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Interaction of cocaine with some central dopominergic and serotonergic mechanisms

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INTERACTION OF COCAINE WITH SOME CENTRAL DOPAMINERGIC AND SEROTONERGIC MECHANISMS

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Abstract of Thesis

The present study in male Wistar rats was designed to rate and analyze six specific cocaine-induced behaviors. These behavioral parameters have been defined by others as either dopaminergic (sniffing, grooming, and locomotor activity) or serotonergic (repetitive head movements, rearing, and Straub tail) in origin.

Results were analyzed by analysis of variance in two ways: (i) as grouped dopaminergic or serotonergic scores, and (ii) as the net behavioral index (dopaminergic scores minus the serotonergic scores). The purpose of approaching the data in this way was to attempt to define the behavioral interactions of the two neurotransmitters.

One conclusion that developed from this study was the indication that dopaminergic behaviors peak at lower doses of cocaine than do serotonergic behaviors. This relationship held true for all the individual parameters in addition to the dopaminergic and serotonergic totals.

A dopaminergic blocker, haloperidol, significantly attenuated all responses elicited by cocaine. When the net behavioral index was analyzed,it was found that the response of the median dose of cocaine was significantly altered from a net dopaminergic score towards a net serotonergic score. In this sense, haloperidol was shown to have the capacity to attenuate dopaminergic-associated parameters to a greater extent than the serotonergic-associated parameters.

Cyproheptadine, an antiserotonergic agent, did not significantly affect the net behavioral index; however, this compound did significantly increase the dopaminergic parameter of grooming at the high doses of cocaine and cyproheptadine. Also at this dose combination, gnawing was elicited --a dopaminergic response seen under no other experimental conditions. Due to the antiserotonergic agent causing an increase in the dopaminergic parameters of grooming and gnawing, it is proposed that the serotonergic influence on these two dopaminergic behaviors is of an inhibitory type.

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INTRODUCTION

History

A classic drug, cocaine, the active alkaloid of the shrub Erythroxylon coca, is perhaps the most euphorogenic as well as the most legislated against drug of all time (Mortimer, 1974).

The coca leaf was believed to be a gift to the Inca people from Manco Ccapac, son of the Sun God, bestowed as a token of esteem and sympathy for their suffering labor. Originally restricted to Inca aristocracy, the destruction of the Incan civilization in the 16th century by Francisco Pizarro led to the widespread use of coca by the masses. The stimulation from coca leaf provided the conquistadores an effective means of low-cost/high-output labor. In practice, coca leaves, bound into a ball (cocada) with guano or cornstarch, were chewed with lime (as the alkaline ash) to release the active alkaloid.

In 1859, the Italian physician, Paolo Mantegazza introduced coca leaves into European medicine. Americans viewed coca leaves as a panacea while the English viewed them as a pharmacological curiosity.

Alfred Niemann isolated and named the active alkaloid in 1860. Producers of tonics, elixirs, and wines soon

added coca extractives to their lists of ingredients, the classic example being 'Vin Mariani' a preparation made from coca leaves and wine (popular with Queen Victoria and Pope Leo XIII) (Gay, 1976).

Sigmund Freud experimented with cocaine and was soon using the alkaloid in the treatment of various physical and psychological disorders. Between 1884 and 1887 he wrote five papers ("Ueber Coca") extolling coca as a wonder drug (Freud, 1884).

Freud's colleague, Karl Koller, is responsible for the first report of the local anesthetic effects on the eye (Mortimer, 1974). Dr. William Halstead of Johns Hopkins in Baltimore performed the first nerve block in 1885. By 1886, Halstead wrote no further about cocaine as he battled his own two gram per day habit!

Coca Cola, when first introduced in 1888 contained cocaine until the Harrison Tax act of 1914 outlawed cocaine as a "narcotic."

Robert Louis Stevenson is said to have composed the first draft of Doctor Jekyll and Mister Hyde in three days while undergoing treatment of tuberculosis with cocaine. Arthur Conan Doyle, the literary genius who created Sherlock Holmes, portrayed Holmes as an advocate of cocaine usage in the novel, The Sign of Four. Doyle himself was reputed to be a user of cocaine.

Admittedly, this is only a cursory look at the long and exotic history of cocaine. Books have been written on the subject and the reader is referred to Mortimer's book on the history of cocaine (Mortimer, 1974) for further information.

General Pharmacology

Cocaine is classified as both a local anesthetic and a central nervous system stimulant. It is the only natural product with both of these pharmacologically different qualities, and the mechanisms for these two simultaneous actions are still far from certain. To some extent cocaine is a direct agonist of adrenergic receptors, although the importance of this action is questionable. Also, cocaine blocks nerve conduction in the periphery and probably centrally as well (Trendelenberg, 1968). Both of these mechanisms may play a role in the central effects that cocaine produces, but by far the most investigated (if not necessarily the most important) is the ability of cocaine to block presynaptic reuptake of norepinephrine, dopamine, and serotonin--the major mechanism of deactivation for the catecholamines and serotonin (Schubert et al., 1970; Taylor and Ho, 1977).

Literature evaluation reveals a lack of older papers dealing with the neuropharmacology of cocaine. The older literature deals with the alkaloid in terms of its local anesthetic qualities, behavioral reinforcing properties or

as an investigational tool taking advantage of its reuptake blocking capabilities *in vitro*.

It is the most recent literature on cocaine which serves as the basis of this thesis. This literature on the behavioral pharmacology of cocaine will be discussed in detail as the last part of this introduction. For completeness, the literature on conditioned behavior, seizure induction, and the biochemical pharmacology will be discussed first.

Effects on Conditioned Behavior

Cocaine acts as a positive reinforcer of conditioned behavior (for a recent review see Schuster and Balster, 1977). In a recent paper by McKenna and Ho (1977), the behavioral reinforcing properties were reduced with chronic cocaine experimentation. This implies a tolerance developing to the reinforcing properties of cocaine over time. The behavioral reinforcing properties of cocaine are attenuated by the neuroleptics pimozide and spiroperidol (Colpaert, *et al*., 1979). It is these types of studies--defining drugs which abolish behavioral reinforcing properties--that serve to elucidate mechanisms of action. Since spiroperidol and pimozide are known to block the dopamine receptor, this is good evidence that cocaine's mechanism of action is mediated through the dopaminergic system.

Cocaine has been shown to function as a discriminative

stimulus in monkeys (Spealman and Kelleher, 1979; Byrd, 1979; Goldberg, 1973; Stretch et al., 1976) and in rats (Colpaert et al., 1978; Ross et al., 1978; McKenna et al., 1979). Usually in these experiments, laboratory animals are trained to emit one response when treated with a stimulus drug, and an alternate response when treated with the drug vehicle, a different dose of the same drug, or a different drug. When acquisition of such a response is reliably established, the drug is said to produce a discriminative stimulus that controls the differential responding in the trained subjects. In all such trained subjects, cocaine can be discriminated from saline and different doses of cocaine can be discriminated from each other (Goldberg, 1973; Byrd, 1979; Colpaert et al., 1978; Ross et al., 1978).

On the other hand, the trained subject may mistake the stimulus drug from other drugs in a similar class. This lack of preference is known as stimulus generalization. Studies with monkeys and rats have shown that d-amphetamine and norcocaine (the N-demethylated metabolite) both produce stimulus generalization with cocaine (Stretch et al., 1976; McKenna et al., 1979).

Chronic administration and seizure development

An interesting phenomenon observed with chronic cocaine administration is the development of seizures. This has

been referred to as reverse tolerance due to the fact that the seizure development is elicited with subseizure doses of cocaine and only occurs after chronic administration of the drug. Post and Kopanada (1975) have implicated catechol involvement in this phenomenon due to the facilitation of these seizures by 6-hydroxydopamine (chemical sympathectomy). Due to the similarity of this chronic effect of cocaine and of low level electrical stimulation of the amygdala, this process has also been referred to as 'kindling' (Stripling and Ellinwood, 1977). The precise developmental mechanism for kindling is not known.

As chronic cocaine administration reaches the seizure threshold, an augmentation of behavioral response--behavioral reverse tolerance--occurs (Schuster et al., 1977). Rackham and Wise (1977) investigated the mechanisms involved in cocaine and electrical kindling by stopping cocaine therapy after behavioral sensitization occurred but before seizures developed. They then continued the experiment with electrical stimulation. They found that cocaine pretreatment did not facilitate the onset of electrically-induced kindling; therefore, these two means of kindling, cocaine and electrical induction, do not share a common ontologic basis.

Biochemical Actions

Cocaine affects the biosynthetic systems of the brain in a number of ways. The serotonergic, dopaminergic and

adrenergic systems are the most affected (Knapp and Mandell, 1972; Patrick and Barchas, 1977; Taylor and Ho, 1978).

In an *in vitro* study, cocaine was shown to block the reuptake of various neurotransmitters. Using rat brain slices, cocaine was seen to be a powerful inhibitor of the reuptake of 5-hydroxytryptamine (serotonin) and less powerful in the inhibition of dopamine and norepinephrine reuptake (Taylor and Ho, 1978).

A comparison of inhibition of monoamine uptake by cocaine, amphetamine and methylphenidate was also conducted (Taylor and Ho, 1978). The latter two compounds were found to be more potent reuptake inhibitors of dopamine and norepinepherine than cocaine in synaptosomal preparations. Cocaine was found to be potent in inhibiting serotonin reuptake in synaptosomal preparations from the septum-caudate. These results agree with previous work done by Scheel-Kruger (1972). A study by Lidbrink *et al*. (1971) suggests that drugs with reuptake inhibiting properties may be of value in the treatment of depression although the authors do not specifically refer to cocaine.

Cocaine, amphetamine and methylphenidate have similarities in their biochemical effects with regard to dopamine. The difference between the apparent inhibitory actions of cocaine on serotonin brain levels and the effects caused by methylyphenidate and amphetamine has been suggested by Yu and Smith (1977) and Taylor and Ho (1978) to be at least

partially responsible for the observed behavioral and clinical differences of these drugs.

Cocaine has been shown to cause weak ipsilateral rotation in animals with unilateral 6-hydroxydopamine lesions of the nigro-striatal dopaminergic pathway (Christie and Crow, 1973). Contralateral rotation in this model is characteristic of direct-acting agonists of the dopamine receptor while ipsilateral rotation is characteristic of indirect acting agonists. Pretreatment with a monoamine oxidase inhibitor caused cocaine to exhibit intense ipsilateral rotation that was sensitive to alpha-methyl, para-tyrosine (dopamine synthesis interrupter) and reserpine (catecholamine depletor). It is interesting to note that two cocaine analogs (Win 35,428 and Win 35,065) cause intense ipsilateral rotation by themselves in such lesioned animals (Heikkila et al., 1979). Therefore, by altering the structure of the cocaine molecule, an aparent increase in dopamine reuptake blockage is obtained.

In two recent papers relating the effects of cocaine on acetylcholine and cyclic AMP levels, Ngai (1979) and Post (1979) report that although cocaine does not affect mean cyclic AMP levels, closer study of the data indicates that cocaine does indeed cause large fluctuations of cyclic AMP levels when examined for each individual animal. Also, while most local anesthetics cause a decrease in brain acetylcholine levels (Stripling and Ellinwood, 1977),

cocaine increases these levels. It seems that this discrepancy between most local anesthetics and cocaine may, in part, be responsible for the stimulant activity of cocaine and the lack of such activity in local anesthetics.

Patrick and Barchas (1977) have shown that the reuptake blocking effect of cocaine primarily affects the newly synthesized and released pool of dopamine. With chronic cocaine treatment, an increase in dopamine receptor binding occurs (Taylor, 1979) implying an increase in dopamine receptor sensitivity. Estevez (1979) has shown that repeated cocaine injection shows reverse tolerance to the stereotyped behavior effect of cocaine and this can be correlated with an increased binding of dopamine to its receptors. Roy et al. (1978) have shown increasing dopamine levels in the caudate nucleus and the diencephalon-midbrain while the serotonin levels decreased in the diencephalon-midbrain and pons-medulla. Roy et al. also reported slight changes in the levels of norepinephrine and acetylcholine. Ross et al. (1978) demonstrated with Skinner boxes an opposite effect. While they found no changes in the levels of dopamine and serotonin, a pronounced cyclic rise and fall of norepinephrine was seen with every reinforcement. The inhibition of serotonin brain levels agrees with the turnover studies by Friedman et al. (1975). These serotonin levels can be correlated with the temporal development of

the cocaine-induced behavior reported by Pradhan et al. (1978b).

The behavioral effects seen with cocaine administration, therefore, can be best characterized as an interplay between the two transmitters, dopamine and serotonin. Further, the supersensitivity of dopamine receptors may explain some of cocaine's chronic effects.

Taylor and Ho (1976) have shown that cocaine affects tryptophan hydroxylase in a dual manner. There are two forms of the enzyme. The soluble form is associated with serotonergic cell bodies, whereas the particulate enzyme is associated with serotonergic nerve endings (Knapp and Mandell, 1972). Mandell (1975) has suggested that the effect of cocaine may be an initial stimulation of serotonin receptors as evidenced by the significant decrease in the soluble tryptophan hydroxylase activity. Continuation of cocaine treatment results in an increased enzyme activity in the nerve endings. This increase could be regarded as a biochemical compensatory mechanism in response to over stimulation following long-term chronic cocaine treatment.

In a study comparing cocaine, d-amphetamine and methylphenidate on tryptophan hydroxylase activity, cocaine was found to have an inhibitory effect of the enzyme resulting in a decreased serotonin content in the septum-caudate and increased hypothalamic norepinephrine and striatal dopamine metabolism (Taylor and Ho, 1977). In the second portion of

that study, d-amphetamine and methylphenidate were found to activate tryptophan hydroxylase, an effect opposite to that found for cocaine. The septum-caudate and striatum are responsible for control of many facets of behavior that are collectively called stereotyped behavior. Therefore, these brain region studies are strong arguments for the implication of serotonin and dopamine as the major mediators of the behavioral actions of cocaine.

Behavioral Pharmacology

Stereotyped behavior and neurotransmitter involvement

Specifically in rats, stereotyped behavior usually consists of constant repetitive movements of the head, body and extremities, and also constant sniffing, licking, and biting (Pechnick et al., 1979). Behavioral rating scales and models of behavior have been developed to characterize various drugs and their effects on the central nervous system. A good example of a rating scale is the apomorphine-induced dopaminergic response (Costall and Naylor, 1973). This scale is a continuous rating of putative dopaminergic behavior. The lowest score is given to sniffing and as dopamine stimulation increases the behavior exhibited continues through exploratory activity with the highest score given for gnawing (considered the most intense component of dopaminergic activity). A behavioral model of serotonergic activity is the quipazine-induced

head twitch response in mice (Green et al., 1976). Here, instead of quantifying the response on a continuum of behavior, the number of head twitches are totaled as a measure of serotonergic activity.

To date, there has been no behavioral scale developed for cocaine-induced stereotypy that specifically measures those stereotyped movements uniquely seen with cocaine (e.g., repetitive head movements both lateral and vertical, rearing, exploratory behavior, sniffing, Straub tail, etc.). An early attempt to rate cocaine-induced behavior was the use of a nine-point rating scale by Ellinwood and Balster (1974), a scale developed for amphetamine. Ellinwood used this scale in a modified form for the stereotypy seen with cocaine. Although this scale was applicable to the behavioral effects seen with cocaine, the scale was too general in defining the parameters and soon other scales, specifically designed for cocaine, were tried.

Pradhan et al., (1978b) used a scale where characteristic cocaine-induced behaviors were given a quantal score. Behaviors such as sniffing, searching, rearing, biting and licking were included in this model. Some studies have used spontaneous motor activity meters exclusively in attempting to quantify cocaine-induced behavior (Wilson and Holbrook, 1979).

Pradhan (1978a) established the time course of behavioral activity after acute cocaine administration. Locomotor

activity developed and peaked within 20 minutes while stereotyped behavior took as long as 50 minutes to peak. Dopamine increases in the caudate nucleus and diencephalon-midbrain were correlated with the increase in locomotor activity. The stereotyped behavior seen was correlated with an initial decrease and subsequent increase of serotonin in the pons-medulla and diencephalon-midbrain. This pattern of behavioral development, an initial increase in spontaneous motor activity then stereotypy, seems typical of cocaine at many doses tested (i.e. 5, 10, 15, and 20 mg/kg, i.p.) (Bhattacharyya and Pradhan, 1979). In a follow-up paper, Bhattacharyya et al. (1979) showed that haloperidol antagonized both the biochemical and behavioral correlates induced by cocaine. Taylor and Ho (1979) correlated increases in sensitivity to chronic cocaine-induced locomotor activity with an increase in dopamine receptor binding. Similar results were found by Schuster et al. (1977) using the running response in mice. Apomorphine, a dopaminergic agonist, at low doses in mice does not induce a compulsion to gnaw, but with cocaine pretreatment, persistent gnawing occurs (Dadkar et al., 1977). These results all seem to implicate an interaction of cocaine with dopaminergic mechanisms. Further support is given by studies demonstrating that alpha-methyl, para-tyrosine and haloperidol (dopaminergic receptor blocker) can abolish this compulsion to gnaw.

Cocaine diverges from other central nervous system stimulant drugs in the sense that in addition to having a major effect on dopaminergic mechanisms, serotonergic mechanisms are also affected. Studies with para-chloro, phenylalanine (serotonin depleter) and electrolytic lesions of the medial raphe nucleus (a major serotonergic ascending tract) have served to define serotonin's role in behavioral effects. Thornton and Goudie (1978) used both these techniques and obtained analogous findings. Their studies revealed that raphe lesions and serotonin depletion both caused increased motor activity, increased responsiveness to stimuli, and improved acquisition of active-avoidance responses, all of which were suggested to be the result of a deficit in an inhibitory system. Jacobs (1976) in a minireview of the animal models used in studying central serotonergic synapses describes the various drug treatments with the capability to induce what is now called the "serotonin syndrome." When compounds which either dramatically increase synaptic serotonin (e.g., monoamine oxidase inhibitors, serotonin precursors, or serotonin releasing agents) or directly stimulate post-synaptic serotonin receptors, a syndrome is produced which consists of resting tremor, Straub tail, hindlimb abduction, lateral head weaving, head shaking, hyperreactivity, hyper-activity and salivation. In many respects, this syndrome is very similar to that seen with the administration of cocaine.

Today, subsets of this awe-inspiring list of symptoms are routinely used to measure serotonin activity. Sloviter et al. (1978), using 5-methoxy-N,N-dimethyltryptamine (a serotonergic agonist) produced the serotonin syndrome but only measured the more convenient parameters of head weaving, splayed hindlimbs and forepaw padding. The serotonergic blockers cyproheptadine and methysergide prevented this syndrome while dopaminergic and adrenergic blockers did not. Honma's work (1979) with methamphetamine, a putative serotonergic agonist, resulted in the demonstration that methysergide and cyproheptadine block the serotonergic component (slow head shaking) of the behavioral syndrome, but not the stereotypic component (gnawing). Apparently, gnawing was not blocked since it is reputed to be regulated by a dopaminergic mechanism (Costall and Naylor, 1975). In separate studies, both cyproheptadine and methysergide were shown to be potent antagonists of serotonergic responses (Clineschmidt and Lotti, 1974; Jacoby et al., 1978).

Cocaine has been shown to decrease serotonin levels in both acute and chronic studies (Friedman et al., 1975; Roy et al., 1978). Pradhan (1978) has shown that pretreatment with the serotonin precursor, 5-hydroxytryptophan eliminates these cocaine-induced changes. Taylor and Ho (1979) obtained similar results when 5-hydroxytryptophan was added to the dietary intake of rats. Therefore, it seems reasonable to conclude that cocaine's behavioral profile is in part

mediated by serotonergic mechanisms.

It has been hypothesized that there is a catecholamine-serotonin interaction that is partly responsible for modulating both so-called pure dopaminergic or pure serotonergic behavioral actions (Jacobs and Wise, 1974). The direct acting serotonergic agonist lysergic acid diethylamide (LSD) has both dopaminergic and serotonergic properties (Silbergeld and Hruska, 1979). The syndrome produced by LSD can be partially blocked by cyproheptadine and potentiated by haloperidol. Jacobs (1974), induced the serotonin syndrome with a l-tryptophan/pargyline combination and found it to be attenuated by low doses of the dopamine receptor blocker spiroperidol but not at all by low or high doses of the neuroleptic pimozide. This observation simply indicated that some dopaminergic blockers have the capacity to block the serotonin syndrome and others do not. Major tranquilizers (e.g., spiroperidol, pimozide, fluphenazine and reserpine) were used in a study by Maj (1975) to induce catalepsy in rats. Catalepsy induced by these drugs is presumably due to the blockage of post-synaptic dopamine receptors. However, Maj found that the antiserotonergic, cyproheptadine, reversed the catalepsy. Furthermore, cyproheptadine potentiated the anti-cataleptic effect of L-dopa. Balsara et al. (1979), using methamphetamine, obtained similar results with the serotonergic agonist methysergide.

Other evidence on an interaction between the central transmitters dopamine and serotonin is the fact that the long regarded pure dopamine agonist, apomorphine, causes an elevation of brain 5-hydroxy-indoleacetic acid, the metabolite of serotonin (Grabowska and Michaluk, 1974). However, these results only indicate an indirect serotonergic effect of apomorphine. Jacobs (1974) demonstrated that strikingly similar syndromes are obtained when pargyline pretreatment is followed by either l-tryptophan or L-dopa. They both display characteristics of the serotonin syndrome. Alpha-methyl, para-tyrosine and pimozide failed to affect either syndrome, whereas para-chlorophenylalanine, cinanserin and methysergide blocked both. Jacobs concludes that at least a portion of the syndrome that emerges following pargyline and L-dopa is mediated by serotonin rather than dopamine.

From the studies presented here, it would be a rather safe conclusion to state that there are many complicated interactions of dopamine and serotonin. Cocaine has been shown to express behavioral parameters of both putative dopamine and serotonergic responses. It is the purpose of this investigation to characterize the behavioral manifestations that cocaine can induce. Further, if both serotonergic and dopaminergic parameters can be characterized and quantitated an attempt will be made to selectively block these behaviors by the use of specific blocking agents.

METHODS

Animals

Animals used throughout this study were drug-naive male Wistar rats (Simonsen Laboratories, Inc.; Gilroy, California) with a weight range of 200-250 g. Food (S/L Custom Lab Diet--G4.5) and tap water were available *ad libitum*. Animals were housed under a consistent light/dark cycle (0700-1900 hours light, 1900-0700 hours dark) at a constant temperature of 21 $\pm$ 1^{o} C. All experiments were begun by 1200 hours and completed by 1600 hours.

Experimental

Animals were allowed to acclimate to their living quarters for one week prior to use. They were placed in viewing cages of clear plastic (20 x 24 x 45 cm.) for at least one hour prior to the start of an experiment and held without access to food and water. The cages were lined with bedding (Willis Lab Litter) to allow any spontaneous or drug-induced gnawing.

Animals were pretreated for 30 minutes with either saline, atropine sulfate (1.25, 2.50, or 5.00 mg/kg), haloperidol lactate (0.125, 0.250, or 0.500 mg/kg) or cyproheptadine maleate (1.0, 2.0, or 4.0 mg/kg) and then challenged with cocaine hydrochloride (10, 20, or 40 mg/kg). There were seven animals in each treatment group. All

dosages are expressed in terms of the salt, except for haloperidol where the doses are expressed in terms of the free base.

Injections were intraperitoneal (i.p.) and delivered in a volume of 1 ml/kg with a 1-ml tuberculin syringe and a 3/8" 25 gauge needle. All solutions were prepared in 0.9% saline just prior to use.

Source of drugs

Cocaine hydrochloride was obtained from Mallinckrodt, Inc., haloperidol lactate from McNeil Inc., cyproheptadine maleate from Merck, Sharp & Dohme Research Laboratories, and atropine sulfate was obtained from the Nutritional Biochemical Corporation.

Stereotyped behavior

Stereotyped behavior was assesed during six, one-minute periods at 10, 20, 30, 45, 60 and 90 minutes after cocaine administration. Behavior was quantified during these one-minute observation periods for the presence or absence of the six behavioral parameters defined in Figure 1. Both blind and non-blind observations were made. The blind observations did not statistically differ from the non-blind, therefore, the rest of the observations were conducted in a non-blind fashion. The haloperidol, atropine and cyproheptadine experiments were conducted separately but within each experimental session, the injections were randomized to reduce any day-to-day experimental bias.

DOPAMINERGIC PARAMETERS

(1) Sniffing (at least 15 seconds per one-minute observation)

(2) Grooming (at least 15 seconds per one-minute observation)

(3) Locomotor Activity (traveling at least one-half the distance of the cage)

SEROTONERGIC PARAMETERS

(4) Rearing (at least 5 rearings per one-minute observation)

(5) Repetitive Head Movements (at least 5 repetitive head movements per one-minute observation)

(6) Straub Tail (carrying of the tail above the bedding for the majority of the one-minute observation)

Figure 1: Behavioral parameters for the quantification of cocaine-induced stereotyped behavior.

The first three parameters (see Figure 1) used were chosen because they are putative indicators of dopamine behavioral responses (Costall and Naylor, 1974), while the second three parameters were selected as putative indicators of serotonergic behavioral responses (Jacobs, 1976).

Analysis of Data

Each behavioral parameter was totaled over the six time intervals (maximum score =6), and the mean response for the seven animals in each treatment group was calculated. The six behavioral parameters were analyzed as dependent variables. This was accomplished via computer analysis of variance. The program used was a library program entitled, "BMD 11V Multivariate General Linear Hypothesis" (Dixon, 1973). The mathematical model was designed to analyze interactions between the blocker and the test agonist. Any comparison that resulted in a higher than critical F value indicates that there is an interaction between the blocker and the agonist that is more than just a simple dose-response relationship. The pooled variance obtained from each parameter was used as a measure of error in a modification of the Student's t-test for statistical significance.

In addition to ANOVA analysis of the six individual behavioral parameters, comparison of relative dopaminergic versus serotonergic behaviors was done by totaling the scores demonstrative of each. Finally to obtain a net score, the mean serotonergic score was subtracted from the

mean dopaminergic score. The result (net behavioral index) was considered to be a measure of net dopaminergic or serotonergic predominance.

RESULTS

The responses obtained in this model are meant to serve as a means for interpreting the degree of dopamine and serotonin involvement in the behavioral response produced by cocaine. Serving as a check of the dose-response validity of this model, Fig. 2 shows the time-response effect of three doses of cocaine. This figure illustrates that the behavioral totals (all six parameters) increase with increasing cocaine administration. The duration of response also increases with increasing cocaine dosage. Significant response differences begin at 30 minutes and by 45 minutes the responses of each of the three doses are significantly different from one another. The maximum differences in time-response were recorded at 60 minutes.

The Cocaine/Haloperidol Experiments

The multivariate ANOVA analysis was computed and the results of the statistical analysis are summarized in Table 1. To facilitate the ANOVA analysis, a mathematical model was constructed to check for effects of haloperidol on cocaine-induced stereotyped behavior other than that due to the dose-response relationship. Summarized in Table I are the pooled mean responses for each parameter used in this study, the

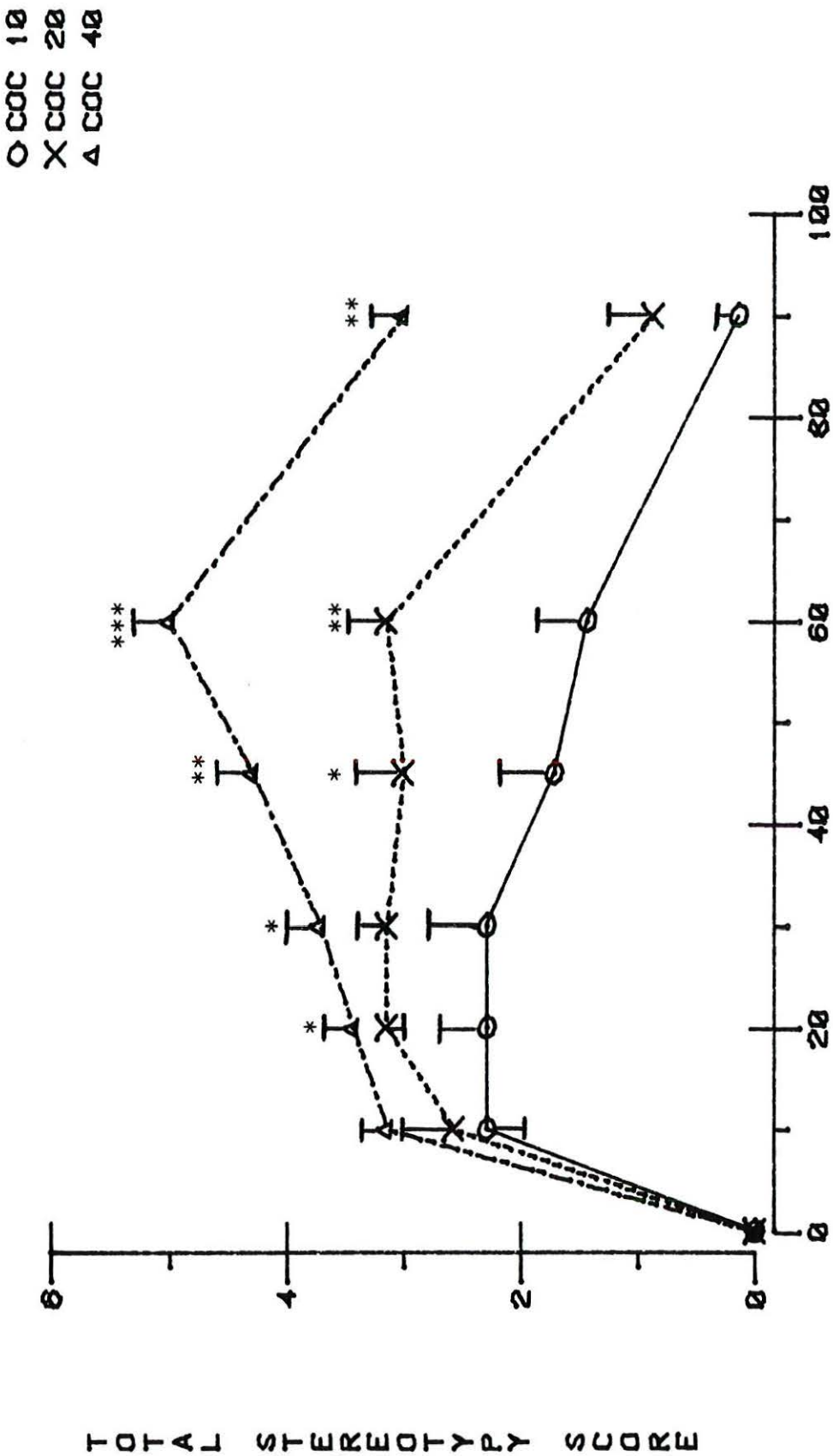

O COC 10
X COC 20
Δ COC 40
TOTAL STEREOTYPY SCORE
TIME IN MINUTES
0
2
4
6
0
20
40
60
80
100

Table I. Summary of Multivariate ANOVA Analysis of the Haloperidol/ Cocaine Experiments.

Parameter	Pooled Mean Response: Saline N=21	Haloperidol N=63	Variance s^2	Calculated F	Observed P
Sniffing	5.24	2.14	2.10	1.77	>0.10
Grooming	1.48	0.50	1.04	2.24	=0.05
Locomotor Activity	3.57	0.49	0.88	8.50	<0.001
Repetitive Head Movements	3.19	1.16	1.82	3.20	<0.01
Rearing	2.95	0.46	1.10	1.43	>0.20
Straub Tail	1.57	0.22	0.63	5.03	<0.001
Dopaminergic Score	3.43	1.05	1.88	4.17	<0.005
Serotonergic Score	2.57	0.65	2.11	4.46	<0.001
Net Behavioral Index	0.86	0.44	1.68	3.19	<0.01

pooled variance for each parameter, the calculated F, and the observed P for the calculated F value.

Tables II, III, and IV summarize the mean scores at each treatment level, broken down into the three behavioral parameters classified as dopaminergic behaviors. Table II shows that the sniffing response peaks at saline/cocaine 20 (pretreatment/challenge mg/kg) and is not significantly different for saline/cocaine 40. Both these treatment levels are significantly different from saline/cocaine 10. Haloperidol can be observed to attenuate the effects of all doses of cocaine to very highly significant levels. Table III shows the grooming response to cocaine administration. The grooming response increases but not significantly from saline/cocaine 10 to saline/cocaine 20. There is, however, a very significant drop in grooming when the dose of cocaine is raised to 40 mg/kg. This observation is to be expected as grooming is eliminated in favor of other, more intense behaviors. It can be seen that haloperidol does not attenuate the grooming response over the doses tested. The locomotor activity response, Table IV, shows a dose-dependent increase over the saline/cocaine doses. Saline/cocaine 10 is significantly lower than saline/cocaine 40 but there is no statistical difference between saline/cocaine 20 and saline/cocaine 40. For these three dopaminergic parameters, the peak response was obtained consistently with the median cocaine dose.

Table II. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Sniffing in Rats.

Cocaine, mg/kg ip	Mean sniffing response[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	4.00	3.14	2.71	0.57[d]
20	5.86	2.86[c]	1.29[d]	1.14[d]
40	5.86	4.00[b]	2.14[d]	1.43[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =2.10; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

Table III. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Grooming in Rats.

Cocaine, mg/kg ip	Mean grooming response[a]			
	Saline control, 1ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	1.86	1.29	1.00	0.14[b]
20	2.29	0.46[b]	0.29[b]	0.43[b]
40	0.29	0.14	0.43	0.29

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.04; d.f. =72

[b] Significantly differs from control ($P < 0.01$)

Table IV. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Locomotor Activity in Rats.

Cocaine, mg/kg ip	Mean locomotor activity response[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	1.29	0.43	0.57	0.00[b]
20	4.43	0.29[c]	0.14[c]	0.14[c]
40	5.00	2.14[c]	0.57[c]	0.14[c]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =0.877; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.001$)

Tables V, VI, and VII summarize the mean scores at each treatment level broken down into the three behavioral parameters classified as serotonergic behaviors. Table V contains the repetitive head movement summary. All three saline/cocaine treatments exhibit a dose-response increase and all three points differ statistically from each other. Haloperidol attenuates the repetitive head movements over all doses tested. Table VI, the summary of cocaine-induced rearing, shows that although there is a statistical difference between saline/cocaine 10 and saline/cocaine 20 and 40, no such statistical difference exists between saline/cocaine 20 and saline/cocaine 40. Here again, haloperidol attenuates the rearing response at all doses of haloperidol tested. The Straub tail response, Table VII, shows that over the dose range tested, saline/cocaine continues to increase, and the results of the three doses tested, 10, 20, and 40 mg/kg cocaine, are significantly different from each other.

In summary, the responses seen with the three dopaminergic-associated parameters peak at the median dose of cocaine. On the other hand, the three serotonergic-associated parameters continue to increase over the three doses of cocaine tested. Haloperidol attenuated all six parameters, although not in an identical manner.

Dopaminergic scores are summarized in Table VIII. This parameter was constructed by totaling the sniffing, grooming

Table V. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Repetitive Head Movements in Rats.

Cocaine, mg/kg ip	Mean repetitive head movement response[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	1.00	0.71	0.29	0.00
20	3.71	1.86[b]	0.86[c]	0.86[c]
40	4.86	4.00	1.57[d]	0.29[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.82; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

Table VI. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Rearing in Rats.

Cocaine, mg/kg ip	Mean rearing response[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	1.86	0.43[b]	0.14[c]	0.00[c]
20	3.29	0.43[d]	0.14[d]	0.43[d]
40	3.71	1.71[c]	0.57[d]	0.29[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.10; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

Table VII. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Straub Tail in Rats.

Cocaine, mg/kg ip	Mean Straub tail response[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	0.14	0.00	0.00	0.00
20	1.57	0.14[b]	0.00[b]	0.14[b]
40	3.00	1.43[b]	0.29[c]	0.00[c]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =0.63; d.f. =72

[b] Significantly differs from control ($P < 0.01$)

[c] Significantly differs from control ($P < 0.001$)

and locomotor activity scores for each animal and then applying these totals to the multivariate ANOVA data analysis. The dopaminergic score is used in this study as an index of total dopaminergic behavior. As in analyses of the individual parameters, the total dopaminergic response peaks at the median dose of cocaine. In fact, the high cocaine dose yields a lower response than did the median dose of cocaine. The effect of haloperidol is similar to that seen with the individual parameters--one of attenuation. Fig. 3 is a graphic illustration of the dopaminergic scores reported in Table VIII.

The serotonergic scores, Table IX, were accumulated in an identical manner as the dopaminergic scores, except that the parameters of repetitive head movements, rearing and Straub tail were used as the indices of total serotonergic response. Similarly to the individual parameters, these scores reflect that the serotonergic totals did not peak and continued to increase over the three doses of cocaine tested. Fig. 4 is a graphic illustration of the results summarized in Table IX.

Table X reports the net behavioral indexes. In this comparison, the new behavioral scores for serotonin were subtracted from the respective mean dopaminergic scores. While this new parameter, the net behavioral index, is not an absolute measure of behavior <u>per se</u>, the net behavioral index does serve to illustrate the relative changes in total

Table VIII. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Dopaminergic Scores in Rats.

Cocaine, mg/kg ip	Mean dopaminergic score[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	2.38	1.62	1.43[b]	0.24[d]
20	4.19	1.19[d]	0.57[d]	0.67[d]
40	3.71	2.09[c]	1.05[d]	0.62[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.88; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

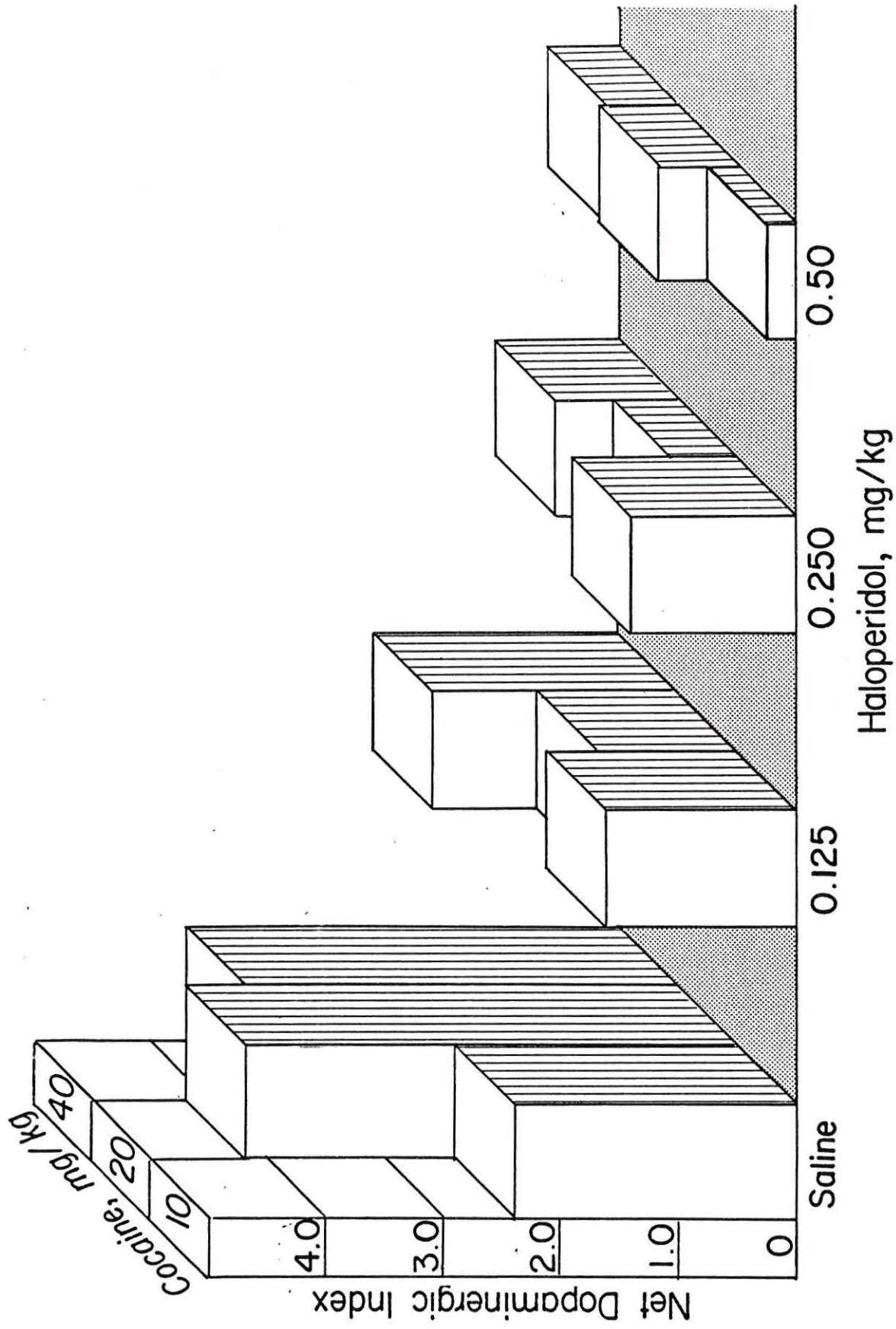

Net Dopaminergic Index
0
1.0
2.0
3.0
4.0
Cocaine, mg/kg
10
20
40
Saline
0.125
0.250
0.50
Haloperidol, mg/kg

Table IX. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Serotonergic Scores in Rats.

Cocaine, mg/kg ip	Mean serotonergic score[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	1.00	0.38	0.14	0.00[b]
20	2.86	0.61[d]	0.62[d]	0.48[d]
40	3.86	2.38[c]	0.81[d]	0.19[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =2.11; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

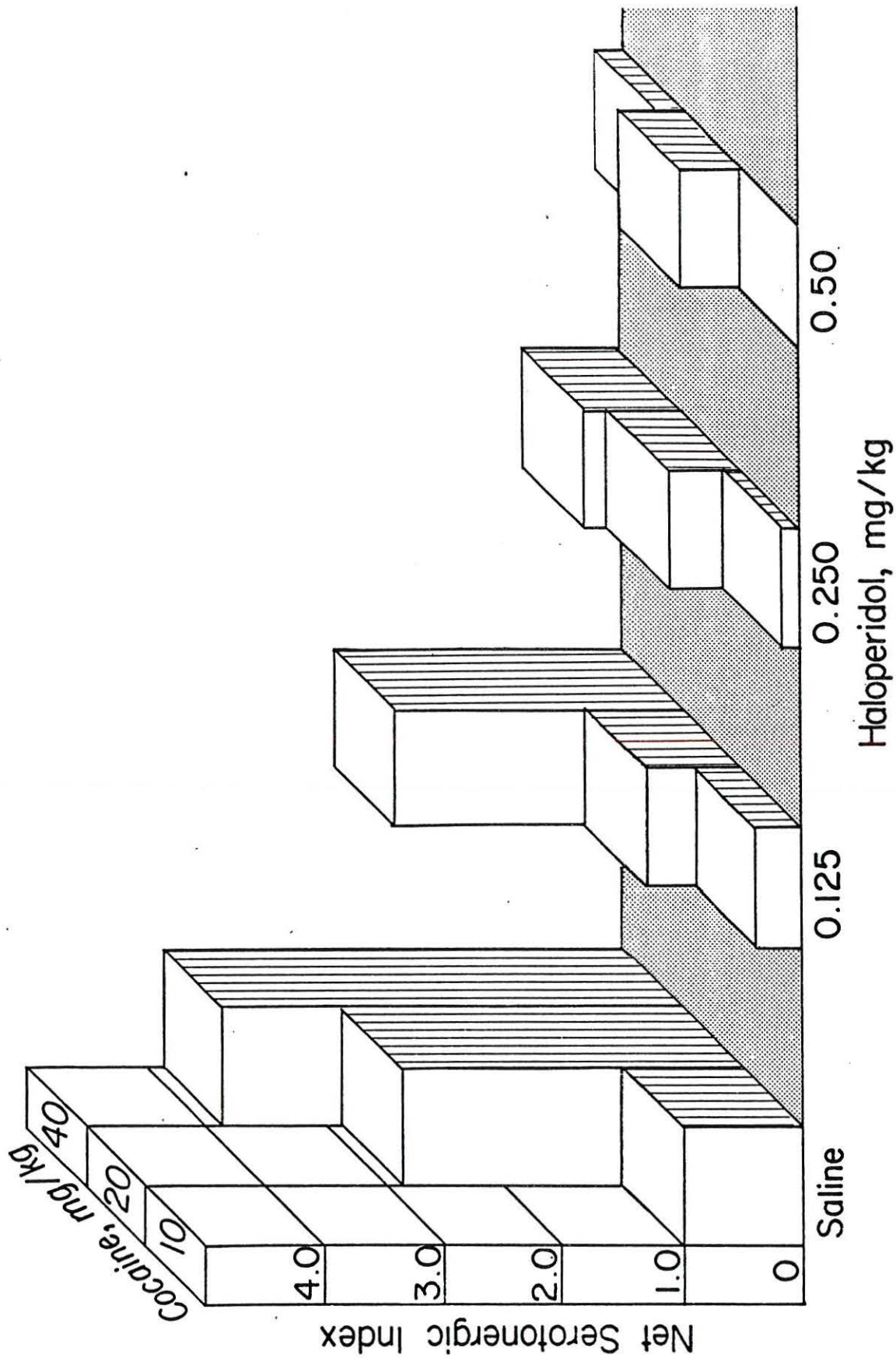

Net Serotonergic Index
4.0
3.0
2.0
1.0
0
Cocaine, mg/kg
40
20
10
Saline
0.125
0.250
0.50
Haloperidol, mg/kg

Table X. Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Net Behavioral Index in Rats.

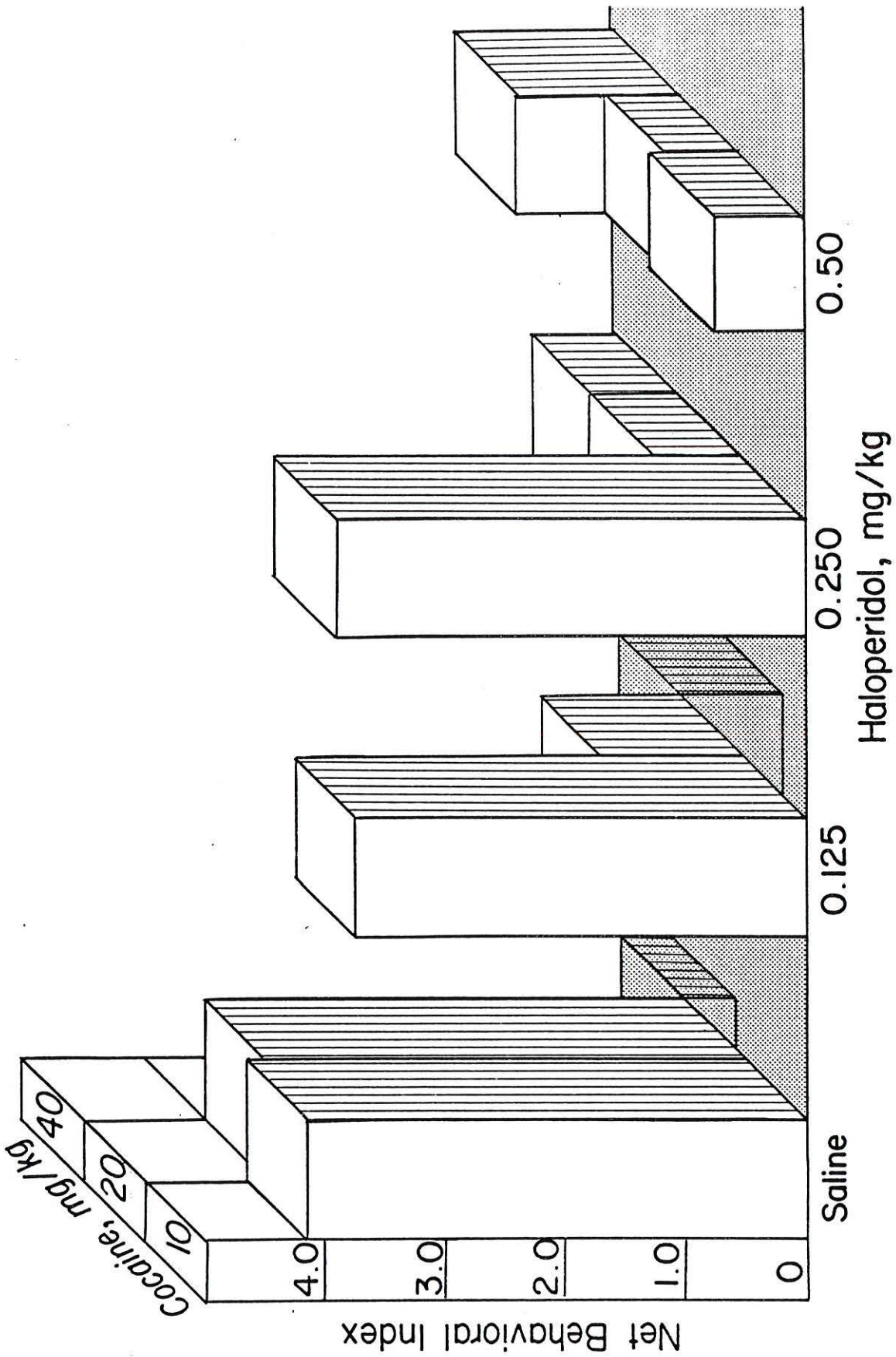
Cocaine, mg/kg ip	Net behavioral index[a]			
	Saline control, 1 ml/kg	Haloperidol, mg/kg ip		
		0.125	0.250	0.500
10	1.38	1.24	1.29	0.24[b]
20	1.33	0.38[b]	0.24[b]	0.19[b]
40	-0.14	0.29	0.24	0.43

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.68; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

Figure 5: Effect of 30-Minute Pretreatment with Haloperidol on Cocaine-Induced Net Behavioral Index in Rats.

This is a graphic illustration of the data summarized in Table X.


Net Behavioral Index
0
1.0
2.0
3.0
4.0
Cocaine, mg/kg
10
20
40
Saline
0.125
0.250
0.50
Haloperidol, mg/kg

dopaminergic and/or serotonergic behavior as the doses of cocaine and haloperidol are altered. The net behavioral indexes of the saline/cocaine treatments show that there was no change of net behavioral signs exhibited from 10 to 20 mg/kg cocaine. At saline/cocaine 40 there was a significant drop in the net index, indicative of increased serotonergic effects or decreased dopaminergic effects. Compared to the saline/cocaine controls, the median dose of cocaine was the only point where the net behavioral index was significantly lowered by the 0.125 mg/kg dose of haloperidol. For the other doses of cocaine, the net behavioral index remained largely unchanged by haloperidol. Fig. 5 is a graphic illustration of the results summarized in Table X.

The Cocaine/Cyproheptadine Experiments

Upon completion of the haloperidol experiments, the effects of cyproheptadine, a serotonergic blocker, were investigated in the same cocaine-induced behavioral model. The results of the computed multivariate ANOVA analysis are summarized in Table XI. The analysis was constructed in an identical manner to the haloperidol experiments.

Tables XII, XIII, and XIV summarize the mean scores at each treatment level broken down into the three behavioral parameters classified as dopaminergic-associated behaviors. Although cyproheptadine decreased the sniffing response (Table XII), this parameter was only significantly decreased

Table XI. Summary of Multivariate ANOVA Analysis of the Cyproheptadine/ Cocaine Experiments.

Parameter	Pooled Mean Response: Saline N=21	Pooled Mean Response: Cyproheptadine N=63	Variance s^2	Calculated F	Observed P
Sniffing	5.24	2.71	1.99	3.03	< 0.025
Grooming	1.48	1.56	0.62	4.17	< 0.005
Locomotor Activity	3.57	1.78	1.62	5.02	< 0.001
Repetitive Head Movements	3.19	0.96	1.27	2.03	> 0.05
Rearing	2.95	1.45	1.22	2.96	< 0.025
Straub Tail	1.57	0.72	0.37	1.99	> 0.05
Dopaminergic Score	3.43	2.02	1.99	4.13	< 0.005
Serotonergic Score	2.57	1.05	1.28	4.21	< 0.005
Net Behavioral Index	0.86	0.97	0.51	3.43	< 0.01

Table XII. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Sniffing in Rats.

Cocaine, mg/kg ip	Mean sniffing response[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	4.00	3.87	3.00	2.43
20	5.86	3.14[b]	2.57[c]	2.57[c]
40	5.86	3.00[b]	2.57[c]	1.57[c]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.99; d.f. =72

[b] Significantly differs from control ($P < 0.01$)

[c] Significantly differs from control ($P < 0.001$)

Table XIII. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Grooming in Rats.

Cocaine, mg/kg ip	Mean grooming response[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	1.86	1.57	1.86	1.87
20	2.29	2.14	1.41	2.47
40	0.29	0.43	0.86	1.43[b]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =0.621; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

Table XIV. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Locomotor Activity in Rats.

Cocaine, mg/kg ip	Mean locomotor activity response[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	1.29	1.13	0.74	0.57
20	4.43	3.29	2.86[b]	2.43[b]
40	5.00	2.71[c]	1.22[d]	1.04[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.62; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

at the 20 and 40 mg/kg cocaine doses. The saline control values are the same as those used in the haloperidol experiments. The significant attenuation seen at 20 and 40 mg/kg was statistically identical across the doses of cyproheptadine tested. The grooming response (Table XIII) remained statistically unaffected throughout most of the experiment. A trend towards increasing grooming was apparent with increasing cyproheptadine dosage for the highest cocaine dose. This increase became significantly greater than control at the high dose of cyproheptadine. This effect of increased grooming with cyproheptadine can be compared with the decreased grooming seen with haloperidol. Table XIV, the locomotor activity response, shows dose-dependent attenuation at all doses of cyproheptadine.

Tables XV, XVI, and XVII summarize the mean scores at each treatment level broken down into the three behavioral parameters classified as serotonergic behaviors. Cyproheptadine was able to attenuate the repetitive head movement response, Table XV, at all doses of cocaine except the low, cocaine 10 dose. The rearing response, Table XVI, was attenuated by cyproheptadine and the responses elicited by cocaine were sensitive to cyproheptadine at all doses of cocaine tested. The Straub tail response, Table XVII, was significantly attenuated by cyproheptadine at the median and high doses of cocaine, although at the low cocaine dose it reamined unaffected. This lack of significant reduction of

Table XV. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Repetitive Head Movements in Rats.

Cocaine, mg/kg ip	Mean repetitive head movement response[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	1.00	0.86	0.59	0.29
20	3.71	1.71[b]	0.73[c]	0.87[c]
40	4.86	1.86[c]	0.97[c]	0.79[c]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.27; d.f. =72

[b] Significantly differs from control ($P < 0.01$)

[c] Significantly differs from control ($P < 0.001$)

Table XVI. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Rearing in Rats.

Cocaine, mg/kg ip	Mean rearing response[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	1.86	1.57	1.41	0.38[b]
20	3.29	2.00[b]	1.55[b]	1.56[b]
40	3.71	2.14[b]	1.67[c]	0.79[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.22; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

Table XVII. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Straub Tail in Rats.

Cocaine, mg/kg ip	Mean Straub tail response[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	0.14	0.14	0.00	0.00
20	1.57	0.86[b]	0.86[b]	1.14
40	3.00	1.14[c]	0.81[c]	1.57[c]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =0.370; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.001$)

response could be due to the fact that a very low response was initially present, and the total elimination of the response therefore failed to be significantly lower.

Total dopaminergic scores are summarized in Table XVIII. This parameter was obtained in an identical manner as in the haloperidol experiments (i.e. totalling sniffing, grooming, and locomotor activity). Cyproheptadine significantly attenuated all doses of cocaine, except 10 mg/kg cocaine. Fig. 6 is a graphic illustration of the dopaminergic scores seen in Table XVIII.

The serotonergic total scores were accumulated in an identical manner as the dopaminergic scores, except that the parameters of repetitive head movements, rearing and Straub tail were used as the indices of total serotonergic response. Cyproheptadine attenuated the responses from all but the low dose of cocaine. Fig. 7 is a graphic illustration of the results seen in Table XIX.

Similarly to the haloperidol experiments, the serotonergic scores were subtracted from the dopaminergic scores to illustrate the changes in net behavioral index. Cyproheptadine did not significantly alter the net behavioral response. Fig. 8 is a graphic illustration of the results summarized in Table XX.

Table XVIII. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Dopaminergic Scores in Rats.

Cocaine, mg/kg ip	Mean dopaminergic score[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	2.38	2.19	1.87	1.62
20	4.19	2.86	2.28[b]	2.39[b]
40	3.71	2.05[b]	1.55[b]	1.35[c]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.99; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

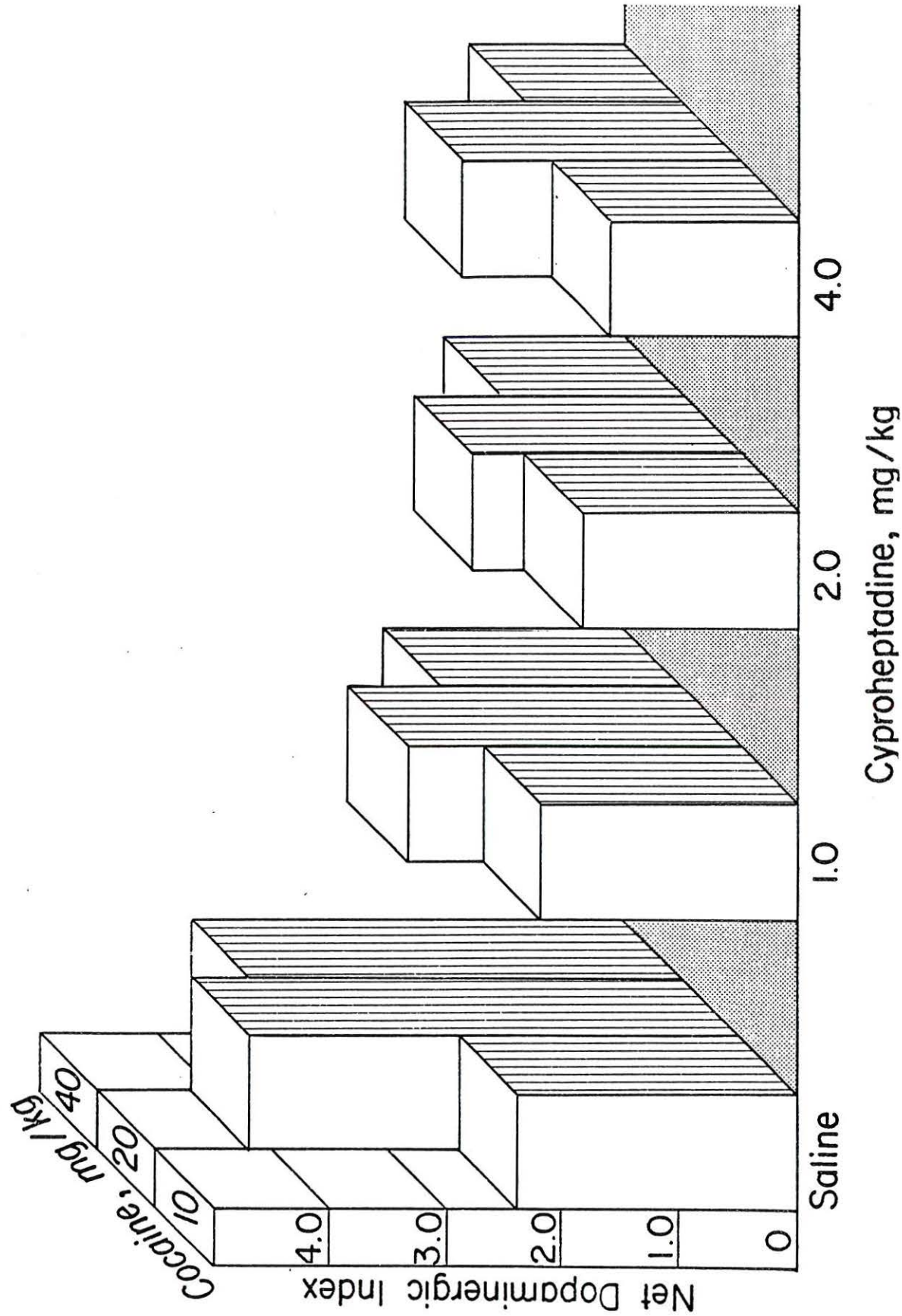

Net Dopaminergic Index
0
1.0
2.0
3.0
4.0
Cocaine, mg/kg
10
20
40
Saline
1.0
2.0
4.0
Cyproheptadine, mg/kg

Table XIX. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Serotonergic Scores in Rats.

Cocaine, mg/kg ip	Mean serotonergic score[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	1.00	0.86	0.67	0.22
20	2.86	1.52[b]	1.05[b]	1.19[b]
40	3.86	1.71[c]	1.15[d]	1.05[d]

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =1.28; d.f. =72

[b] Significantly differs from control ($P < 0.05$)

[c] Significantly differs from control ($P < 0.01$)

[d] Significantly differs from control ($P < 0.001$)

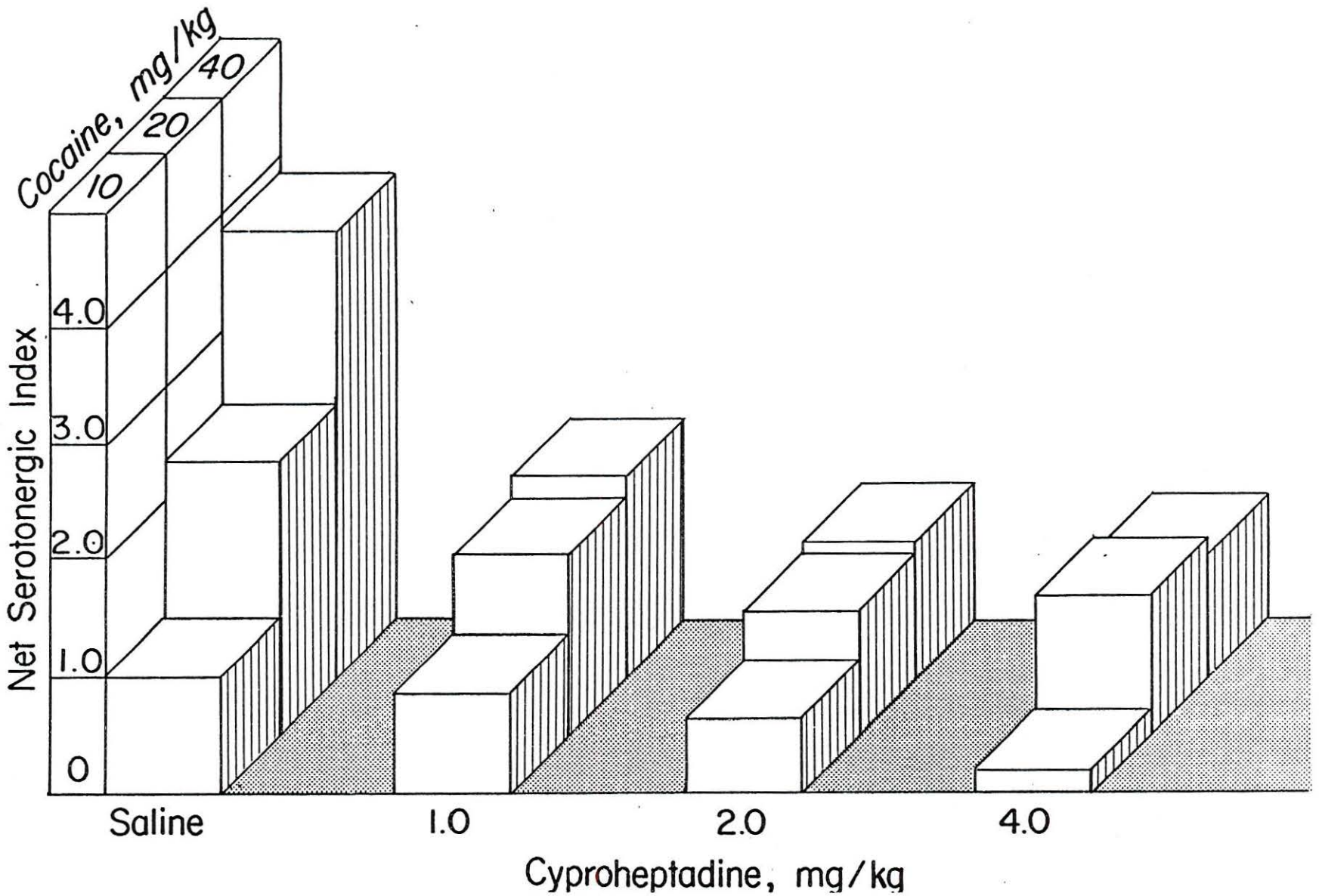
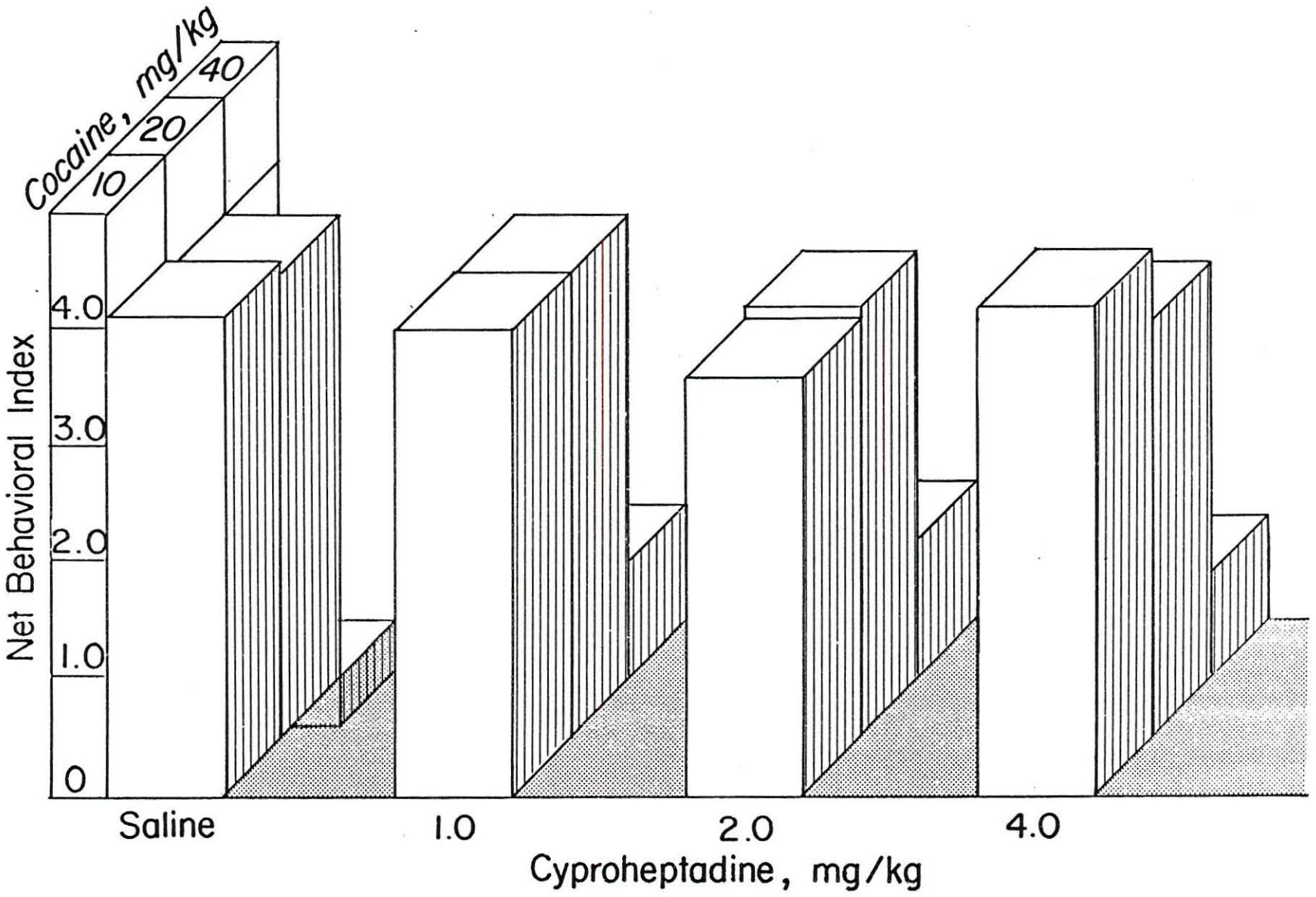

Cocaine, mg/kg
40
20
10
Net Serotonergic Index
4.0
3.0
2.0
1.0
0
Saline
1.0
2.0
4.0
Cyproheptadine, mg/kg

Table XX. Effect of 30-Minute Pretreatment with Cyproheptadine on Cocaine-Induced Net Behavioral Index in Rats.

Cocaine, mg/kg ip	Net behavioral index[a]			
	Saline control, 1 ml/kg	Cyproheptadine, mg/kg ip		
		1.0	2.0	4.0
10	1.38	1.33	1.20	1.40
20	1.33	1.33	1.23	1.20
40	-0.14	0.33	0.40	0.30

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =0.51; d.f. =72


Cocaine, mg/kg
40
20
10
Net Behavioral Index
4.0
3.0
2.0
1.0
0
Saline
1.0
2.0
4.0
Cyproheptadine, mg/kg

DISCUSSION

The results obtained in this study confirm previous findings that cocaine induces stereotyped behavior (Pradhan et al., 1978; Bhattacharyya, 1979). Additionally, the present study is the first attempt to define cocaine's dose-related dual relationship with serotonin and dopamine neurotransmitters in stereotyped behavior. Examination of the saline/cocaine data illustrates the dual neurohumoral involvement in behavioral induction as a function of cocaine dose. At the lower doses used in this study, cocaine displays predominantly dopaminergic behaviors. Serotonergic behaviors continue to increase in intensity as the doses of cocaine are increased. The dopaminergic maximum agrees with previous studies by Bhattacharyya and Pradhan (1979) who found that 20 mg/kg cocaine was sufficient to obtain a peak response on a modification of Costall and Naylor's (1974) dopaminergic scale of behavior. However, the present study is unique in that serotonergic behaviors have been concurrently analyzed with dopaminergic behaviors. The result of this analysis shows that the serotonergic behaviors continue to increase up to the 40 mg/kg dose of cocaine.

Studies of rodent stereotypy have generally utilized continuum scales to evaluate the intensity of drug-induced

stereotypy. These scales are often weighted on a progression of behavioral intensity, going from increased locomotor activity to sniffing to repetitive head, body, and extremity movements to licking, biting, and gnawing (Costall and Naylor, 1973, 1974; Scheel-Kruger, 1970). Such scales have two weaknesses. First, they imply a linear progression of intensity of stereotypy, suggesting, for example, that a rating of 4 represents twice as much stereotypy as a rating of 2, an assumption of questionable validity. Second, although a progression of behaviors does indeed occur with increasing doses of cocaine, the relative predominance of a given behavior and its duration differ between doses. Thus an analysis of specific behaviors, rather than a continuum scale, may be the most appropriate in comparing the stereotypic effects of cocaine.

The application of a quantally-constructed model in this study was done for several reasons. The three dopaminergic (sniffing, grooming, and locomotor activity) and three serotonergic (repetitive head movements, rearing, and Straub tail) parameters could not fit into the concept of a continuum scale of behavior considering the fact that one of the purposes of this study was to individually analyze the presence/absence of cocaine-induced behaviors. On a continuum scale, discrete behavioral responses would be lost. It was decided instead, to total each of the six one-minute observation periods as an index of the parameter's intensity.

This method analyzed all behaviors exhibited and accounts for the consideration that certain behavioral manifestations occur that tend to preclude others (e.g., locomotor activity precludes rearing and/or grooming). With this style of data gathering, net behavioral parameters could be created for each test animal. The three dopaminergic and serotonergic parameters were totaled for each test animal and ANOVA analysis carried out on these new parameters. These new totals facilitate the analysis of the relative activities used as a comparison of dopaminergic or serotonergic dominance.

There are drawbacks to any kind of behavioral model. The model used in this study, for example, implies that each parameter is equal in weight. This may not necessarily be the case. However, the fact that the parameters are looked at individually and as totals allows for interpretation of the results. It should be kept in mind that the total behaviors and net behavioral index are only meant to serve as aids in interpretation of obtained results.

The results of the present study indicate several novel developments regarding the investigative work done with cocaine. By examining both serotonergic and dopaminergic behavioral development, some unifying facts describing neurotransmitter involvement can be stated. The locomotor activity seen with cocaine begins with a dose as low as 5 mg/kg (Bhattacharyya and Pradhan, 1979). In this study,

locomotor activity was seen at 10 mg/kg along with various components of stereotyped behavior. The total dopaminergic behavior did not significantly increase past 20 mg/kg cocaine. This, in part, is explained through the decrease in the grooming response seen at the high dose of cocaine, but the sniffing and locomotor activity responses both showed a maximum at the median and high doses of cocaine. Thus, it can be stated that dopaminergic behaviors seem to reach a maximum expression around 20 mg/kg cocaine. The serotonergic behavior seen with cocaine continues to increase throughout all doses of cocaine tested (10, 20, and 40 mg/kg cocaine) without apparently reaching a peak response. In other words, dopaminergic behavioral expression exhibits a shift to the left compared to serotonergic behavioral expression on a dose-response behavioral representation. The doses tested are at the high end of the dose-response scale for dopaminergic behavior and at the middle of the dose-response scale for serotonergic behaviors. This last statement may be a bit misleading since a higher dose of cocaine (i.e. 50 mg/kg) leads to convulsions in greater than 50 percent of the animals tested (unpublished observations in this laboratory). However, no animals died at the highest dose used in this study (i.e. 40 mg/kg cocaine).

Haloperidol did attenuate all behavioral parameters. However, the doses of haloperidol needed to establish a highly significant attenuation were rather high when compared

to doses of haloperidol used to block apomorphine-induced stereotypy (Rotrosen et al., 1972). This could be expected since the doses of cocaine used in this study were relatively high when compared to previous studies (Bhattacharyya, 1979). The doses of haloperidol had two effects on the results of this study. First, since the doses used were high from the outset, this fact tends to show that the intense stereotyped behavior that cocaine elicits at higher doses is less sensitive to attenuation by haloperidol than the stereotyped behavior seen at the lower doses. Simple kinetics may, in part, account for this observation. However, the behavioral scenario at the two doses is dissimilar and this should be taken into account. Second, a possible explanation for the haloperidol attenuation of serotonergic responses may include the non-specific antagonism of serotonergic receptors that haloperidol shows at higher doses.

Examination of the net behavioral index of the cocaine/haloperidol experiments yields two interesting points. The indexes of saline/cocaine 10 and saline/cocaine 20 definitely illustrate that these two doses of cocaine show a net dopaminergic dominance. Saline/cocaine 40 however, has a net negative index, indicative of a net serotonergic dominance. What this shows is that at lower doses of cocaine the dopaminergic influence is stronger than the serotonergic influence and at the high dose used in this study,

the influence of the transmitters is reversed. Also, it seems that the 20 mg/kg cocaine dose may be near a transition point inasmuch as the lowest dose of haloperidol was able to switch the net dominance of the median dose of cocaine from dopaminergic to serotonergic. This transition effect was unique to the median dose of cocaine.

The antiserotonergic agent cyproheptadine was effective in blocking both serotonergic and dopaminergic responses. The attenuation seen with cyproheptadine was essentially non-specific for all parameters analyzed except for grooming which will be discussed separately. With this exception, cyproheptadine consistently attenuated all parameters, affecting the dopaminergic, serotonergic and net behavioral index in similar manners.

The grooming induced by cocaine is an interesting phenomenon. Grooming is considered a low level dopaminergic activity (Ellinwood and Balster, 1974). The grooming seen upon cocaine administration is biphasic. Grooming increases from the low dose of cocaine to the median dose, although not significantly. From the median dose to the high dose of cocaine, however, there was a very significant drop in the grooming response. As the intensity of stereotypy increases, decreases in lower order stereotyped activities should be expected. Haloperidol was able to block the grooming response in all but the high dose of cocaine, probably as a consequence of the low initial grooming score

seen at this dose. Cyproheptadine did not attenuate the grooming response at all. In fact, the animals receiving the high dose of cocaine actually demonstrated a significant increase in grooming with the high dose of cyproheptadine. This observed finding agrees with the opinions found in the literature (Balsara et al., 1979) that serotonin possesses a negative influence on dopaminergic behavior.

In the present study, cocaine alone never induced stereotyped gnawing. However, upon pretreatment with the high dose of cyproheptadine, the high cocaine challenge dose allowed the expression of gnawing to occur (5 out of a group of 7 animals). Serotonin is reputed to have a negative influence on dopaminergic activity (Scheel-Kruger, 1970); therefore, it seems reasonable to infer that cocaine, at high doses, shows a strong serotonergic influence which prevents the expression of gnawing. This observed result is supported by other investigators. Apomorphine, when administered in a dose which normally does not induce gnawing, can induce gnawing when cocaine is used as a pretreatment (Dadkar et al., 1977). Other investigators have shown that with respect to the biochemical basis of gnawing, it may be that gnawing behavior represents a more 'pure' dopaminergic effect than other stereotyped behaviors.

The literature on dopaminergic and serotonergic involvement in cocaine-induced stereotypic behavior is far from complete. In agreement with the results of this investigation,

other studies have shown that haloperidol antagonizes both the biochemical and behavioral effects induced by cocaine (Bhattacharyya, 1979). 5-Hydroxytryptophan methyl ester likewise blocks the biochemical changes that cocaine can induce (Pradhan, 1978). The present study also found a cocaine-induced serotonergic influence. However, the present study showed that antagonism occurs with a serotonergic blocker while Pradhan's study (1978) demonstrated behavioral antagonism with a serotonergic agonist.

Some of the results obtained in this investigation illustrate that serotonin possesses a negative influence on dopaminergic behavior. The grooming increase that was seen with cyproheptadine/cocaine 40 shows that by blocking serotonin receptors, an increase in a dopaminergic response can occur. Additional evidence for the inhibitory action of serotonin was gained quite accidentally. While the six parameters were recorded with great care, other behaviors were noted as they occurred. As was stated earlier, gnawing never occurred throughout the doses of cocaine tested, but when the series of experiments with cyproheptadine 4/ cocaine 40 was completed, it was noticed that five out of the group of seven animals exhibited an intense gnawing compulsion. Costall and Naylor (1974) report that gnawing is probably the most intense dopaminergic behavior. Since this study elicited gnawing behavior with a serotonergic antagonist, this is very strong evidence for a negative

regulatory role for serotonin in cocaine-induced dopaminergic behavior. It has been shown (Silbergeld and Hruska, 1979) that direct dopamine receptor stimulation with apomorphine does not potentiate or affect the serotonin syndrome while dopaminergic and serotonergic blockers do attenuate this syndrome. Therefore, Foldes and Costa (1975) have speculated that the serotonin syndrome can be blocked by dopaminergic and serotonergic antagonists but remains unaffected by agonists of these receptors.

This mutual interdependence of the dopaminergic and serotonergic systems has also been demonstrated morphologically. Serotonergic fibers arising from the raphe nuclei have been shown to make synaptic contact with dopaminergic cells in the substantia nigra (Parizek et al., 1971; Aghajanian and Bunney, 1975). Further, the neostriatum and limbic striatum brain areas which receive dopaminergic input have also been shown to receive serotonergic input from the raphe nuclei (Fuxe and Jonsson, 1974).

In summary, the morphologic evidence plus the behavioral evidence from this and other studies supports the theory of an interplay between the serotonergic and dopaminergic systems. The present study has demonstrated that cocaine's effects on dopaminergic behaviors peak at a lower dose of cocaine than do serotonergic behaviors. The initial goals of this study hoped to demonstrate that cyproheptadine and

haloperidol could preferentially block serotonergic and dopaminergic behaviors respectively. This was not demonstrable due to the truly complicated nature of the neurotransmitter interactions. What was shown was that haloperidol changed the response to the median dose of cocaine from a net dopaminergic dominance to a net serotonergic dominance, an effect seen only at this transitional dose. The induction of gnawing and the increase in grooming seen with the high dose of cocaine and cyproheptadine supports the hypothesis that cocaine-induced dopaminergic behavior is inhibited by serotonergic activity.

CONCLUSIONS

1. In this study, dopaminergic behaviors in rats peaked at lower doses (20 mg/kg, i.p.) of cocaine while serotonergic behaviors did not peak until higher doses (40 mg/kg, i.p.) had been administered. In this study, dopaminergic behaviors are defined as sniffing, grooming and locomotor activity while serotonergic behaviors are defined and repetitive head movements, rearing and Straub tail.
2. The dopamine antagonist, haloperidol (0.125-0.500 mg/kg, i.p.) appears to change the balance of behavior at the median dose of cocaine (20 mg/kg) from a net dopaminergic to a net serotonergic response.
3. The serotonin antagonist, cyproheptadine (1-4 mg/kg, i.p.) facilitated cocaine-induced grooming and allowed the expression of gnawing to occur. It is proposed that the serotonin-related effects of cocaine may have a negative influence on dopamine-mediated behaviors.

APPENDIX

The following six tables represent a summary of the experiments done with an atropine pretreatment followed by the cocaine challenge. The pooled variances obtained in this series precludes any statement on statistical significance. Therefore, the tables are here only as a record of the obtained results.

Table A-I. Effect of 30-Minute Pretreatment with Atropine on Cocaine-Induced Sniffing in Rats.

Cocaine, mg/kg ip	Mean sniffing response[a]			
	Saline control, 1 ml/kg	Atropine, mg/kg ip		
		1.25	2.50	5.00
10	4.00	3.86	4.14	4.14
20	5.86	5.14	4.86	5.00
40	5.86	4.86	4.14	3.86

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =3.50; d.f. =72

Table A-II. Effect of 30-Minute Pretreatment with Atropine on Cocaine-Induced Grooming in Rats.

Cocaine, mg/kg ip	Mean grooming response[a]			
	Saline control, 1 ml/kg	Atropine, mg/kg ip		
		1.25	2.50	5.00
10	1.86	1.29	0.86	0.43
20	2.29	1.86	1.14	1.43
40	0.29	0.43	0.46	0.86

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =3.06; d.f. =72

Table A-III. Effect of 30-Minute Pretreatment with Atropine on Cocaine-Induced Locomotor Activity in Rats.

Cocaine, mg/kg ip	Mean locomotor activity response[a]			
	Saline control, 1 ml/kg	Atropine, mg/kg ip		
		1.25	2.50	5.00
10	1.29	1.43	1.86	2.14
20	4.43	4.43	3.86	3.14
40	5.00	6.00	4.86	4.86

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =3.41; d.f. =72

Table A-IV. Effect of 30-Minute Pretreatment with Atropine on Cocaine-Induced Repetitive Head Movements in Rats.

Cocaine, mg/kg ip	Mean Repetitive head movement response[a]			
	Saline control, 1 ml/kg	Atropine, mg/kg ip		
		1.25	2.50	5.00
10	1.00	1.29	0.86	1.43
20	3.71	2.29	2.14	2.43
40	4.86	3.86	4.86	3.14

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =3.62; d.f. =72

Table A-V. Effect of 30-Minute Pretreatment with Atropine on Cocaine-Induced Rearing in Rats.

Cocaine, mg/kg ip	Mean rearing response[a]			
	Saline control, 1 ml/kg	Atropine, mg/kg ip		
		1.25	2.50	5.00
10	1.86	2.14	2.43	1.29
20	3.29	2.86	3.43	2.71
40	3.71	3.86	3.14	2.86

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =2.75; d.f. =72

Table A-VI. Effect of 30-Minute Pretreatment with Atropine on Cocaine-Induced Straub Tail in Rats.

Cocaine, mg/kg ip	Mean Straub tail response[a]			
	Saline control, 1 ml/kg	Atropine, mg/kg ip		
		1.25	2.50	5.00
10	0.14	0.29	0.43	0.00
20	1.57	1.86	2.29	1.14
40	3.00	1.57	1.86	1.86

[a] Each score represents the mean of a scoring system ranging from 0-6. Number per test group =7. Pooled variance (s^2) =2.99; d.f. =72

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