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EXAMINATION OF THE ROLE OF BRAIN AMINES AND CYCLIC AMP IN BENZODIAZEPINE-INDUCED SUPPRESSION OF AGGRESSION

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ABSTRACT

The behavioral depressant property of chlordiazepoxide (CDP) has been traced to reduced turnover of brainstem norepinephrine. Since tolerance to these effects develop within 5-6 administrations of the drug, chronic administration and repeated testing provides a means to separate the adrenergic behavioral depressant effect from more persistent anti-anxiety or disinhibitory, or antiaggression effects which have been traced to blockade of brainstem serotonin turnover and increased levels of cyclic AMP by phosphodiesterase inhibition.

Muricidal activity of rats is blocked by CDP. The effect is reversed by repeated testing, by pretreatment with CDP, and by concomitant dosing with caffeine. This points to the adrenergically mediated general behavioral depressant action of CDP as being responsible for its anti-muricidal activity.

The attenuation of shock-induced fighting by CDP had previously been shown to persist despite chronic administration of the drug and repeated testing. Thus behavioral depression is not important in reducing shock-induced aggression (SIA) and a role for serotonin was suggested. However, concomitant administration of 5-hydroxytryptophan, the precursor of serotonin, or α-methyl tryptamine, a serotonergic receptor stimulator, did not reverse the effect of CDP on SIA.

An alternate hypothesis suggested that phosphodiesterase inhibition might be the property essential to the reduction of SIA. A phosphodiesterase stimulator, imidazole acetic acid, which did not have any effect on SIA alone, but when administered with CDP reduced it further rather than increasing the level of fighting. Thus a neurochemical explanation for CDP's action on SIA is not presently possible.

While CDP was found to reduce septal lesion-induced rage, pretreatment before lesioning did not seem to alter the effectiveness of the drug. Thus it seems that the attenuation of septal rage is neither dependent on the adrenergically mediated behavioral depressant effect of CDP nor could any serotonergic mechanism be reliably related to the effect. A more specific anti-aggression effect is suspected.

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Chlordiazepoxide (CDP) has for many years been known to have a profound effect on aggressive behavior (Randall, 1960; 1961). The effects of CDP as well as the other benzodiazepines have been extensively examined in a variety of aggressive paradigms. CDP was found to depress mouse killing in rats (Loiselle and Caparrel, 1966), to have taming effects on vicious cynomolgus monkeys (Heise and Boff, 1961), to produce "calming" of septal lesion-induced rage (Horovitz et al., 1963), to reduce shock-induced aggression of mice (Tedeschi et al., 1959), and to reduce fighting of previously isolated mice (Cole and Wolf, 1966). Although a reduction in various aggressive behaviors occurs under many conditions, the results of a number of experiments are contradictory. For example, several authors have found that CDP causes an increase in spontaneous aggression in grouped male mice (Fox and Snyder, 1969; Fox, Tockosh, and Wilcox, 1970). Reports on the effect of benzodiazepines on hostile-aggressive behavior in humans also contain contradictory results. While a number of experimenters report a reduction in various aggressive behaviors (Gleser et al., 1965; Podobnikar, 1971), others report an increase in overtly hostile behaviors (DiMascio et al., 1969; Salzman et al., 1969). This increase in aggression in the clinical setting is most often attributed to the release of behaviors previously under aversive control.

Examination of the literature suggests that there are several variables which may explain the discrepancies in experimental results. First, the species examined may be a significant factor in the determination of anti-aggressive effects of the benzodiazepines. Species variations have been demonstrated not only behaviorally but neurophysiological differences in response to benzodiazepine administration have also been reported (Nagy and Desci, 1973). Second, the dose of the drug and the route of administration clearly have an effect upon the efficacy of the drug in altering aggression (Christmas and Maxwell, 1970). Third and perhaps most important is the aggression paradigm used. It is generally assumed that various testing situations elicit different aggressive behaviors having different underlying physiological substrates (Moyer, 1968). If this indeed is the case, then it should not be surprising that different drugs are effective in altering different aggressive behaviors, or that the same drug is not equally effective in reducing the various forms of aggression. Finally, the duration of treatment with the benzodiazepines, i.e., acute vs. chronic administration, is an important factor in the effect of the drugs on aggression (Fox et al., 1970; DiMascio, 1973).

Of considerable importance in evaluating the effectiveness of the benzodiazepines in reducing aggression is the fact that they cause some general behavioral depression. Previously the determination of the role of behavioral depression in the reduction of aggression has led to ambiguous results. For instance, Horovitz

et al. (1965) found a reduction in mouse killing in rats after treatment with CDP but only at doses which impaired locomotor ability. In most cases in order to deal with this problem an index of the effective dose (ED50) at which aggression was reduced was compared to the effective dose at which locomotor ability was impaired. The determination of the ED50 of locomotor impairment, however, varies significantly among laboratories and for this reason may represent a major variable in the estimation of the significance of behavioral depression to the reduction in aggressive behavior.

A more effective way of identifying the contribution of behavioral depression to the attenuation of aggression is to allow a tolerance to the behavioral depressant effect to develop over several days of administration of the drug. This technique is based on the finding of Stein et al. (1973) who demonstrated that the behavioral depressant effect of oxazepam, a drug in the benzodiazepine class, is correlated with the reduction of NE turnover in the midbrain-hindbrain region of the rat, an effect previously reported for all brain regions except the medulla-pons region by Taylor and Laverty (1969) and Corrodi et al. (1971). Both the change in NE turnover and the general depression of behavior as measured in the Geller-Seifter test, disappeared after 5-6 daily administrations of the drug. Therefore, the technique of repeated administration of the drug eliminates the necessity to

determine the level of behavioral depression by using a measure such as locomotor ability which may be quite unrelated to the aggression paradigm used.

A second significant behavioral effect of benzodiazepine administration is an increase in behaviors under aversive control. This potent "anti-anxiety" effect has been demonstrated in a number of paradigms (Feldman and Green, 1967; Feldman, 1968; Cook and Kelleher, 1962; Geller, Kulak and Seifter, 1962; Feldman, Kaada, and Langfeldt, 1973; and others) and in each case, the drug seems to have reduced the significance of punishment as demonstrated by an increase in responding under conditions of negative reinforcement. Stein et al. (1973) has shown that the anti-anxiety effect of oxazepam is correlated with the significant reduction in 5HT turnover, an effect found by Chase et al. (1970) after administration of diazepam, a congener of CDP. Neither the potent anti-anxiety actions of the drug nor the reduced 5HT turnover seem to undergo tolerance with repeated administration of the drug.

From the foregoing discussion it seems that the most effective way to measure the contribution of behavioral depression to the decrease in aggression is to use chronic administration and repeated testing. This method would allow the examination of the initial effectiveness of the drug as well as changes in effectiveness as tolerance to the behavioral depressant effect develops. It follows, first, that any anti-aggression effects of benzodiazepines that diminish or disappear with repeated testing are

probably due to the alteration of an adrenergic mechanism. Second, the persistence of an anti-aggressive effect during repeated administration of the drug would give strong support for the involvement of a serotonergic mechanism.

Experiment 1

While mouse killing by rats is one of the aggressive behaviors that has been suppressed by CDP, recently Wnek et al. (1974) induced an increase in mouse killing in rats treated with low doses of CDP. However, even in cases where higher doses of CDP were used and mouse killing was attenuated, the importance of the behavioral depressant effect for the reduction of aggression is not clear. Horovitz and his co-workers (1965, 1966) and Karli (1961) determined that mouse killing is diminished by benzodiazepines only at doses that produce ataxia. These studies, however, determined the drug effect only after acute administration. Therefore, differences in the determination of the role of behavioral depression on muricide activity may once again only have reflected differences in methods of measuring behavioral depression.

The following experiments have determined the effects of daily treatment with CDP on mouse killing in rats in an attempt to resolve the discrepancies previously described and to determine the role of behavioral depression in CDP's effect on mouse killing. It was hypothesized, on the one hand, that if behavioral depression

plays a significant part in the reduction of mouse killing, the frequency of killing should be lowest, or the latency to kill mice should be highest during initial drug tests. Then as testing continues the killing frequency should increase and killing latency should decrease as the tolerance to the behavioral depressant effect gradually develops. On the other hand, if there were an anti-muricide factor independent of behavioral depression, then the frequency of mouse killing should remain low or the latencies of mouse killing should be high for the duration of the drug tests.

If tolerance to a CDP-induced anti-muricide effect were found, it would also be of interest to measure the duration of the tolerance effect. Ideally, the duration of tolerance would involve comparisons among groups that would be retested with the drug after different time intervals of drug abstinence. However, the difficulty of obtaining large numbers of muricidal subjects which would be necessary for such tests precluded this procedure. Instead, subjects in our experiments were retested for the effect of CDP on muricide after a number of different time intervals of drug abstinence. The complication here is the possibility that a series of drug tests might contribute to the duration of the tolerance effect. Nevertheless, this procedure was carried out because it would at least provide an initial estimate of the duration of tolerance.

Method

The subjects in the first experiment were 10 male Sprague-Dawley rats 4-6 months old and weighing 350-475 g. They were housed individually and fed and watered ad libitum. The animals were maintained on a 12 hour alternating light-dark cycle with light from 7 P.M. to 7 A.M. throughout the experiment. These animals quickly and reliably killed mice placed in their home cages prior to any drug treatment. During each test session a live mouse was placed in the living cage of each rat and the latency to kill the mouse was recorded. If the mouse was not killed within 15 minutes it was removed from the cage. Mice killed during the session were removed promptly from the cage to prevent the rat from eating it. After three daily no-drug muricide tests, each rat received 50 mg/kg CDP i.p. 30 minutes before 8 successive daily muricide tests.

Following the eight consecutive tests each rat was retested for muricide activity under drug conditions after 7 days of neither drug administration nor muricide testing. Then another drug test was given after an additional 14 days; and a final test after 26 additional days.

Results and Discussion

Three of the 10 subjects were removed from the analysis of the results. Two of the 3 rats never seemed to be affected by

the drug and killed mice consistently with a low latency throughout the drug tests. The third animal appeared to be severely depressed and this behavioral depression persisted throughout the 8 days of drug administration with no muricide activity. These animals were eliminated from the analysis because their response to the drug was clearly aberrant since it was consistent throughout the testing procedure and could not be due to procedural variation. Moreover, the addition of this data for the most part adds a constant to the measurement of muricide and latency since their responding is relatively stable over the 8 days of drug treatment. Finally, adding these subjects does not alter the significance level of the overall findings.

The results of the remaining animals are shown in Fig. 1. It is clear from the figure that the initial administration of CDP on Day 4 significantly reduced the number of killing responses. Although two of the 7 animals represented did kill mice on the first day of drug administration, both of these animals were non-killers on the second day. It can also be seen that the number of kills increases over successive days under drug conditions and that by Day 10, all animals are again killing mice placed in their home cages. The one non-killer on Day 11 appeared sick and died two days later.

The latency of mouse-killing is shown in Fig. 2. Non-killers

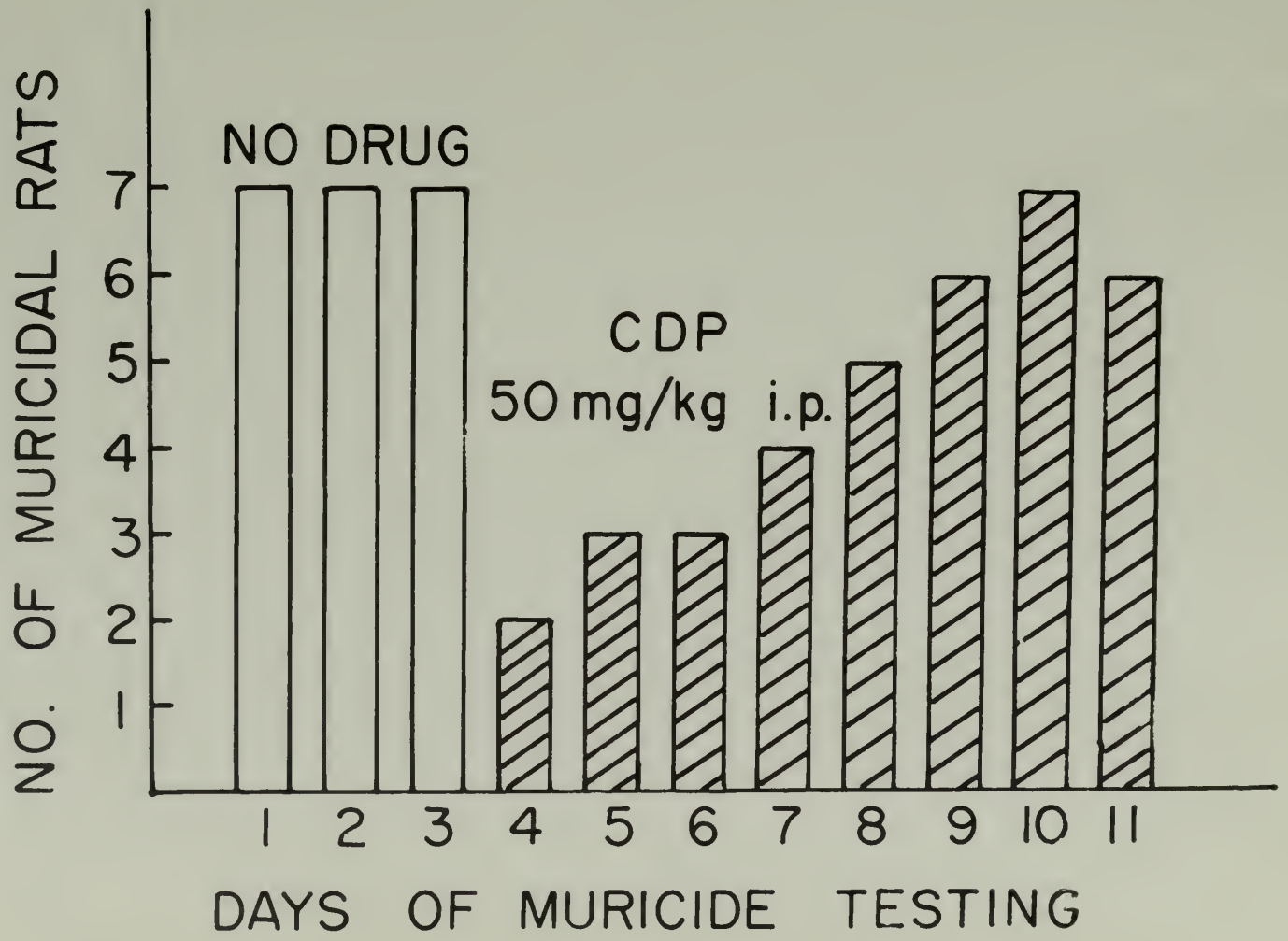

Figure 1. Number of muricidal rats under no drug (saline) and drug (CDP) conditions. Rats had to kill a mouse within 15 min. to be designated as muricidal.

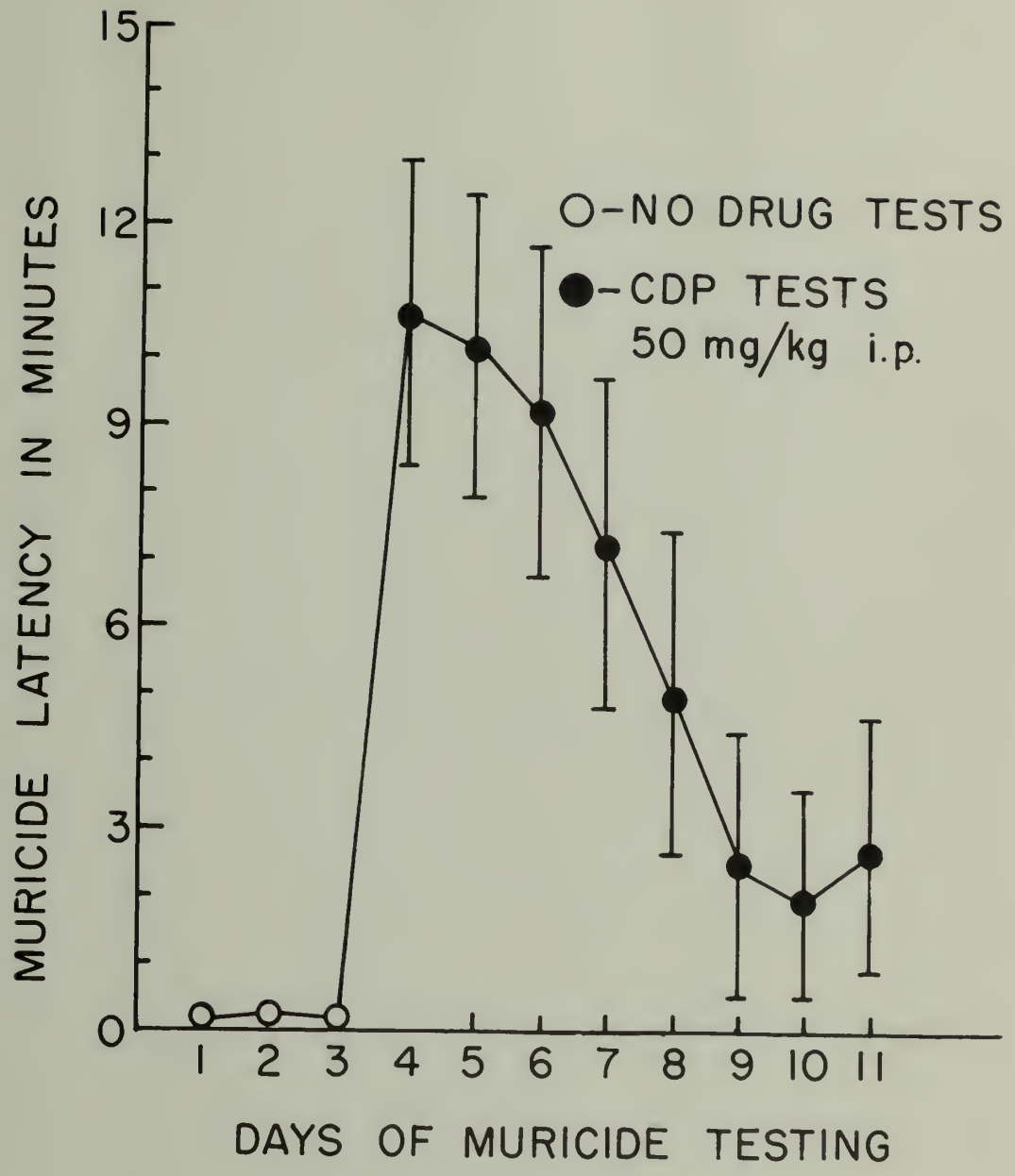

Figure 2. Latency of muricidal activity under no drug (saline) and drug (CDP) conditions. Non-muricidal rats were assigned a latency of 15 min. The vertical bars through the data points indicate the standard error of the mean.

were assigned the maximum latency of 15 minutes. A t-test for paired observations showed that there is a significant increase in latency on Day 4 (the first Drug day) as compared to the last No-drug test day (Day 3) ($t = 4.04$, $p < .01$). Comparison of the mean latencies for the first 4 drug days with the last 4 drug days also shows a significant difference ($t = 3.53$, $p < .02$). However, the difference in latency between Day 3 (the last No-drug day), and Day 11 (the eighth Drug day) was not significant. These results clearly show suppression of mouse killing by rats after initial treatment with CDP, but there also was a gradual increase in killing and a decrease in latency with repeated administrations of the drug. This strongly suggests that the behavioral depressant effect of the drug, which usually shows a tolerance after repeated doses, is the significant factor that reduces mouse killing. Examination of the data of individual animals show that each animal developed tolerance to the effect of the drug at a different rate and this difference accounts for the variance at each data point in Fig. 2. Two of the 7 subjects returned to killing mice after only one drug administration. Three of the 7 did not return to killing until after receiving drug on 3 consecutive days. One subject did not kill for 4 drug-test sessions and one subject showed an absence of mouse killing behavior for 6 out of the 8 drug tests.

A discussion of the data concerning the duration of tolerance will be postponed until the discussion of Experiment 2 since subjects from both experiments were used.

Experiment 2

Drug tolerance to the behavioral depressant effect in these experiments is either physiological or behavioral. With respect to a physiological tolerance, there is a drug disposition tolerance which is caused by an increased rate of elimination or deactivation of the drug. There is also a pharmacodynamic tolerance in which neural tissue apparently returns to normal function in spite of continuing high plasma levels of the drug. This reaction is characteristic of morphine tolerance (Jaffe, 1970). Behavioral tolerance is characterized as an acquired accommodation to the drug probably through learning experiences and a return to normal functioning (Dews, 1962). There was the possibility that the tolerance that occurred in Experiment 1 was the behavioral type and was merely a manifestation of the animals' acquired ability to kill mice in the presence of the drug. To investigate that possibility muricidal rats were given CDP daily for a number of days, then tested for muricide for one day under drug conditions. It was hypothesized on the one hand that if the animals in this experiment would show undiminished muricidal activity on the drug-test days, this would argue for a drug disposition or pharmacodynamic tolerance. On the other hand, since these rats would have had no opportunity to learn to kill mice under the influence of CDP and if they were suppressed by the drug and no muricide occurred, this would argue for a behavioral tolerance in Experiment 1.

Method

Nine male Sprague-Dawley rats from 5-7 months old and weighing 375-500 g. were used as subjects. Each of the rats used consistently killed mice placed in his home cage. On days 1-3 each rat was given a saline injection followed 30 min later with a muricide test as described in Experiment 1. On days 4-10, each rat was given 50 mg/kg i.p. CDP, but no muricide tests were given. On day 11, each rat was given the usual CDP dose followed 30 min later by a muricide test. Latency to kill the mouse was recorded. A maximum of 15 minutes was allowed for each test. As in Experiment 1, these animals were also tested after an interval of 7, 14 and 26 days to get an estimate of the duration of the tolerance.

A control group of 6 mouse killers were tested with saline instead of CDP on days 4-10 and then tested with CDP (50 mg/kg) on day 11. This was done to eliminate the possibility that the interval without mouse killing after the first 3 day period of mouse killing had in itself an effect on muricide on day 11.

Results and Discussion

The results of Experiment 2 were that the 9 subjects that killed mice on the first 3 no-drug test days also killed mice during the muricide test under drug conditions (day 11). A comparison of the average latency to kill on the third no-drug day and the drug-test day (day 11) also showed no significant difference.

These data, along with subjective observation of the activity of these chronically dosed subjects in response to the presentation of the mouse on day 11 strongly suggests that tolerance to the behavioral depressant effect of the drug had developed during the daily administration of the drug. These observations are consistent with the findings of Hoogland et al. (1966), Goldberg et al. (1967) and Margules and Stein (1968). Also, the high rate of mouse killing among these chronically dosed rats indicates that there is probably no specific anti-muricidal effect of CDP, and the initial suppression of this form of aggression in Experiment 1 was due to general behavioral depression. Furthermore, the fact that all the subjects killed on their first test day under the drug condition eliminates the possibility that the rats in Experiment 1 had acquired a behavioral tolerance and merely needed practice under the drug state to again become consistent killers.

The 6 rats in the control group first killed mice in muricide tests for 3 days. They were then given saline on days 4-10, and CDP and a muricide test on day 11. On the drug-test day 4 rats did not kill at all during the 15 minute test, and 2 killed after relatively long latencies, 70 and 90 seconds. Assigning a latency of 15 minutes to the non-killers, a t-test for paired observations showed that the latency difference between day 11 for the control and experimental groups were also significant ($t = 4.33$, $p < .01$). This result shows that the no drug-no test interval (days 4-10) did

not itself increase the probability of muricide on drug day 11, and that the sole determinant of mouse killing on drug day 11 was whether or not drug was given on days 4-10.

Finally, as in Experiment 1 the chronically dosed animals in Experiment 2 were also given a drug-muricide test after 7, 14, and 26 day intervals. Combining the results of these two groups, muricide latency after each of these 3 intervals was compared with the latency for the third no-drug test day and the last drug test day of Experiment 1. No significant difference was detected in each comparison after 7 days of drug abstinence. Fourteen days after that, a t-test for paired observations showed that the differences in both comparisons were significant ($t = 2.38$, $p < .05$; $t = 2.17$, $p < .05$) as were the differences after a further interval of 26 days ($t = 6.75$, $p < .001$; $t = 6.48$, $p < .001$). This data suggests that the tolerance to the behavioral depressant effect of CDP lasts for at least a week and is lost sometime in the next 2 weeks of drug abstinence when tested in this manner.

Experiment 3

In order to further support the earlier finding that behavioral depression is the significant factor in reducing mouse killing with CDP, a CNS stimulant was used to antagonize the behavioral depressant effect of CDP. Caffeine has previously been found to

effectively counteract the behavioral depressive effect of CDP on spontaneous activity (Quenzer et al., 1974). Also caffeine has been shown to have no effect itself on muricide (Sofia, 1969).

Method

Ten male albino rats (5 Sprague Dawley and 5 Holtzman), approximately 6 months old and weighing 400-500 g. served as subjects. Each of the rats was a reliable mouse killer when tested for several days in his home cage under no drug conditions. They were housed separately and fed and watered ad libitum. Each subject was given muricide tests for a total of 6 days in the manner described in Experiment 1. However, saline (0.9%, 1 cc/kg i.p.) was administered 30 min before testing on Days 1 and 2, caffeine (75 mg/kg) on Days 3 and 4, and CDP (50 mg/kg) plus caffeine (75 mg/kg) on Days 5 and 6.

Results and Discussion

The data for the 2 strains of animals were grouped because there were no differences in frequency, latency or drug effects between them. All subjects killed mice within 15 min on each of 6 tests whether they were administered saline, or caffeine, or caffeine plus CDP. This result markedly contrasts the almost complete suppression of mouse killing after initial tests with 50 mg/kg CDP in Experiment 1. However, a comparison of the latencies of mouse killing showed that on Days 5 and 6 (CDP plus caffeine) lat-

encies were longer than those on Days 1 and 2 (saline). The mean values were 52.0 and 11.4 seconds for the drugged and saline test respectively. A t-test for paired observations showed that this difference was significant ($t = 2.60$, $p < .05$). But a Student t-test showed that a comparison of the average latencies for the first two days of CDP administration in Experiment 1 with latencies on Days 5 and 6 in Experiment 3 produced a highly significant difference in latency of muricide ($t = 6.88$, $p < .001$). The mean values were 52.0 and 636.8 seconds for the latencies in Experiment 3 and 1 respectively. This shows that the concommitant addition of caffeine strongly affected the anti-muricide effect of CDP, but in the doses used, the CDP effect was not completely antagonized by caffeine. Our observations were that the rats made numerous attempts to catch the mice but showed a degree of incoordination that often delayed the kill. Caffeine administered on Days 3 and 4 did not noticeably interfere with muricidal activity.

General Disscussion

In the present experiments, repeated testing with CDP altered the effect of the drug on mouse killing. The fact that the rats resumed muricidal activity under large doses of CDP suggests that the adrenergically mediated behavioral depressant effect of CDP was responsible for the non-specific initial suppression of muricide. The antagonism of the anti-muricide effect by caffeine,

which is known to reverse the behavioral depressant effect of CDP in an activity box, provides further support for this hypothesis. It is also known that the xanthines and the catecholamines increase peripheral concentrations of cAMP (Ritchie, 1966). It follows that the caffeine-induced increase in cAMP would reverse the possible reduction of cAMP brought about by a CDP-induced decrease in catecholamine turnover. This argument lends credence to the notion that the adrenergic behavioral depressant effect is antagonized by caffeine.

A number of muricide experiments manipulating brain levels of 5HT indicate a role for this amine as well. Both Sheard (1969) and DiChiara et al. (1971) showed that the blockade of 5HT synthesis with pCPA is followed by muricide behavior in rats that previously did not kill mice. Grant et al. (1973) showed that after dorsal and median raphe lesions, when the forebrain content of 5HT was approximately 70% below normal, rats showed increased frequency and decreased latency for lethal attacks on mice. The time course of this behavioral effect paralleled the reduction in forebrain 5HT suggesting that 5HT exerts inhibitory control over mouse killing. Further support for the inhibitory effect of 5HT is presented by Kulkarni (1968) who found a significant suppression of mouse killing in rats pretreated with 5HTP. Our results are also compatible with the notion that 5HT has an inhibitory effect on

mouse killing. CDP blocks 5HT turnover as well as reducing NE turnover, and this reduction in 5HT activity may actually increase muricide. This latter effect, however, is masked by CDP-induced behavioral depression and emerges only after tolerance to this adrenergic, muricide-suppressant effect occurs. Thus our conclusion is that the balance between NE and 5HT may be the significant factor in determining the level of mouse killing. This idea may account for some anomolous effects of CDP that were found when protocols of individual rats were examined. Grouping the results of individual rats showed a steady increase in muricide activity during consecutive drug tests as seen in Fig. 1. However, some animals fluctuated between killing and not killing during early drug tests. This may be due to a delicate balance between the depressant action of CDP mediated by lowered adrenergic activity and a muricide stimulant action mediated by lowered serotonergic activity.

PART II

The technique of chronic administration of CDP with repeated testing was formerly used in the shock-induced aggression (SIA) paradigm (Quenzer et al., 1974). In those experiments it was found that CDP suppressed SIA and repeated testing had no further effect. Thus, it is fairly clear that the CDP-induced change in NE turnover and the correlated behavioral depression was not responsible for the decrease in SIA since tolerance to behavioral depression, with the restoration of normal turnover rates of NE, was not accompanied by increased fighting. A more likely alternative is that the CDP-induced reduction in 5HT turnover, for which no tolerance seems to occur was responsible for the decrease in this type of aggression. This alternative hypothesis, however, must be weighed in light of other evidence presented by Eichelman and his coworkers (1972a, 1972b, 1972c) who suggested an inhibitory role for NE in this type of aggression. They found that depletion of brain NE with either 6-hydroxydopamine or 6-hydroxydopa significantly increased the amount of SIA without altering jump thresholds to electric shock, and that the increase in attack over time seemed to be correlated with the time course of adrenergic nerve terminal degeneration. However, at least in some cases 5HT was also significantly reduced (Eichelman et al., 1972a). Thus, in some ways we have examined the possibility that the reduction in SIA

following CDP administration is related to the reduction in 5HT turnover induced by the drug.

In order to test this hypothesis a serotonergic agonist which increases neural activity in serotonergic neurons was used to antagonize the CDP-induced attenuation of SIA. The restoration of brain levels of 5HT by 5HTP, the biologic precursor of 5HT, after depletion with such agents as pCPA has been well documented (Geller and Blum, 1970; Tenen, 1967) and is a technique commonly employed to substantiate the efficacy of serotonergic depletion on behavior. Thus, the CDP-induced reduction in 5HT turnover may be reversed by concomitant administration of 5HTP although an increase in the levels of the amine does not necessarily imply an increase in utilization. With this technique several other potential problems have been identified (Tenen, 1967). One such problem is that although 5HT levels of total brain are returned to within the normal range, regional distribution of 5HT might be quite different from normal. Also, the distribution and amount of "bound" and "free" 5HT might be quite different. Finally, even if distribution were restored to normal, the sudden, rapid increase in 5HT at those sites that have had reduced serotonergic activity for several days might result in different functional effects than normal, such as a pharmacologically induced denervation supersensitivity.

Further, if the CDP reduction in turnover were due to a decrease in the release of 5HT rather than in the rate of synthesis,

then increasing endogenous levels of transmitter with administration of 5HTP may not reverse the physiological effect of CDP nor its effect on SIA. In this case a 5HT agonist such as ∝-methyl tryptamine which directly stimulates post-synaptic receptor sites, would more readily reverse the CDP effect. It should be mentioned that earlier attempts to change the behavior of pigeons on multiple food and punishment schedules using ∝-methyl tryptamine have not been entirely successful (Graeff and Schoenfeld, 1970). They found a reduction in most behaviors examined, but only at doses that produced "gross behavioral changes" such as a refusal of food, marked tremors and diarrhea. Also, an attempt to reverse the CDP-induced facilitation of two-way avoidance with varied doses of ∝-methyl tryptamine did not proved to be successful (Espino, 1974). Nevertheless, an antagonism of the CDP-induced reduction of SIA by either concomitant 5HTP or ∝-methyl tryptamine administration could indicate a role for 5HT in SIA.

Experiment 1

The subjects were 48 male Sprague Dawley rats approximately 5 months old, weighing between 350-400 g. They were paired according to body weight at the start of the experiment. They were housed individually and fed and watered ad libitum.

The apparatus used was a modification of that used by Ulrich and Azrin (1962) and has been described elsewhere (Quenzer et al.,

1974). Each test session consisted of the presentation of 100 2 mA. shocks of ½-sec duration applied through the grid floor every 2 seconds. The number of characteristic fighting responses made by each pair of rats was recorded. Each pair of subjects was tested for 9 days after 3 initial priming sessions. On days 1-3 each subject received a saline injection (0.9% saline, 1 cc/kg) 30 min before testing. On days 4-6, 15 mg/kg CDP was administered to each subject 30 min before testing. The pre-test drug treatment on days 7-9 was 15 mg/kg CDP plus 15 mg/kg 5HTP for Group 1 and 15 mg/kg plus 30 mg/kg 5HTP for Group 2. Group 3 received 6 mg/kg α-methyl tryptamine plus 15 mg/kg CDP on days 7-9 and on days 10-12 pretest treatment consisted only of 6 mg/kg α-methyl tryptamine. For the last two days this group received only saline before testing.

It was hypothesized that if the CDP-induced reduction in SIA is due to a decrease in 5HT turnover, then concomitant treatment with 5HTP or α-methyl tryptamine should return the number of fighting responses to control levels. However, if no change in the aggression level is determined, it is likely that some effect of CDP other than that of altering 5HT turnover is responsible for the reduction in SIA. Moreover, if CDP effects are not reversed by 5HTP but are reversed by α-methyl tryptamine, it strongly suggests that CDP blocks turnover of 5HT at some point other than at synthesis.

Results and Discussion

The results of Experiment 1 are illustrated in Fig. 3. A t-test for paired observations showed that CDP signfficantly reduced SIA in Groups 1 and 2 respectively ($t = 6.28$, $p < .001$; $t = 3.82$, $p < .01$) confirming earlier studies (Quenzer et al., 1974). Clearly, neither dose of 5HTP administered concomitantly significantly reversed the CDP-induced attenuation of SIA. However, despite the fact that numerous experimenters report behavioral effects at these doses, it has been suggested that the drug may not be reaching the brain due to rapid peripheral decarboxylation to 5HT (Cook, personal communication). While it may be desirable to reexamine the effects of 5HTP on the CDP-induced attenuation of SIA in the presence of a peripheral L-aromatic amino acid decarboxylase inhibitor, the additional drug-behavior interactions and the attendent difficulties in identifying the neurochemical effects of the new drug combination would make the results of this procedure subject to considerable speculation.

Figure 4 shows the amount of shock-induced fighting for Group 3 which was treated with α-methyl tryptamine. An analysis of variance detected a significant difference in the amount of fighting after the various drug treatments ($F = 34.60$, $p < .01$). A t-test for paired observations of the first 3 saline treatment days as compared to the last 2 saline treatment days showed no significant

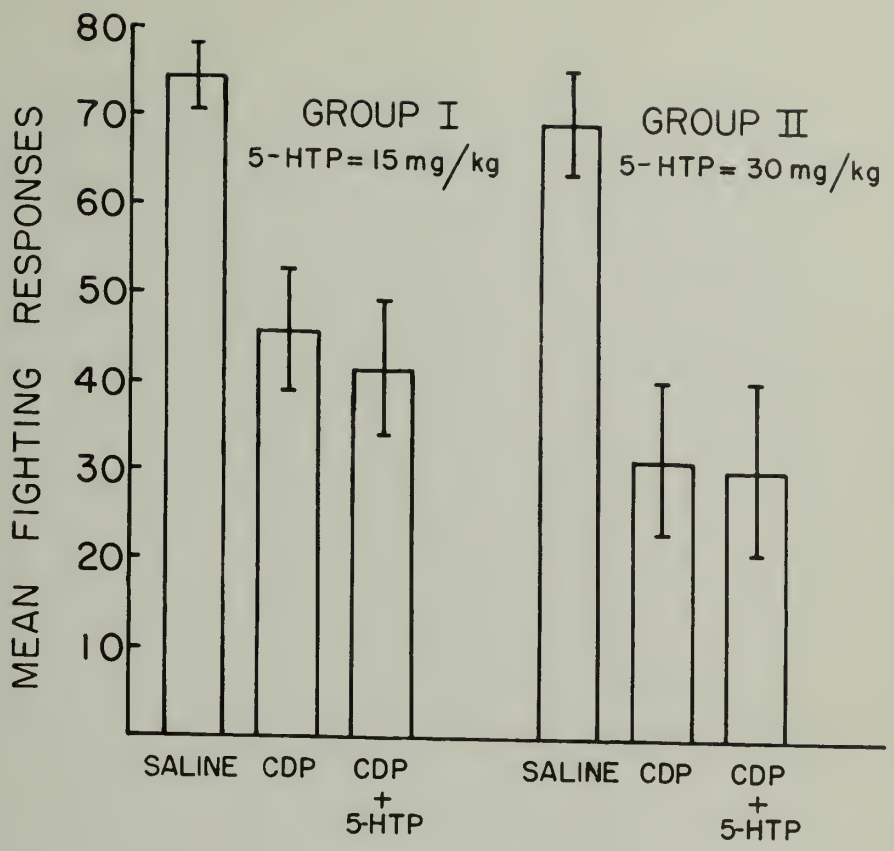

Figure 3. Number of fighting responses after treatment with saline, CDP, and CDP plus 5HTP (Group 1 = 15 mg/kg; Group 2 = 30 mg/kg).

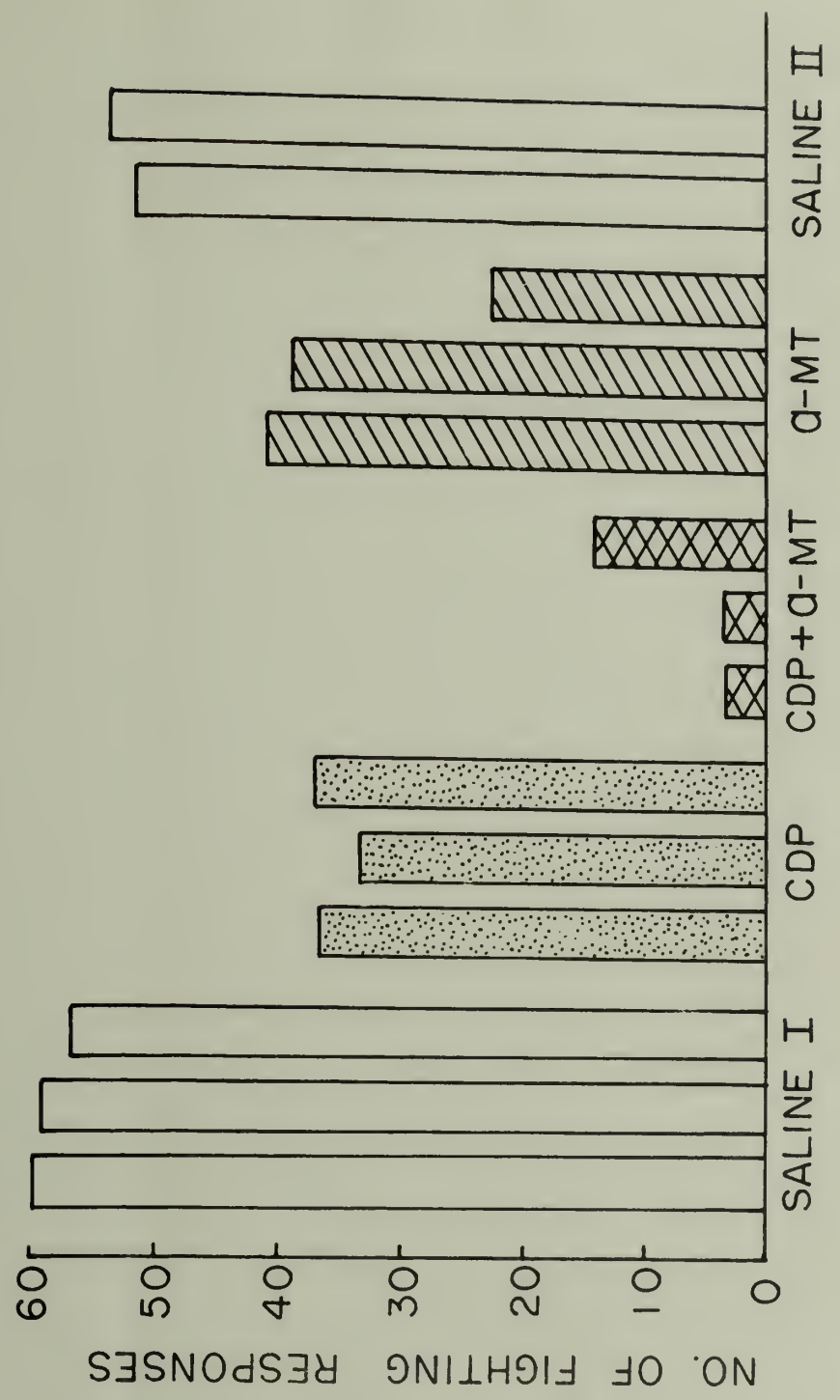

Figure 4. The effects of CDP and α-methyl tryptamine, alone and in combination, on shock-induced fighting.

difference suggesting that prolonged periods of daily testing and multiple drug treatments probably did not have any effect on the base rate of fighting.

A Newman Keul's test of contrasts demonstrated that CDP significantly reduced SIA as compared to saline treatment ($p < .01$). The fighting score after the combined treatment of CDP plus α-methyl tryptamine also was significantly different from saline alone ($p < .01$) as well as from both the CDP alone treatment ($p < .01$) and the α-methyl tryptamine alone treatment ($p < .01$). Finally, the number of fighting responses after α-methyl tryptamine alone was significantly different from baseline (saline) levels ($p < .01$). These data show that while CDP alone significantly reduced shock-induced fighting, this attenuation of aggression is not antagonized by the serotonergic agonist, α-methyl tryptamine; rather, there was an additive effect.

Why α-methyl tryptamine should itself reduce SIA and further reduce SIA already diminished by CDP is not clear. Although the dose of the drug used was quite high, none of the "gross behavioral changes" described by Graeff and Schoenfeld (1970) for pigeons were evident. Further, rats given similar doses and tested in a 2-way avoidance paradigm did not in any way impair performance nor reverse CDP facilitation of acquisition of avoidance responses (Espino, 1974). These results, in combination with the inability

to detect significant effects of 5HTP on the reduction of SIA by CDP as shown in Groups 1 and 2, suggest that a serotonergic mechanism may not be central to the CDP-induced attenuation of SIA. Such a conclusion supports the findings of Connor et al. (1970) who found that 5HT depletion with pCPA had no effect on the level of SIA.

Experiment 2

An alternative mechanism by which CDP may attenuate SIA is by inhibiting cAMP phosphodiesterase. The finding by Quenzer et al. (1974) that caffeine as well as CDP alone reduce SIA and in combination had an additive effect, further suggests that a property held in common by caffeine and CDP, i.e. cAMP phosphodiesterase inhibition, may be responsible for the decrease in SIA. Inhibition of phosphodiesterase, the enzyme responsible for the degradation of cAMP to 5'AMP, produces an increase in the level of endogenous cAMP. Cyclic AMP has been identified as the mediator of numerous hormonal responses, and as having a role as a "second messenger" in synaptic transmission (Rall and Gillman, 1971; Kebabian and Greengard, 1971; McAfee and Greengard, 1972).

It has been known for some time that caffeine and other drugs in its class, the methylxanthines, are potent phosphodiesterase inhibitors (Robison et al., 1971). Recently the same property has been identified <u>in vitro</u> for CDP as well (Beer et al., 1972;

Crowley et al., 1974). Beer et al. (1972) further showed that this effect was correlated with anti-anxiety properties of the substances during behavioral testing. Also, antagonism of the CDP-induced anti-anxiety effect has been demonstrated by stimulating phosphodiesterase with imidazole acetic acid (IMA) a naturally occurring metabolite of histamine (Horovitz et al., 1972). Imidazole acetic acid selectively increases phosphodiesterase activity in brain, kidney, intestine and liver but not in heart or skeletal muscle (Roberts and Simonsen, 1970).

In order to test the hypothesis that CDP-induced phosphodiesterase inhibition may be responsible for attenuating SIA, IMA was concomitantly administered with CDP with the expectation that IMA would stimulate phosphodiesterase activity and the CDP-induced reduction of SIA would be reversed. Further, assuming a common mechanism, caffeine's attenuation of SIA should also be reversed by IMA.

Method

The subjects were 24 male Sprague Dawley rats from 6-8 months of age. They weighed between 375-450 g. at the start of the experiment and were paired according to body weight. They were housed individually and fed lab chow and watered ad lib.

The apparatus and testing procedure was the same as that used in Experiment 1. Five pairs of subjects were first tested for SIA on 3 initial daily priming sessions. Subsequently, on days 1-3

each subject received an i.p. saline injection (0.9% saline, 1 cc/kg) 30 min before testing. On days 4-6, 15 mg/kg CDP i.p. was administered 30 min before testing. The drug treatment on days 7-9 was 125 mg/kg IMA i.p. 1 hour before and 15 mg/kg CDP 30 min before SIA testing. The dose and time parameters were suggested by Beer (1974, personal communication). IMA alone was the pretest drug treatment administered 1 hour before SIA testing on days 10-12. To examine the possibility that repeated testing may in itself alter the number of fighting responses, the Ss were again tested under saline conditions for 3 days (Days 13-15) after the imidazole alone treatment. The injected volume was the same for saline and drugs.

The SIA testing procedure was the same for 7 pairs in Group 2. However, following three saline test days, these subjects received on days 4-6, 50 mg/kg caffeine benzoate i.p. 30 min before testing. On days 7-9 the pre-test drug treatment included 125 mg/kg IMA 1 hour before and 50 mg/kg caffeine 30 min before testing. On days 10-12 each subject received 50 mg/kg caffeine plus 250 mg/kg IMA.

Results

The results are shown on the left in Fig. 5. A within subjects' analysis of variance of the data for Experiment 1 showed a significant effect for treatments ($F = 37.10$, $p < .005$). There was no significant change over days within treatments; nor was there an interaction between the various treatments and days of testing within each treatment.

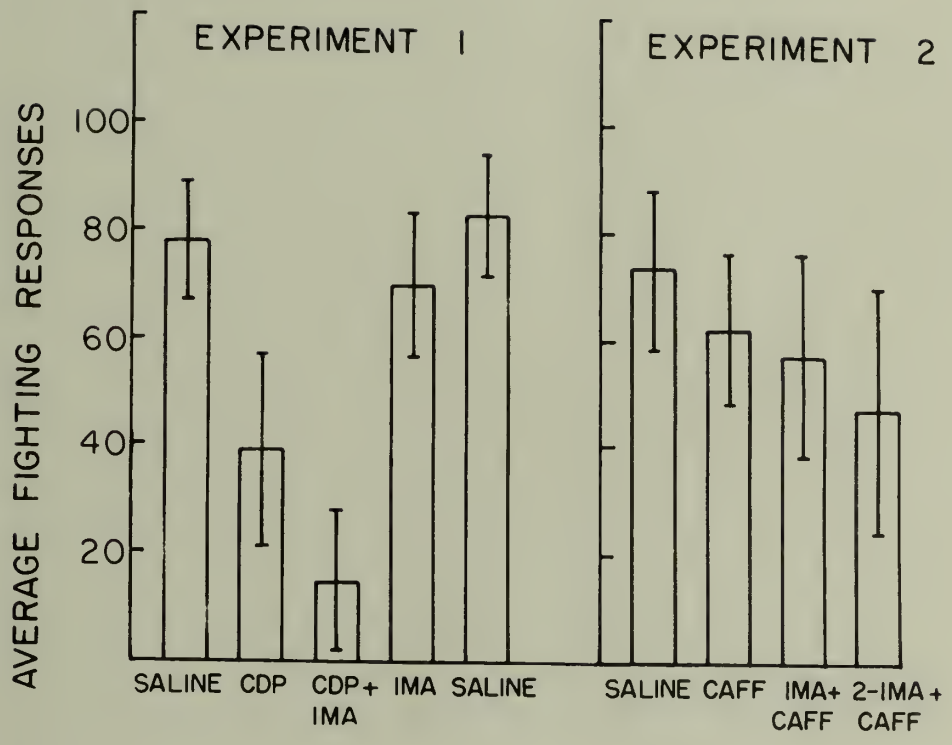

Figure 5. Comparison of the number of fighting responses under saline and drug conditions. Vertical bars represent the standard deviation.

A Newman Keul's test of contrasts for group 1 showed that the number of fighting responses after IMA alone was not different from the number of fighting responses after either of the saline pretreatments. After the CDP treatment, the amount of fighting was significantly less than that after saline ($p < .01$). The combined treatment, CDP + IMA, similarly produced a significant reduction as compared to saline ($p < .01$) as well as compared to the CDP alone treatment ($p < .01$).

The results for group 2 are shown on the right in Fig. 5. Analysis of the data for group 2 again showed a significant effect of treatment ($F = 27.23$, $p < .01$) as well as a days effect within treatments ($F = 6.72$, $p < .05$). An analysis of daily effects showed this latter effect probably is due to an initial depression of the fighting rate each time a new caffeine treatment was administered; then the rate increased slightly and leveled off over the second and third days of each treatment. Why this effect occurred with caffeine and did not with CDP is not known.

A Newman Keul's test of contrasts for group 2 showed that caffeine significantly reduced the number of fighting responses as compared to saline days ($p < .01$). The aggression scores after caffeine and IMA were also different from saline pretreatment days ($p < .01$). However, the amount of fighting after caffeine plus IMA as compared to caffeine alone was not significantly different.

The final test with 50 mg/kg caffeine and 250 mg/kg of IMA was done in an attempt to find an effective dose of IMA to interact with caffeine. This drug combination led to a further reduction in SIA. However, prior to SIA tests these animals showed signs of catalepsy; they could be placed in awkward positions without resistance and remained so without restraint. Despite this effect their response to grid shock did not seem impaired, but because of the suspicion of catalepsy, this test was not included in the statistical analysis.

Discussion

The significant findings of this investigation were that the phosphodiesterase stimulator, IMA, had no effect by itself yet it potentiated the effect of CDP on SIA rather than antagonizing it. Also, IMA had virtually no effect on caffeine-induced reduction of SIA. Therefore, it would seem that the anti-SIA properties of CDP and caffeine are not related to their ability to inhibit phosphodiesterase.

The fact that IMA itself produced no change in the level of SIA but potentiated the CDP-induced reduction of SIA suggests that there may be a competition at some point in the metabolic degradation of these substances. That is, IMA may compete for the enzymes that metabolize CDP, thereby raising plasma levels of the parent drug or its many active metabolites and potentiating their

effects. The measurement of plasma levels and rate of degradation of CDP in the presence of IMA should answer this question. However, this hypothesis is weakened somewhat by the fact that 15 mg/kg CDP yielded a level of suppression of SIA that could not be increased by doubling the dose (Quenzer et al., 1974), which presumably would also raise plasma levels. Consequently any increase in plasma levels of CDP would not be expected to be more effective.

With respect to caffeine, the fact that IMA does not potentiate the anti-aggression effect of this drug suggests that the metabolic pathways for IMA and caffeine are different. This hypothesis is still consistent with the finding that caffeine and CDP have additive effects in suppressing SIA.

Finally, the possibility must be considered that the in vitro findings of phosphodiesterase stimulation by IMA (Roberts and Simonsen, 1970), may not be applicable to in vivo conditions.

General Discussion

Considering the results of Experiments 1 and 2 together, it is clear that we have not yet elucidated the neurochemical mechanism by which CDP and caffeine reduce shock-induced fighting in rats. Our previous work using repeated administration of CDP and testing, suggested that the decrease in SIA is not related to the reduction in NE turnover. Failure to antagonize the CDP-induced reduction in SIA with either 5HTP or α-methyl tryptamine casts considerable doubt on the hypothesis that the reduced 5HT turnover after CDP is

the neurochemical event responsible for the attenuation of shock-induced fighting. Finally, Experiment 2 raises a considerable question about the importance of phosphodiesterase inhibition as a mechanism responsible for the reduction of shock-induced fighting by CDP.

No current hypothesis of neurochemical action of CDP presents a plausible explanation for the reduction of SIA by the drug. However, Lidbrink and Farnebo (1973) suggest that the reduced amine turnover induced by CDP may be due to a reduction in the flow of nerve impulses, rather than altering synaptic activities such as uptake, retention or stimulation-induced release of neurotransmitter. Also, very recently it has been found that diazepam, in a dose dependent manner, inhibited the post-tetanic potentiation of the excitatory post-synaptic potential in sympathetic ganglia (Suria, 1974). Further evidence indicated that the action is mediated by prostaglandins of the E series (PGE_1 and PGE_2). Thus, it is conceivable that the reduction in SIA induced by CDP may be due to altered nerve impulse traffic or prostaglandin-mediated changes in membrane potentials or ion flow, and therefore the manipulation of various neurotransmitters would not effectively antagonize the drug's action.

PART III

A third reliable method of inducing aggressive behavior in rats in the laboratory is to bilaterally lesion the septal area of the brain. The increased reactivity or hyperirritability reaction toward inanimate stimuli, auditory or tactual stimulation is well known (Brady and Nauta, 1953). A number of experimenters have tried to alter the aggressive behavior induced by septal lesioning with various pharmacologic agents including various depressant drugs (Stark and Henderson, 1966), anti-depressants (Horovitz et al., 1963) as well as minor tranquilizers such as CDP (Christmas and Maxwell, 1969). However, their results have been varied and contradictory. For instance, in at least one case, CDP was found to selectively reduce septal lesion-induced rage (SIR) (Christmas and Maxwell, 1969) while others report a decrease in SIR only at doses that produce ataxia (Sofia, 1969; Horovitz et al., 1963). The discrepancies in these results can be explained by some of the factors discussed earlier. However, the significance of behavioral depression and the various measures used by these experimenters to determine behavioral depression once again seems to be the most important factor in their different results.

An effective way to determine the importance of behavioral depression as well as to compare the role of NE and 5HT turnover in reducing septal rage is to use again chronic administration of CDP

and repeated testing so that tolerance to the adrenergically mediated behavioral depressant effect would develop. If after such prolonged treatment, the same dose of CDP on the aggression test day (post-lesion) still reduced septal rage, it would indicate that the attenuation of SIR by CDP was not due to behavioral depression but more likely would be due to the reduced turnover of 5HT which persists throughout the chronic administration.

Method

The subjects were 27 male, albino Sprague-Dawley rats weighing between 360-450 grams at the start of the experiment. The animals were maintained on a 12 hour alternating light-dark cycle with light from 4:00 A.M. to 4:00 P.M. throughout the experiment. Ten days prior to surgery the animals were randomly divided into four groups. Groups 1 and 2 were administered saline injections (0.9% saline, 1 cc/kg) each day for 10 days while groups 3 and 4 were given 25 mg/kg CDP i.p. for the same period of time. On the 11th day the animals were given septal lesions, on the 12th day they were allowed to recover in their home cages. On the 13th day irritability scoring and drug tests began and continued for 3 days.

On the drug test days, each rat served as its own control and the series of tests were applied immediately before, ½ hour after and 1 hour after the appropriate drug treatment. Groups 1 and 4 received saline injections at this time, while groups 2 and 3 were

administered 25 mg/kg CDP i.p. The procedures are shown in Table 1. Irritability was evaluated by a series of tests consisting of (1) response to nose poke, (2) response to flank poke, (3) resistance to capture, and (4) amount of vocalization upon capture. Scoring was based on a scale of 0 for no reaction to 3 for a very violent reaction. Rats scoring less than 8 out of a possible 12 were not used in the drug tests.

After 3 days of testing the rats were sacrificed with an overdose of Diabutal and perfused with saline and 10% formalin. The brains were removed and fixed in Formalin. Three of the brains were embedded in sucrose-formalin. Frozen serial sections were then made in a frontal plane at 80μ. Every second section was mounted and included the full extent of the lesion. The tissue was stained with cresyl violet. Because of the delicate nature of the tissue the remaining brains were embedded in gelatin and frozen sections were made. The lesion size was determined visually by two independent observers.

Results and Discussion

Out of 29 rats, 11 had almost total destruction of the medial and lateral septal nuclei. Ten more had over 80% destruction, while two showed only 50-75% destruction. Two of the 29 rats had less than 50% destruction but these had been eliminated from the analysis very early because of their low rage scores. For 4 rats the damage

GROUP	PRETREATMENT DAYS 1-10	LESION DAY 11	DRUG TESTS DAYS 12-15
1	saline	lesion	saline
2	saline	lesion	CDP
3	CDP	lesion	CDP
4	CDP	lesion	saline

Table 1. Summary of procedures for pretreatment, septal lesioning and post-lesioning drug test days for four groups.

was indeterminate since the rats died and the brains had not been perfused. Consequently the tissue was very fragile producing poor histology.

There were a number of additional problems in determining the extent of the lesion. First of all, since the objective of making lesions was to destroy as much of the septum as possible, there was the danger that a number of adjoining structures were also destroyed. These structures might include the anterior commissure, nucleus acumbens septi, bed nucleus of the anterior commissure, corpus callosum and hippocampal commissure. However, since the septal nuclei are bounded laterally by the lateral ventricles, dorsally by the corpus callosum, and ventrally by the anterior commissure, there is a natural boundary which seems to keep the lesion within bounds.

Second, since there was a very short interval between lesioning and necropsy, gliosis was not well advanced, again making lesion determinants difficult especially in ragged tissue. It was not known whether the missing tissue was effectively lesioned or merely torn away and lost during tissue preparation.

A within and between subjects analysis of variance was used to examine the groups that were administered saline on days 13-15 (groups 1 and 4) as compared to those which received CDP on those days (groups 2 and 3). The results are illustrated in Fig. 6. A significant main effect for Groups indicated that treatment

with CDP reliably reduced SIR ($F= 27.04$, $p < .001$).

A significant Trials effect ($F= 175.67$, $p < .001$) and a Groups x Trials interaction ($F= 58.67$, $p < .001$) demonstrate that not only were pre-drug scores different from post-drug scores for the groups, but also that the post-drug reduction in rage for the CDP-treated groups were different from the saline-treated groups. Fig. 6 clearly shows that rage scores after receiving CDP are significantly lower than pre-drug scores, and those of saline controls.

The significant Days main effect ($F= 12.59$, $p < .001$) in combination with a lack of a signficant Groups x Days interaction suggests that there was some reduction in rage over days of testing but it was not a function of group. This gradual disappearance of the rage reaction in septal rats has been well documented (Brady and Nauta, 1955) and has been shown to be a function of time as well as amount of handling (Gotsick and Marshall, 1972). No Groups x Days x Trials interaction was detected.

A Student-t test comparing the first post-lesion pre-drug rage score of groups 3 and 4, who were treated with CDP before lesioning, with groups 1 and 2 who were treated with saline before lesioning showed no significant difference between the two. This comparison was considered of importance because it has been recently shown that certain presurgical drug treatments modify the recovery period that follows neurological damage including that of septal

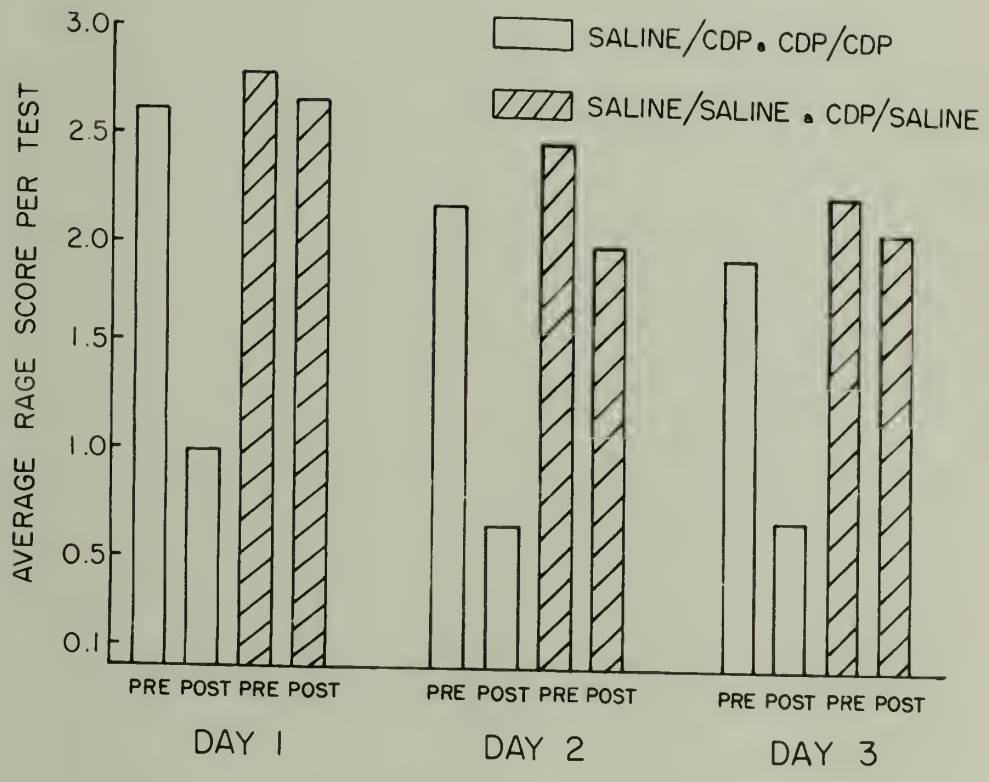

Figure 6. Comparisons between CDP and saline effects on septal rage. Pre = preinjection rage test, Post = postinjection rage test.

lesions (Harrell and Balagura, 1974). It is interesting that in the case of CDP treatment preceding septal lesioning, no modification of the lesion-induced rage could be found.

Now that it was demonstrated that CDP does significantly reduce SIR, a second analysis of variance compared the results of groups 2 and 3 which were treated with CDP after lesioning but which were treated differentially with CDP or saline before lesioning. The results are illustrated in Fig. 7. There was no significant difference between these groups indicating that the difference in pre-lesion drug treatment did not have an effect on the CDP-induced post-lesion reduction of rage scores.

There was a significant Trials effect ($F= 159.83$, $p < .001$) reflecting the reduction in rage scores after drug treatment, but the lack of a Groups x Trials interaction suggests that the rage is diminished by the drug treatment in the same way regardless of pre-lesion drug treatment. From this it can be concluded that CDP is effective in reducing SIR in rats even after tolerance to the behavioral depressant effect has developed. Thus, behavioral depression is not likely to be a significant factor in the suppression of septal rage.

The significant Days main effect for Groups 2 and 3 indicated a change in rage over days of testing ($F= 16.93$, $p < .001$). Since the Groups x Days interaction was insignificant, it can be assumed that the groups decreased in rage over the three days of testing

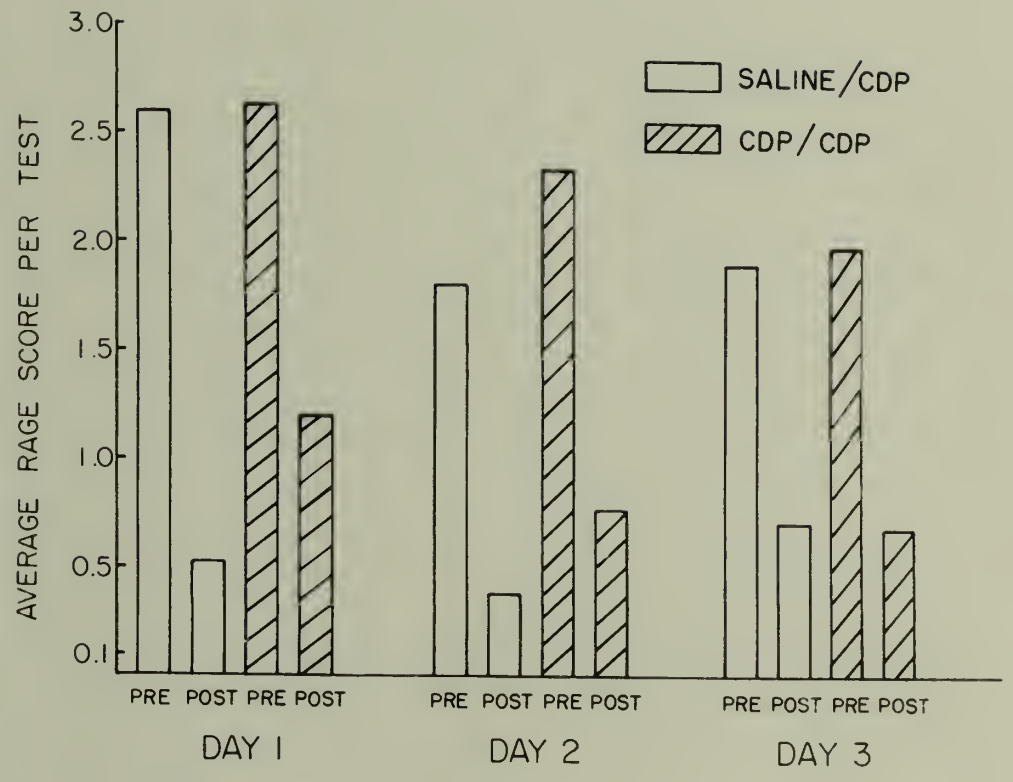

Figure 7. Comparisons between effects of CDP and saline pretreatment on CDP-induced suppression of septal rage. Pre = predrug rage test, Post = postdrug rage test.

in a similar way. No interaction between Groups x Days x Trials was detected indicating that the differences in pre- and post-drug rage scores for each group changed similarly over the three days of testing.

It is interesting to note in Fig. 7 that while the post-drug rage scores of the saline/CDP animals decline on the second day, as compared to the first, on the third day there is a slight return toward levels comparable to day 1. The CDP/CDP animals, however, show a more consistant trend toward lower rage scores over each of the three days of testing. Even though these differences were statistically insignificant they occur in an expected direction. The general downward trend in rage scores in the CDP/CDP animals probably is a reflection of the typical habituation that septal rats show to rage provoking stimuli (Brady and Nauta, 1955). In the case of the saline/CDP animals the increase in septal rage with repeated testing might be expected to occur as tolerance to the depressive effect of CDP developed. Meanwhile, this increase in rage would be antagonized by the habituation to the noxious stimuli. The figure suggests that by the third day these two effects canceled each other, leading to virtually identical post-drug rage scores for the CDP/CDP and saline/CDP groups.

An additional point to be considered is the neurochemical basis for the attenuation of SIR by CDP. Although the septal syndrome is well known, the neurochemical correlates of SIR

have not yet been determined. It has been shown that septal lesions significantly decrease whole brain levels of 5HT while causing an insignificant decrease in whole brain NE levels (Heller, Harvey and Moore, 1962; Lints and Harvey, 1969). However, Dominguez and Longo (1970) found that depletion of 5HT with pCPA produced an immediate taming of septal animals. Harrell and Balagura (1974) found that serotonergic depletion induced by pCPA administration for 5 days before lesioning, also reduced the post-lesion rage response to various stimuli. Finally our CDP-induced attenuation of SIR after chronic treatment with CDP indicates that reduced adrenergic activity is probably not responsible for the reduction in rage and suggests that the persistent reduction in 5HT turnover may be the effect responsible for the behavioral change. In each case, however, it is difficult to explain how serotonergic depletion or inactivity could act in suppressing the rage when studies have shown that 5HT reduction is not correlated with septal irritability (Heller, Harvey and Moore, 1962).

However, one must also be aware of the fact that measures of whole brain 5HT do not reflect regional activity. Further, the lesion might not only differentially alter serotonergic activity but the drugs that were tested might also interact in a selective manner at various serotonergic centers. Evidence for the latter effect has recently been presented. Harvey and Gal (1974) found

that pCPA causes a depletion of tryptophan hydroxylase, the enzyme that converts tryptophan to 5HTP, but this effect was found in all areas of the telencephalon except the septum.

Finally, it is interesting to note that CDP has a somewhat paradoxical effect in terms of septal lesion-induced behaviors. Feldman et al. (1973) have found that CDP enhances the disinhibitory effects of septal lesions in rats in an appraoch-avoidance conflict as measured by reduced response latency; while in contrast, the present experiment has shown an attenuation of septal hyperirritability by CDP. In regards to the anti-aggression effect, it is possible that the effect is mediated via the amygdala since intracranial administration of diazepam in the amygdaloid complex has been reported to eliminate the aggression induced by hypothalamic carbachol injection (Nagy and Desci, 1973). It is quite clear that much more research on the neurochemical correlates of septal rage is necessary. It also appears that the regional specificity of action of CDP within the central nervous system needs greater clarification with respect to its behavioral correlates.

Summary

In summary it can be said that three major conributions have been made by this research. First, CDP clearly has differential effects on several aggressive behaviors. Our experiments have lent support to the hypothesis that "aggression" is not a global concept but consists of a variety of different behaviors with distinct physiological, anatomical and neurochemical substrates (Moyer, 1968).

Second, the paradigm employed has, for the first time, provided a means of determining the contribution of behavioral depression to the reduction of aggressive behaviors. This is significant because earlier discrepancies in the literature stemmed from inconsistant results in determining locomotor impairment because diverse measures of ataxia that were completely unrelated to the aggression paradigm were used. It is clear that adrenergically mediated behavioral depression is instrumental in the CDP-induced suppression of muricide in rats but not a significant factor in the suppression of shock-induced aggression or septal rage.

Finally, an examination of the possible role of serotonin or cyclic nucleotides in the reduction of SIA by CDP was found to be indeterminate by manipulating the levels of serotonin and cAMP. It suggests the possibility that these agents are not cental to

the anti-aggression properties of CDP. However, it is to be noted that we have deliberately refrained from assuming a direct relationship between the anti-anxiety or disinhibitory effects of CDP and the reduction of SIA. Since manipulating 5HT and cAMP was ineffective, such a distinction seems warranted. Thus there is the possibility that specific anti-aggression properties independent of the other effects exist and can be more completely specified by further research.

REFERENCES

Beer, B., M. Chasin, D. Clody, J. Vogel, and Z. Horovitz. Cyclic adenosine monophosphate phosphodiesterase in brain: Effect on anxiety. Sci. 176: 428-430 (1972).

Beer, B., personal communication (1974).

Brady, J. and W. Nauta. Subcortical mechanisms in emotional behavior: Affective changes following septal forebrain lesions in the albino rat. J. Comp. Physiol. Psychol. 46: 339-346 (1953).

Brady, J. and W. Nauta. Subcortical mechanisms in emotional behavior: The duration of affective changes following septal and habenular lesions in the albino rat. J. Comp. Physiol. Psych. 48: 412-420 (1955).

Chase, T., R. Katz, and I. Kopin. Effect of diazepam on fate of intracisternally injected serotonin-C^{14}. Neuropharmacol. 9: 103-108 (1970).

Christmas, A. and D. Maxwell. A comparison of the effects of some benzodiazepines and other drugs on aggressive and exploratory behavior in mice and rats. Neuropharmacol. 9: 17-29 (1970).

Cole, H. and H. Wolf. The effects of some psychotropic drugs on conditioned avoidance and aggressive behaviors. Psychopharmacologia 8: 389 (1966).

Connor, R., J. Stolk, J. Barchas, W. Dement and S. Levine. The effect of pCPA on shock-induced fighting behavior in rats. Phsyiol. Beh. 5: 1221-1224 (1970).

Cook, L. and R. Kelleher. Drug effects on the behavior of animals. Ann. N.Y. Acad. Sci. 96: 315-335 (1962).

Corrodi, H., K. Fuxe, P. Lidbrink, and I. Olson. Minor tranquilizers, stress, and central catecholamine neurons. Bran. Res. 29: 2-16 (1971).

Crowley, H. C. Dalton, W. Schallek, and H. Sheppard. Benzodiazepine inhibition of cyclic nucleotide phosphodiesterase in the central nervous system (CNS). Fed. Proc. 33: 493 (1974).

Dews, P.B. Psychopharmacology. In: Experimental Foundations of Clinical Psychology, A.J. Bachrach (ed.) New York: Basic Books, pp. 423-441 (1962).

DiChiara, G., R. Camba, and P. Spano. Evidence for inhibition by brain serotonin of mouse killing behavior in rats. Nature 233: 272-273 (1971).

DiMascio, A. The effects of benzodiazepines on aggression: Reduced or increased? In: The Benzodiazepines, S. Garattini, E. Mussini, and L.O. Randall (eds.). New York: Raven Press, pp. 433-440 (1973).

DiMascio, A., R. Shader and J. Harmatz. Psychotropic drugs and induced hostility. Psychosomatics 10: 47-50 (1969).

Dominguez, M. and V. Longo. Effects of pCPA, α-methyl tyrosine and of other indole- and catechol-amine depletors on the hyperirritability syndrome of septal rats. Physiol. Beh. 5: 607-610 (1970).

Eichelman, B., N. Thoa, and K. Ng. Facilitated aggression in the rat following 6-hydroxydopamine administration. Physiol. Beh. 8: 1-3 (1972a).

Espino, Conchita. The role of brain amines in the conditioned avoidance response: Chlordiazepoxide facilitation of 2-way avoidance, Unpublished Master's Thesis, Univer. of Mass., 1974.

Feldman, Robert S., The mechanism of fixation prevention and "dissociation" learning with CDP. Psychopharmacol. 12: 384-399 (1968).

Feldman, R.S. and K.F. Green. Antecedents to behavior fixations. Psychol. Rev. 74: 250-271 (1967).

Feldman, R.S., B.R. Kaada, and T. Langfeldt. Effects of septal lesions and chlordiazepoxide (Librium) on avoidance behavior in rats. Pharmacol. Biochem. Beh. 1: 379-387 (1973).

Fox, K. and R. Snyder. Effect of sustained low doses of diazepam on aggression and mortality in grouped male mice. J. Comp. Physiol. Psychol. 69: 633-666 (1969).

Fox, K., J. Tockosh, and A. Wilcox. Increased aggression among grouped male mice fed chlordizepoxide. Eur. J. Pharmacol. 11: 119-121 (1970).

Geller, I., J. Kulak, and J. Seifter. The effects of CDP and chlorpromazine on a punishment discrimination. Psychopharmacologia. 3: 374-385 (1962).

Gleser, G., L. Gottschalk, R. Fox and W. Lippert. Immediate changes in affect with CDP. Archives of Gen. Psychiatry. 13: 291-295 (1965).

Goldberg, M., A, Manian, and D. Efron. A comparative study of certain pharmacological responses following acute and chronic administration of CDP. Life Sci. 6: 481-491 (1967).

Gotsick, J. and R. Marshall. Time course of the septal rage syndrome. Physiol. Beh. 9: 685-687 (1972).

Graeff, F. and R. Schoenfeld. Tryptaminergic mechanisms in punished and non-punished behavior. J. Pharmacol. Exper. Ther. 173: 277-283 (1970).

Grant, L., D. Coscina, S. Grossman, and D. Freedman. Muricide after serotonin depleting lesions of midbrain raphe nuclei. Pharmacol. Biochem. Beh. 1: 77-80 (1973).

Harrell, L. and S. Balagura. Septal rage: Mitigation by surgical pretreatment with pCPA. Submitted for publication, 1974.

Harvey, J. and E. Gal. Septal tryptophan-5-hydroxylase: Divergent response to raphe lesions and pCPA. Sci. 183: 869-871 (1974).

Heise, G. and E. Boff. Taming actions of chlordiazepoxide. Fed. Proc. 20: 393 (1961).

Heller, A., J. Harvey and R. Moore. A demonstration of a fall in brain serotonin following CNS lesions in the rat. Biochem. Pharmacol. 11: 859-866 (1962).

Hoogland, D., T. Miya and W. Bousquet. Metabolism and tolerance studies with chlordiazepoxide-2-^{14}C in the rat. Toxic. Appl. Pharmacol. 9: 116-123 (1966).

Horovitz, Z., A. Furgiuele, L. Brannick, and B. Craver. A new chemical structure with specific depressant effects on the amygdala and on the hyperirritability of the 'septal rat'. Nature 200: 369 (1963).

Horovitz, Z., B. Beer, D. Clody, J. Vogel and M. Chasin. Cyclic AMP and anxiety. Psychosomatics 13: 85-92 (1972).

Horovitz, Z., J. Piala, J. High, J. Burke, and R. Leaf. Effects of drugs on the mouse killing (muricide) test and its relationship to amygdaloid function. Int. J. Neuropharmacol. 5: 405-411 (1966).

Horovitz, Z., P. Ragozzino and R. Leaf. Selective block of rat mouse killing by anti-depressants. Life Sci. 4: 1909 (1965).

Jaffe, Jerome. Drug addiction and drug abuse. In: The Pharmacological Basis of Therapeutics, L. Goodman and A. Gilman (eds.) New York: The Macmillan Co. pp. 276-313 (1970).

Karli, P. Action du methaminodiazepoxide ("Librium") sur l'agressivite interspecifique rat-souris. Soc. Biologie, Paris, Comptes Rendus. 155: 625-627 (1961).

Kebabian, J. and P. Greengard. Dopamine-sensitive adenyl cyclase: Possible role in synaptic transmission. Sci. 174: 1346-1348 (1971).

Kulkarni, A.S. Muricidal block produced by 5-HTP and various drugs. Life Sci. 7: 125-128 (1968).

Lidbrink, P. and L.-O. Farnebo. Uptake and release of NA in rat cerebral cortex in vitro: No effect of benzodiazepines and barbiturates. Neuropharmacol. 12: 1087-1095 (1973).

Lints, C. and J. Harvey. Altered sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. J. Comp. Physiol. Psych. 67: 23-31 (1969).

Loiselle, R. and H. Capparell. Effects of chlorpromazine HCl and chlordiazepoxide HCl on 'instinctual' aggressive behavior in rats. Presented at EPA meeting, April, 1966.

Margules, D. and L. Stein. Increase of "anti-anxiety" activity and tolerance of behavioral depression during chronic administration of oxazepam. Psychopharmacologia 13: 74-80 (1968).

McAfee, A. and P. Greengard. Adenosine 3',5'-monophosphate: Electrophysiological evidence for a role in synaptic transmission. Sci. 178: 310-312 (1972).

Moyer, K.E. Kinds of aggression and their physiological basis. Comm. Beh. Biol. A, 2: 65-87 (1968).

Nagy, J. and L. Desci. Location of the site of the tranquilizing action of diazepam by intralimbic application. Neuropharmacol. 12: 757-768 (1973).

Podobnikar, I. G. Implementation of psychotherapy by Librium in a pioneering rural-industrial psychiatric practice. Psychosomatics 12: 205-209 (1971).

Quenzer, L., R. Feldman, and J. Moore. Toward a mechanism of anti-aggression effects of chlordiazepoxide in rats. Psychopharmacologia. 34: 81-94 (1974).

Rall, T. and A. Gilman. The role of cyclic AMP in the nervous system. In: Neurosciences Research Symposium Summaries. vol. 5. Schmitt, Adelman, Melnechuk and Worden (eds.), Cambridge: The MIT Press (1971).

Randall, L.O. Pharmacology of methaminodiazepoxide. Dis. Nerv. Sys. 21(Suppl.): 7-10 (1960).

Randall, L.O. Pharmacology of chlordiazepoxide (Librium). Dis. Nerv. Sys. 22 (Suppl.): 7-15 (1961).

Ritchie, J.M. Central nervous system stimulants II. The xanthines. In: The Pharmacological Basis of Therapeutics L. Goodman and A. Gilman (eds.) New York: The Macmillan Co. pp. 354-366 (1966).

Roberts, E. and Simonsen, D. Properties of cyclic-3',5'-nucleotide phosphodiesterase of mouse brain: Effects of imidazole-4-acetic acid, chlorpromazine, cGMP and other substances. Brn. Res. 24: 91 (1970).

Robison, G., R. Butcher, and E. Sutherland. Cyclic AMP. New York: Academic Press, p. 307 (1971).

Salzman, C.,A. DiMascio, R. Shader, and J. Harmatz. Chlordiazepoxide expectation and hostility. Psychopharmacologia. 14: 38-45 (1969).

Sheard, M. The effect of p-chlorophenylalanine on behavior in rats: Relationship to brain 5HT and 5HIAA. Brain Res. 15: 524-528 (1969).

Sofia, R.D. Effects of centrally active drugs on four models of experimentally-induced aggression in rodents. Life Sci. 8: 705-716 (1969).

Stark, P. and J. Henderson. Differentiation of classes of neurosedatives using rats with septal lesions. Int. J. Neuropharmacol. 5: 385-389 (1966).

Stein, L., C. Wise and B. Berger. Antianxiety action of benzodiazepines: Decrease in activity of serotonin neurons in the punishment system. In: The Benzodiazepines, S. Garattini, E. Mussini and L. Randall (eds.). New York: Raven Press, pp. 229-325 (1973).

Suria, Amin. Diazepam and post-tetanic potentiation: Possible role of prostaglandins. Fed. Proc. 33: 590 (1974).

Taylor, K. and R. Laverty. The metabolism of tritiated dopamine in regions of rat brain in vivo: The significance of the neutral metabolites of catechlamine. J. Neurochem. 6: 1367-1376 (1969).

Tedeschi, R., D. Tedeschi, A. Mucha, L. Cook, P. Mattis and E. Fellows. Effects of various centrally acting drugs on fighting behavior of mice. J. Pharmacol. Exper. Ther. 125: 28-34 (1959).

Tenen, S.S. The effects of pCPA, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. Psychopharmacologia 10: 204-219 (1967).

Thoa, N., B. Eichelman and L. Ng. Shock-induced aggression: Effects of 6-hydroxydopamine and other pharmacological agents. Brn. Res. 43: 467-475 (1972c).

Thoa, N., B. Eichelman, J. Richardson and D. Jacobowitz. 6-hydroxydopa depletion of brain NE and the facilitation of aggressive behavior. Sci. 178: 75-77 (1972b).

Ulrich, R. and N. Azrin. Reflexive fighting in response to aversive stimulation. J. Exper. Anal. Beh. 5: 511-520 (1962).

Valzelli, L. Activity of benzodiazepines on aggressive behavior in rats and mice. In: The Benzodiazepines. S. Garattini, E. Mussini, and L. Randall (eds.), New York: Raven Press, pp. 405-417 (1973).

Wnek, D., P. Gay, and R. Leaf. Effects of CDP and diazepam on mouse killing by rats. Fed. Proc. 33: 465 (1974).