NSB denotes nigrostriatal bundle.

### C. Chronic Haloperidol

If it is assumed that morphine-withdrawal aggression reflects a dopaminergic supersensitivity produced as a result of dopamine receptor blockade by morphine, then certain similarities should be noted between chronic morphine and chronic haloperidol, since haloperidol is generally regarded to block dopamine receptors (Carlsson and Lindqvist, 1963; Anden et al., 1970a). For this reason, haloperidol was administered to rats chronically and observations were made after the drug was discontinued.

Following the cessation of chronic treatment with haloperidol, rats exhibited an apparent supersensitivity to the stereotypy-producing actions of apomorphine (Table 24) or amphetamine (Table 25). It is widely believed that stereotypy produced by these drugs is dependent upon dopaminergic stimulation (Ernst, 1967; Snyder et al., 1970). Doses of apomorphine (1.25 mg/kg) and amphetamine (7.5 mg/kg) were selected which produced a low to moderate degree of stereotypy in normal rats on the basis of pilot experiments. When these drugs were administered to rats three days after stopping chronic treatment with haloperidol, the stereotypy was significantly increased. When the rats treated chronically with haloperidol were grouped after discontinuation of the drug, no spontaneous aggression was observed (Table 26). However, when these rats received small doses of apomorphine (0.63-2.5 mg/kg), considerable aggression was

TABLE 24

APOMORPHINE<sup>1</sup> INDUCED STEREOTYPY IN RATS AFTER CHRONIC  
TREATMENT WITH HALOPERIDOL

Group	N	Stereotypy Score (Median $\pm$ S. E.)
Control <sup>2</sup>	16	7 $\pm$ 1.3
Chronic Haloperidol <sup>3</sup>	18	12 $\pm$ 1.3*

\* Significantly different ( $p < 0.025$ ) from control (Mann-Whitney U-Test).

<sup>1</sup> 1.25 mg/kg, 10 minutes before testing.

<sup>2</sup> Naive rats.

<sup>3</sup> 72 hours after last haloperidol injection.

TABLE 25

AMPHETAMINE<sup>1</sup> INDUCED STEREOTYPY IN RATS AFTER CHRONIC  
TREATMENT WITH HALOPERIDOL

Group	N	Stereotypy Score (Median $\pm$ S. E.)
Control <sup>2</sup>	16	8 $\pm$ 0.7
Chronic Haloperidol <sup>3</sup>	10	14 $\pm$ 1.4*

\* Significantly different ( $p < 0.05$ ) from control (Mann-Whitney U Test).

<sup>1</sup> 7.5 mg/kg, 30 minutes before observations

<sup>2</sup> Naive rats.

<sup>3</sup> 72 hours after last haloperidol injection.

TABLE 26

## APOMORPHINE INDUCED AGGRESSION FOLLOWING CHRONIC HALOPERIDOL ADMINISTRATION

Drug	Dose (mg/kg)	Days of With- drawal <sup>1</sup>	N <sup>2</sup>	Aggressive Responses/Hour (mean + S. E.)		
				Attacks	Rearing	Vocalizations
Isolated Controls <sup>3</sup>						
Saline	-	-	4	0	0	0
Apomorphine	2.5	-	4	1 ± 1	22 ± 22	19 ± 3
Chronic Haloperidol <sup>4</sup>						
Saline	-	3	5	0	7 ± 7	4 ± 4
Apomorphine	1.25	2	3	0	0	0
	2.5	2	2	13 ± 10	1606 ± 1475	895 ± 830
	1.25	3	3	1 ± 1	17 ± 17	6 ± 6
	2.5	3	8	59 ± 13	1734 ± 303	1359 ± 310
	0.64	7	3	6 ± 3	678 ± 340	440 ± 259
	1.25	7	3	22 ± 6	2668 ± 349	1730 ± 666
	2.5	7	4	78 ± 17	2747 ± 154	1951 ± 313
	2.5	18	4	8 ± 8	528 ± 512	312 ± 310

TABLE 26 continued

- <sup>1</sup> Refers to number of days after terminal injection of haloperidol.
- <sup>2</sup> Number of groups tested (3 rats per group).
- <sup>3</sup> Housed one per cage for three weeks.
- <sup>4</sup> Animals injected chronically with haloperidol as described in Methods.

observed (Table 26). This indicated that rats who exhibit dopaminergic supersensitivity, exhibit a supersensitivity to the aggression eliciting effects of apomorphine as well, which would be consistent with the explanation proposed in morphine withdrawal. However, in contrast to morphine-withdrawal aggression, there was no spontaneous aggression after chronic haloperidol. The aggression produced by apomorphine was particularly intense seven days after "withdrawal" but had subsided by 18 days (Table 26). If amphetamine was injected instead of apomorphine, aggression was observed infrequently and inconsistently (Table 27), in contrast to the effect seen during morphine withdrawal. There was virtually no aggression even with a dose of amphetamine as high as 16 mg/kg.

Despite the fact that aggression was not elicited by amphetamine in rats after chronic haloperidol, the locomotor stimulation produced by amphetamine was increased. It can be seen in Figure 5 that a normally ineffective dose of amphetamine (0.2 mg/kg) produced a significant stimulation of locomotor activity after chronic treatment with haloperidol. This effect of amphetamine was further analyzed in Figure 6. It should be noted, first of all, that rats which had been treated with haloperidol chronically exhibited an increased spontaneous activity after drug administration was discontinued when compared with drug-free rats. (This was also noted when chronically haloperidol treated rats were compared with rats treated chronically with 0.3% tartaric



TABLE 27

## FAILURE OF AMPHETAMINE TO INDUCE AGGRESSION IN RATS CHRONICALLY TREATED WITH HALOPERIDOL

Drug	Dose (mg/kg)	Days of With- drawal <sup>1</sup>	N <sup>2</sup>	Aggressive Responses/Hour (mean + S.E.)		
				Attacks	Rearing	Vocalizations
Saline	-	3	4	0	0	0
d-amphetamine	4	3	3	0	0	0
	8		5	2 ± 2	72 ± 72	69 ± 44
	16		1	0	0	5
l-amphetamine	8	3	3	5 ± 5	772 ± 772	96 ± 90
	16		2	2 ± 1	84 ± 62	65 ± 18
d-amphetamine	8	7	2	0	0	0
	16		2	0	0	0
l-amphetamine	8	7	2	0	0	6 ± 6
	16		2	0	0	8 ± 6
apomorphine	2.5	7	3	72 ± 19	3016 ± 341	1801 ± 295

TABLE 27 continued

<sup>1</sup> Refers to days since last haloperidol injection.

<sup>2</sup> Number of groups tested (3 rats per group).

<sup>3</sup> Amphetamines were injected 30 minutes prior to observations. Apomorphine was injected 5 minutes before testing.

Fig. 5--Effect of amphetamine on locomotor activity in normal and chronic haloperidol treated rats. Amphetamine was injected, at the indicated doses, to normal rats and to rats who had received their last of a series of haloperidol injections three to six days prior to testing. Activity was measured for one hour, 60 minutes after the amphetamine injection. Each point is the mean  $\pm$  S. E. for 10 rats. Asterisks (\*) indicate that the dose significantly increased locomotor activity ( $p < 0.05$ ) compared with the corresponding saline (0 mg/kg) control (Dunnet's Test, Winer, 1971).

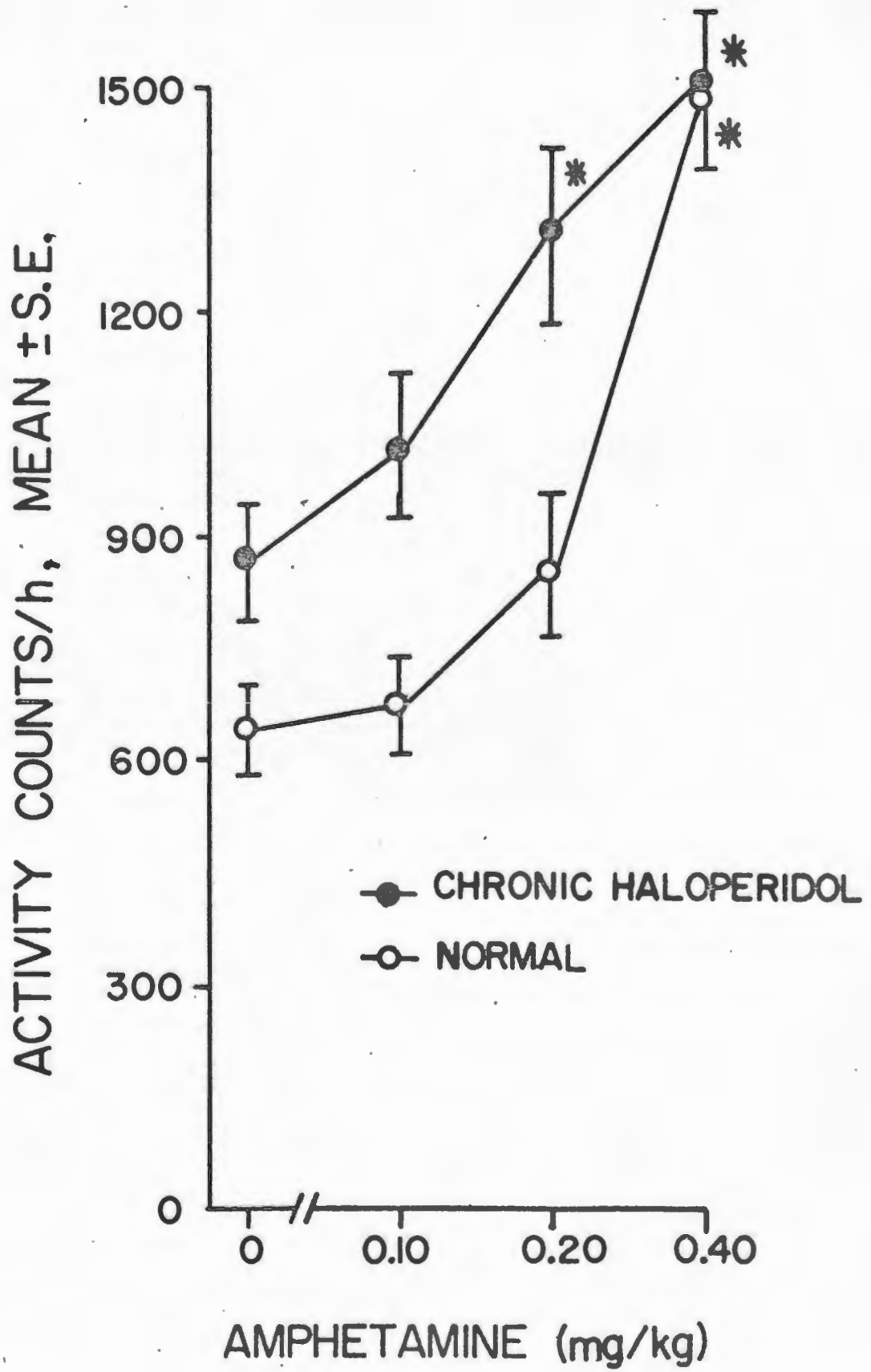
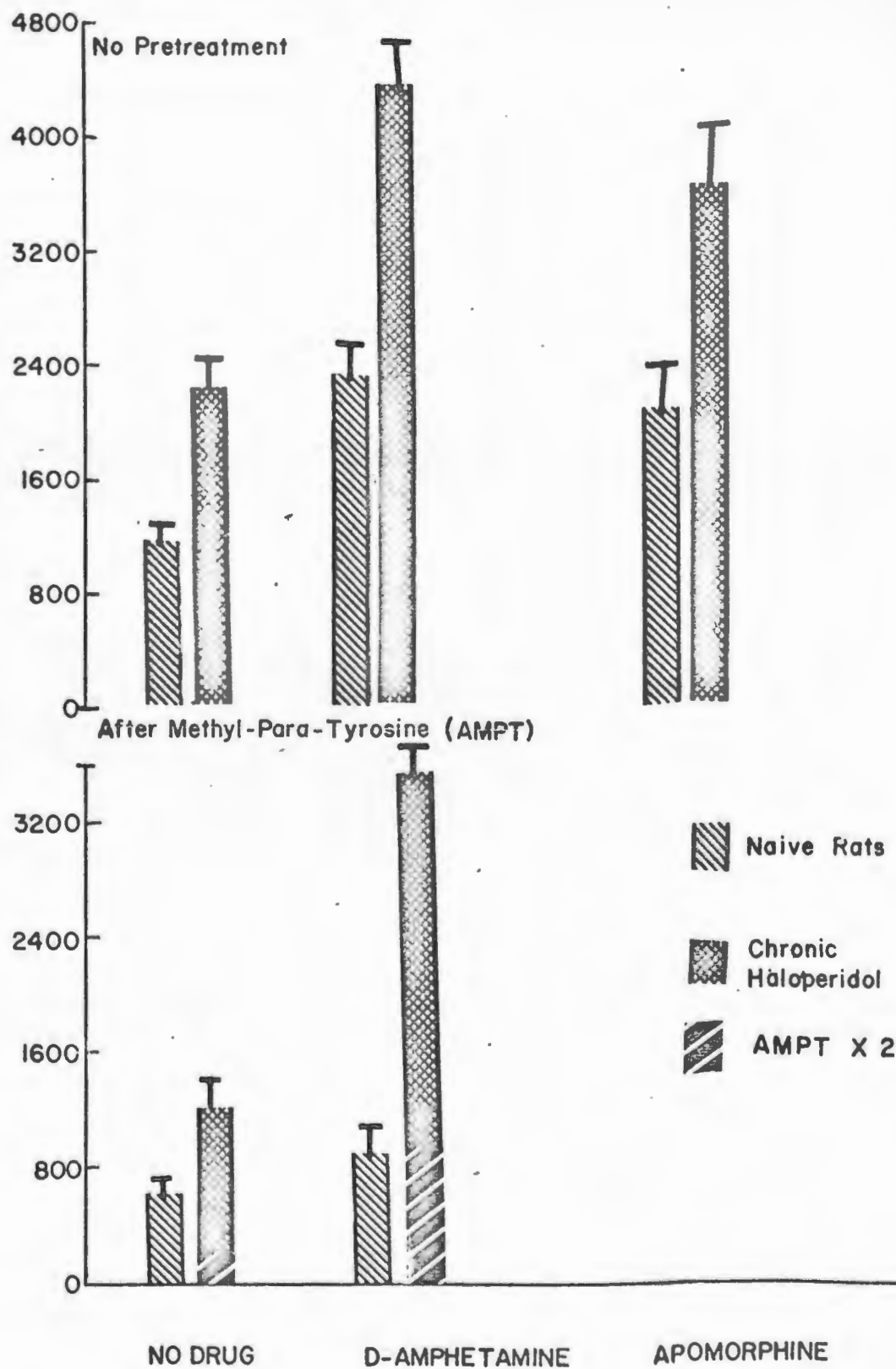


Fig. 6--Effect of amphetamine, apomorphine or alpha-methyl-p-tyrosine (MPT) on locomotor activity in normal and chronically haloperidol treated rats. The rats were chronically treated with haloperidol, then withdrawn for three to five days before locomotor activity testing. Amphetamine (1.6 mg/kg) was injected 60 minutes before testing, while apomorphine (0.5 mg/kg) was injected 10 minutes before testing. MPT was injected either 4 hours or 4 and 16 hours before testing. Locomotor activity in haloperidol treated rats was significantly higher than control rats in each comparison ( $p < 0.01$ ). MPT significantly reduced locomotor activity in all groups (MPT vs. no MPT,  $p < 0.01$ ). After MPT treatment, amphetamine induced locomotor stimulation was not higher than the control activity of normal rats, but was significantly higher ( $p < 0.01$ ) in haloperidol treated rats, except when these rats were given two doses of MPT (stripped bars). Apomorphine failed to increase locomotor activity in normal rats ( $p > 0.05$ ), but did produce stimulation in the chronic haloperidol group ( $p < 0.05$ ).

ACTIVITY COUNTS/HOUR (MEAN  $\pm$  S.E.)



acid, the vehicle for haloperidol, in pilot experiments.) In addition, they exhibited an enhanced response to the stimulation produced by apomorphine and to the maximally effective dose of amphetamine (1.6 mg/kg). When alpha-methyl-p-tyrosine (MPT) was administered (200 mg/kg, four hours before testing), it reduced the spontaneous locomotor activity and blocked the stimulation produced by amphetamine in normal rats (Figure 6). However, amphetamine was still capable of producing a marked stimulation after MPT in the chronic haloperidol group, and only a very large dose of MPT (two doses of 200 mg/kg each, 12 hours apart) was capable of blocking the amphetamine in these rats. These results further support the idea of a dopaminergic supersensitivity after chronic haloperidol.

The influence of drugs having an effect on acetylcholine was also studied. Dexetimide was more effective in producing locomotor stimulation following chronic haloperidol. As shown in Figure 7, normally ineffective doses of dexetimide produced a significant increase in locomotor activity when tested four to six days after termination of repeated haloperidol treatment. In contrast, pilocarpine was less effective in reducing locomotor activity after chronic haloperidol as illustrated in Figure 8. Pilocarpine normally depresses activity at a dose of 4 mg/kg, but this dose did not reduce activity in the chronic haloperidol group. These results are suggestive of a cholinergic hyposensitivity after chronic haloperidol. Pentobarbital was equally

Fig. 7--Effect of dexetimide on locomotor activity in normal and chronic haloperidol treated rats. Dexetimide was injected at the indicated doses into normal rats and to rats who had received their last of a series of haloperidol injections three to six days before testing. Activity was measured for one hour, 30 minutes after injection. Asterisks (\*) indicate that the dose significantly increased ( $p < 0.05$ ) locomotor activity compared with the corresponding saline (0 mg/kg) control group (Dunnet's Test, Winer, 1971).



ACTIVITY COUNTS/h, MEAN  $\pm$  S.E.

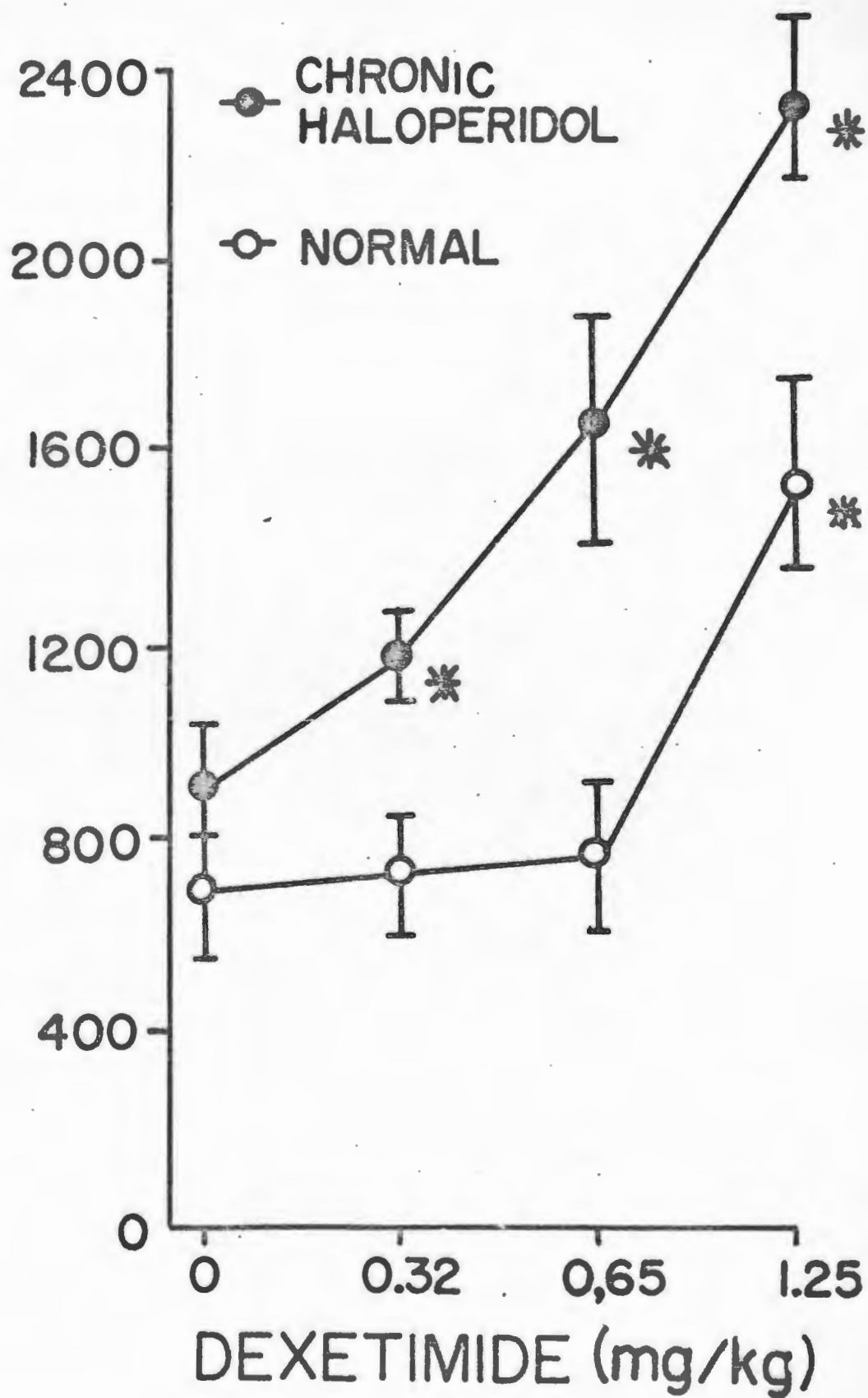
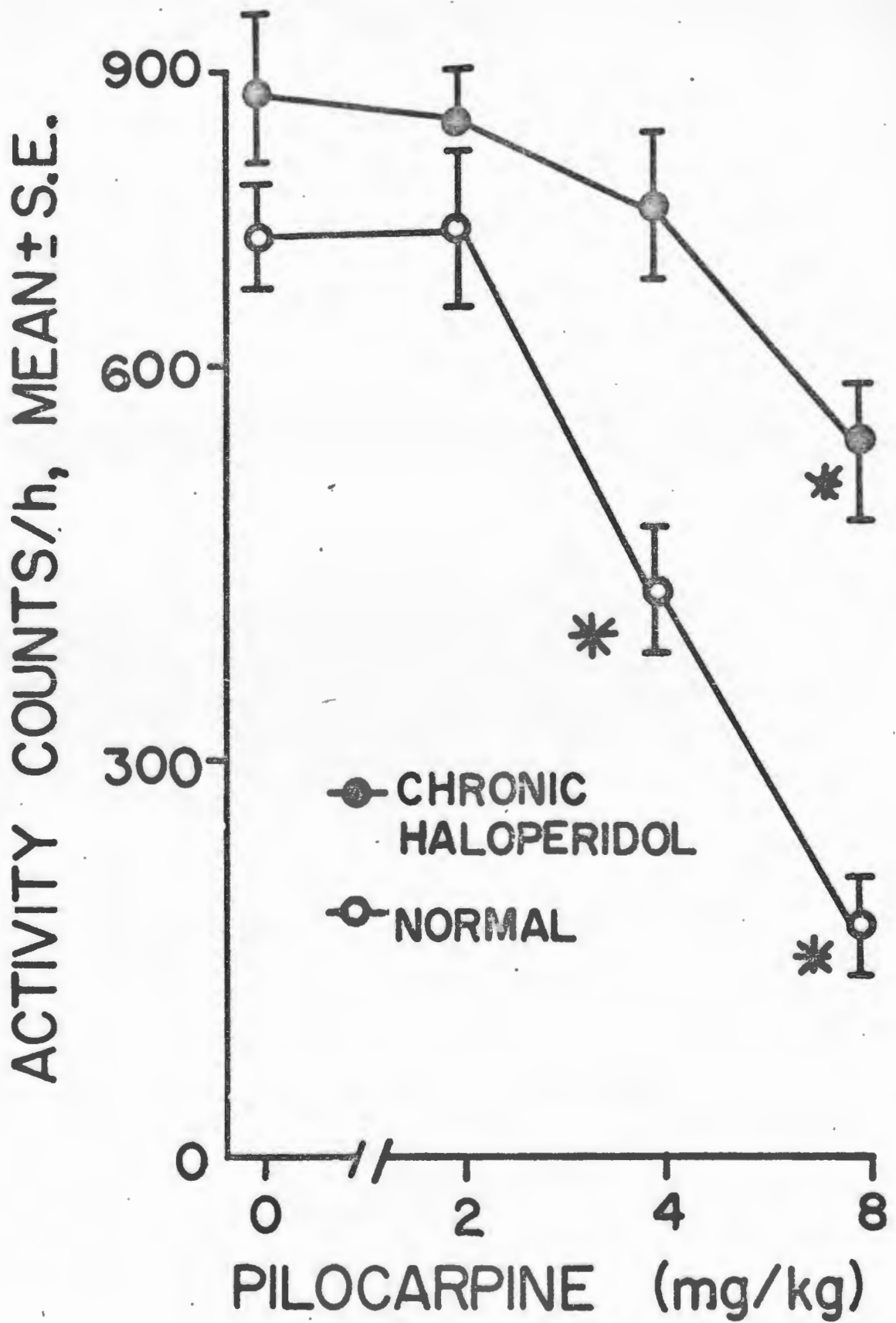


Fig. 8--Effect of pilocarpine on locomotor activity in normal and chronic haloperidol treated rats. Pilocarpine was injected at the indicated doses to normal rats and to rats who had received their last of a series of haloperidol injections three to six days before testing. Activity was measured for one hour, ten minutes after the injection. Asterisks (\*) indicate that the dose significantly ( $p < 0.05$ ) decreased locomotor activity compared with the corresponding saline (0 mg/kg) control group (Dunnet's Test, Winer, 1971).



effective in both normal and haloperidol "withdrawn" rats (Table 28).

Lastly, the dynamics of striatal dopamine were directly measured following termination of chronic treatment with haloperidol, in order to determine if neurochemical substantiation for the dopaminergic supersensitivity could be obtained. It can be seen from Table 29 that neither the steady state level nor the turnover of striatal dopamine differed in the chronic haloperidol group when compared with naive rats. However, doses of apomorphine which produced no change in dopamine turnover in normal rats, significantly reduced striatal dopamine turnover when given seven days after the terminal dose of haloperidol (Figure 9), supporting a concept of dopaminergic supersensitivity.

TABLE 28

EFFECT OF PENTOBARBITAL ON LOCOMOTOR ACTIVITY IN  
RATS AFTER CHRONIC TREATMENT WITH HALOPERIDOL

Group	N	LOCOMOTOR ACTIVITY COUNTS/HOUR (Mean + S. E.)	
		Saline	Pentobarbital <sup>1-</sup>
Control <sup>2</sup>	5	690 ± 68	432 ± 48 *
Chronic Haloperidol <sup>3</sup>	5	891 ± 66	656 ± 82 *

\* Significantly different ( $p < 0.05$ ) from corresponding saline control (Student's t test).

<sup>1</sup> Pentobarbital (10 mg/kg) was injected 10 minutes before testing.

<sup>2</sup> Naive rats.

<sup>3</sup> Three to six days after last haloperidol injection.

TABLE 29

## EFFECT OF CHRONIC HALOPERIDOL TREATMENT ON STRIATAL DOPAMINE

	CONTROLS <sup>1</sup>	CHRONIC HALOPERIDOL <sup>2</sup>
Striatal Dopamine Level (nmol/gram)	43.7 ± 2.7	39.9 ± 4.8
Dopamine Turnover (nmol/g/hour)	11.7 ± 1.3	12.3 ± 1.7

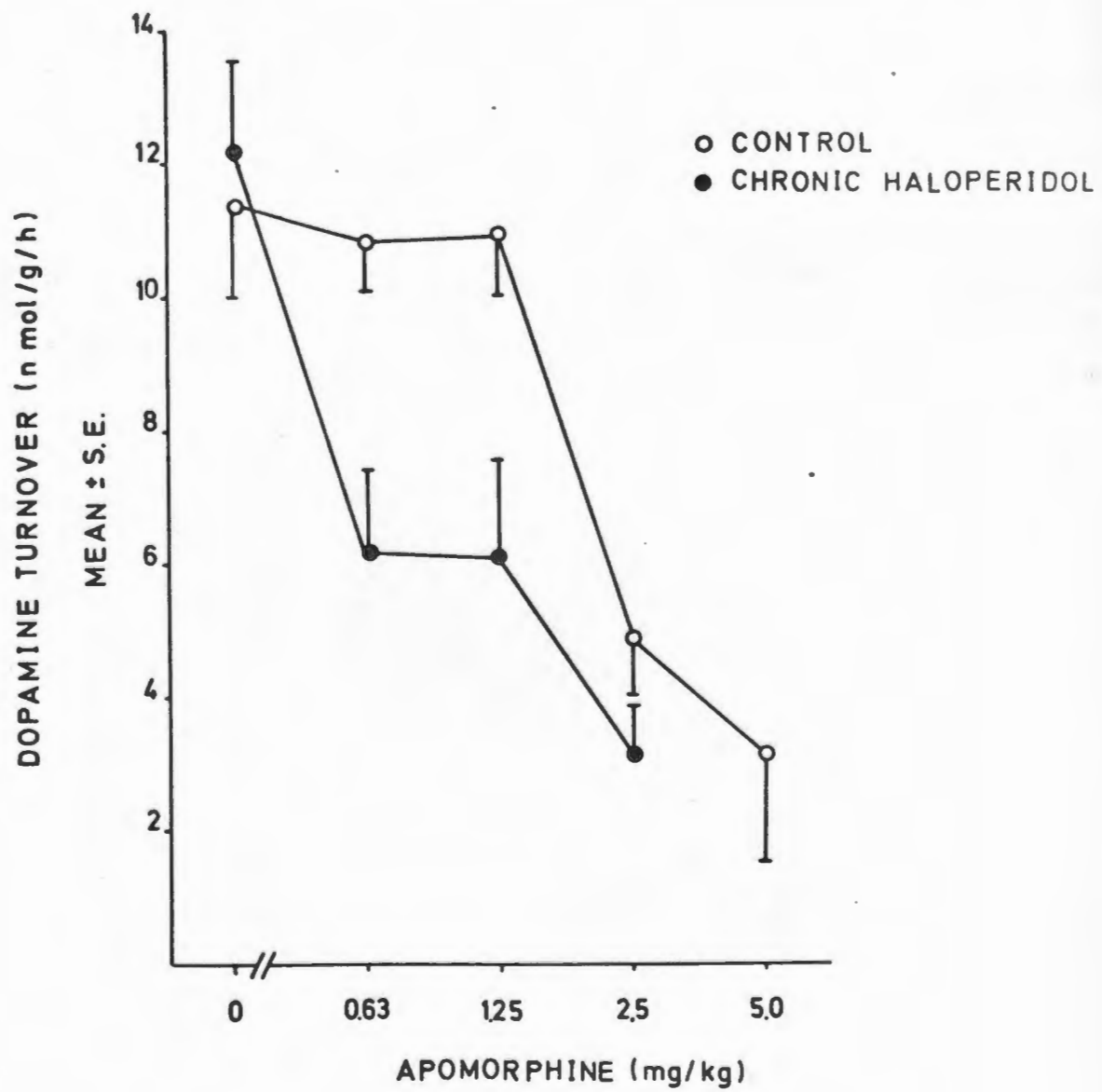
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Values are mean and standard error based upon four animals used in each group. There was no difference ( $p < 0.05$ ) between controls and chronic haloperidol groups on either measure (Student's  $t$  test).

<sup>1</sup> Naive rats.

<sup>2</sup> Rats treated chronically with haloperidol and then left drug free for seven days before testing.

Fig. 9--Effect of apomorphine on striatal dopamine turnover in normal rats and after chronic haloperidol. Rats were sacrificed 30 minutes after apomorphine or saline. The drugs were injected into either normal rats or rats who had received haloperidol for 17 days. Tests were made seven days after the last haloperidol injection. The dose of 0.63 mg/kg significantly reduced the striatal dopamine turnover in the chronic haloperidol group ( $p < 0.05$ ) while the dose of 2.5 mg/kg was the minimum effective dose in normal rats. Points are mean  $\pm$  S. E. for four rats. Dunnet's Test was used for statistical evaluations.





## V. DISCUSSION

The discussion of the results will be divided into the same three sections used in the results (i.e., apomorphine-induced aggression, morphine withdrawal aggression, chronic haloperidol) examining the role of the various neurotransmitters in the behavior. In addition, there will be a section which will specifically compare haloperidol with morphine.

In brief, the postulated mechanisms behind drug-induced aggression may be summarized as shown in Table 30. Dopamine plays a major role in the initiation and maintenance of the behavior, with acetylcholine and serotonin having an antagonistic effect. Stimulation by norepinephrine appears to facilitate the aggression, but further experimentation is necessary to clearly establish this effect. In general, apomorphine-induced aggression and morphine-withdrawal aggression appear to operate under the same mechanisms.

During morphine withdrawal, there is spontaneous and amphetamine stimulated aggression in contrast to the behaviors noted after chronic haloperidol, despite the fact that after either treatment there is evidence of dopaminergic supersensitivity. Several possibilities for this apparent contradiction will be discussed. In brief, these

TABLE 30  
EFFECT OF BRAIN NEUROTRANSMITTERS IN DRUG INDUCED  
AGGRESSION

Transmitter	TYPE OF AGGRESSION	
	Apomorphine Induced	Morphine Withdrawal
DOPAMINE	*	*
ACETYLCHOLINE	-	-
SEROTONIN	-	-
NOREPINEPHRINE	+ ?	+ ?

\* This type of stimulation is necessary for aggression to occur.

- This type of stimulation opposes dopamine effect.

+ This type of stimulation facilitates dopamine effect.

? Evidence is insufficient to make a decision or contradictory.

explanations are that chronic haloperidol may lead to an inhibition of neurotransmitter release in selected brain areas; the supersensitivity after each drug may be qualitatively or quantitatively different; morphine may directly interfere with compensatory mechanisms to alter the normal balance in brain function; or any combination of these effects.

#### A. Apomorphine-Induced Aggression

##### 1) Role of Dopamine

Apomorphine induced aggression in a dose-dependent manner. This was reliably established using three different parameters of aggression. It is generally accepted that apomorphine stimulates dopamine (DA) receptors on the basis of biochemical (Anden et al., 1967), histochemical (Anden et al., 1967), behavioral (Ernst, 1967) and neuroanatomical (Price and Fibiger, 1974) studies. In addition, large doses of apomorphine have pre-synaptic actions on dopamine neurons, causing both an inhibition of DA uptake and a dose-dependent release (Ferris et al., 1974).

These mechanisms may be considered to be responsible for the aggression produced by apomorphine, since it was found that the aggression was blocked by neuroleptics, drugs which are believed to block DA receptors with little if any direct effect on norepinephrine (Nyback et al., 1968; Anden et al., 1970a). In addition the aggression was shown to be intensified by amphetamine. Previous reports have also shown that

the aggression produced by apomorphine plus reserpine is increased by intraventricular dopamine (Patni and Dandiya, 1974).

Evidence for a dopaminergic basis for drug-induced aggression is also supported by several reports in the literature. For example, the aggression in mice elicited by amphetamine is blocked by neuroleptics while drugs which inhibit the synthesis of norepinephrine (NE), such as FLA-63, or block NE receptors, such as phenoxybenzamine do not have a clear-cut blocking action (Hasselager et al., 1972). Similarly, fighting was observed in rats after treatment with DOPA plus reserpine and a peripheral decarboxylase inhibitor, which produced a substantial elevation of brain DA (Benkert et al., 1973a). Elevation of brain NE levels with pargyline plus dihydroxyphenylserine failed to produce fighting (Benkert et al., 1973a). In addition, the aggression produced by ET-495 was increased by L-DOPA (Butterworth et al., 1975). A detailed search of the literature revealed that drug-induced aggression not associated with elevated DA receptor activity is rarely, if ever, observed. Therefore, it may be concluded that DA plays a primary role in drug-induced aggression.

## 2) Cholinergic - Dopaminergic Interaction

Cholinergic activity in the CNS produces a variety of effects on aggression, depending on the model studied. For example, local injection of cholinergic compounds into the

hypothalamus (Myers, 1964) or amygdala (Allikmets et al., 1969) of cats produces a rage response which includes attacks on the experimenter. Similarly, anticholinergic drugs are potent inhibitors of isolation-induced aggression in mice (Janssen et al., 1960). On the other hand, cholinergic drugs suppress the hyperirritability resulting from septal lesions (Stark and Henderson, 1972), while the anticholinergic, scopolamine, when combined with an inhibitor of monoamine oxidase, results in aggression-like responses in rats (Randrup and Munkvard, 1969). In the present experiment, pilocarpine blocked both the aggression which occurs during morphine withdrawal and the aggression induced by injections of apomorphine, while dexetimide, an anticholinergic compound (Janssen et al., 1971) devoid of serotonergic or noradrenergic blocking properties (Janssen, 1971) enhanced both forms of aggression. Furthermore, moderately effective doses of oxyperomide and pilocarpine were synergistic. These results would argue that the effect of cholinergic stimulation in drug-induced aggression is antagonistic to dopaminergic stimulation. The blockade of aggression by pilocarpine appeared to be centrally mediated since it was partially reversed by anticholinergic drugs having a central action but was unaffected by the peripherally acting anticholinergic isopropamide (Janssen and Niemegeers, 1967) at a dose which blocked the pilocarpine-induced diarrhea.

The extensive evidence for a balance between acetylcho-

line and dopamine in the CNS (see Section II.C.1) suggests an explanation for the apparent paradoxical effects of cholinergic stimulation on aggression. Acetylcholine (ACh) has a widespread distribution in the CNS and it may be possible that activity in certain brain areas enhances, while activity in other brain areas reduces aggression. It is interesting in this regard that cholinergic drugs injected into the central nucleus of the amygdala suppress the rage response produced by cholinergic stimulation of the hypothalamus (Decsi et al., 1969). It is possible that the proposed cholinergic inhibitory system directly interacts with a dopaminergic system which initiates the aggressive responding. Therefore, aggression elicited by DA hyperactivity - as a result of apomorphine injection or morphine withdrawal - is modulated by the activation of a cholinergic inhibitory system in a manner analogous to the control of motor movements proposed by Klawans (1968). Pilocarpine would synergize with this cholinergic system, while dexetimide would antagonize it. It should be remembered from Section II.C.1 that many other behaviors produced as a result of DA stimulation are antagonized by ACh, such as stereotypy and locomotor stimulation (Mennear, 1965; Arnfred and Randrup, 1968; Klawans et al., 1970) while the locomotor stimulation produced by anticholinergics is dependent on DA (Thornburg and Moore, 1973b). However, neurochemical support for this hypothesis is lacking. Dopaminergic drugs reduce the release of ACh in the striatum (Sethy and Van Woert, 1974) while anticholinergics

reduce the utilization of DA (O'Keefe *et al.*, 1970; Haubrich and Goldberg, 1975). It would be expected from the above hypothesis that DA stimulation should facilitate the release of ACh. It should be pointed out that the neurochemical analyses were made only in the striatum, which may not be the area responsible for aggression. Interestingly, amphetamine causes an increase in ACh output from the cortex, which is prevented by alpha-methyl-p-tyrosine (Nistri *et al.*, 1972). Alternatively, it may be hypothesized that the reduced ACh as a result of DA stimulation is the mechanism which triggers the aggression, or that still other interactions are necessary.

In addition to the effects enumerated above, low doses of anticholinergic drugs were also able to partially reverse the blockade of aggression produced by the neuroleptics but not the blockade produced by morphine. Anticholinergics also reverse the increase in dopamine turnover produced by the neuroleptics (Anden, 1972) as well as some of the anti-psychotic activity of these drugs (Singh and Smith, 1973).

### 3) Dopaminergic-Serotonergic Interaction

Evidence in favor of a serotonergic system which antagonizes aggression was also provided by this study. It was predicted that amphetamine, which releases dopamine (Besson *et al.*, 1971; Chiueh and Moore, 1973), would intensify apomorphine induced aggression. Although amphetamine did in-

crease the aggression occurring after threshold doses of apomorphine, no aggression was elicited by the combination of amphetamine and sub-threshold doses of apomorphine. It should be remembered that release of serotonin by amphetamine reduced the locomotor stimulation produced by the drug (Breese et al., 1974). It was demonstrated that the combination of amphetamine plus cyproheptadine, a serotonin receptor blocker, and a sub-threshold dose of apomorphine did result in significant aggression, while cyproheptadine at the dose used had no effect of its own. This suggests that serotonin release antagonizes aggression. However, cyproheptadine is non-specific in its effect, since it is anticholinergic and antihistaminic in addition to being anti-serotonergic and these other effects would have to be ruled out before making a definite conclusion.

An additional test of this hypothesis utilized p-chloroamphetamine (PCA) which produces a long-lasting depletion of brain serotonin (Sanders-Bush et al., 1972). This drug produced a marked reduction in the threshold dose of apomorphine necessary for eliciting aggression. Recent evidence (Yunger et al., 1974) suggests that large doses of PCA cause a degeneration of substantia nigra cells, so that it may be interpreted that the apomorphine effect is due to a dopaminergic denervation supersensitivity after PCA. Alternatively, it may be hypothesized that PCA causes reduced activity in a serotonin-containing inhibitory system and therefore removes an inhibition of dopaminergic activity. Consistent



with the latter interpretation are studies which show that parachlorophenylalanine (PCPA), an inhibitor of serotonin biosynthesis (Koe and Weissman, 1966), elicits aggression after treatment with DOPA in mice (Lycke et al., 1969) and rats (Benkert et al., 1973b). Furthermore, three days after an injection of 20 mg/kg of PCA, there is spontaneous aggression in mice (Gianutsos and Lal, 1975).

Apomorphine causes a transient elevation in serotonin turnover which is antagonized by neuroleptics (Grabowska et al., 1973). Furthermore, activity in the dopaminergic caput nuclei caudati rostromedialis of the caudate nucleus is abolished by activation of serotonergic nuclei in the caudate (Cools, 1974). These results suggest that dopaminergic activity activates a serotonergic system which attenuates the effects of the dopaminergic stimulation that initiated the cycle.

In addition, the disruption of behavior produced by scopolamine is antagonized by elevating brain serotonin with 5-hydroxytryptamine (Swonger and Rech, 1972), while pilocarpine increases brain serotonin turnover (Haubrich and Reid, 1972). These findings suggest a co-operation between ACh and serotonin in the CNS. Since both neurotransmitters appear to reduce drug-induced aggression, such a cooperative relationship would be consistent with the data presented.

#### 4) Effect of Clonidine

Clonidine dose-dependently and reliably elicited aggression when combined with a sub-threshold dose of apomorphine. It was previously reported that a very high dose of clonidine (1 mg/kg) could induce some aggression in rats after apomorphine or ET-495 (Senault, 1974), while a smaller dose (0.2 mg/kg) increased the "ferocity" and motor activity of rats produced by ET-495 while inhibiting the stereotypy (Butterworth et al., 1975). The present results confirmed and extended the subjective impressions gained by these fixed dose experiments. Clonidine, by itself, did not produce aggression in rats.

Clonidine is considered to be a centrally acting noradrenergic stimulant since it decreases the rate of depletion of norepinephrine in the brain after alpha-methyl-*p*-tyrosine (Anden et al., 1970b). This effect was measured both biochemically and histochemically. However, clonidine in vitro is also reported to inhibit the stimulation induced release of norepinephrine from brain slices (Starke and Montel, 1973) and block the increase in cyclic AMP formation by norepinephrine (Vetulani et al., 1975), which suggests that clonidine may have an anti-adrenergic effect. Similarly, clonidine in low doses decreased the self-stimulation of the medial forebrain bundle, which is usually enhanced by norepinephrine (Vetulani et al., 1975). Clonidine does not affect the turnover of dopamine in the striatum (Anden et al., 1970b; Rochette and Bradet, 1975).

If it is assumed that clonidine is a noradrenergic agonist, it may be argued that dopaminergic activity initiates noradrenergic activity (or vice versa) and that this combination leads to the expression of aggression. Some evidence which would support this notion is as follows: Apomorphine-induced aggression is blocked by large doses of the alpha-adrenergic receptor blocker, phenoxybenzamine (Senault, 1974) or the beta noradrenergic receptor blocker, L-propranolol (Butterworth et al., 1975). In mice, a very large dose of clonidine induces aggression by itself, but this aggression is blocked by haloperidol and not by phenoxybenzamine. Neurochemically, apomorphine increases the turnover of norepinephrine, apparently by an indirect action through stimulation of dopamine receptors (Persson and Waldeck, 1970). On the other hand, Geyer and Segal (1974) reported that intracerebral injections of dopamine enhance shock induced aggression, while intracerebral norepinephrine reduces the aggression. Similarly, it was shown in REM-sleep deprived rats that aggression could be elicited by apomorphine, L-DOPA or phenoxybenzamine (Carlini and Lindsey, 1974). These latter results suggest that norepinephrine reduces aggression.

An alternative explanation may be that the clonidine effect is mediated by serotonin. Clonidine, at a dose which does not affect serotonin metabolism on its own (1 mg/Kg), antagonized the increase in the accumulation of the serotonin

metabolite, 5-hydroxyindoleacetic acid produced by apomorphine (Maj et al., 1973). Thus, it may be argued that, as above, stimulation of dopamine receptors produces a compensatory antagonistic activation of serotonin neurons. Clonidine, therefore, would exacerbate the aggression induced by apomorphine by preventing the release of serotonin which would normally antagonize the aggression in a manner analogous to the argument for PCA (above).

#### B. Morphine Withdrawal Aggression

Aggression during morphine withdrawal has been considered to reflect a dopaminergic supersensitivity in the CNS since it is enhanced by apomorphine (Puri and Lal, 1973a), amphetamine (Thor, 1969; Lal et al., 1971) and L-DOPA (Puri and Lal, 1973a) and reduced by haloperidol (Puri and Lal, 1973a). The degree of aggression was shown to be dependent on the amount of morphine administered during the addiction process, since increasing the terminal dose or the duration of addiction increased the amount of aggression seen during withdrawal. The aggression reaches its maximum intensity at 72 hours of withdrawal, in contrast to other signs of withdrawal which peak earlier. However, the onset of withdrawal hyperactivity was found to be well correlated with the appearance of the aggression.

The aggression was reduced by morphine, haloperidol or lesions of the dopaminergic nigro-striatal bundle, which pro-

vides further support for the role of dopamine in the withdrawal aggression. The withdrawal aggression was affected in qualitatively the same way as apomorphine-induced aggression. It was found to be blocked by morphine, haloperidol or pilocarpine and increased by dexetimide, apomorphine, amphetamine and clonidine, implying that the same mechanisms responsible for apomorphine-induced aggression also govern morphine-withdrawal aggression. However, in contrast to these effects in naive rats, anticholinergics failed to antagonize the blockade of aggression produced by neuroleptics in addicted rats. This will be discussed further below.

#### 1) Elicited Aggression

Naloxone precipitated withdrawal in addicted rats when administered within a few hours of the last morphine injection, but the symptomatology had some differences from the withdrawal produced by withholding of morphine injections. In the naloxone precipitated withdrawal, there was evidence of teeth chattering and lacrimation which are not seen during spontaneous withdrawal and the naloxone treated rats showed more diarrhea and fewer "wet shakes" than spontaneously withdrawn rats (see Gianutsos et al., 1975). In addition, the naloxone-treated rats failed to consistently show aggression during the time in which they exhibited other signs of withdrawal. It should be pointed out that rats undergoing either naloxone-precipitated or spontaneous withdrawal are hyperirritable and will vocalize and struggle violently

and bite the experimenter when handled. However, when grouped, the naloxone treated rats did not consistently fight with each other. Since the onset of withdrawal aggression in spontaneously withdrawn rats occurs later than other signs, it perhaps may be expected that aggression during naloxone-precipitated withdrawal may occur at a later time as well. Furthermore, the possibility remains, as yet untested, that naloxone may compress the withdrawal period such that aggression may emerge earlier than normal after naloxone treatment (that is to say before 48-72 hours of withdrawal) but not during the time of peak drug effect. Naloxone's action could not be attributed to a direct anti-aggression action since it failed to affect either the 72 hour withdrawal aggression or the weak elicitation of aggression produced by amphetamine at four hours of withdrawal. However, it is interesting that naloxone causes an increase in brain dopamine levels in morphine dependent rats (Iwamoto et al., 1973), which is suggestive of decreased transmitter release. Clearly, such an effect would prevent the expression of withdrawal aggression.

In contrast to the effect of naloxone, apomorphine administered as little as four hours after the last morphine injection was capable of eliciting intense aggression in addicted rats without producing other signs of withdrawal. The aggression was produced either in the presence or absence of naloxone. These results strongly suggest that the

addicted rats have a latent dopaminergic supersensitivity which remains masked during the addiction process while morphine injections are continued. Despite the fact that there are supersensitive DA receptors at the time of withdrawal, the aggression still does not emerge until 48-72 hours later. It is possible, although untested, that the supersensitivity functionally increases in intensity over the course of withdrawal as a compensation for other withdrawal signs. It is interesting that directly and indirectly acting dopaminergic agonists reduce the severity of withdrawal, except for aggression which is intensified (Gianutsos, Hynes and Lal, in preparation). Exactly how this may take place is highly speculative, but Marshall and Smith (1974) showed that, in mice, caudate tyrosine hydroxylase activity is depressed at 36 hours of withdrawal, but returns to normal by 72 hours. Thus, one could predict that aggression emerges as the biphasic changes in tyrosine hydroxylase activity return towards normal.

## 2) Protracted Aggression

Following the chronic administration of morphine, spontaneous aggression as well as the supersensitivity to apomorphine was evident at 30 days of withdrawal. This suggests that the neuronal changes associated with narcotic dependence and withdrawal are long-lasting phenomena. Lesioning of the nigro-striatal bundle (NSB), which is the major dopaminergic fiber tract, reduced the aggression, while lesioning the ad-

jacent medial forebrain bundle was ineffective. When a normally ineffective dose of apomorphine was administered to dependent rats with the NSB lesion, the aggression was reinstated which demonstrated that the normal pre-synaptic nerve stimulation of receptors could be successfully substituted by a direct chemical stimulation in the lesioned rats and further demonstrated the presence of supersensitive dopamine receptors. The supersensitivity was also demonstrated neurochemically since rats which were withdrawn for 30 days required a significantly smaller dose of apomorphine to produce a decrease in striatal dopamine turnover (reported in Gianutsos et al., 1974).

### C. Chronic Haloperidol

Following prolonged treatment with haloperidol, rats exhibited an enhanced sensitivity to the actions of apomorphine and amphetamine in stereotypy and locomotor activity. Since these effects are believed to be dependent on DA stimulation (Ernst, 1967; Snyder et al., 1970; Costa et al., 1972; Thornburg and Moore, 1973a), it may be proposed that a dopaminergic supersensitivity develops during chronic haloperidol treatment. Since haloperidol is believed to block dopamine receptors (Nyback et al., 1968; Anden et al., 1970a; Koe, 1974), this supersensitivity may reflect a compensatory adaptation to the prolonged receptor blockade. This supersensitivity was also demonstrated neurochemically by a shift to the left in the apomorphine dose-response curve for re-



ducing dopamine turnover in the striatum. Normally ineffective doses of apomorphine were capable of reducing striatal dopamine turnover after chronic haloperidol treatment.

In addition, there was a marked shift to the left of the apomorphine-induced aggression dose-response curve after repeated injections with haloperidol. However, in contrast to the effect of chronic morphine, there was no spontaneous or amphetamine-induced aggression after chronic haloperidol, despite the presence of supersensitive dopamine receptors. It is interesting that amphetamine induces aggression after chronic morphine (Thor, 1969; Lal et al., 1971) or chronic ethanol (Mann and Lal, 1975) both of which are abused drugs, but failed to induce aggression after prolonged treatment with haloperidol, a non-abused drug. The reason for these effects is not clear, but several explanations may be offered. For example, it may be proposed that haloperidol stabilizes the pre-synaptic dopamine neuronal membrane (Janssen, 1967). Such a stabilization may prevent the release of dopamine by stimulation of the nerve or by amphetamine so that the amount of dopamine reaching the receptor is insufficient to produce aggression. Such an effect, if it in fact occurs, would have to be long-lasting and specific for brain areas which are responsible for aggression, since other actions of amphetamine (stereotypy, locomotor stimulation) are enhanced after chronic haloperidol. A long-lasting alteration of pre-synaptic dopamine mechanisms may be an important aspect of the haloperidol action, working in concert with receptor

blockade for the alleviation of schizophrenia. Clearly, it would not be beneficial to treat schizophrenics with haloperidol only to produce supersensitive receptors, requiring ever increasing doses of an antagonist and which, in the absence of receptor blockade, would result in an exacerbation of symptoms when the drug was discontinued.

Evidence to support this hypothesis is lacking. However, Seeman and Lee (1975) demonstrated that neuroleptics block the coupling between nerve impulse and dopamine release, which was correlated with anti-psychotic potency. Alternatively, the interaction between DA and other neurotransmitters (e.g. serotonin, ACh) may be different after chronic haloperidol or morphine (see below). Lastly, the supersensitivity occurring after chronic haloperidol may be qualitatively or quantitatively different from the supersensitivity occurring after chronic morphine (see below).

Long-term neuroleptic therapy reportedly results in tardive dyskinesias in humans which is associated with dopaminergic supersensitivity and a cholinergic subsensitivity (Klawans, 1973; Gerlach et al., 1974). A similar relationship was demonstrated with drug effects on locomotor activity in rats following chronic haloperidol in the present experiment. The stimulation produced by dopaminergic agonists (amphetamine, apomorphine) was enhanced in these rats. A similar effect was reported in mice (Thornburg and Moore, 1974; Von Voightlander et al., 1975). In addition, the

stimulation of locomotor activity produced by the anticholinergic, dexetimide, was likewise enhanced. Conversely, the decrease in locomotor activity produced by cholinergic stimulation (pilocarpine) was antagonized after chronic treatment with haloperidol. A generalized increase in CNS excitability could be proposed as the mechanism responsible for these effects, except that the effect of pentobarbital in reducing locomotor activity was identical in normal and in chronically haloperidol treated rats. This would also rule out an explanation based on interference with drug metabolism.

The locomotor stimulation produced by both amphetamine (Costa et al., 1972; Thornburg and Moore, 1973a; Hollister et al., 1974) and anticholinergics (Thornburg and Moore, 1973b) is dependent on dopaminergic stimulation. However, this is not a general phenomenon, since the improvement of shuttle-box avoidance by amphetamine requires catecholamines, while the similar effect of the anticholinergic, scopolamine, is not dependent on catecholamines (Rech and Moore, 1968). Several anticholinergics are known to block the reuptake of dopamine (Coyle and Snyder, 1969) and, although this has not been studied with dexetimide, it could provide an explanation for the enhanced stimulatory effects since the dopamine which would be prevented from returning to the pre-synaptic neuron for inactivation would react with supersensitive receptors. However, it has been demonstrated that anticholinergics produce their effects on locomotor stimulation by an anticholinergic rather than an uptake-blocking mechanism (Thornburg

and Moore, 1973c). Furthermore, the decreased effectiveness of pilocarpine could not be accounted for by a mechanism which is based on blockade of catecholamine uptake. Since cholinergic drugs antagonize the stimulation produced by dopaminergic drugs (Mennear, 1965), while anticholinergics apparently facilitate the expression of DA activity held in check by cholinergic stimulation, it may be concluded that the results can be explained by a reduced sensitivity of the cholinergic receptor as a result of chronic neuroleptic treatment (also see below).

#### D. Comparison of Haloperidol and Morphine

Haloperidol and morphine share a number of similar behavioral and biochemical actions related to dopamine. For example, both drugs produce a state of immobility (Costall and Naylor, 1974); block the stereotypy produced by dopaminergic agonists (Janssen et al., 1965; Puri et al., 1973); block jumping produced by a combination of DOPA + amphetamine (Colpaert et al., 1975); inhibit conditioned avoidance responding (Niemegeers et al., 1964); inhibit brain self stimulation (Wauquier et al., 1974; 1975); and inhibit the aggression produced by DOPA + amphetamine (Lal et al., 1972) by shock (Lal et al., 1975a) and, as shown here, the aggression produced by apomorphine or morphine withdrawal. In addition, both morphine and haloperidol increase the turnover of brain dopamine (see II.B.3); increase the activity of dopamine stimulated adenylate cyclase

(Iwatsubo and Clouet, 1975); and increase plasma prolactin levels while decreasing plasma luteinizing hormone levels (Clemens et al., 1974; Dobrin and Mares, 1974). After chronic treatment with either drug, behavioral and neurochemical evidence of dopaminergic supersensitivity is noted (this thesis and Puri and Lal, 1973a; 1974b; Tarsy and Baldessarini, 1973). In addition, repeated treatment with either drug results in locomotor stimulation during withdrawal (this thesis) and facilitates recovery from lesions of the lateral hypothalamus (Hynes et al., 1975). Moreover, morphine withdrawn rats are tolerant to the increase in striatal dopamine turnover produced by either drug (Puri and Lal, 1974a).

However, equally striking are the differences between the two drugs. Most notable, of course, is the well recognized ability of morphine to produce physical dependence and reinforcing properties. Recently, Glick and Cox (1975) demonstrated that very small doses of haloperidol would be self administered by the rat, but it is generally recognized that haloperidol is not abused and does not produce physical dependence. In addition, the behavioral effects of morphine and haloperidol are differentially antagonized by different compounds. Anticholinergics reverse the blockade of aggression produced by neuroleptics, but not that produced by morphine. On the other hand, morphine's actions were antagonized by naloxone, but haloperidol's were not. Similar results have been demonstrated for the catalepsy produced by narcotics and neuroleptics (Kuschinsky and Hornykiewicz,

1972; Costall and Naylor, 1974) and for the blockade of mouse jumping induced by DOPA + amphetamine (Colpaert et al., 1975) and the blockade of brain self-stimulation (Wauquier et al., 1974; 1975) produced by either drug. This points up an important difference in the dopaminergic/cholinergic balance between these two compounds and suggests that the reduction of dopaminergic activity by morphine may be indirect. Furthermore, the ability of anticholinergics to reverse the neuroleptic-induced blockade of aggression was lost after chronic treatment.

It was pointed out previously that chronic treatment with either haloperidol or morphine produces measurable dopaminergic supersensitivity, but only treatment with the latter results in spontaneous aggression. One possible explanation has already been offered (see previous section). Alternatively, there may be differences in the supersensitivity produced by the two drugs. Kuschinsky (1975) observed that morphine dependent rats failed to show an enhanced sensitivity to apomorphine-induced stereotypy, while a dopaminergic supersensitivity was demonstrated neurochemically. Some unpublished, preliminary observations in our laboratory would be consistent with these results. However, following chronic haloperidol, enhanced apomorphine-induced stereotypy is readily observed (this thesis and Schelkunov, 1967; Tarsy and Baldessarini, 1973). Furthermore, the supersensitivity produced by morphine appears to be longer lasting

than the comparable supersensitivity produced by haloperidol (N.B. apomorphine-induced aggression appeared to fade 18 days after stopping haloperidol, while the morphine effect lasted for at least 30 days of withdrawal). This suggests that morphine and haloperidol may produce a qualitatively different supersensitivity, perhaps by interacting with different types or groups of receptors.

It should be remembered that after chronic haloperidol, a subsensitivity to cholinergic stimulation was noted. Since neuroleptics, as a result of DA receptor blockade, increase the release of ACh (Stadler et al., 1973; Guyenet et al., 1975; McGeer et al., 1974) one may hypothesize a three stage process of adaptation to CNS DA receptor blockade. In the first stage, since blockade of DA receptors by neuroleptics reduces the stimulation of those receptors (and increases the release of ACh), the post-synaptic neuron would attempt to compensate by sending messages to the dopamine neuron to increase the synthesis and release of DA in order to override the effect of the neuroleptic or other drug. There is ample evidence that this occurs after neuroleptics (Anden et al., 1971; Carlsson and Lindqvist, 1963). Since this method is inefficient over the long-term, the post-synaptic neuron could also compensate by becoming supersensitive to stimulation by DA. In the meantime, the cholinergic sensitive receptors could also compensate for the unusually large number of messages reaching them by becoming less sensitive to cholinergic stimulation. By these adaptations, a new

homeostatic balance is developed. (Incidentally, if one was to speculate that an enhanced release of ACh from DA sensitive sites was responsible for the anti-aggression action of the neuroleptics, it would explain the antagonism by anticholinergics of the neuroleptic effect.) Although this hypothesis was presented in terms of DA and ACh, it would work equally well for DA and serotonin (i.e., DA receptor blockade would reduce the need for serotonin to antagonize DA and so the serotonin neuron would decrease its activity. In the long run, one would predict that the serotonin receptors per se would become supersensitive due to a lack of stimulation and this would contribute to the lack of aggression during "withdrawal" of haloperidol).

Morphine, on the other hand, may be thought of as interfering with and modifying the normal compensatory processes, so that a more drastic or entirely different mechanism for adaptation would be called into play. There are some differences in the effects of morphine and haloperidol on ACh. It is believed, for example, that morphine blocks the release of ACh (Beleslin and Polak, 1965), although this effect has been questioned (Mullin and Phillis, 1974). A resulting cholinergic supersensitivity has been proposed as being responsible for narcotic withdrawal (Paton, 1969). Although it is known that ACh utilization is increased during withdrawal of narcotics (Domino and Wilson, 1973), this effect does not appear to follow the temporal pattern of the



abstinence syndrome (Mullin and Phillis, 1974). It has been noted that neuroleptics accelerate the turnover of ACh in the striatum, but do not affect cortical ACh turnover (Trabucchi et al., 1974), while morphine decreases the turnover of ACh in the cortex, with no effect on the striatum (Cheney et al., 1974). This has been attributed to a combined direct effect on morphine on ACh neurons and an indirect effect mediated by DA (Cheney et al., 1974). Furthermore, Yarbrough (1974) was unable to demonstrate an increased sensitivity of cortical neurons to ACh when measured six hours after a terminal injection of morphine, but the efficacy of atropine in blocking the effects of ACh was reduced (another apparent distinction between morphine and haloperidol, since the behavioral effects of anticholinergics were increased after chronic haloperidol).

In spite of the seeming cholinergic supersensitivity, there are some inconsistencies. The ratio of the effective anti-aggression dose of pilocarpine to haloperidol in blocking apomorphine-induced aggression in naive rats was 2:1 (5 mg/kg:2.5 mg/kg). The ratio of these drugs in blocking morphine withdrawal aggression was 16:1 (10 mg/kg:0.64 mg/kg). Thus, despite the fact that there is supposed to be a dopaminergic supersensitivity and a cholinergic supersensitivity during narcotic withdrawal, pilocarpine (cholinergic stimulation) was relatively much less effective than dopaminergic receptor blockade in decreasing morphine withdrawal aggression

than in decreasing apomorphine-induced aggression in naive rats. Thus it may be proposed that the normal compensatory balance between ACh and DA is interfered with during morphine addiction and the central cholinergic stimulation may not be relied upon following chronic morphine. In other words, while cholinergic receptors in the simpler circuitry of the peripheral nervous system may adapt by becoming more sensitive to cholinergic stimulation, central cholinergic neurons, which are under a more complex regulation, may be bypassed and become functionally inoperative. If so, withdrawal aggression may be evident because of the functional lack of a cholinergic inhibitory system.

In addition, morphine is known to increase the turnover of serotonin in the brain (Way et al., 1968; Yarbrough et al., 1971; Haubrich and Blake, 1973). There is apparently no tolerance to this effect when rats are made dependent by morphine injections (Bowers and Kleber, 1971). Administration of PCPA prevents the development of morphine tolerance and physical dependence (Way et al., 1968). If one proposes that the increased turnover of serotonin is a consequence of increased release of serotonin directly produced by morphine, this may contribute to the anti-aggression action of the drug and provide an explanation for the aggression during withdrawal. It may be further assumed that this continual release produces a decrease in sensitivity of serotonin receptors, analogous to the argument made for subsensitive cholinergic receptors after chronic haloperidol (above), and/or an addi-

tional further compensatory increase in sensitivity of dopamine receptors. Under this assumption, very sensitive DA receptors would be associated with subsensitive serotonergic receptors during morphine withdrawal, in contrast to the assumption made for chronic haloperidol in which both sets of receptors were assumed to be supersensitive.

Lastly, the recent discovery of endogenous morphine-like peptides in the brain (Hughes, 1975) introduces another possible mechanism for the direct or indirect actions of narcotics. Although the exact significance of these endogenous compounds is unclear at this time, their importance cannot be ruled out.

## VI SUMMARY AND CONCLUSIONS

1. Apomorphine induced aggression in a dose-dependent manner in naive rats. The aggression was blocked, dose-dependently, by haloperidol, oxyperomide and morphine and increased by amphetamine. These results suggest that the aggression is the result of hyperactivity of dopaminergic systems in the brain.
2. The blockade of aggression by haloperidol or oxyperomide was reversed by anticholinergics, but not by naloxone. Morphine's anti-aggression action was reversed by naloxone but not by anticholinergics. This suggests that the neuroleptics and morphine produce a blockade of aggression by different mechanisms, perhaps involving a cholinergic component in the case of neuroleptics.
3. Apomorphine-induced aggression was blocked dose-dependently by pilocarpine and increased by dexetimide. In addition, it was enhanced by pretreatment with p-chloroamphetamine or clonidine, while the amphetamine effect was enhanced by cyproheptadine. These results suggest that the aggression is modulated by activity in cholinergic, serotonergic and, possibly, noradrenergic areas. Cholinergic and serotonergic stimulation appears to inhibit aggression while noradrenergic stimulation may facilitate the aggression.

4. Morphine withdrawal aggression was noted when withdrawal was initiated by withholding of morphine injections. The aggression was blocked by morphine, haloperidol or by lesions of the dopaminergic nigro-striatal bundle and was enhanced by apomorphine. When withdrawal was precipitated by naloxone, aggression was not observed when other signs of withdrawal stress were noted. However, a small dose of apomorphine was able to elicit aggression when given as little as four hours after the last morphine injection. These results further provide evidence that chronic morphine administration results in dopaminergic supersensitivity and that this is reflected in the withdrawal aggression, but suggests that mere removal of morphine from its "receptors" by naloxone is insufficient to elicit the aggression.

5. Morphine withdrawal aggression was noted even 30 days after withdrawal was initiated and was enhanced by small doses apomorphine and blocked by nigro-striatal lesions, suggesting that the supersensitivity produced during narcotic dependence is a long-lasting phenomenon.

6. Morphine withdrawal aggression pharmacologically resembled apomorphine-induced aggression since both were enhanced by dexetimide, amphetamine and clonidine and both were reduced by haloperidol, morphine and pilocarpine. However, the blockade of aggression produced by haloperidol was reversed by anticholinergics in naive rats, but not in morphine-withdrawnrats, suggesting that an alteration in a cholinergic

inhibitory system or in the balance between dopaminergic and cholinergic activity develops during the course of narcotic addiction.

7. Following discontinuation of long-term treatment with haloperidol, enhanced apomorphine or amphetamine induced stereotypy or locomotor stimulation was observed, leading to the conclusion that this treatment induces supersensitivity of dopamine receptors. This was further supported by neurochemical evidence which showed that the dose response curve for apomorphine-induced reduction in striatal dopamine turnover was shifted to the left after chronic treatment with haloperidol was discontinued.

8. The dose of apomorphine required to elicit aggression was also markedly reduced after cessation of chronic haloperidol injections, supporting the hypothesis of dopaminergic supersensitivity as the mechanism for morphine withdrawal aggression. In contrast to the effect of chronic morphine, there was no spontaneous or amphetamine-induced aggression after chronic haloperidol. These results suggest either that other mechanisms besides dopaminergic supersensitivity are necessary for morphine withdrawal aggression; or that the supersensitivity produced after these two treatments are dissimilar; or that after chronic haloperidol treatment there is interference with the process of transmitter release; or that there are differences in the balance between dopamine and other transmitters after treatment with the two drugs; or any combination of these factors.

9. Following chronic haloperidol, the stimulation of locomotor activity produced by dexetimide was increased and the locomotor depression produced by pilocarpine was decreased, while pentobarbital was equally effective in naive and chronically haloperidol treated rats. These results suggested that a cholinergic subsensitivity develops during chronic haloperidol treatment, perhaps as a compensatory mechanism.

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