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1, 1-DIMETHYLHYDRAZINE EFFECTS ON CENTRAL EXCITATORY AND INHIBITORY MECHANISMS IN CATS

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FOREWORD

The research was performed under Contract AF 41(609)-2329 by the Brain Research Institute, Center for the Health Sciences, University of California, Los Angeles, School of Medicine. The work was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology and Biochemistry," from January 1964 to June 1965 for the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

These experiments were conducted jointly by M. D. Fairchild, PhD, Research Pharmacologist, Long Beach Veterans Administration Hospital, Long Beach, California, and Assistant Professor in Residence, Department of Pharmacology, UCLA and M. B. Sterman, PhD, Research Psychologist, Sepulveda Veterans Administration Hospital, Sepulveda, California and Assistant Research Anatomist, Department of Anatomy, UCLA. Kenneth C. Back, PhD, was contract monitor for the Aerospace Medical Research Laboratories.

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This technical report has been reviewed and is approved.

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ABSTRACT

Experiments, using cats with chronically implanted brain electrodes, were performed to explore the influence of subconvulsive doses of 1,1-dimethylhydrazine (UDMH) on certain excitatory and inhibitory mechanisms in the central nervous system (CNS). The cats were stimulated electrically in the midbrain reticular activating system, the basal forebrain inhibitory area, and both areas simultaneously while the animal was tested for performance in a positively reinforced experimental situation. UDMH was compared with amphetamine, chlorpromazine and phenobarbital both in the presence and absence of CNS stimulation. UDMH acted in a manner similar to chlorpromazine in subconvulsive doses in these tests. The most interesting and consistent effect of UDMH was to abort performance when the basal forebrain inhibitory area was stimulated. The animals resumed performance when the stimulus was terminated. UDMH has detectable CNS effects at doses well below convulsive levels.

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I. INTRODUCTION

This report is concerned with experiments designed to explore the influence of subconvulsive doses of UDMH on certain excitatory and inhibitory mechanisms within the central nervous system. Cats with electrodes chronically implanted in the midbrain reticular activating system (RF) and the basal forebrain inhibitory area (BF) were trained to negotiate a runway in order to obtain a food reward. Electrical stimulation of the RF was found to produce a statistically significant increase in the speed with which this act was performed while stimulation of the BF had the opposite effect. Simultaneous stimulation of these two brain sites produced effects on the rate of locomotion which were mutually antagonistic, since resultant run times were not significantly different from non-stimulated controls.

The influence of UDMH on this particular excitatory-inhibitory interaction within the central nervous system was tested at several dose levels and these results were compared with the effects of certain other centrally active compounds.

II. THE RUNWAY PERFORMANCE OF THE NON-DRUGGED CAT

A. Methods

The experimental procedures and special behavioral apparatus employed in these studies have been described in detail elsewhere (M.D. Fairchild & M.B. Sterman, Behavioral and Neurophysiological Studies of UDMH in the Cat, AMRL-TDR-64-72). Briefly, the apparatus consisted of a narrow runpath 6 centimeters wide and 4 meters long which served as a bridge over a water moat separating two enclosures. Each of the enclosures was equipped with a motor-operated door and contained a feeding device for delivering small quantities of milk. Photocell units located at various points along the runway operated clocks capable of timing performance with an accuracy of .01 seconds. Specially designed programming equipment controlled the delivery of milk, opening and closing of the doors, and the intertrial interval time, so that once placed within the apparatus the trained animal moved alternately between the two boxes without being further disturbed. It was possible to stimulate or record from electrodes chronically implanted within the animal's brain at any time during the running cycle.

Eight adult cats were surgically prepared under deep anesthesia with indwelling electrodes stereotaxically placed in the mesencephalic reticular formation (RF) and in the basal forebrain area (BF). In some of these animals additional

electrodes were placed in the thalamus, hypothalamus, amygdala and dorsal hippocampus. After recovery, the animal was placed in the runway apparatus described above, and trained to perform in the manner indicated. When stable runway performance was achieved, direct brain stimulation was introduced, and its effects upon the velocity of this performance determined. Prior to the opening of the delay box door, and during the presentation of a cue-masking tone, stimulation was applied to the site or sites called for by a previously determined counterbalanced design. It was terminated when the animal reached the food cup of the opposite box. Pilot experimentation had provided information with regard to such factors as optimal stimulation parameters, amount of food deprivation and reinforcement, and test design. Stimulation parameter tests indicated that a high frequency stimulus (300 cps) with a duration of 0.1 msec. was most effective when appropriate current was determined for RF and BF stimulation.

B. Results

Four of the animals were employed in the final experiments. The others were eliminated due to difficulty in training or consistently less stable performance. The experimental design employed provided unstimulated control trials in relation to each experimental site tested and, additionally, considered sequence and order effects. Twelve replications of each experimental stimulation were obtained. The most stable segment of runway performance proved to be the actual runway time, excluding time consumed in leaving the start box and in entering the opposite goal box. The data obtained were converted to velocity measures and treated statistically.

The results of brain stem and forebrain tests are summarized in Table 1. An inspection of this table will reveal that stimulation of BF produced a significant decrease in running velocity in all animals tested while RF stimulation was successful in causing the opposite effect in three out of the four cats. Simultaneous stimulation (interaction) resulted in complete cancellation of effects in two animals (cat 1 and 2). In cat 3 the interaction of RF (which in itself had no significant effect) with BF resulted in a slightly slower velocity than that observed with stimulation of the latter area alone, indicating a negative RF electrode placement in this animal. The two

experiments with cat 4 illustrate the exquisite sensitivity of the mechanisms which mediate interaction during simultaneous stimulation. In 4(a) interaction resulted in a decreased velocity relative to control and indicated dominance of the BF effect over that of the RF. In 4(b) stimulus current was decreased in BF and increased in RF; interaction now produced an increase in velocity indicative of RF dominance. A graphic demonstration of some of these various interaction effects is presented in Figures 1 and 2.

Stimulation of the basal medial amygdala and midline thalamus at these and other stimulation parameters did not alter running velocity in the animals tested. Stimulation of the dorsal hippocampus resulted in a marked slowing in one animal. When hippocampal stimulation was paired with RF stimulation, interaction was not obtained. On the contrary, an even greater decrement was observed. Therefore, the decrease in velocity resulting from hippocampal stimulation was probably due to disruption, rather than to functional suppression.

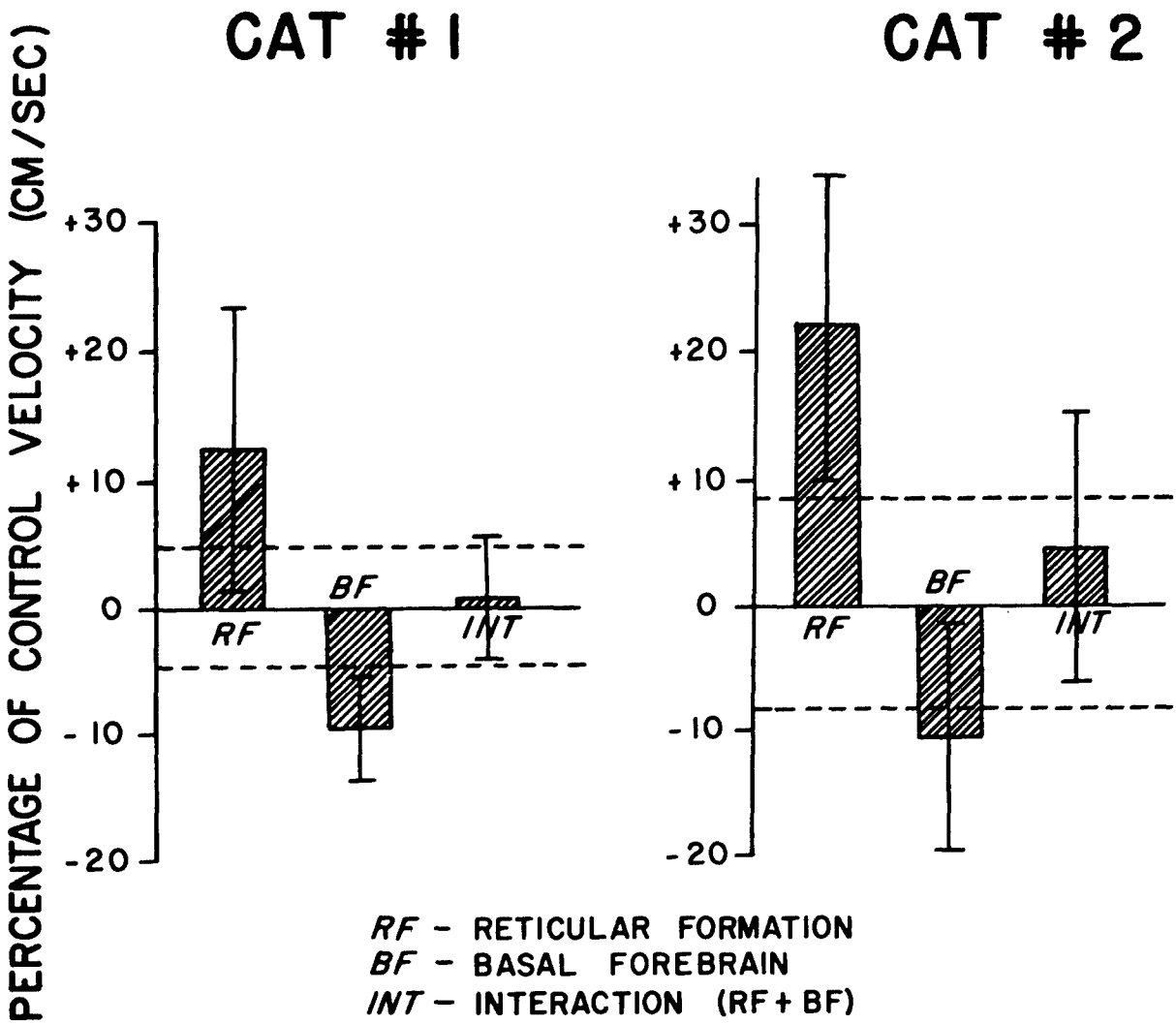

Figure 1. Graphic presentation of statistical velocity data from two cats in which interaction was reliably achieved. The abscissa and broken parallel lines reflect the mean control velocity and its standard deviation, respectively. Solid vertical lines represent standard deviations of the stimulation conditions.

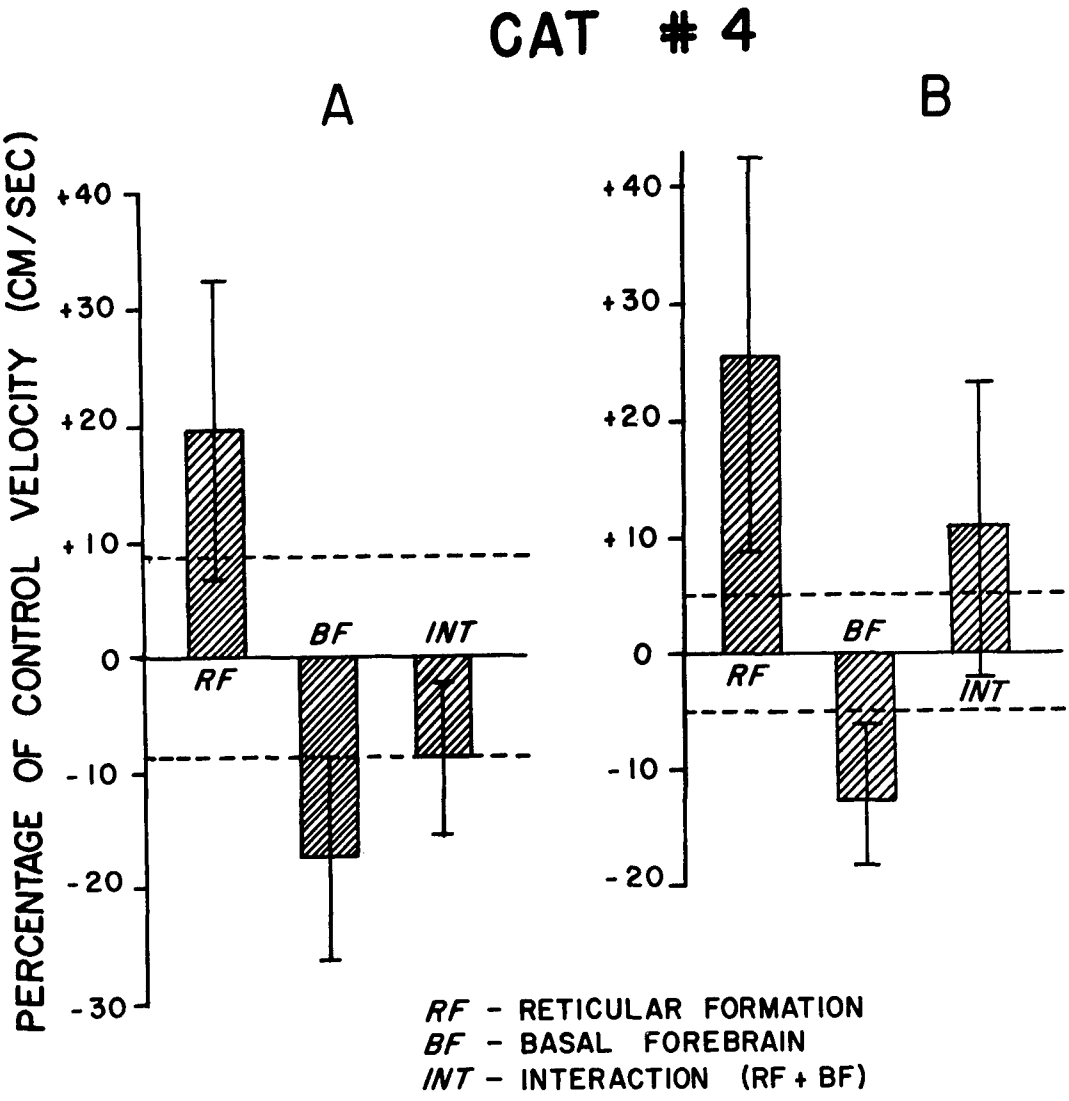

Figure 2. Graphic representation similar to figure 1, but here demonstrating the relationship between stimulation currents and resultant interaction effects (see text).

III. EFFECTS OF UDMH, AMPHETAMINE, PHENOBARBITAL AND CHLORPROMAZINE ON RUNWAY PERFORMANCE

A. Introduction

In this section of the report UDMH and several other centrally active drugs are discussed from the standpoint of their influence on various aspects of the runway performance described in the preceding section.

In the first series of experiments the effects of a central stimulant, a central depressant and a tranquilizing agent are described on the response of cats to brain stimulation during a single test session starting one hour after drug injection.

The influence of UDMH on response to brain stimulation was investigated in a fashion analogous to that above except that two test sessions were employed, one starting at 45 minutes and the second 3 1/2 hours post injection.

A third set of experiments was designed to test UDMH and the other three compounds for their influence on the number of trials an animal would make in the runway prior to reaching satiety without being subjected to brain stimulation.

B. Drug Effects with Brain Stimulation

1) The Effects of Amphetamine, Chlorpromazine and Phenobarbital.

a. Method

A total of four cats, trained to perform in the runway were used in these experiments. A testing session

consisted of 72 trials or 36 "cycles". A cycle was defined as one left-to-right and one right-to-left trial. During every third cycle of the test session either BF, RF or Interaction (INT = BF + RF) stimulation was presented to the animal. The order of stimulation was rotated in a systematic fashion and response was judged by comparing the velocity of running to that of an immediately preceding non-stimulated trial.

The test compounds d-amphetamine SO_4, Chlorpromazine HCl, and Phenobarbital Na were injected intraperitoneally one hour prior to experimental sessions. Doses are reported as milligrams of the salt per kilogram of body weight. Normal saline was injected prior to control runs.

b. Results

d-Amphetamine SO_4

Amphetamine was injected in all animals at a dose of 1.8 mg/kg. This amount produced noticeable hyperactivity, piloerection and mydriasis and most cats responded with an increase in running velocity of approximately 25% during non-stimulated trials. The effectiveness of both BF and RF stimulation in altering running velocity was reduced. Although the response was generally less in degree, it was perhaps slightly more stable following administration of amphetamine.

Chlorpromazine HCl

Chlorpromazine was administered in doses between 0.75 and 1.25 mg/kg. The most consistant effect of this compound was a reduction of running velocity during non-stimulated trials; this occurred at doses which produced no obvious deleterious effects on motor ability. At higher doses it was not unusual for the animals to fail to complete the normal number of trials, and occasionally they would refuse to perform at all.

The response to BF anf RF stimulation was potentiated by chlorpromazine in a number of instances. When this occurred, there was a corresponding increase in variability of the stimulated trials.

Phenobarbital Na

Phenobarbital was injected in doses ranging between 10 and 40 mg/kg. Generally speaking, this compound had little effect on running velocity, except at doses which produced ataxia. When there was obvious locomotor impairment running velocity was, of course, reduced.

Phenobarbital given in doses which were sub-threshold for the production of ataxia had little consistent effect on the animals' response to brain stimulation. However, the inter-trial variability was apparently increased.

The ataxia produced by higher doses of phenobarbital

was of long duration. One animal in which 40 mg/kg severely disrupted motor performance on the day of injection exhibited run times 24 hours later which were almost twice those considered normal for this cat. By the second day following injection no drug effects were apparent.

c. Discussion

The degree to which the performance of the cats varied following drug administration made it difficult to obtain a quantitative evaluation of the activity of these compounds. Thus, only qualitative changes have been discussed. Considerable variability in the response to drugs might be expected in this particular situation, since normal intra-species variation in drug-sensitivity could be compounded by many contingencies, such as slight differences in stability of behavioral performance, degree of drive (hunger) and placement of electrodes within the brain. A continuing effort is being made to bring all possible sources of variation under experimental control. As more experience concerning the effect of drugs on runway performance accumulates, quantitative description of their activity should be possible.

It is of interest to note that the central stimulant amphetamine and the tranquilizing agent chlorpromazine had quite opposite effects on the performance of cats in the runway. Amphetamine caused an increase, while chlorpromazine

administration resulted in a decrease in running speed. Under the influence of the former drug, both BF and RF stimulation were less effective, whereas, following the latter, the converse appeared true. In addition, inter-trial variability was decreased by amphetamine and increased by chlorpromazine.

The ataxia produced by phenobarbital was a reflection of the central nervous system activity of this compound. It was, however, difficult to characterize this effect in terms of running velocity, either in the presence or absence of brain stimulation. Since the runpath in the apparatus is only 2 inches wide and is suspended over a water moat, even a slight degree of locomotor impairment could have quite marked and variable effects on running velocity.

Since an animal usually completed the requisite number of runs for a milk reward and eagerly consumed supplemental rations of solid food given after a drug test session, none of these compounds, in the doses given, appeared to be decreasing the cat's drive level. It had been anticipated that the anorexic effect of amphetamine might operate to reduce the number of trials made for a food reward. Such was not the case.

2) The Effects of UDMH

a. Method

In a previous study (M.D. Fairchild & M.B. Sterman, Behavioral and Neurophysiological Studies of UDMH in the Cat AMRL-TDR-64-72) it was found that the onset of toxic symptoms following UDMH administration was usually delayed by a number of hours. In order to investigate the possibility of a similar delay in behavioral disturbances and to check also for the occurrence of more immediate alterations in runway performance which might be produced by UDMH it was deemed advisable to run the cats twice rather than once per day. To accomplish this goal it was necessary to retrain the animals employed in the previously described drug series. Two of these four cats adapted easily to the new situation and were therefore selected for UDMH testing. A total of 40 trials (20 cycles) were given during each of the two runs comprising a test-session, and either BF, RF or INT stimulation was presented in a manner similar to that employed in the drug series.

UDMH was given intraperitoneally in doses of 7, 14 and 21 mg/kg and the animals were tested starting at 45 minutes (Run 1) and 3 1/2 hours (Run 2) post-injection. Each dose was administered to each cat on at least two occasions with no less than 48 hours elapsing between test sessions.

b. Results

An analysis of variance performed on the data from three control days, during which normal saline rather than UDMH was administered, revealed that the only significant source of variation in the performance of the two animals selected for UDMH testing was that produced by the three different stimulus conditions. This indicated that response to brain stimulation between each run and between replications of stimulus conditions during the three control days was relatively consistent and could logically be used to estimate UDMH effects (see Table 2).

A significant increase in the variability of response to brain stimulation occurred following UDMH administration, reminiscent of that observed with phenobarbital and chlorpromazine. This effect precluded any possibility of treating the data quantitatively. The problem of response variability was compounded during UDMH testing, because injection of this compound often resulted in different behavior during run 1 and run 2, a situation which did not occur during saline control sessions. This prevented pooling of the data from the two runs, and in effect, reduced the number of trials which could be utilized to judge UDMH effects.

The results of UDMH testing will be reported below for each cat separately in order to emphasize both the similarities and differences in effects which were observed.

Cat No. 1

UDMH at 7 mg/kg

No detectable alterations occurred in either response to brain stimulation or in running speed during non-stimulated trials; there were no toxic symptoms and the electroencephalogram (EEG) pattern appeared normal both in the initial experiment and when the injection was repeated 48 hours later.

UDMH at 14 mg/kg

Following the first injection of this dose behavior was relatively normal except that in Run 2 presentation of INT stimulation caused a disruption of performance and the animal would not move until the stimulus was terminated. As mentioned above, considerable variability in response to all brain stimulation was evident.

A second injection of UDMH at 14 mg/kg administered 48 hours after the first produced dramatically different results. Performance during Run 1 was slower but relatively normal during both non-stimulated and RF stimulation trials. Upon presentation of the first BF stimulation the animal stopped and would not perform further although only 10 of the normal 40 trials had been completed. This cat subsequently completed all 40 trials of the run, but 3 of 4 INT and 1 of 4 BF stimulations caused cessation of running until the stimulus was terminated. RF stimulation was not disruptive, but non-

stimulated trials were noticeably slower.

UDMH at 14 mg/kg produced no obvious signs of toxicity and locomotor ability remained normal. The EEG contained long runs of low voltage, fast activity indicative of an alert state. As usual, a supplemental ration of solid food given at the end of the experiment was eagerly consumed.

UDMH at 21 mg/kg

Only 4 of the usual 40 trials were completed during Run 1 of the first experiment, since the animal refused to continue following presentation of the first INT stimulation. As in test sessions with 14 mg/kg all 40 trials of Run 2 were completed although most INT and BF stimulations disrupted behavior as before. Non-stimulated trials were noticeably slower and, once again, RF stimulation was not disruptive.

The second experiment at 21 mg/kg resulted in effects somewhat similar to the first except that all 40 trials were completed in both runs and BF stimulation markedly slowed but did not actually disrupt running behavior.

The only obvious toxic symptom was that of depression. The EEG did not exhibit slow wave activity characterisitc of depressed states but, on the contrary, was highly desynchronized. Supplemental food rations were eagerly consumed.

Cat No. 2

UDMH at 7 mg/kg.

Non-stimulated run times were slower and one INT and RF stimulation disrupted behavior during Run 1 of the first experiment. Non-stimulated run times were also slow in Run 2, but brain stimulation was not disruptive.

A second experiment with 7 mg/kg was essentially negative with non-stimulated trials and response to brain stimulation appearing relatively normal during both runs.

There were no toxic symptoms and both locomotor ability and EEG recordings were normal. Supplemental food rations were eagerly consumed.

UDMH at 14 mg/kg.

During Run 1 of the first experiment non-stimulated run times were considerably slower but response to brain stimulation was essentially normal. Only 5 trials of Run 2 were completed since on the first presentation of BF stimulation the animal attempted to escape from the runway and would not continue to perform.

The second injection of 14 mg/kg resulted in fewer behavioral changes than did the first, a phenomenon which was also observed in this animal during tests with 7 mg/kg (see above). Non-stimulated run times were slower. Except for a single disruption of behavior by BF during Run 2,

response to brain stimulation appeared normal.

Depression was the only symptom to toxicity; locomotion was slower but appeared coordinated, and food was readily consumed. The EEG was desynchronized with long runs of low-voltage fast activity.

UDMH at 21 mg/kg.

The animals' performance in the runway was grossly altered. Initial non-stimulated trials during Run 1 of the first experiment were performed slowly and with much hesitation. BF stimulation disrupted behavior, although the animal continued to run when the stimulus was terminated. A series of RF stimulations were tolerated. However, on presentation of the first INT stimulation the cat stopped and would complete no further trials. The animal refused to perform at all during Run 2 and remained quietly in the start box before finally being removed from the apparatus.

The second experiment with 21 mg/kg was characterized by obvious symptoms of toxicity. The animal appeared depressed and disorganized and was salivating slightly at the start of Run 1. A total of 8 non-stimulated and 2 BF stimulated trials were completed but a subsequent RF stimulation permanently ended performance. Behavior during Run 2 was very similar to that during Run 1; non-stimulated trials were made with considerable hesitation, BF stimulation disrupted behavior, but

not permanently, and the animal refused to continue performance following RF stimulation.

Approximately 4 hours post-injection, and after being removed from the runway, this cat experienced a generalized seizure which had a duration of approximately 1 1/2 minutes. Recovery seemed complete but 1 1/2 hours later a second convulsion occurred and pentobarbital was administered.

c. Discussion

A significant individual difference in sensitivity to UDMH was observed in the two animals tested here. For instance, 7 mg/kg did not effect the performance of cat No. 1, and, although 21 mg/kg produced definite behavioral alterations, this dose was not accompanied by symptoms of marked toxicity in this animal. On the other hand, 7 mg/kg did result in detectable changes in the performance of cat No. 2, and 21 mg/kg produced complete behavioral disruption and toxicity which culminated in a convulsive episode.

Another aspect of individual difference in response to UDMH was observed in relation to the degree of behavioral effect associated with the time sequence on a given testing day. Cat No. 2 was more profoundly affected by UDMH during Run 2, 3 1/2 hours post-injection, than during Run 1, starting at 45 minutes post-injection. This animal also experienced a

series of convulsions approximately 4 hours after injection of 21 mg/kg. Conversely, cat No. 1, which experienced no convulsions, generally exhibited a greater UDMH effect during the early run.

Several effects of UDMH, which appeared to be dose related, were observed in both cats. Non-stimulated run times were slower in both animals after 14 mg/kg of the compound, but no deleterious influence on locomotor ability was observed. Brain stimulation, which would normally speed or slow performance, often resulted in complete behavioral disruption following UDMH injections. Disruption was most frequently observed following BF and interaction stimulation. RF stimulation would occasionally cause an animal to stop performing, but in general this stimulation continued to be effective and was much better tolerated following UDMH. The basal forebrain is known to be involved in a descending inhibitory pathway connecting frontal and orbital cortical areas with bulbar nuclei which mediate motor, autonomic, and cortical excitability (R. Hernandez-Peón, and M.B. Sterman, Brain Function. In: Annual Review of Psychology, 1966). In this regard, it is interesting to note that adequate UDMH administration produced a tonic depression of control velocities and caused BF and interaction stimulation to be disruptive. This disruption involved a disinterest or refusal to run on the part of the animal. Both of these effects could reflect an enhancement of activity within the above-mentioned forebrain inhibitory

system, resulting from UDMH administration. The fact that RF stimulation was still effective and that long periods of desynchronized EEG patterns were simultaneously observed further suggests that the effect was limited to the inhibitory mechanism and, perhaps, resulted in a compensatory increase in the activity of the excitatory system. Since the inhibitory pathway is thought to require neurochemical mediation (e.g. acetylcholine or serotonin) it may be speculated that the chemical release of these compounds by UDMH causes the enhanced inhibitory action and, if prolonged or intense, could lead to the eventual depletion of the chemical transmitter substance. The net effect would be a functional disequilibrium, with increased excitatory activity unchecked by reciprocal inhibition. Hyper-activity and seizure could be a consequence of these events. We are presently considering this interpretation.

C. Drug Effects Without Brain Stimulation

1) Introduction

As discussed in the section above, brain stimulation which normally decreased running velocity resulted in complete disruption of behavior following administration of UDMH. Although frequently encountered in conjunction with UDMH, behavioral disruption from brain stimulation was only rarely observed following administration of amphetamine, chlorpromazine or phenobarbital. These observations raised an additional question.

What influence might the four test compounds have upon the duration of runway performance when brain stimulation was not employed in the experiments? More specifically, what unique effects might be produced by UDMH under these circumstances? A series of experiments were, therefore, instituted in which cats were allowed to run in the apparatus until they reached satiety and spontaneously stopped performing. The influence of the test compounds on both the running velocity and number of trials completed prior to satiation was investigated.

2) Method

Two cats were selected for the relative stability of their running performance during non-stimulated trials. These animals were allowed to run to satiation on alternate days. Except for supplemental rations given following some drug experiments and regularly on week-ends their only source of nutrition was the fortified milk obtained in the runway. This schedule provided for at least 48 hours of food deprivation prior to each test session. Either normal saline or a test compound was injected intraperitoneally one hour prior to each test.

3) Results

The experimental design called for a saline control session to be run before and after each drug test. An analysis of variance of each of these sets of three experiments was performed to examine the significance of the variation contributed by mean differences in running velocity and by linear

and quadratic regressions of velocity on trial number both on the interaction between controls and control versus drug.

One animal (cat no. 8) was consistent throughout these experiments in that pre- and post-drug control sessions did not significantly differ. Changes occuring in the runway performance of this cat following drug administration could therefore be compared quantitatively. The second experimental subject (cat no. 3), while initially exhibiting stable performance, soon developed significant variation between control sessions and as a result could not be employed for quantitative comparison of drug activity. This animal was therefore employed in estimating the qualitative effects of wider dose ranges of the test compounds.

Cat No. 8

Analysis of variance revealed that with adequate doses of the test compounds there were significant differences between control and experimental conditions in mean running velocity and in both linear and quadratic regression of velocity on trial number. Linear regression, however, most consistently reflected minimum variance between controls and maximum variance between control and experimental conditions. Therefore, the linear regression coefficient was employed to quantitatively express drug activity. A summary of these results is presented in Table 3. Amphetamine, chlorpromazine, and phenobarbital are reported in single, non-toxic doses which were above threshold

for producing observable physiological effects in this animal. The results of UDMH testing are given for three sub-convulsive dose levels.

Linear, and in most cases, quadratic regression on trial number accounted for a significant amount of variability when the animals were allowed to run until satiated. A tendency to move progressively slower with increasing trial numbers is understandable, both on the basis of simple fatigue and reduction of hunger drive. That the test compounds were capable of significantly altering these relationships can be appreciated from an inspection of Table 3.

The central stimulant amphetamine caused a significant decrease in the linear regression coefficient which reflected a more consistent and faster running pattern. Surprisingly, phenobarbital, a central depressant, also produced more consistent and slightly faster running which resulted in a decreased linear regression coefficient. Chlorpromazine, a tranquilizing agent, markedly altered the running pattern by reducing the total number of trials completed and causing a sharp decrease in running speed with increasing trial numbers; a large increase in the linear regression coefficient resulted. UDMH administration produced a progressive increase in the linear regression coefficient which was dose-related; this reflected completion of fewer trials with a marked decrease in running speed with increasing trial numbers and was analogous to the effects produced by chlorpromazine.

Cat No. 3

Results obtained with cat No. 3 were qualitatively similar to those observed with cat No. 8 but, as previously mentioned, control run variability did not permit a similar quantitative presentation of the data.

Amphetamine was administered several times in doses between 0.92 and 7.36 mg/kg (.005 and 0.4 mmol/kg). Lower doses produced increased running speed over a greater number of trials, analogous to the effects observed with cat No. 8, but at the highest dose tested this compound disrupted running performance. In this latter experiment 9 cycles of running were completed in an essentially normal fashion but at this point the animal abruptly stopped and refused to continue.

Phenobarbital was injected between 10 and 50 mg/kg and, as with cat No. 8, a decrement in running behavior was not observed until ataxia produced by the drug began to limit the animal's ability to negotiate the narrow runpath. In one experiment 30 mg/kg of phenobarbital resulted in only minimal motor involvement and cat No. 3 proceeded to run an amazing 141 cycles, a total at least twice that normally observed on controls. A subsequent dose of 50 mg/kg induced severe ataxia and, although obviously willing to perform, the animal was unable to negotiate the runpath and was removed from the apparatus.

Chlorpromazine produced effects similar to those with cat No. 8; a dose of 1 mg/kg resulted in marked reduction of running speed with fewer trials being completed.

Cat No. 3 appeared more sensitive to the effects of UDMH than did cat No. 8. In No. 3, 10 mg/kg slowed and disrupted runway performance to the point where only 4 cycles of running were completed while in No. 8, 35 cycles of slower running were managed following a dose of 20 mg/kg. A subsequent challenge with 10 mg/kg approximately three weeks after the first did not result in as severe a disruption, although effects were obviously greater than those produced by a comparable dose in cat No. 8. UDMH at 5 mg/kg had no obvious effects.

4) Discussion

Although adequate doses of UDMH may produce an enhancement of CNS excitation leading to convulsions, it is of interest to note that this compound at subconvulsive levels did not manifest any amphetamine-like stimulation of motor activity. On the contrary, a significant depression of locomotion was observed. This effect was not related to a general malaise with a resulting decrease in appetite, as indicated by the animal's general appearance and eager consumption of subsidiary rations given outside the runway. These lower doses of UDMH, associated with noticeable locomotor depression, did

not result in any general toxicity; the animals appeared normal or, in some cases, somewhat more hyperactive and alert.

UDMH, unlike the central depressant phenobarbital, did not produce locomotor ataxia. Phenobarbital produced a decrement in runway performance only at doses which resulted in obvious ataxia and apparently had little influence on the animal's desire to perform. Sub-ataxic doses, on several occasions, actually resulted in marked increases in the number of trials completed. This effect was never observed with UDMH, which produced an increasing performance decrement with increasing dose.

The locomotor depression resulting from UDMH administration resembled that observed with the tranquilizer chlorpromazine. Both compounds resulted in slower individual run times and fewer total trials completed. Chlorpromazine also produced an obvious general sedation, which was in contrast to the normal or somewhat hyperactive appearance following lower doses of UDMH. These results again indicate a locomotor depression associated with some indication of general excitation, an observation which is consistent with our earlier speculation.

IV. GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

A. The Experimental Method

The concept of diffusely projecting excitatory and inhibitory mechanisms in the central nervous system mutually and antagonistically interacting to influence momentary and diurnal variations in levels of activity provides an interesting model with which to investigate certain neurophysiological and neuropharmacological correlates of behavior.

The present investigations have demonstrated that the mesencephalic reticular activating system and the basal forebrain inhibitory area are, indeed, capable of mutual and antagonistic interaction at some level in the complex neuronal mechanisms whose totality of function controls the rate at which an animal negotiates a runway to seek a food reward. The selection of this response contingency, while neglecting many other possible effects of reticular formation and basal forebrain stimulation, was adopted with the express hope of obtaining a relatively precise quantitative measure of at least one aspect of the interaction of diffuse excitatory and inhibitory mechanisms within the central nervous system. It is felt, that in this task, some measure of success was achieved.

The application of these quantitative measurements to the study of drug effects on these systems proved to be

somewhat difficult. The major problem was a tendency for the performance of the animals to become more variable once drugs were introduced into the situation. Under conditions in which only saline injections were given, non-stimulated run times and responses to brain stimulation normally remained stable for a number of weeks. As drug test sessions continued, however, all aspects of performance tended to become more labile. Reasons for this drug-induced increase in variability are not clear; reasonable periods of time were allowed between drug tests and there was no obvious change in the general physiological condition, weight or feeding patterns of the cats.

Future work will concentrate on training animals to run in two sessions per day with either normal saline or a test compound being administered in the interval between them. This will insure that each animal serves as its own control for each day of drug testing, and provides a means of assessing gradual changes in performance. This design was avoided originally because of an obvious disadvantage which stems from the fact that a cat will complete only a certain number of trials on a given day. If one-half of the available trials are employed as controls, the number of replications of stimulus conditions following administration of a test compound is necessarily limited. Experiments with partial reinforcement schedules have been instituted in an attempt to extend the period of running during each session.

B. Results of UDMH Testing

The most interesting and consistent effect of UDMH was an apparent increase in the degree to which brain stimulation involving the BF disrupted performance in the runway. In a well trained animal with the correct amount of current flow across electrodes, total disruption of behavior from brain stimulation was a rare event. However, following injection of UDMH in relatively small, subconvulsive doses, stimulation would frequently cause the animal to "freeze" in the startbox or at some point in the runway. Less often, an attempt to escape from the apparatus would result. The cats often resumed running when the stimulus was terminated. However, on a number of occasions, no further running would occur during that session. These effects were most commonly observed with simultaneous stimulation of basal forebrain and reticular formation and with basal forebrain stimulation alone. This type of behavioral disruption, while occasionally observed following injection of amphetamine, phenobarbital and chlorpromazine, was not a prominent feature of the effects of these compounds on runway performance. These findings indicate that UDMH has detectible central nervous system effects at doses well below convulsive levels. Future work is contemplated to more clearly define the anatomical and physiological parameters of this UDMH activity, and to explore the implications of the present findings.

Table 1. Summary of velocity changes induced by test stimulation of the reticular formation and basal forebrain area in the cat. These results represent the analysis of 12 counterbalanced replications. Stimulation was delivered at 300 cps, 0.1 msec, with current as indicated.

Animal No.	Stim. Sites	Stim. Current	Velocity Change	F Ratio (All condit.)	t Ratio (Cont. vs stim.)
1	Retic. Form.	30 μA	(+)		6.719***
	Basal Forebr.	220 μA	(-)	307.3**	9.797***
	RF + BF	same	no change		0.319
2	Retic. Form.	28 μA	(+)		4.747***
	Basal Forebr.	120 μA	(-)	151.1**	3.139***
	RF + BF	same	no change		1.393
3	Retic. Form.	28 μA	no change		1.109
	Basal Forebr.	140 μA	(-)	14.7**	2.467*
	RF + BF	same	(-)		2.891**
4(a)	Retic. Form.	25 μA	(+)		8.544***
	Basal Forebr.	220 μA	(-)	125.8**	9.141***
	RF + BF	same	(-)		5.405***
4(b)	Retic. Form.	28 μA	(+)		9.958***
	Basal Forebr.	175 μA	(-)	78.5**	10.413***
	RF + BF	same	(+)		6.185***

*** $p < .001$
** $p < .01$
* $p < .05$

Table 2. Analysis of variance of three control days (saline injection) on paired data. Control for each stimulus condition (BF, RAS, INT.) was an immediately preceding non-stimulated trial. Two runs with 12 replicates each comprized a daily session (Total N = 72).

Cat No. 1

Source	df.	Sum of Squares	Mean Square	F	P
Between Stimulus Condit.	2	744510.3333	372255.16666	407.8674	<.001
Between Replicates	11	17696.3750	1608.76136	1.7613	<.05
Between Runs	1	1292.0138	1292.01380	1.4145	<.05
Interactions					
Cond. x Rep.	22	37201.6670	1690.98486	1.8513	<.05
Cond. x Runs	2	16368.7779	8184.38890	8.9602	<.01
Rep. x Runs	11	9522.4862	865.68056		
Error Term	22	20095.0968	913.41349		

Cat No. 2

Source	df.	Sum of Squares	Mean Square	F	P
Between Stimulus Condit.	2	25002.8611	12501.4305	10.1052	<.001
Between Replicates	11	6026.4445	547.8586		
Between Runs	1	3280.5000	3280.5000	2.6517	<.05
Interactions					
Cond. x Rep.	22	35574.4722	1617.0215	1.3071	<.05
Cond. x Runs	2	1624.7500	812.3750		
Rep. x Runs	11	13870.1666	1260.9242	1.0192	<.05
Error Term	22	27216.5834	1237.1174		

Table 3. Linear regression coefficients in analysis of variance found not to be significantly different between pre- and post-drug controls but significant between control and drug at P > .001.

Cat No. 8

Drug	Dose (mg/kg)	Linear Regression Coefficient				
		Pre-Drug Control	Post-Drug Control	Controls	Drug	% Change
Amphetamine	1.84	57.4	66.7	62.1	19.2	- 69
Phenobarbital	20.0	52.3	33.4*	42.8	21.7	- 49
Chlorpromazine	1.0	14.7	6.0	10.3	84.5	+720
UDMH	10.0	49.0	42.9	45.9	82.8	+ 80
UDMH	15.0	18.9	17.2	18.0	63.6	+253
UDMH	20.0	21.0	21.2	21.1	107.9	+411

*Pre-drug and post-drug controls differ significantly. (P > .01)

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13. ABSTRACT

Experiments, using cats with chronically implanted brain electrodes, were performed to explore the influence of subconvulsive doses of 1,1-dimethylhydrazine (UDMH) on certain excitatory and inhibitory mechanisms in the central nervous system (CNS). The cats were stimulated electrically in the midbrain reticular activating system, the basal forebrain inhibitory area, and both areas simultaneously while the animal was tested for performance in a positively reinforced experimental situation. UDMH was compared with amphetamine, chlorpromazine and phenobarbital both in the presence and absence of CNS stimulation. UDMH acted in a manner similar to chlorpromazine in subconvulsive doses in these tests. The most interesting and consistent effect of UDMH was to abort performance when the basal forebrain inhibitory area was stimulated. The animals resumed performance when the stimulus was terminated. UDMH has detectable CNS effects at doses well below convulsive levels.

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