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SUBCONVULSIVE EFFECTS OF MONOMETHYLHYDRAZINE ON RUNWAY PERFORMANCE IN THE CAT

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Foreword

This research was performed under Contract AF 33(615)-2822 by the Department of Anatomy and the Brain Research Institute, School of Medicine, University of California, Los Angeles, California 90024. The work was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202," Pharmacology and Biochemistry," Work Unit 018, "Research on the Subconvulsive Effect of Air Force Compounds on the Nervous System," for the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio. This report covers research performed between January 1967 and November 1968.

The experiments were conducted jointly by M. B. Sterman, PhD, Chief, Neuropsychology Research, Veterans Administration Hospital, Sepulveda, California, and Assistant Professor, Departments of Anatomy and Physiology, UCLA, M. D. Fairchild, PhD, Research Pharmacologist, Veterans Administration Hospital, Long Beach, California, and Assistant Professor, Department of Pharmacology, UCLA, and H. B. Van Twyver, PhD, Neuroanatomy Trainee, Department of Anatomy, UCLA. Kenneth C. Back, PhD, was contract monitor for the Aerospace Medical Research Laboratory.

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Abstract

Previous neurophysiological and behavioral studies of the toxic propellant UDMH have indicated that its subtle low-dose influences can be most effectively evaluated in the cat by reference to trained locomotor performance. To determine similar fundamental information in evaluating monomethylhydrazine (MMH), a related derivative of hydrazine, this same technique was employed. Cats were trained and tested in a special runway apparatus to provide a reliable indication of performance changes over a 6-hour period following the administration of 1, 2, and 4 mg/kg MMH. These low doses significantly altered locomotor performance, both during drug session testing and saline control testing carried out 24 hours later. Within 30 minutes after injection of all three doses of MMH, runway performance was depressed. At 2 and 4 mg/kg, this influence was profound and was associated with overt physiological symptoms of toxicity. A total disruption of performance occurred with 4 mg/kg doses when tested 2-5 hours after administration. Performance was still depressed after 24 hours following 4 mg/kg, but was actually facilitated at this same point following 1 and 2 mg/kg doses.

Section I

INTRODUCTION

Neurophysiological and behavioral studies of 1, 1-dimethylhydrazine (UDMH) in the cat have disclosed a number of significant facts concerning the central nervous system (CNS) action of this toxic substance. The cat is particularly well-suited for such studies due to the extensive literature available on its advanced mammalian nervous system, and the action upon it of convulsive and other important pharmacologic agents. Moreover, the cat's capacity for mastering complex motor and perceptual tasks in unrestrained test situations allows for the assessment of subtle influences throughout the full range of more-or-less natural performance.

In the cat, UDMH produce significant dose-related alterations in brain electrical activity, sensory and motor excitability, and performance in a learned motor task (Fairchild and Sterman, 1964, 1965, 1967; Goff *et al.*, 1967). Specifically, convulsive doses of this substance resulted in a graded increase in cortical excitability, as reflected by enhanced neuroelectric responses in cortical sensory projection areas, and decreased threshold for centrally-induced motor seizures. The increased sensory response at cortical levels was attributed to a progressive blocking of inhibitory interneurons which normally modulate the excitability of axodendritic synapses.

Motor facilitation resulted from the dual influence of increased axodendritic excitability in motor cortex pyramidal cells and a dramatically increased afferent corticopetal bombardment of these cells. Thus, it was proposed that a sensorimotor positive feedback situation gradually emerged as a result of both an increased sensory excitability and bombardment, and a decreased inhibitory regulation of cortical motor centers. At doses of 20 mg/kg and above this situation resulted inevitably in generalized CNS seizures.

Neurophysiological studies of subconvulsive doses of UDMH showed changes in the same direction; however, recovery to normal levels always occurred before critical excitability was reached. Emesis and mild motor ataxia were observed, also, with higher subconvulsive doses. Behavioral studies involving the execution of a well-learned locomotor task proved to be a most sentive index of low-dose effects. Performance was significantly altered with doses as low as 4 mg/kg. Recovery occurred within 5 hours, and performance was significantly enhanced after 5 hours with 4-8 mg/kg exposures. Evidently a systematic alteration of central depression and excitation accompanies low-dose exposure to UDMH. The time course of these effects, like the more dramatic effects of convulsive doses, is directly related to dose. Moderate degrees of excitation, without other side effects, appeared to enhance motor performance.

In the present study, this highly sensitive behavioral approach was again utilized in an evaluation of subconvulsive doses of another derivative of hydrazine, monomethylhydrazine (MMH). Additional studies of this compound in the cat have compared its toxicity and other aspects of its central action with UDMH (Sterman, Fairchild and LoPresti, 1968). They show that MMH is substantially more toxic than UDMH and indicate several other differences in its physiological effects. They suggest, also, as did the studies of UDMH, that its action in the realm of motor function is of particular importance.

Section II

METHODS

Five adult cats were trained to stable performance in a runway apparatus designed to detect subtle changes in locomotor performance related to experimental manipulations of the central nervous system. Integrated behavior is quantified in this runway by measurement of the time required to run, alternately, between two enclosed chambers. This apparatus, and its application in the study of centrally-acting chemical compounds, has been described in detail elsewhere (Fairchild and Sterman, 1964, 1965; Sterman and Fairchild, 1967).

After being trained to stable performance in this runway, the five animals were tested daily in a predetermined sequence which spanned a period of approximately 6 hours following the intraperitoneal injection of either 1 cc normal saline, or three different subconvulsive doses of MMH. Animals were started in this sequence at periods 30, 90, 150, 210, and 270 minutes following injection. Each of the five cats was run 46 consecutive trials (23 in each direction); and their starting time sequence was rotated in a counterbalanced design which provided for the appearance of each animal in one of the five time blocks near the beginning, middle, and end of the post-injection period during the testing of each of the three subconvulsive doses of MMH.

Test sessions were initially conducted each 24 hours following saline injections until five days of control performance data were collected. On the sixth day of the experiment 4 mg/kg of MMH was administered to all animals and the same test sequence initiated. Saline control data were again obtained 24 and 48 hours after drug injection. A second and third test of this dose was obtained consecutively in the same manner, utilizing, in each instance, a changed order of running for the five experimental animals. Three replications each of tests involving 2 mg/kg and 1 mg/kg injections of MMH were obtained employing this same experimental design.

Performance measures, in terms of the time required for the animals to negotiate the runway between the two chambers, were converted to reciprocals (1/T) and subjected to statistical analysis. Paired comparison t-tests, Analysis of Variance, and trend analysis were employed for this purpose.

Section III

RESULTS

Table I shows the results of a paired comparison t-test between pre-drug saline performance and the effects of 4, 2 and 1 mg/kg doses of MMH. The probability values listed under the far-right column in this table indicate that 4 and 2 mg/kg doses significantly depressed performance, and suggest that 1 mg/kg had a similar but less-profound influence. These results are displayed graphically in figure 1. Whereas performance is slowed by all three of these very low-dose exposures to MMH, the effect is particularly increased between 2 and 4 mg/kg, suggesting that some threshold for the composite CNS effects of this drug is defined at this level.

Behavioral changes noted in association with altered performance were variable, depending upon dose. With 2 and 4 mg/kg doses the animals showed many of the symptoms described in previous behavioral studies of both MMH and UDMH (Fairchild and Sterman, 1964; Sterman et al., 1968). At 4 mg/kg doses, these included vomiting and a general emesis, confusion, salivation, piloerection, and alternating motor depression and hyperactivity. Most animals were unable to complete the day's test session. Testing was terminated by a refusal to perform further in the runway. Some animals displayed motor ataxia, whereas others showed relatively normal motor performance. Similarly, approximately half of the test group lost interest completely in the food reward and the other half drank normally. These symptoms were most dramatic 2-4 hours after injection. In only one instance of 15 test sessions at this dose did they culminate in a full-blown seizure episode. This animal, however, appeared to be recovered after 48 hours.

TABLE I

PAIRED COMPARISON t-TEST OF PRE-DRUG SALINE AND MMH DRUG PERFORMANCE MEANS (N=5)

	Means			
Dose	*Pre*	*Drug*	*t*	*p*
4 mg.	0.137	0.049	9.53	0.0006
2 mg.	0.158	0.143	6.67	0.0026
1 mg.	0.175	0.168	2.44	0.0710

Residual effects of 4 mg/kg doses were clearly and consistently detected 24 hours after administration. At 2 mg/kg doses, the primary symptoms were mild emesis and confusion. All animals completed testing, but behavior was often erratic and confused. Although vomiting occurred in two of the animals, food consumption was not altered at this dose. Performance, however, was frequently sluggish. At 1 mg/kg doses, no specific symptoms were detected; the animal's performance appeared normal and undisturbed. Several of the animals, however, seemed somewhat sensitive to handling and were very active. In spite of this essentially normal picture, behaviorally, statistical analysis showed decremental effects upon performance from 1 mg/kg doses of MMH.

A consideration of dose-time effects for these doses is shown in figure 2. In this analysis, drug performance is compared with saline control performance obtained 48 hours after administration

of MMH. Drug effects are noted within 30 minutes of injection at all doses, but are most profound with 4 mg/kg. The peak disturbance following 1 and 2 mg/kg doses occurred after 3 hours, with a trend towards recovery after 5 hours for 2 mg/kg, and an apparently complete recovery within 5 hours for 1 mg/kg. Although the depression of performance with 4 mg/kg was significant within 30 minutes, the decremental effect of this dose continued to mount throughout the 5-hour test period. Some oscillation was suggested by the tendency towards recovery after 2-3 hours in these data, but this tendency is imposible to evaluate within the scope of these experiments.

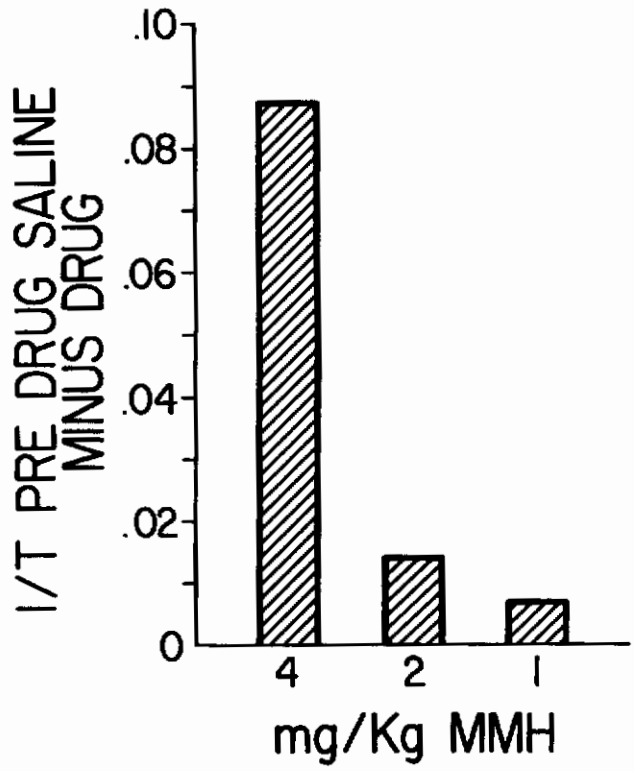

Figure 1. Differences in Runway Performance Time (in seconds) between Pre-drug Saline Control Tests and Tests of 4, 2 and 1 mg/kg MMH. Reciprocals (1/T) were taken to reduce the influence of extreme scores in the statistical analysis shown in Table I. Height of bars indicates the relative increase in time required to negotiate the runway. The range of performance times was 2-10 seconds. N=15 at each dose.

A comparison of saline control performance during the five pre-drug days and in the two control tests separating each drug test indicated a complex alteration of performance detectable for a prolonged period after MMH injection at these very low doses (figure 3). This comparison showed that 4 mg/kg doses reliably depressed control performance, whereas 2 and 1 mg/kg doses progressively increased the velocity of control performance. A trend analysis of the curve generated in figure 3, by these control data, provided a significant quadratic component ($F=36.5$) related to the 4 mg/kg doses and a significant linear component ($F=14.8$) associated with the sequence of 2 mg/kg and 1 mg/kg dose tests. Thus, drug effects continued to influence behavior for at least 24 hours following the test situation. It is unlikely that the two lower-dose effects are cumulative; rather, they indicate an increasing influence in the same direction with decreasing dose.

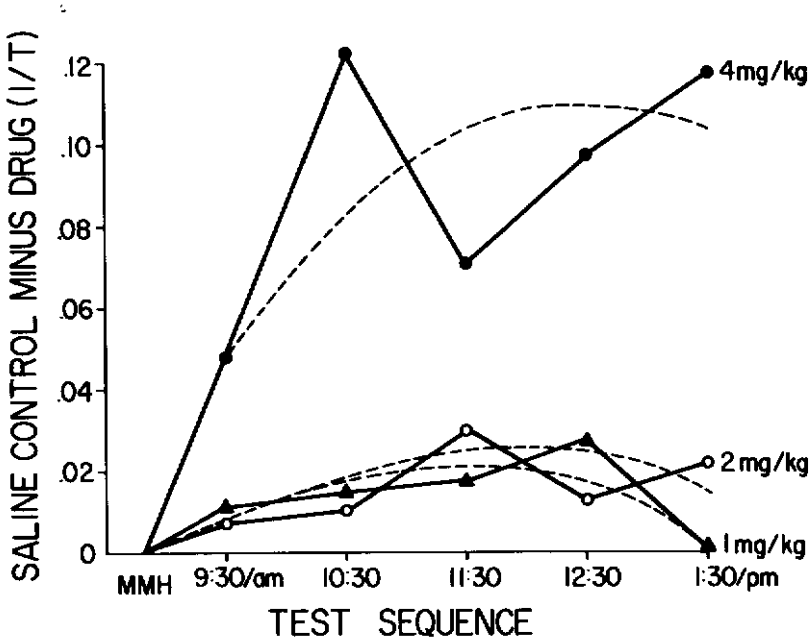

Figure 2. Mean differences in performance time reciprocals between three MMH doses and saline control values obtained 48 hours later are shown here as a function of order in the test sequence. Each point represents difference scores obtained from three replications run at each time slot for each dose. Estimated curves of best fit are shown superimposed over actual data points.

MEAN SALINE CONTROL PERFORMANCE
BEFORE AND DURING MMH DRUG TESTS

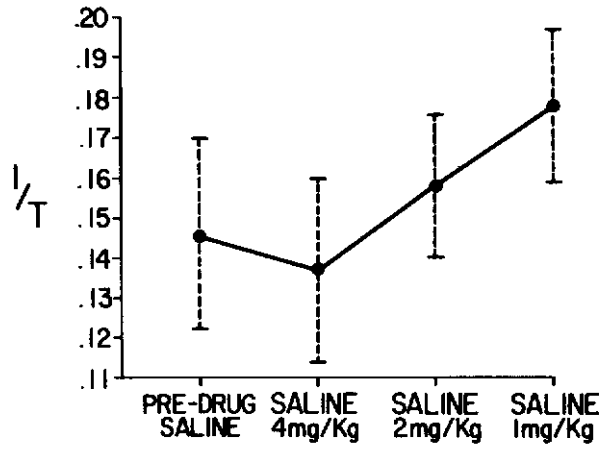

Figure 3. Comparison of mean performance time reciprocals obtained from 15 animals during saline control tests before and after MMH injection. Three replications of each dose were carried out in the sequence of 4, 2 and 1 mg/kg with saline control data obtained at 24 and 48-hour intervals between each drug test. Note depressed performance during saline tests after 4 mg/kg doses and increasingly enhanced performance during saline tests after 2 and 1 mg/kg doses, as compared with pre-drug saline tests. Trend analysis provided statistical verification of these effects.

Section IV

DISCUSSION

Monomethylhydrazine significantly interfered with locomotor performance in the cat at doses of 1, 2 and 4 mg/kg, administered intraperitoneally. Within 30 minutes of exposure and lasting for a succeeding 6-hour period, animals displayed a significant decrement in performance. This decrement was related to the appearance of some symptoms of toxicity at 2 and 4 mg/kg, including emesis, salivation, hyperactivity, and confusion. At 1 mg/kg, behavior was essentially normal in animals whose performance was generally slower. The disturbance was still clearly detectable after 24 hours in animals receiving 4 mg/kg doses. At 1 and 2 mg/kg, there was evidence of an enhancement of performance during tests obtained 24 hours after drug administration.

As indicated earlier, studies of UDMH under these same test circumstances had shown that low sub-convulsive doses of this compound (4 mg/kg and 8 mg/kg) actually enhanced runway performance at 270 and 320 minutes post-injection (Sterman and Fairchild, 1967). This was attributed to a cycling of central depression and excitation resulting from this drug and related in its period to the dose administered. These levels of UDMH produced cycles whose biphasic characteristics could be observed within the 5-hour period of drug evaluation.

It is possible that a similar process resulted from MMH exposure. However, because of the more potent effect of this compound on the CNS, resulting excitability cycles could have been longer in duration. With MMH, the curves shown in figure 3 indicated, after 5 hours, a complete recovery at 1 mg/kg doses, a partial recovery at 2 mg/kg, and no recovery at 4 mg/kg. These data suggest a different dose-time relationship than seen with low doses of UDMH. Performance data obtained 24 hours after MMH administration showed an opposite direction of effect for 4 mg/kg, as compared with 1 and 2 mg/kg. Altered performance 24 hours after MMH administration could, therefore, have reflected a continuing oscillation of excitability within the motor system, which affected performance differentially as a function of dose.

It is possible, however, that the three doses tested here merely had singular decremental influences differing, not only in magnitude, but also in duration. This interpretation, however, would not explain the specific shifts seen in post-drug saline control performance. These changes cannot be attributed to some systematic drift in baseline performance, since data obtained from these and many other animals over long periods of runway testing have established conclusively the stability of performance in this regard.

It is apparent from behavioral and neurophysiological studies that regulation in motor function is profoundly influenced by both UDMH and MMH. The delicate balance between proprioceptive afferent and motor efferent activity, which normally provides for graded motor discharge and the resulting motor coordination, is disturbed, most likely, due to the failure of inhibitory feedback. Studies with both of these compounds have shown that the suppression of motor activity can significantly delay or prevent the debilitating consequences of CNS seizures (Goff et al., 1967; Sterman et al., 1968).

It can be assumed, therefore, that the motor task required in runway performance had the opposite influence, namely to enhance the detrimental influences of MMH. The general occurrence of intense pre-dromal symptoms, and at least one full-blown seizure at the subconvulsive dose of 4 mg/kg tends to support this conclusion. Unfortunately, it appears that exposure to these compounds not only disrupts the performance of motor tasks but that, in turn, the performance of motor tasks facilitates the pathological process set in action by these compounds.

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13. ABSTRACT

Previous neurophysiological and behavioral studies of the toxic propellant UDMH have indicated that its subtle low-dose influences can be most effectively evaluated in the cat by reference to trained locomotor performance. To determine similar fundamental information in evaluating monomethylhydrazine (MMH), a related derivative of hydrazine, this same technique was employed. Cats were trained and tested in a special runway apparatus to provide a reliable indication of performance changes over a 6-hour period following the administration of 1, 2, and 4 mg/kg MMH. These low doses significantly altered locomotor performance, both during drug session testing and saline control testing carried out 24 hours later. Within 30 minutes after injection of all three doses of MMH, runway performance was depressed. At 2 and 4 mg/kg, this influence was profound and was associated with overt physiological symptoms of toxicity. A total disruption of performance occurred with 4 mg/kg doses when tested 2-5 hours after administration. Performance was still depressed after 24 hours following 4 mg/kg, but was actually facilitated at this same point following 1 and 2 mg/kg doses.

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