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The mechanism of suppression of shock-induced fighting by chlordiazepoxide.

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THE MECHANISM OF SUPPRESSION OF SHOCK-INDUCED FIGHTING BY CHLORDIAZEPOXIDE

A Thesis Presented

by

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THE MECHANISM OF SUPPRESSION OF SHOCK-INDUCED FIGHTING BY CHLORDIAZEPOXIDE

LINDA F. QUENZER

Chlordiazepoxide (CDP), one of a number of drugs in the class known as the benzodiazepines, is known to have central muscle relaxant effects, anticonvulsant effects against electroconvulsive shock and chemical agents, depressive effects on the duration of electrical afterdischarges in the limbic system, and attenuation of fear in avoidance and conflict situations. It also has been found to have a suppressive effect on a number of aggressive behavioral responses. CDP was found to depress mouse killing in rats (Loiselle and Caparell, 1966), to have taming effects on vicious cynomolgus monkeys (Heise and Boff, 1961), to produce calming of "septal rats" (Horovitz, Furgiuele, Brannick, Burke and Craver, 1963); to reduce shock-induced fighting of mice (Stille, 1962), and to lessen fighting of previously isolated mice (Cole and Wolf, 1966). Reduction of aggressive behavior, then, would seem to be quite evident.

However, a problem arises when one asks whether suppression of aggressive behavior is due to an anti-aggressive pharmacologic property of CDP or a secondary effect due to general behavior depression. Evidence supporting the behavioral depressive effect of CDP as the primary cause of reduced aggression is presented by Horovitz, Raggozzino and Leaf (1965) and Horovitz, Piala, High, Burke, and Leaf (1966).

It was found that the dose of CDP that was effective in suppressing mouse killing was approximately six times the dose that effectively impaired rotarod[1] performance. In order to provide a fair measure of nonspecific depressant action, CDP was also evaluated for its effects on a conditioned avoidance response (pole climbing to avoid shock) and was found again to have a depressant effect on the avoidance response at a dose lower than that at which mouse killing was significantly reduced.

A study by Gray, Osterberg and Rauh (1961) as cited in Randall and Schallak (1968), supports the finding of Horovitz and his coworkers (1965, 1966). They found that CDP did not reduce isolation-induced fighting behavior of mice except at doses more potent than that which caused ataxia.

Opposite results were reported in the previously cited study of Loiselle and Capparell (1966) who found CDP significantly decreased mouse killing by rats. They found that significantly fewer CDP treated rats killed mice than rats treated with chlorpromazine; and a greater percentage of rats in the no-drug control group killed mice than the chlorpromazine treated group. In order to determine the extent of general behavioral depression, general activity level was measured for each of the subjects in both an activity wheel and a pivital "jiggle box". The group receiving CDP was

1. Rotarod is a slowly rotating rod upon which the animals attempt to maintain balance.

significantly more active than the chlorpromazine group, while not differing significantly from the controls. It was concluded, therefore, that CDP decreased mouse killing behavior and that the anti-aggressive effect of the drug could not be attributed to general depression of behavior.

Similar results were reported by Heise and Boff (1961) who devised a check list of behaviors in order to differentiate between aggressive behaviors and more general activity. Once the motor activity, ataxia, and response to auditory and visual stimuli were examined, a ratio of the amount of activity was determined. "Tame" animals were designated as those whose aggression-activity ratio was 0.5 or less, meaning that activity was at least twice as frequent as aggressive behaviors. They found that "tame" animals were not significantly less active. Behaviorally they were not sluggish or ataxic, but did not attack when handled or poked. "Taming" was confirmed for animals receiving doses of 5 mg/kg p.o. chlordiazepoxide.

Stille (1962) found CDP blocked shock-induced fighting in mice at a dose which did not impair the righting reflex. It took approximately eight times the dose that suppressed shock-induced fighting between paired mice to cause loss of righting reflexes. The ratio between anti-fighting effect and lethal dose was about 1:20. Thus, careful consideration of the available data still leaves unanswered the question of whether suppression of aggressive behavior is due to specific pharmacologic anti-aggressive properties of CDP or is due to general behavioral depression.

The determination of the role of behavioral depression as it relates to other drug effects has proved a formidable task primarily because any behavioral test is confounded by drug effects on motor coordination, response threshold to stimuli of different motivational significance, and so on. Perhaps the ideal method of determining the extent of behavioral depression would be to assess the depression level in terms of a neural correlate. Schallek and Kuehn (1965) demonstrated a significant increase in the frequency of the spontaneous EEG in cats after a 10 mg/kg dose of CDP, but they did not find a significant change in the threshold for behavioral arousal with stimulation of the reticular formation after administering CDP. However, when the more potent congener, diazapam, was used the arousal threshold was elevated. These results suggested to the authors, "that depression of the cephalic outflow of the reticular formation plays only a minor role in the action of these drugs." The correlation of the EEG recordings and threshold of arousal to the more subjective evaluation of behavioral depression caused by benzodiazepines has thus not been clearly determined.

While a neural correlate has not been determined, more recently Wise, Berger, and Stein (1972) have determined a <u>neurochemical</u> correlate of the depressive effect of the benzodiazepines. Using oxazepam and the Geller-Seifter conflict test[1] with rats they separated the behavioral depressive and the anti-anxiety effects of the drug and found a

1. A leverpress produces an inescapable foot shock as well as food reward.

correlation with NE and 5HT turnover respectively in the mid-brain-hindbrain region. Moreover, the depressant action in the conflict test disappeared after six doses of oxazepam; and similarly, the reduction in the turnover rate of NE after six doses of oxazepam could no longer be detected. This work, then, seems to establish rather conclusively that tolerance to the depressive effect of the benzodiazepines occurs with repeated testing, i.e., after five or six doses.

Thus, one way of eliminating the behavioral depressive effect of CDP would be to allow drug tolerance of the effect to develop during chronic administration of the drug. Margules and Stein (1968) have already shown that the behavioral depressant action of oxazepam, a drug in the benzodiazepine class, on a VI schedule with food reinforcement, declined over repeated doses and disappeared almost completely. However, another study was done which showed that CDP's behavioral depressant effect did not diminish with repeated administration of the drug. Ralph (unpublished master's thesis) showed that tolerance of behavioral depression on spontaneous activity did not develop after repeated testing with a dose of 15 mg/kg. Upon increasing the dose to 100 mg/kg he did find some tolerance of the drug-induced depression of spontaneous activity. This result supported similar findings by Goldberg, et al (1967). Hoogland, et al (1966) suggested that tolerance in this case is a drug disposition tolerance; that is, it is due to an increased rate of CDP disappearance

from tissues and excretion of the drug from the body. This increase involves a drug-induced stimulation of hepatic microsomal enzymes found to be responsible for CDP metabolism. If this hypothesis is valid, it could then be supposed that 15 mg/kg doses were not sufficient to stimulate the production of the metabolic enzymes and therefore no tolerance developed with that dose.

A second explanation for the discrepancy between the results of Ralph, and those of Margules and Stein is that motivational levels within the testing situation may determine whether tolerance effects will be detected. For example, one could assume that exploratory behavior in the relatively barren environmental space of an activity cage, comparatively speaking, would not be very highly motivated. After a 15 mg/kg dose of CDP there was a significant suppression of exploratory behavior (Ralph). Even if some tolerance did develop it is still possible that there was no observable behavior change because the dose was sufficiently large to obscure the gradual increase in general activity. On the other hand, in Margules and Stein's procedure, because the rats were food deprived they were probably motivated enough to respond normally as soon as the depressive effects of the drug were diminished.

In the present study anti-aggression properties of CDP were sought using shock-induced fighting in rats as the behavioral test. The possible identification of the anti-aggressive

pharmacologic properties of CDP immediately posed the problem of isolating the effects of generalized behavioral depression. First, the similarities and differences in the physical features of the test for behavioral depression as compared to the physical parameters of the anti-aggression test must be considered. In the former studies that examined shock-induced fighting the commonly used methods for determining behavioral depression, such as the rotarod, inclined screen, and activity cage, seem to be of questionable relevance in evaluating the role of behavioral depression with respect to fighting. Since tolerance has been shown to occur or not to occur under different experimental conditions (Margules and Stein, 1968; Ralph, 1969) it would seem reasonable to use a tesing situation for behavioral depression that is at least similar in some respects to the shock-induced fighting paradigm.

It would appear, then, that a shock assay method which indicates the rat's response to various grid shock intensities under drug and no drug conditions would be a preferred method of testing behavioral depression in relation to the shock-induced fighting tests. Although this method appears to have greater similarity not only to the physical parameters but also to the motivational features of the shock-induced fighting situation than any of the tests previously used, there is, none the less, an important difference between them. Recently, Williams and Eichelman, (1971) have shown

that the physiological concomitants of the fear response elicited in the shock assay test and those of the aggressive response elicited in the fighting test are distinctly different. Measuring blood pressure using a tail cuff, they found blood pressure elevation during the jump-flinch test[2] while the pressure showed a significant drop during the fighting test. This difference in blood pressure change suggests that the underlying physiological responses may be significantly different. It is quite clear that although the shock assay test best approximates a number of parameters of the fighting test, direct comparison of the two is of questionable validity.

On the other hand, the jump-flinch test is useful in demonstrating the time course of tolerance of behavioral depression over several days and might also be an effective way of determining the extent of acute tolerance that may build up within a single day of testing. Also this paradigm represents a technique that possibly permits the detection of the interaction of levels of motivation and the development and detection of tolerance.

2. the jump-flinch test measures the degree to which a rat responds to increasing levels of grid shock.

EXPERIMENT Ia.

The effect of CDP on the jump-flinch test.

In this test the effect of repeated administration of CDP on responding to varying levels of foot shock was determined.

METHOD

Subjects

The subjects were 30 Charles River, Sprague-Dawley strain, male albino rats, between 90 and 120 days old at the start of the procedures.

Apparatus

The apparatus was a Grason-Stadler rat test chamber, 29 x 23 x 9 cm. The grid floor of the box was wired to a Grason-Stadler shock source, model E1064GS and scrambler unit. A Plexiglas door gave the experimenter a clear view of the animals. The experimenter sat quietly 3 to 4 feet from the window and recorded responses to the shock. The ventilating fan for the test chamber also served to mask the sound of the programming apparatus.

Procedure

The subjects were randomly divided into two groups, one receiving 15 mg/kg i.p. CDP 30 minutes before each test session and a second which received no drug. Each subject was placed in the test chamber and allowed to explore freely for a few minutes. When the rat stopped excessive exploratory behavior

the test session was started by administering a series of 19 1-second foot shocks of varying intensities (see below) and recording the rat's response. The response was evaluated on a three point scale. A zero (0) was recorded for the trials in which the animal made no noticeable response to the shock. An evaluation of "1" was made if the rat raised only one paw from the grid or noticeably flinched without raising a paw. A designation of "2" corresponded to the movement of two or more paws by the rat from the grid floor.

The footshocks varied in intensity from .025 to .475 mA. with step intervals of approximately .025 mA. between each intensity. A pilot study had shown that this range provides sufficient diversity in shock intensities to elicit the complete range of responses in most rats. The duration of the shock was 1 second. The interval between shocks was variable and was regulated by the experimenter in order to allow time for the animal to stop moving after the previous shock stimulus. However, a timing device provided a minimum intertrial interval of 5 seconds by opening the recording circuit. The nineteen shocks were administered in a fixed random order and constituted one series. Each rat received 10 consecutive series for 5 consecutive days. The rats returned to their individual living cages immediately after testing. Their daily ration of food (lab chow) was then given. Water was always available except in the test chamber.

Results

An analysis of variance for Within and Between subjects' effects was performed on the data for Experiment Ia, the jump-flinch test. The results are shown in Table 1. With respect to the main effect of Shock Level the analysis showed a highly significant effect ($p<.001$), indicating that the Ss responded differently at different shock levels. This is clearly shown in Fig. 1 which reveals a higher mean value of responses as shock intensity increases. The Groups main effect was also clearly significant ($p<.01$), indicating a difference in responding to the various levels of foot shock for the Drug and No-drug groups. A comparison of the Drug and No-drug groups shown in Fig. 1 indicates principally that at all shock intensities for both day 1 and day 5 the Drug group had a significantly lower value of average responses than the No-drug group. Fig. 1 also shows that for the No-drug group there is virtually no difference in response level for the first and fifth day over all shock levels. But, for the Drug group it is seen that the response level approaches the No-drug values as a function of shock levels. The above findings are statistically reliable as indicated by a significant Days effect ($p<.025$) and a Groups x Days interaction ($p<.025$).

Further, Fig. 1 shows for the Drug group that at low shock levels (25-125 mA) the response level was depressed and tended to remain so even through the fifth day, while at the higher shock levels by the fifth day the response level increased almost to control values. As will be seen, this

Table 1 - Analysis of variance of responses to varying foot-shock intensities for Drug and No-drug group (the jump-flinch test)

Source of Variance	df	MS	F	p<
Between Group Variance				
G (Groups--Drug vs. No-drug)	1	611.47	8.77	p<.01
S/G	26	69.69		
Within Group Variance				
D (Days)	4	17.97	3.05	p<.025
GD	4	17.88	3.04	p<.025
SD/G	104	5.91		
T (Trials)	9	1.46	1.48	N.S.
GT	9	1.05	1.01	N.S.
ST/G	234	1.01		
L (Shock levels)	18	317.77	288.9	p<.001
GL	18	1.00	.9	N.S.
SL/G	468	1.05		
DT	36	1.00	1.15	N.S.
GDT	36	1.54	1.72	p<.01
SDT/G	936	.87		
DL	72	.68	1.66	p<.005
GDL	72	.78	1.90	p<.005
SDL/G	1872	.41		
TL	162	.44	1.26	N.S.
GTL	162	.34	1.00	N.S.
STL/G	4212	.35		
DTL	648	.30	.90	N.S.
GDTL	648	.35	1.01	N.S.
SDTL/G	16848	.33		

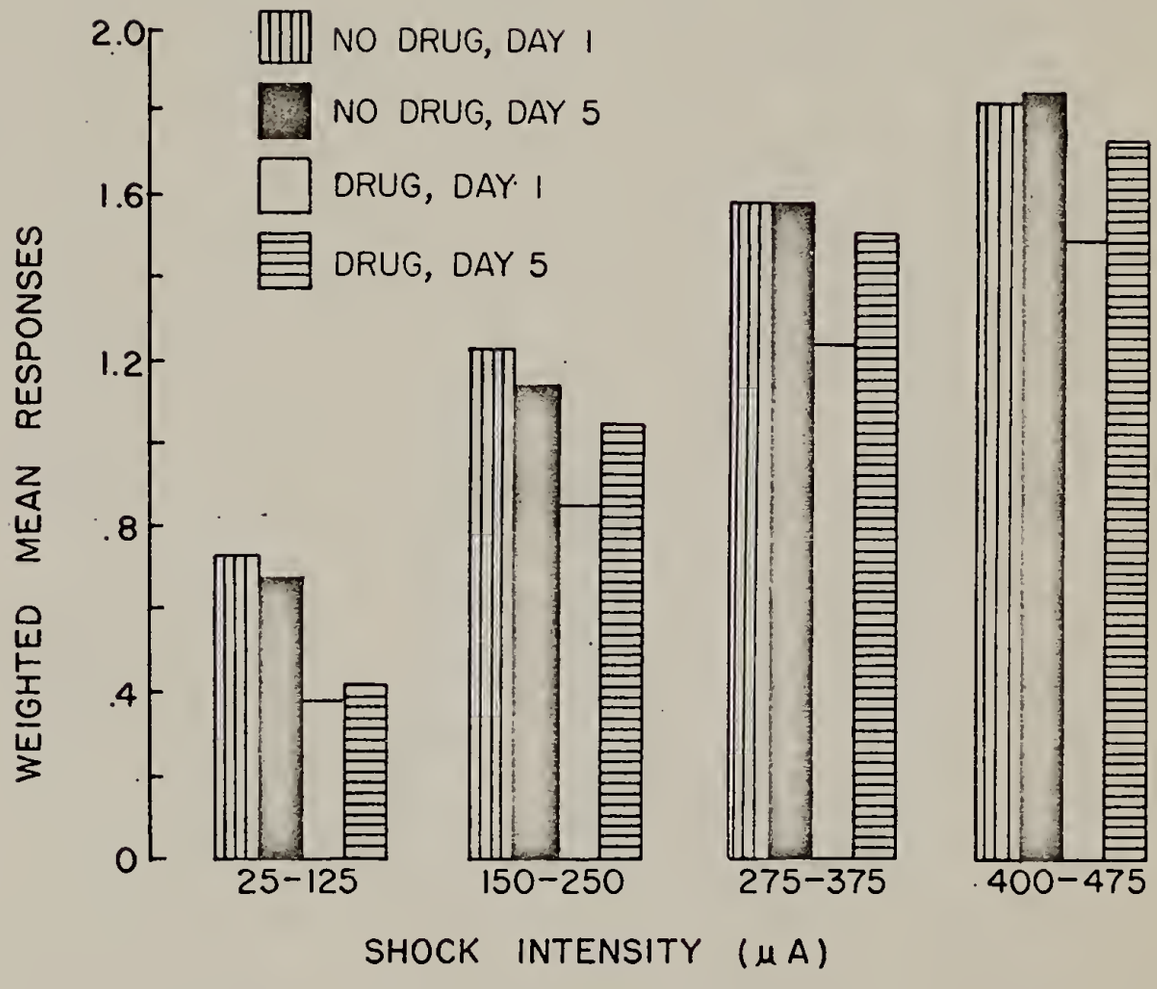

Fig. 1. Comparisons of the effect of CDP and no drug on responses to varying levels of foot shock intensity for the first and fifth days of testing.

finding is related to the detection of tolerance effects under different motivational levels.

Last, a Groups x Days x Trials effect was found to be significant ($p<.01$). It is quite possible that this interaction was a chance occurrence owing to the large number of interactions that were possible. A graphic representation of these Groups x Days x Trials variables was found to be exceedingly complex, and the only discernable trend that could be detected was that during early trials there was more variance for the drugged animals for the first few days of testing. Then as days progressed the variance over trials diminished. For the non-drugged animals, the proportion of responses over trials was more stable from day to day.

DISCUSSION

The significant findings in this study were: (a) higher values of responses for higher levels of foot shock, (b) overall lower response value for CDP treated rats, (c) virtually no change in response values over days for the control rats, and, (d) increasing response values over days for the drugged rats as a function of foot shock intensity. Thus, the data demonstrated that any attenuation of responding due to general behavioral depression that occurred with the initial dosing with CDP had almost disappeared by the fifth day of drug testing when the test utilized higher levels of foot shock. Further, it appears as though the presence of tolerance effects requires comparatively high levels of foot shock.

Having established that tolerance occurs after repeated administration of CDP, the next step was to observe the effect of tolerance to CDP-induced general behavioral depression on CDP-induced suppression of shock-induced aggression.

EXPERIMENT Ib.

The effects of CDP on shock-induced aggression.

The purpose of this experiment was to determine the effects of CDP on shock-induced aggression. There was a further attempt to isolate and eliminate the effects of general behavioral depression by repeated testing permitting tolerance to occur.

METHOD

Subjects

The subjects were 48 Charles River, Sprague-Dawley strain, male white albino rats, from 80 to 100 days old.

Apparatus

The apparatus for this experiment was the same as for experiment 1. A cumulative recorder was attached to the fighting response counter to provide a running record of the amount of fighting for each session.

Procedure

The 48 rats were randomly paired and assigned to four groups. Each group received 10 consecutive daily tests followed by one day of rest and then ten additional test days. Group 1 (No Drug) received no drug. Ten days of shock-induced

aggression testing was followed by one rest day and then ten consecutive days of shock-induced aggression testing still without drug.

Group 2 (Drug before) received 15 mg/kg i.p. 30 minutes before each of the shock-induced fighting tests on the first 10 days. After one day of rest, the rats had 10 consecutive days of shock-induced fighting without receiving drug.

The third group (Drug - no test) received 15 mg/kg CDP i.p. 30 minutes before each test session during which the animals were placed in the experimental chamber but received no shocks. After 10 days of drug and one rest day the animals had ten days of aggression testing but did not receive drug.

Group 4 (Drug after) was tested using the same fighting paradigm for 10 days and received 15 mg/kg CDP immediately after the completion of the shock-induced fighting test. After one day of rest, this group was subjected to the shock-induced fighting but received no drug for 10 additional days. These procedures are summarized in Table 2.

For each fighting test one pair of rats was placed in the experimental chamber and allowed to move about freely. After a few minutes the rats were subjected to repeated 2.0 mA. grid shocks with a duration of 0.5 seconds. Thirty shocks per minute for 10 minutes (300 shocks) constituted one testing session. The experimenter meanwhile recorded fighting responses by operating a handswitch. Responses designated as fighting responses were any aggressive striking or poking by either rat while it was on its rear limbs in the typical

Table 2

Procedure for Experiment 2

Group	Stage 1 10 daily tests of 300 trials each	Rest	Stage 2 10 daily tests of 300 trials each
Group 1 (No Drug)	SIA*	1 day	SIA No drug
Group 2 (Drug before)	SIA + pre-CDP	1 day	SIA No drug
Group 3 (Drug - no test)	CDP	1 day	SIA No drug
Group 4 (Drug after)	SIA + post-CDP	1 day	SIA No drug

*shock-induced aggression

fighting posture (see Ulrich and Azrin, 1962). A fighting response was not recorded if the animal made threatening gestures without making contact, or if the animals stood with their front paws in contact but were not striking. The aggressive fighting posture was generally easy to determine since it consisted of quite characteristic behavior. However, cases did occur when evaluation was more difficult such as when the animals were standing in the aggressive posture and were moving their paws but not in the typical aggressive fashion. Questionable instances such as these were infrequent and on the whole, inter-rater reliability for judging fighting responses was high (approximately 95%).

Results

The results of the shock-induced fighting test are reported in Table 3 and illustrated in Fig. 2. Using an analysis of variance, a comparison was made among the No-drug control group, the Drug group which received CDP 30 minutes before testing, and the group which received 15 mg/kg CDP immediately after testing. The results demonstrate a clearly significant difference in the amount of fighting during the first 10 days of testing ($p<.001$). When multiple comparisons were made a highly significant difference was found between the Drug-before group and the average scores of the No-drug and Drug-after groups ($p<.001$). A significant difference was also found between the No-drug and Drug-after group ($p<.001$) demonstrating that not only did pre-treatment with CDP reduce

Table 3 - Analyses of variance of the number of fighting responses for the no drug control group, drug-before group, drug-after group, and drug- no test groups for the first and second ten-day fighting tests.

Analysis of variance #1 - comparison of no-drug, drug-before, and drug-after groups for the first 10 days of fighting tests.

Source of variance	df	MS	F	p<
G (groups)	2	304226.1	34.5	p<.001
S/G	15	8827.2		
D (days)	9	4176.9	3.09	p<.005
GD	18	1570.2	1.2	N.S.
SD/G	135	1354.0		

Analysis of variance #2 - comparison of the 4 groups over test 2.

Source of variance	df	MS	F	p<
G	3	3628.1	.37	N.S.
S/G	20	9656.9		
D	9	5122.1	4.5	p<.001
GD	27	3333.7	2.9	p<.001
SD/G	180	1134.8		

Analysis of variance #3 - within subjects comparison of the drug-before group over tests 1 & 2.

Source of variance	df	MS	F	p<
T (first 10 days vs. 2nd 10 days testing)	1	285480.1	24.4	p<.005
ST	5	11685.3		
D	9	1404.7	1.02	N.S.
SD	45	1383.6		
TD	9	526.5	.5	N.S.
SPD	45	1047.3		

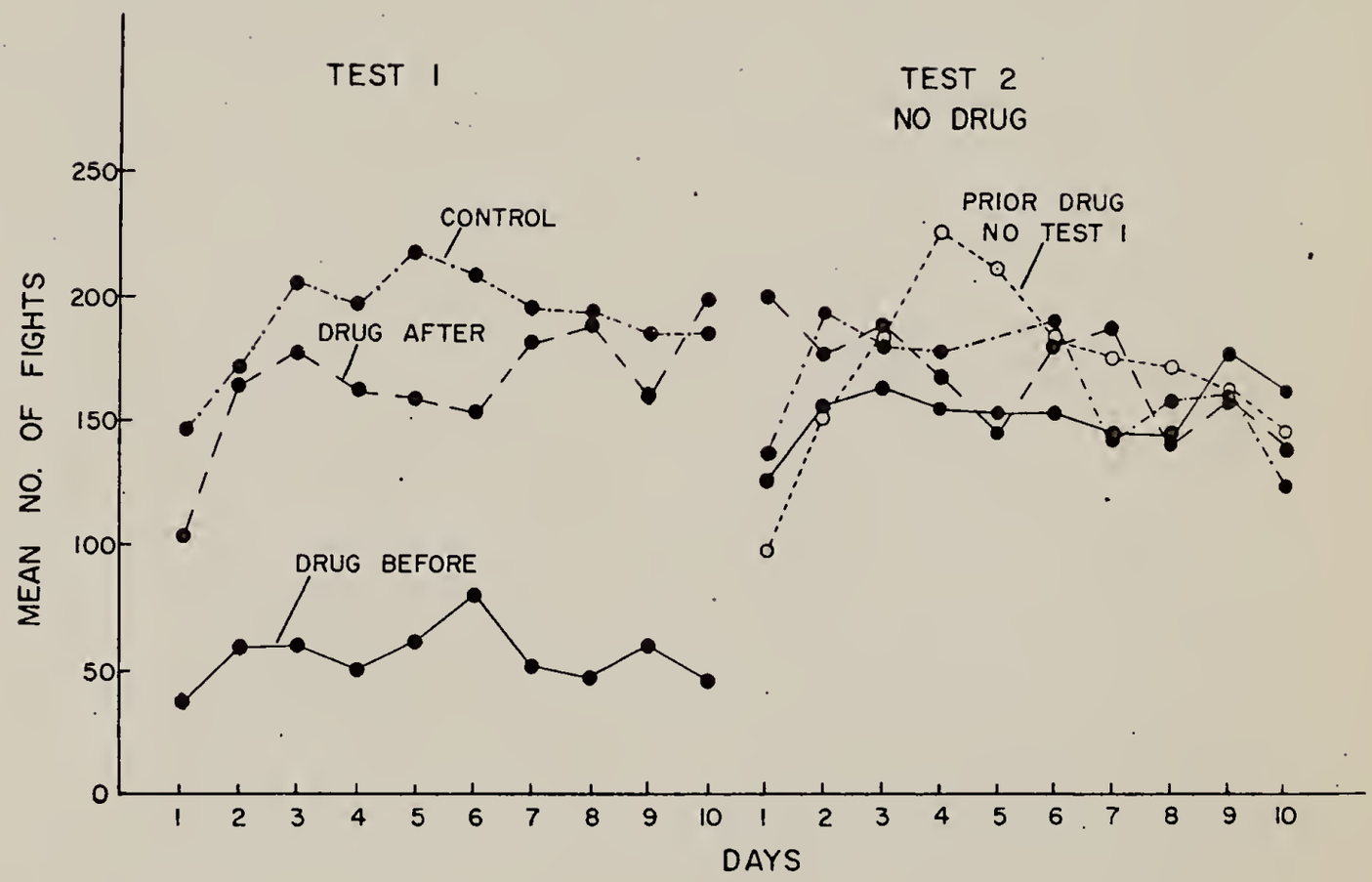

Fig. 2. The effect of CDP on shock-induced aggression. The mean number of fighting responses over days of shock-induced aggression testing for control and CDP-treated groups. One group received only drug administration for 10 days, and was tested on shock-induced aggression for the first time in Test 2.

fighting but also that treatment with CDP immediately after the fighting test significantly reduced fighting on subsequent days. A Days main effect, indicating a change in the amount of fighting over the ten days of the first fighting test, was also highly significant ($p<.001$). No significant Groups x Days interaction effect could be detected suggesting that the change over days was not a function of group.

An examination of the four groups over the second ten days of fighting tests revealed no difference in the overall average amount of fighting. The suggestion here is that despite the different treatments administered during the first ten days, when all groups were tested without drug treatment during the second ten day test all the groups showed about the same amount of fighting. However, fighting was found to change over the second ten day test and this Days effect ($p<.001$) was matched by a highly significant Groups x Days interaction ($p<.001$). These effects imply that the change in responding over days was not the same for all groups.

A Within Subject's analysis of the fighting responses for the Drug-before group over both the first and second 10-day test showed that there was a difference in their amount of fighting for the first 10 days as compared to the second ($p<.005$). This can be seen in Fig. 2. The abrupt rise in the amount of fighting after the first ten days is clearly evident.

The Drug-no test group which received drug without fighting during the first 10 day period showed an unusual peak of

fighting responses when it was tested during the second 10-day test. This suggested that receiving the 10-day drug treatment produced responding which was different from that of the other groups over the second test period. As mentioned previously an analysis of the four groups over the second 10-day test showed a significant Groups x Days interaction ($p < .001$) indicating a difference between groups over days of testing. Since the Drug-no test group received fighting tests for the first time during the second 10-day period, its fighting scores were compared with those of the control group for the first 10-day test. The curves of the two groups were virtually overlapping.

DISCUSSION

As might have been predicted the group treated with CDP before testing fought significantly less than either of the other groups tested over the first ten day period. The significant Days effect was probably due to the lower levels of fighting for the first two days of tests. It should be noted that no significant Groups x Days effect was found, implying that the three groups varied over the ten days in a similar way.

The significant increase in fighting during the second 10-day testing period after withdrawal of the drug from the Drug-before group suggests that no transfer had occurred between the drug and no-drug state. It seems reasonable that the drugged rats might have "learned" to suppress fighting in

the first 10-day test, and this would have transferred to the no-drug state. But this obviously did not occur.

The unexpected significant difference between the No-drug group and the Drug-after group during the first 10-day test suggests that CDP administered after the shock-induced fighting test had some effect on subsequent fighting tests. The possibility that the drug had an effect on fighting 24 hours after administration seemed unlikely since previous studies have shown that CDP has a half-life of four to six hours in the rat and is significantly metabolized within 24 hours after administration (Koechlin, Schwartz, Krol, and Oberhausli, 1965). An alternate possibility is that the levels of fear and anxiety remaining after the fighting test were reduced by the post-test administration of CDP and this in turn reduced the overall aversiveness of subsequent tests. This could occur because the reduction of anxiety produced by the drug injection immediately following the aggression testing became conditioned to the cues of the fighting situation. Thus, on subsequent days the fighting situation elicited less fear.

The analysis of the fighting responses of the four groups over the second ten days of testing showed a significant Days effect and a significant Groups x Days interaction. This suggests that the change over days was not uniform over groups. Looking at Fig. 2 it can be seen that the number of fighting responses for the Drug-no test group (i.e., the rats that received drug without SIA testing during the first 10 days)

rises to a sharp peak in four days of testing and falls off steadily from that point. This group alone seems to deviate from the rather constant level of fighting seen in the other groups. But the results showed that when this group was compared to the pattern of fighting for the control group during the first 10 day test, the curves were virtually identical. Therefore, it would appear that since the Drug-no fighting group was not tested until the second ten days, the change in that group over days is merely characteristic of a group run for an initial ten days.

Finally, it was generally observed that even though CDP-dosed animals did not fight, they actively jumped and scrambled about the test chamber in response to the shocks. From this viewpoint it was quite evident that behavior depression played, at best, a very minor part in the reduction of fighting by CDP.

The results of the jump-flinch test suggested that tolerance to any depressive effect of CDP in the SIA tests would have occurred after five days of SIA testing. If this reduction in depressive effect had occurred during SIA testing, a rise in the level of fighting after approximately five days of aggression testing would have been expected. However, no such change was seen during the fighting tests at any point in the ten day sequence of drug testing. On the other hand, the absence of any tolerance effect might have been due to the fact that 2.0 mA of electric current used in the fighting

tests brought about extremely vigorous behavior on the part of the subjects, so that any CDP-induced depressive effect was not great enough to be detected behaviorally. Consequently, neither the depressive effect of CDP nor the tolerance to it could be observed.

In summary, this experiment attempted to eliminate CDP-induced behavioral depression by repeated administration of the drug in the expectation that tolerance to the depressive effect would occur. The results point to the conclusions that either there was no depressive effect to begin with, or the depressive effect and tolerance to it were obscured by the motivational features of the fighting test.

The next experiments attempted to evaluate the possibility of antagonizing the depressive effect of CDP with the stimulant drug, caffeine. First, the effects of caffeine and CDP alone and in combination were evaluated in a spontaneous activity test. Then the drugs were evaluated in shock-induced fighting tests.

PART II.

The interactive effects of caffeine and CDP

It was suggested earlier that one way to eliminate a depressive effect of CDP was to administer concomitantly a CNS stimulant, such as caffeine. If behavioral depression is at least partly responsible for the CDP-induced suppression of fighting, and if the depression were counteracted by the stimulant effect of caffeine, there should be an increase

in fighting responses in pairs of rats treated with both drugs.

However, a recent study by Beer et al. (1972) showed that caffeine as well as the other methylxanthines has a very significant anti-anxiety effect. They argued that this effect is related to the interference of cyclic AMP phosphodiesterase activity in the brain. Their argument was based on the observation that when the compound SQ 20, 009[1], a potent inhibitor of phosphodiesterase, was given to rats, cats, and monkeys it released behavior that was suppressed by punishment in a way similar to that after administering CDP and diazepam, both of which also are phosphodiesterase inhibitors. Cyclic AMP phosphodiesterase is an enzyme that regulates brain levels of cyclic AMP but it is not known precisely how this latter substance is related to behavior except that it acts in an intermediate step between presynaptic exocytosis of transmitter substance and the resultant postsynaptic neural response.

While it was pointed out previously that the depression of spontaneous activity by CDP is not of much value in assessing the role of depression by CDP under different motivational states, i.e., during shock-induced fighting, it would be of value to show that while caffeine would reverse CDP-induced depression of spontaneous activity, it would potentiate CDP-induced suppression of fighting. This would be of value because

1. 1-ethyl-4 (isopropylidine-hydrazino)-1H-pyrazolo (3,-4-b) pyridine-5-carboxylic acid, ethyl ester, hydrochloride

it suggests that the anti-aggressive properties of CDP are not due to its depressant property but rather to the property it has in common with the methylxanthines which are CNS stimulants, namely inhibiting phosphodiesterase activity. This argument, however, does not rule out the possibility that some other unknown property held in common by those substances may be responsible for any anti-aggressive effects that may be found, but it seems to vitiate the argument that the sedative action of CDP is largely responsible for its anti-aggressive effects.

It would also be of value to show that while caffeine in the experiments by Beer et al. (1972) released behavior that was suppressed by punishment, it would also suppress behavior that was elicited by punishment, such as shock-induced fighting. This would demonstrate that the stimulant effect of caffeine was not primarily responsible for the release of punishment-suppressed behavior, but rather that the general motivational properties of punishment are diminished by caffeine.

To study the effect of caffeine on CDP-induced behavioral depression, two more experiments were done. The first experiment showed that caffeine reversed the CDP-induced depression of spontaneous activity, and the second demonstrated that caffeine potentiated CDP-induced suppression of shock-induced fighting.

EXPERIMENT IIa.

The effect of caffeine and CDP on spontaneous activity.

The purposed of this experiment was to determine the extent to which caffeine counteracted the CDP-induced suppression of spontaneous activity. A Within Subjects design was used. In this way the effects of caffeine and CDP administered singly as well as concomitantly were assessed for each animal.

Since both drugs are rapidly metabolized, no cumulative effect is likely from one day to the next. However, to further reduce the possibility of confounding the data with cumulative drug effects, testing was done on alternate days to make doubly sure that all drugs were completely metabolized. It will be seen that in the shock-induced fighting tests in Experiment IIb the subjects were tested on successive days. This was done because (1) pilot studies showed that rest days result in a decrease in fighting activity on the following day while daily testing results in stable fighting rates; and (2) the responding to the high shock level in fighting tests are probably not as likely to be affected by slight residual amounts of unmetabolized drugs.

METHOD

Subjects

The subjects were 15 Charles River, Sprague-Dawley strain, male white rats, approximately 100 days old when the tests started.

Apparatus

The apparatus for this experiment was a cylinder 62 cm. in diameter and 42 cm. deep with a mesh floor (Lehigh Valley activity box, model #145-03). Counters provided a record of the animal's activity as it interrupted light beams that were detected by photocells located along the perimeter of the cage. The box was kept in a quiet, darkened room away from the recording and programming apparatus.

Procedure

Each animal received one of the four treatments each day for four days. One rest day was allowed between each test day in order, as was mentioned earlier, to make doubly sure that there was complete metabolism of the drugs. The treatments were randomly ordered and assigned to give each rat all of the treatments without repetition of treatment. The four treatments were: (1) no drug, (2) 15 mg/kg CDP i.p., (3) 50 mg/kg caffeine benzoate i.p., and (4) 15 mg/kg CDP plus 50 mg/kg caffeine benzoate i.p. Thirty minutes after receiving the appropriate treatment the subject was placed in the activity box and allowed to move about freely for 20 minutes.

Results

The principal results of the spontaneous activity test are shown in Figure 3. A Wilcoxon Matched-Pairs Signed-Ranks Test was used to evaluate the data. A significant difference between the No-drug control group and CDP-treated group ($p < .01$) shows the typical depression of behavior after CDP administration.

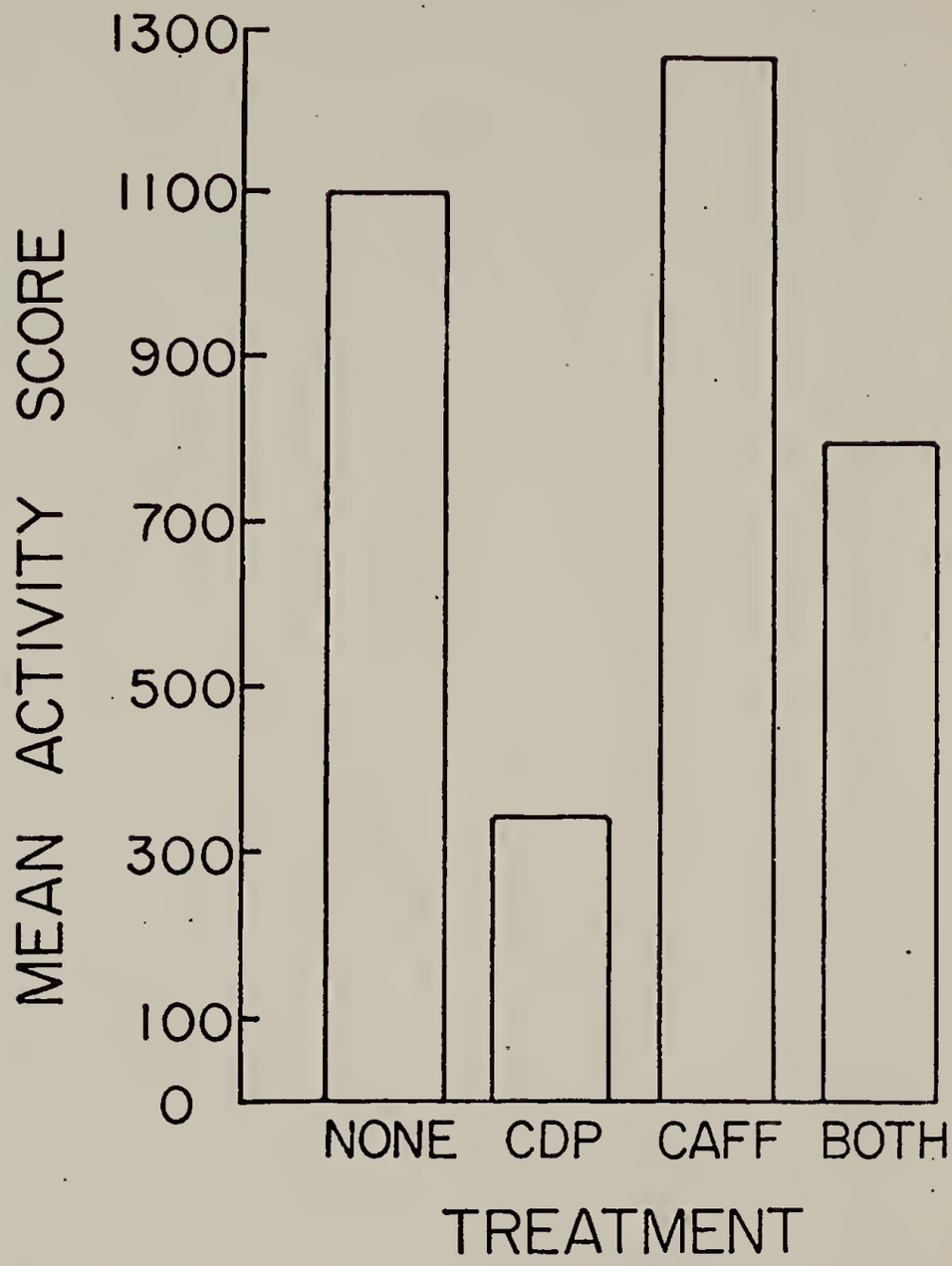

Fig. 3. Comparisons among the mean locomotor test scores on days on which Ss received no drug, CDP, caffeine, and both drugs.

Although treatment with caffeine generally increased the spontaneous activity, a Wilcoxon Test did not indicate a significant difference between the Caffeine group and the No-drug control group. However, the more sensitive correlated t-test did prove significant ($p<.05$).

Administered in combination, the two drugs appeared to have a antagonistic effect. The Wilcoxon test indicated that the activity under both drugs was greater than that of the CDP-alone group ($p<.01$) and significantly less than the Caffeine-alone group ($p<.01$). The combined drug score was also found to be significantly lower than control levels ($p<.01$), but the combined drug score was almost exactly midway between the CDP and the caffeine score. The difference between the combined drug score and the CDP and Caffeine score was 442 and 474 activity counts respectively.

DISCUSSION

The results of experiment IIa clearly demonstrate that treatment with CDP and caffeine, a CNS stimulant, have antagonistic effects upon spontaneous activity. While CDP was seen to severely depress spontaneous activity, caffeine significantly increased it. Combining 15 mg/kg CDP with 50 mg/kg caffeine did not restore activity to the no-drug control level. Rather it produced an activity score almost exactly mid-way between the scores of each drug alone. It seems probable that with manipulation of dose size a complete cancelling effect could be achieved resulting in a combined drug activity score equal to that of control treatment. While this result might be

desirable, it is not a primary concern of this study. More importantly these results clearly show that CDP and caffeine have an antagonistic effect on spontaneous activity.

EXPERIMENT IIb.

The effect of CDP and caffeine on shock-induced fighting.

The purpose of this experiment was to determine if caffeine will increase the suppression of shock-induced fighting when administered with CDP. A Within Subjects (pairs) design was used as in Experiment IIa.

METHOD

Subjects

The subjects of this experiment were 14 of 15 subjects used in Experiment IIa. Pairs were formed on the basis of the effectiveness of the drugs for each subject as measured by the subject's spontaneous activity under drug and control conditions. A measure of drug effectiveness which is called the drug effectiveness index (DEI) was constructed as follows. The difference between the no-drug control score (conditon A) and the activity score of the animal after treatment with CDP (condition B) represents the effectiveness of the CDP treatment. Similarly the efficacy of the caffeine treatment (condition C) was determined by finding the difference between the animal's no-drug control score (A) and the activity score recorded while under the influence of caffeine. The sum of these two scores (A-B) + (C-A) gives an indication of the effectiveness of CDP and caffeine for depression and stimulation of spontaneous activity respectively.

Ideally, with concurrent treatment with both drugs (condition D) caffeine should counteract the effect of CDP on spontaneous activity, making the difference between the control activity score (A) and the double drug score (D) very small. Because the sum of the first two differences tends to be large while the combined drug effect (A-D) ideally should be small, the former constitues the numerator of the index while the latter, to increase the value of the index, is placed in the denominator.

Thus:

$$(1) \quad \frac{(A-B) + (C-A)}{(A-D)}$$

Formula (1) can be simplified to:

$$(2) \quad \frac{(C-B)}{(A-D)}$$

Thus DEI = $\frac{C-B}{A-D}$

If the score for the combined drug effect (D) was greater than the control score (A), the denominator was arbitrarily set at 1.00. This was done to avoid a negative score in the denominator and thus a negative DEI. A negative DEI would be misleading because it would suggest that one or both drugs were not effective in the expected way. If, for example, caffeine more than compensated for the CDP effect the D score could be higher than the A score. Thus making A-D negative, yet the drugs would have had their expected effects. The rat with the lowest DEI was eliminated, thus providing 14 rats for 7 pairs.

Apparatus

The apparatus for this experiment was the same as for experiment Ib. Again the experimenter activated a counter and a cumulative recorder to provide a record of the number of fighting responses within each session.

Procedure

Each of the seven pairs was subjected to the fighting paradigm used in experiment Ib for three days in order to determine the baseline of fighting. Each pair then received a random sequence of four treatments, one treatment each day over four consecutive days. At the completion of the four day treatment sequence, a second four day sequence of treatments was given using a different order of drug administration. The four treatments used were: no drug, 15 mg/kg CDP i.p., 50 mg/kg caffeine benzoate i.p., and 15 mg/kg CDP plus 50 mg/kg caffeine benzoate i.p. All drug treatments were administered 30 minutes before SIA testing by another experimenter. A double-blind technique was used; the experimenter recording the fighting did not know the drug treatment used for any pair.

Results

A Within Subjects analysis of variance was done on the fighting scores for each pair of animals under the four treatments. A nonsignificant Days effect suggests that each drug had a similar effect regardless of the amount of previous testing or its relative position in the random sequence.

Despite the use of a highly conservative test of significance[1], a highly significant Treatment effect was found ($p<.005$) indicating that there was a difference in the amount of fighting after the various treatments. Further analysis of the Treatments effect using the Newman-Keul's test (Winer, 1962) revealed that the CDP treatment (B), the caffeine treatment (C), and the combined drug treatment (D) were each found to be different from the No-drug controls ($p<.01$). The results of the Newman-Keul's test are shown in Table 4, and Fig. 4 clearly illustrates these differences. The figure shows, as well, the difference between the effects of combined drug treatment with the effects of CDP and caffeine given alone. ($p<.01$). Comparison of the CDP treatment scores and those of the caffeine treatment revealed no significant difference in the amount of suppression of fighting between these two treatments.

Further examination of the data represented in Fig. 4 shows that with administration of caffeine there was a 34% reduction in the number of fighting responses. After treatment with CDP, fighting was reduced by 41%. The combined drug treatment, produced a nearly additive effect by reducing fighting by 69%.

1. Assuming non-additivity and heterogeneity of variance and covariances, the F score was assessed against the F required for significance on 1 and n-1 degrees of freedom. (Myers, J., Fundamentals of Experimental Design, Allyn and Bacon, Inc., 1971, p. 162.

Table 4 - Newman-Keul's analysis of the difference in amount of fighting for all subjects under four conditions: (1) no drug (A), (2) 15 mg/kg CDP (B), (3) 50 mg/kg caffeine (C), (4) caffeine and CDP (D)

		D	B	C	A
	Totals	716	1275	1425	2155
D	716	---	599*	709*	1439*
B	1275		---	150	880*
C	1425			---	730*
A	2155				---
	$q_{.99}(r,18)$		4.07	4.70	5.09
	$\sqrt{nMS_{res}}q_{.99}(r,18)$		382.17	441.33	477.95

* significant ($p < .01$)

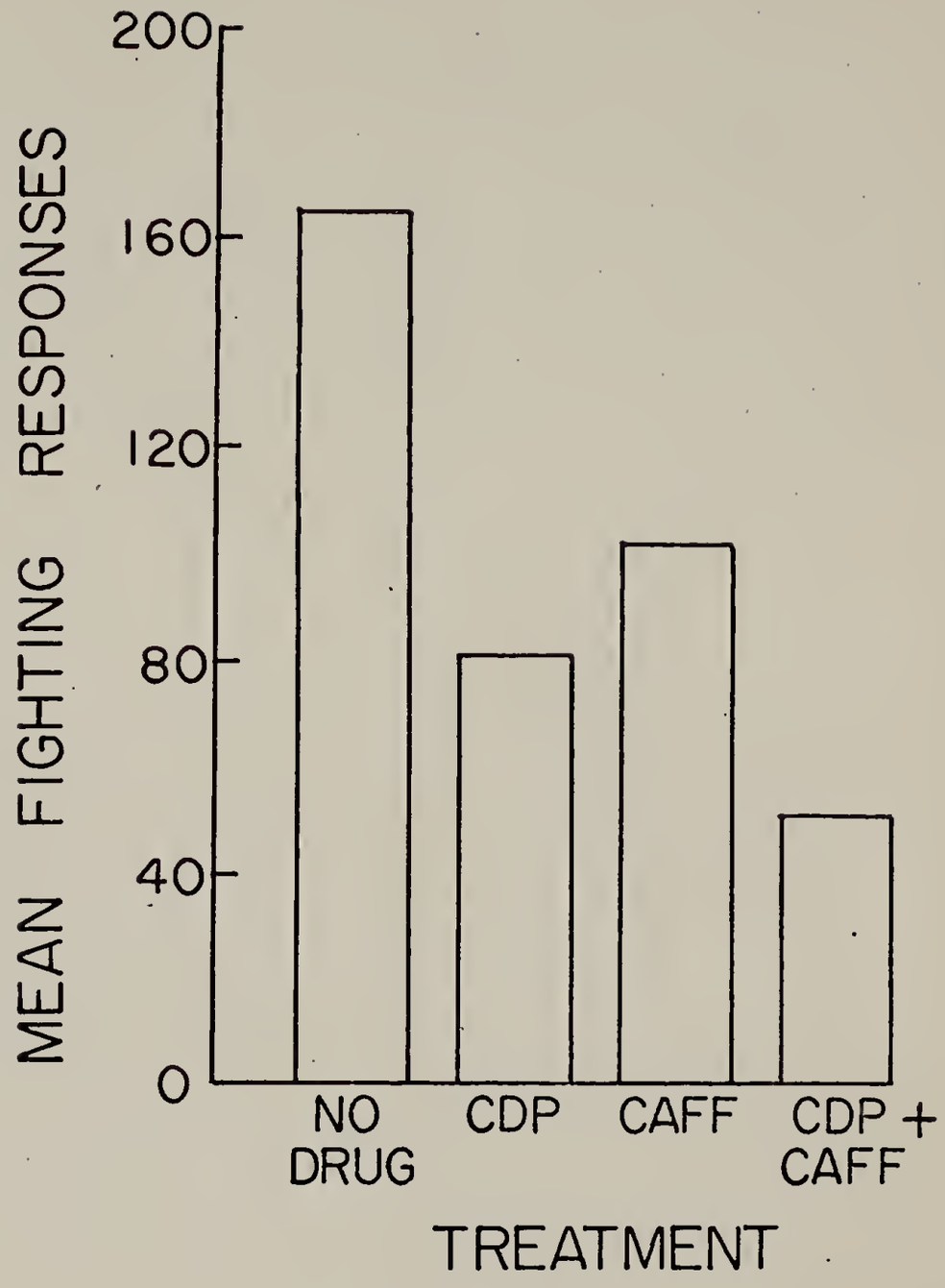

Fig. 4. Comparisons of the mean number of fighting responses occurring on no drug, CDP, caffeine, and combined drug treatment days.

DISCUSSION

The results of experiment IIb clearly demonstrated again the effectiveness of CDP in reducing shock-induced fighting in rats. Overall, fighting after treatment with CDP was reduced by 41%. An equally significant anti-aggressive effect of the caffeine-alone treatment was found, although fighting was reduced by only 34%. Also, a real difference between each of these single treatments and the combined drug treatment was found. In fact, the combined drug effect was almost equal to the sum of each of the two individual effects. Contrary to the antagonistic action of caffeine on CDP's depressive effect on spontaneous activity shown in experiment IIa, the two drugs have a similar effect on the suppression of shock-induced fighting.

These findings provide the strongest argument to date against the notion that the depressive effect of CDP is responsible for the suppression of aggression in rats. The data clearly showed that caffeine did antagonize the depressive effects of CDP in the spontaneous activity test. If the suppression of fighting with CDP were due to general depression, and if caffeine had the same effect of antagonizing depression during aggression testing, then CDP plus caffeine should have produced more rather than less fighting than that found in rats treated with CDP alone. If caffeine and CDP both had only a suppressing effect on aggression, one might assume that the two effects would be completely additive. On

the other hand, if CDP induced depression accounted for part of the reduction of fighting and if caffeine antagonized this depression, it could account for the difference between the combined effect of the drugs (69%) and the sum of the separate effects (75%), a difference of only 6%. Even if the difference of 6% only represents half of the effects of depression, the role of depression is still quite small. The data of experiment Ia likewise suggest that even if the suppression of aggression by CDP were due at least in part to a general depressive effect, the depressant effects of the drug would probably be minimal after five days of drug administration.

To explain the anti-aggression effects of CDP in neurochemical terms the following facts should be considered. First, the benzodiazepines are effective in releasing behavior that is suppressed by punishment (Wise, et al, 1972; Beer et al, 1972; Margules and Stein, 1968; Feldman and Green, 1967). The present study shows they are equally effective in suppressing irritable aggression, i.e., shock-induced fighting. Second, oxazepam, a benzodiazepine, reduced the turnover rates of 5HT (Wise et al, 1972), thus presumably blocking the brain stem 5HT mechanisms associated with punishment. Also the time course of the change in 5HT turnover correlated well with the time course of the anti-anxiety effects of oxazepam. Third, the benzodiazepines as well as caffeine, theophylline, and SQ 20, 009 are phosphodiesterase inhibitors and these also are anxiety suppressing compounds (Beer et al, 1972). The results of the present study also support this view.

The question that remains is whether blocking 5HT turnover has anti-anxiety effects under some circumstances while phosphodiesterase inhibition is responsible for anti-anxiety effects under other circumstances. One possible explanation is that phosphodiesterase inhibition is indirectly responsible for brain 5HT depletion which could have the same effect as a mechanism that would lead to a reduction of 5HT turnover. An explanation might be as follows. It is known that the level of cAMP represents the balance between the activities of two opposing enzymes, adenyl cyclase and phosphodiesterase. The former catalyzes the conversion of adenosine triphosphate (ATP) to cAMP, the latter catalyzes the hydrolysis of cAMP to 5'AMP (Robinson, Butcher and Sutherland, 1972). Thus, the inhibition of phosphodiesterase would presumably lead to an increase of cAMP levels. Since cAMP is considered to be a second messenger in synaptic transmission involving norepineprine (NE) (Robinson et al, 1972), a high build-up of cAMP might then by a negative feedback mechanism, reduce or block the release of NE from presynaptic terminals and lead to higher brain levels of this amine. Everett and Borcherding, (1970) have shown that injections of L-Dopa not only produce a rise in brain levels of dopamine but also a significant drop in brain levels of 5HT and increase urine levels of the serotonin metabolite 5-HIAA. This suggests that a rise in dopamine leads to a loss of vesticular 5HT and its deamination by MAO. It follows that the presynaptic build-up and increased brain levels of catacholamines by a phosphodiesterase inhibitor might ultimately

lead to a depletion of brain 5HT. Thus, it seems that while CDP blocks serotonergic transmission directly as suggested by Wise et al (1972) the xanthines might have the same effect by lowering the amount of available 5HT. However, the findings by Connor et al (1970) that the lowering of brain 5HT with pCPA had no effect on shock-induced fighting cannot presently be explained by this argument. Hopefully, future research will resolve these difficulties.

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