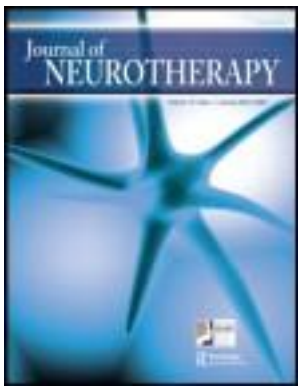

Journal of Neurotherapy: Investigations in Neuromodulation, Neurofeedback and Applied Neuroscience

Electroencephalographic and Behavioral Studies of Monomethyl Hydrazine Toxicity in the Cat

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Published online: 25 Nov 2010.

To cite this article: M. B. Sterman PhD , R. W. LoPresti PhD & M. D. Fairchild PhD (2010) Electroencephalographic and Behavioral Studies of Monomethyl Hydrazine Toxicity in the Cat, Journal of Neurotherapy: Investigations in Neuromodulation, Neurofeedback and Applied Neuroscience, 14:4, 293-300, DOI: 10.1080/10874208.2010.523367

To link to this article: http://dx.doi.org/10.1080/10874208.2010.523367

Journal of Neurotherapy, 14:293–300, 2010

ISSN: 1087-4208 print/1530-017X online
DOI: 10.1080/10874208.2010.523367

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PROLOG. During the 1960s the United States and the Soviet Union were engaged in an intense competition to develop the means for human travel into space. Because of physics and biology the requirements necessary to achieve this goal were shared by the two nations. As a result both were experimenting with high-thrust propellants and seeking protection for technicians and crews exposed to these toxic substances. Consisting mainly of hydrazine compounds, the fuels, shown through accidents in handling, proved to be convulsants at relatively low exposure doses via any route and fatal at higher doses. The U.S. Air Force initiated a scientific program to determine the biochemical mechanism of this toxicity and to search for protection and postexposure treatment in the case of accidental exposures. They also became particularly interested in the possible performance consequences of low-dose exposure in crew members while in flight.

Through a remarkable convergence of events, including the sudden death of a famous UCLA pharmacologist who had received a significant grant as a part of this Air Force program, my good friend and his pharmacologist postdoctoral student Dr. David Fairchild asked me to join him in taking over his laboratory and grant project. The timing could not have been more propitious, as my laboratory had just begun to explore the possibility of utilizing operant conditioning feedback to train animals in the voluntary control of EEG rhythms. Specifically, we had discovered an EEG rhythm in the sensorimotor cortex of cats that was clearly correlated with the suppression of motor excitability, which we named the ''Sensorimotor Rhythm,'' or SMR. We found that training this rhythm brought the motor suppression response under experimental control. As a result we could objectively confirm reductions in muscle tone, reflex amplitudes, and cellular discharge in motor pathways that were strictly associated with the learned EEG response. It was only a matter of time before some of these animals became subjects in our studies of hydrazine effects on the EEG and behavior and, most significantly, of SMR training effects on the convulsive outcomes of hydrazine exposure. The rest, as they say, is history.

The reports from this work were the property of the U.S. Air Force and were not available to the public. The following reproduced paper was the first to report clinical effects with EEG operant conditioning, which in this case were anticonvulsant.

FOREWORD. This research was performed under Contract AF33(615)-2822, by the Department of Anatomy and the Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024. This work was performed in support of Project 6302, ''Toxic Hazards of Propellants and Materials,'' Task 630202, ''Pharmacology and Biochemistry,'' from January 1967 to November 1968 for the Toxicology Branch, Toxic Hazards Division,

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Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

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 Psychology, UCLA, R. W. LoPresti, PhD, Post-doctoral Fellow, Brain Research Institute, UCLA, and M. D. Fairchild, PhD, Research Pharmacologist, Veterans Administration Hospital, Long Beach, California, and Assistant Professor, Department of Pharmacology, UCLA. Kenneth C. Back, PhD, was contract monitor for the Aerospace Medical Research Laboratories.

This research was sponsored by Aerospace Medical Laboratory, Aerospace Medical Division, Air Force System Command, Wright-Patterson Air Force Base, Ohio.

This technical report has been reviewed and is approved.

Wayne H. McCandless
Technical Director
Biomedical Laboratory
Aerospace Medical Research Laboratories

ABSTRACT. The toxicity of monomethyl hydrazine (MMH) administered intraperitoneally in the cat was studied by reference to behavioral and neurophysiological indices. The acute lethal toxicity value (LD_{50}) for MMH was established at 15 mg/kg, and the convulsive toxicity value (CD_{50}) at 7 m/kg. Doses of 18, 9, and 5 mg/kg were then studied systematically in an effort to classify lethal, convulsive, and subconvulsive symptoms. For these doses, a preconvulsive syndrome was described involving recurrent and sustained symptoms, including vomiting, panting, rapid respiration, viscous salivation, hyperactivity, and subcortical seizure activity. The onset latency of these symptoms was directly related to dose. Several lines of evidence suggested at least a partial independence between biochemical and neurophysiological events responsible, on one hand, for convulsions and, on the other, for this preconvulsive syndrome. Convulsions were specifically delayed or prevented in animals trained to suppress movement through the use of a special EEG conditioning technique.

KEYWORDS. Operant conditioning, sensory motor rhythm, monomethyl hydrazine, seizures, convulsion

INTRODUCTION

Valuable information concerning the central nervous system (CNS) and behavioral effects of the toxic propellant 1–1, dimethylhydrazine (UDMH) has been gained from a battery of neurophysiological and performance tests developed in our laboratory. Current studies represent the initial stages of a similar evaluation of monomethyl hydrazine (MMH). The performance studies have been reported in a separate communication (Sterman, Fairchild, & VanTwyver, 1968). Evaluation of changes in cerebral neuroelectric responses is scheduled for the near future.

The objectives of the present experiments were twofold. Our primary goal was to determine the acute, convulsive, and subconvulsive toxicity values for MMH administered intraperitoneally in the cat. The acute toxicity LD_{50} values for intravenous administration in mice and rats is approximately 33 mg/kg, and for dogs 12 mg/kg ("Physiological & Pharmacological," 1955). We were interested in this and more information with regard to the cat. Specifically, we wished to determine the range of characteristic behavioral and electroencephalographic effects of MMH toxicity and to classify their order of occurrence as a function of dose.

Our secondary objective was to test a hypothesis derived from our previous studies of UDMH. It was proposed that learned

inhibition of motor behavior could significantly delay or prevent the occurrence of CNS seizure activity in cats exposed to convulsive doses of MMH.

Neurophysiological studies of UDMH have shown that convulsive doses result in a graded increase in the excitability of cortical neurons responding to afferent sensory bombardment (Goff, Allison, Matsumiya, Sterman, & Fairchild, 1967). This process was attributed to a progressive failure of the inhibitory interneurons, which normally modulate the excitability of axodendritic synapses. In this regard, UDMH appears to act like strychnine by blocking the release of inhibitory transmitter substances from cortical interneurons (Eccles, 1963). Studies of seizure thresholds in limbic and other subcortical structures indicated a similar increase in excitability at this level (Fairchild & Sterman, 1964). The picture that emerged with UDMH was a gradual increase in sensory excitability coupled with a gradual decrease in motor inhibition. It was proposed that a sensorimotor positive feedback condition thereby resulted and led inevitably, at doses of 20 mg/kg and above, to central seizures and convulsions.

These findings suggested that an enhancement of the central process of motor inhibition might effectively delay the occurrence of seizures by interruption of this situation. Studies of paralyzed cat preparations showed that the absence of proprioceptive sensory feedback significantly delayed the occurrence of electrocortical seizure activity (Goff et al., 1967). Paralysis, however, is an impractical therapy for this condition. An approach to the problem in normal animals would be of greater value. Assuming that a similar process occurs with MMH exposure, this drug would be administered to normal, behaving cats previously trained to suppress movement by feedback reward for a specific EEG rhythm.

METHODS

Pilot studies of MMH administered intraperitoneally in the cat established the CD_{50} at 7 mg/kg and the LD_{50} at approximately 15 mg/kg. In order, therefore, to determine lethal, convulsive and subconvulsive symptoms, animals were studied systematically with doses of 18, 9, and 5 mg/kg.

Eight adult cats, weighing 2.5 to 5 kg each, were surgically prepared for chronic electrophysiological and behavioral study. Under deep barbiturate (Nembutal) anesthesia, the animals were placed in a sterotaxic instrument and small burr holes drilled in the skull over sensory and motor cortex and at appropriate locations for insertion of deep recording electrodes. Small stainless steel screws (1/16-in. diameter) were threaded into the skull for cortical recording, and pairs of 10-mil. insulated stainless steel wires, separated by 1 mm, were lowered sterotaxically into a variety of subcortial nuclei. Particular emphasis was placed on thalamic sensory and motor structures and on pathways and way-stations in pyramidal and extrapyramidal motor systems. Leads from these electrodes were connected to a 20-contact plug and the entire assembly fixed to that cat's skull with a crown of dental cement. Wound margins were sutured, and the animals were allowed to recover with careful postoperative care.

After recovery, 8-hr recordings were obtained in each animal from both cortical and deep electrodes with simultaneous observations of behavior. Animals were then administered 5 (subconvulsive), 9 (convulsive), and/or 18 (lethal) mg/kg doses of MMH by intraperitoneal injection. Some animals received all three doses in ascending order, and others received only one or two doses. A total of six animals received the 9 mg/kg dose. Three of these animals were from the group prepared for these studies, and three were drawn from a different experiment. This experiment was concerned with the neurophysiology of behavioral inhibition.

Our laboratory has described a specific EEG rhythm that can be recorded over the sensorimotor cortex of the cat whenever spontaneous or trained suppression of movement occurs (Roth, Sterman, & Clemente, 1967). This "sensorimotor rhythm," or SMR, appears to be a direct indicator of the operation of a central process of motor

inhibition. The most profound evidence for this is derived from experiments in which this EEG rhythm is specifically reinforced by milk in hungry animals. A conditioned EEG response develops in association, behaviorally, with a total suppression of movement (Sterman & Wyrwicka, 1967; Wyrwicka & Sterman, 1968).

Thus, the three animals drawn from these experiments had, several months earlier, received extensive SMR conditioning experience. This training has been found to profoundly influence EEG and motor patterns over long periods of time (Sterman, Wyrwicka, & Roth, 1969).

RESULTS

The characteristic sequence of events attendant upon the intraperitoneal administration of MMH in the cat consisted initially of an abrupt period of restlessness followed shortly by vomiting. This was followed, in turn, by a succession of definitive symptoms with both recurrent and continuous manifestations. The recurrent symptoms included persistent vocalization motor hyperactivity, and subcortical seizure discharge. Vomiting and defecation were seen often during these periods. During the intervals between these recurrent episodes the animals were extremely alert but inactive. They appeared distressed and unwilling to move. The continuous manifestations were EEG desynchronization, panting, rapid respiration, and viscous salivation. At 9 and 18 mg/kg the characteristic sequence was terminated by wild escape behavior leading directly into convulsions. These symptoms of MMH toxicity were dependant in degree and in latency of onset upon the dose administered (Table 1). It can be seen from Table 1 that the impact of the drug, at all three dose levels, was registered within the first 30 min following injection.

5 mg/kg

In addition to the symptoms described in Table 1, the most striking feature of this subconvulsive dose was its effect upon the electrical activity of the brain. Following the occurrence of vomiting, and for a continuous period of 4 to 7 hr thereafter, the EEG showed a sustained pattern of low-voltage fast (desynchronized) activity characteristic of extreme attention and alertness (Figure 1). Although the animal showed frequent alternation between active and quiet behavior, there was no evidence of relaxation or sleep. Reference to data from control animals (Sterman, Knauss, Lehmann, & Clemente, 1965) indicated that such extended periods of desynchronized EEG activity and the lack of relaxation or sleep were abnormal.

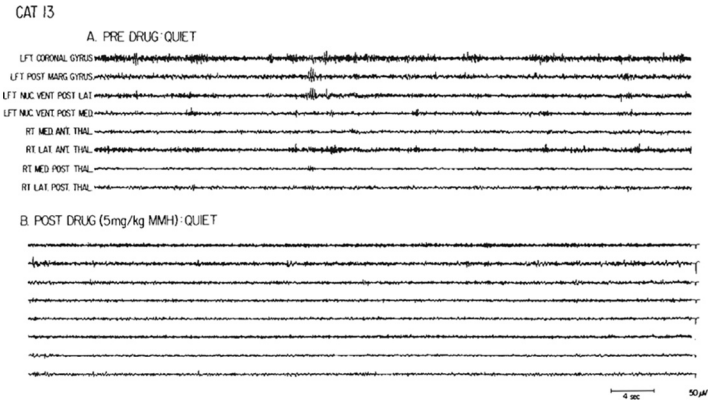
At this dose there was no evidence of seizure activity in the cerebral cortex. However, subcortical leads in several nuclei of the thalamus showed periodic local seizure discharge (Figure 2). These were accompanied often by strange motionless postures, with the animal staring off into the distance or

TABLE 1. Characteristic symptoms of MMH toxicity: relationship of dose to onset latency (Time in Minutes).

Symptoms	5 mg/kg (N = 3)	9 mg/kg (N = 3)	18 mg/kg (N = 3)
Restlessness	28.0 ± 3.6	10.0 ± 11.3	5.3 ± 0.6
Vomiting	30.3 ± 2.1	22.0 ± 5.0	7.0 ± 2.6
Vocalization	38.3 ± 7.2	30.5 ± 1.5	21.0 ± 3.0
Panting	52.5 ± 4.5	35.7 ± 2.1	24.3 ± 5.0
Salivation	62.5 ± 4.5	43.5 ± 13.5	25.5 ± 8.5
Hyperactivity	67.3 ± 15.0	40.3 ± 10.3	24.3 ± 3.0
Subcortical Seizure	145.0 ± 77.5	*	–
Escape Behavior	–	54.0 ± 13.5	28.0 ± 1.7
Convulsions	–	54.7 ± 13.1	29.0 ± 1.7

*Unclear.

FIGURE 1. Typical segments of cortical and subcortical electrical activity recordings in a quiet animal before and 1 hr after the intraperitoneal injection of 5 mg/kg MMH. Note the dramatic absence of any slow-wave activity in the latter.

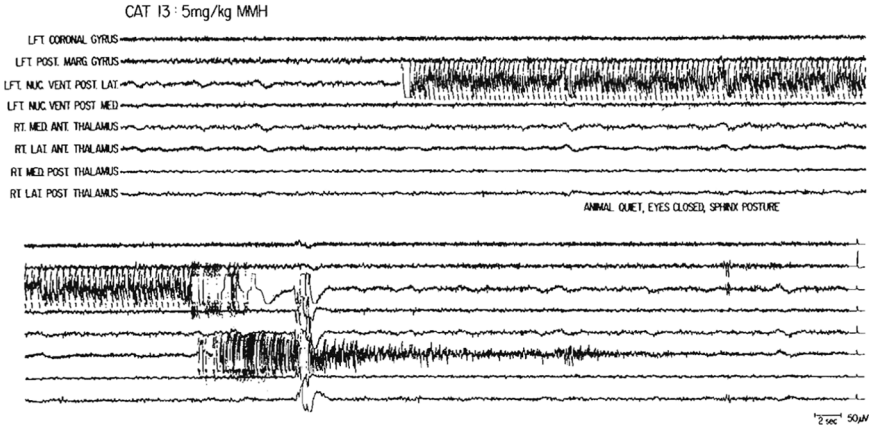


gazing intently at its own lifted paw. Occasionally, this was followed by a brief period of hyperactive behavior. After a period of 7 hr, both EEG and behavior patterns appeared to be normal once again.

9 mglkg

The pattern of symptoms associated with this dose of MMH was identical to that just described for 5 mg/kg; however, their latency of onset and magnitude were altered (Table 1). The events which constitute the characteristic symptoms of toxicity were significantly intensified and appeared with approximately two thirds the delay seen at 5 mg/kg. They included, additionally, escape behavior and convulsions. With 9 mg/kg the animals displayed, in addition, a moderate ataxia together with, in later stages, a persistent generalized motor tremor. Barbiturate anesthesia was typically administered after convulsions, but the post-ictal period was studied in several animals without this treatment. Recurrent convulsions and

FIGURE 2. Spontaneous subcortical seizure discharge in a quiescent animal after administration of 5 mg/kg MMH. In this instance the seizure discharge is seen to shift from the left ventrolateral thalamus to the right anterolateral thalamus. During the latter phase the animal was seen to raise his forelimb slowly and remain immobile for several seconds. Such subcortical seizure patterns were not always distinguishable because of the movement artifacts caused by accompanying motor activity.



protracted symptoms of toxicity persisted for up to 24 hr.

18 mg/kg

With the exception of vomiting, which occurred within the first 10 min following the injections at this dose, acute toxic symptoms appeared abruptly and continuously after approximately 20 min. Ataxia was a consistent feature of the pattern seen at this dose. These symptoms were intense and sustained until convulsions, which occurred with remarkable consistency after 27 to 31 min. The course of events was so rapid that several of the characteristic symptoms appeared only briefly, or not at all, in advance of the seizures. In animals not treated with barbiturate convulsions occurred repeatedly within intervals of approximately 5 min. In some instances seizure discharge was initiated in subcortical sites and propagated rapidly throughout the brain (Figure 3). Five or six tonic-clonic convulsions occurred in rapid succession and were followed by death within 1 hr after injection.

A comparison between untrained cats and animals with SMR training at doses of 9 mg/kg showed a dramatic difference in the onset latency of convulsions. Whereas the latency of toxic symptoms, such as vomiting, panting, and so on, did not differ reliably between these groups, escape behavior and convulsions were delayed substantially (Table 2). EEG recordings from pretrained animals showed a dramatic enhancement of the SMR when compared to records obtained from untrained animals (Figure 4). This enhanced SMR activity was associated with the delay in development of general CNS seizures. It is apparent, therefore, that learned facilitation of motor inhibition through techniques directly influencing its central representation in the brain can effectively delay, or perhaps prevent, the incapacitating consequences of exposure to convulsive doses of MMH.

DISCUSSION

These studies have established the acute toxicity LD_{50} value for intraperitoneal administration of MMH in cats at 15 mg/kg. The CD_{50} value was 7 mg/kg. Characteristic symptoms of toxicity at lethal, convulsive, and subconvulsive doses included vomiting, vocalization, panting and rapid respiration, viscous salivation, and motor hyperactivity. At the subconvulsive dose tested here, subcortical seizures were an additional feature appearing usually 1 to 2 hr after administration. With convulsive and lethal doses escape behavior and generalized CNS seizures terminated this characteristic sequence of symptoms. The onset latency of these symptoms was determined also, and found to be directly related to dose in a near linear fashion. Moreover, the latency values obtained were remarkably stable, particularly at the convulsive and lethal dose levels.

It was interesting to note that subconvulsive exposure to MMH induced the entire sequence of symptoms short of convulsions themselves, and did so within the 1st hr following administration. In this respect MMH differed in kind as well as degree from

FIGURE 3. Generalized CNS seizure discharge associated with convulsions following the administration of 18 mg/kg MMH. Although diffuse spiking activity was seen preceding overt convulsions, the onset of generalized seizures was occasionally initiated at subcortical sites. Note post-ictal depression following seizure discharge.

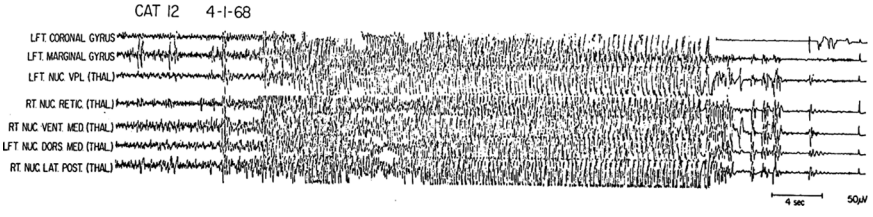


TABLE 2. Comparison of onset latency of MMH toxicity symptoms with 9 mg/kg in untrained cats and cats given motor inhibitory training (Time in Minutes).

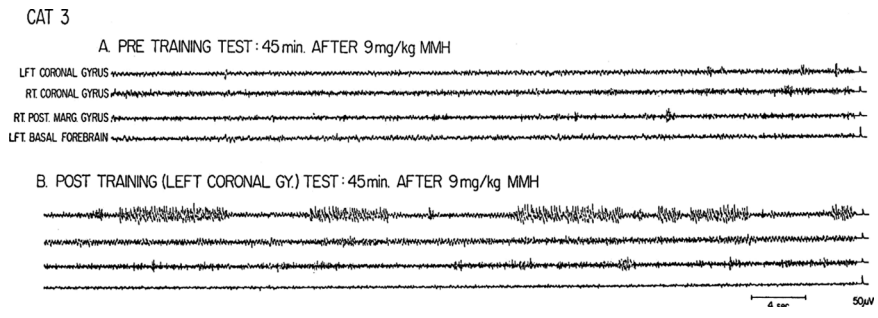
Symptoms	Mean + S.D. Untrained (N = 3)	Mean + S.D. SMR Trained (N = 3)
Restlessness	10.0 ± 11.3	17.0 ± 12.5
Vomiting	22.0 ± 5.0	19.3 ± 15.2
Vocalization	30.5 ± 1.5	33.3 ± 8.5
Panting	35.7 ± 2.1	39.0 ± 11.8
Salivation	43.5 ± 13.5	33.5 ± 4.5
Hyperactivity	40.3 ± 10.3	*
Subcortical Seizures	*	*
Escape Behavior	54.0 ± 13.5	164.5 ± 74.1
Convulsions	54.7 ± 13.1 (range = 40–67)	167.6 ± 74.1 (range = 80–220)

*Unclear.

UDMH (unsymmetrical di-methyl hydrazine). With UDMH a similar sequence of symptoms was recorded in the cat (Fairchild & Sterman, 1964). With convulsive exposures their onset latency was similarly related to dose, although these latencies were more than twice those for MMH. However, unlike MMH, subconvulsive doses of UDMH produced none of the preconvulsive symptoms and deleterious effects could only be disclosed with sensitive behavioral testing. These related toxic compounds probably initiate the same chain of biochemical and neurophysiological events that lead to convulsions. However, preconvulsive symptoms can occur without eventual convulsions at 5 mg/kg doses of MMH. Moreover, several animals tested with 18 mg/kg MMH showed only vomiting, hyperactivity, and seizures without vocalization, panting, or salivation. These facts suggest that some of the preconvulsive symptoms may be at least partially independent from the convulsions themselves.

From the standpoint of protection, perhaps the most interesting finding of these studies was the significant delay of convulsions resulting from the learned suppression of movement. It may be recalled that the animals used in this phase of the experiment had not received SMR training for over 3 months prior to the drug tests. In recent pilot studies we have specifically trained animals for several weeks following tests of convulsive doses of MMH and then retested immediately afterward. These animals showed *no* spontaneous convulsions in spite of the fact that the preconvulsive syndrome occurred intact. It appears, therefore, that this training can provide an effective protection against the most devastating consequences of this compound. It should be stressed, however, that this training involved a more or less direct intervention into the central nervous system,

FIGURE 4. Comparison of sensorimotor rhythm activity from left coronal gyrus following administration of MMH in a cat before and after conditioning of this rhythm. In the pretraining test this animal showed convulsions 57 min after injection, whereas the posttraining test yielded convulsions 220 min after injection.



as it was dependant upon reinforcement of a central electrical event and not merely motor behavior. We are presently seeking similar EEG phenomena in man.

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