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EFFECTS OF CNS MANIPULATIONS ON SEIZURE LATENCY FOLLOWING MONOMETHYLHYDRAZINE ADMINISTRATION IN THE CAT

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FOR THE COMMANDER

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The objective of this study was to determine the influence of intracranial electrode placement upon the seizure response to monomethylhydrazine, and to replicate previous findings on the effects of EEG operant conditioning on this response. Thirty cats were studied, 10 in each of 3 different experimental groups, all of which were exposed to 10 mg/kg doses of intraperitoneally administered monomethylhydrazine. The dependent variable was latency in minutes to generalized motor seizures. The three experimental groups were: (1) an

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered) unoperated group, (2) an operated group with cortical and subcortical electrodes, and (3) an operated group as in (2), but provided additionally with sensorimotor rhythm (SMR) EEG operant conditioning. The operated group without EEG conditioning showed a significantly reduced and more stable latency to seizures when compared to the other two groups. These findings suggested that (1) some aspect of the procedure associated with central nervous system electrode implantation increased susceptibility to MMH-induced seizures, (2) unoperated animals had individual differences in seizure susceptibility, but were significantly more resistant to MMH toxicity than operated animals, and (3) SMR-trained operated animals had individual differences in response to training, but were also more resistant to MMH toxicity.

PREFACE

This research was initiated by the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory, under Project 7163. Experiments were performed under Contract AF F33615-72-C-1855 by the School of Medicine, University of California, Los Angeles, California 90024.

The experiments were conducted by M. B. Sterman, PhD, of the Veterans Administration Hospital, Sepulveda, California, S. J. Goodman, MD, of Harbor General Hospital, Torrance, California 90509, and M. D. Fairchild, PhD, of the Veterans Administration Hospital, Long Beach, California 90804. Kenneth C. Back, PhD, was contract monitor for the Aerospace Medical Research Laboratory.

INTRODUCTION

In a previous report, directed to a determination of acute convulsive and subconvulsive toxicity values for intraperitoneally administered monomethylhydrazine (MMH) in the cat, we established a CD_{50} value of 7 mg/kg and found that a 9 mg/kg dose of this compound produced motor seizures reliably after a mean latency of 54.7 ± 13.1 min (Sterman et al., 1969). We also reported that in animals provided with EEG operant conditioning of a 12-16 cps sensorimotor cortical rhythm, the sensorimotor rhythm or SMR, which is associated with movement suppression, showed a greater tolerance to MMH by virtue of a mean seizure latency after 9 mg/kg of 167.6 ± 74.1 min. This finding was consistent with neurophysiologically demonstrated motor inhibitory influences correlated with the SMR (Wyrwicka and Sterman, 1968), and with the observation that paralyzed cats showed significantly delayed electroencephalographic seizure latencies following exposure to MMH (Sterman et al., 1972a).

All of the cats employed in this and related studies, however, were prepared surgically with indwelling cortical and subcortical electrodes implanted to provide for EEG monitoring in operant conditioning and seizure evaluation. In subsequent studies of MMH seizure latencies, we occasionally utilized unoperated animals as convenient control subjects. Much to our surprise, we consistently observed prolonged seizure latencies in these animals when compared with operated experimental animals. This observation suggested that the cranial invasion associated with experimental placement of electrodes was exerting a potentially significant influence upon seizure susceptibility in the cat.

The objective of the present study was to test this possibility. A systematic comparison of seizure latencies following administration of a convulsive dose of MMH was obtained from cats with and without brain electrode implants. Moreover, the previously observed effects of SMR operant conditioning were extended and re-examined in relation to this comparison.

METHOD

Thirty adult male or female cats weighing between 2.5 - 5.5 kg were employed in this study. Three independent experimental groups of 10 animals each were established and labeled as (1) No Implant Group, (2) Control Implant Group, and (3) SMR Implant Group, respectively. These groups will be described in detail below.

The seizure latency characteristics of 10 mg/kg MMH, intraperitoneal injection, were tested identically in all 3 groups. This procedure consisted of weighing the animal for dose determination, placing it in a standard observation chamber for a 1-hour adaptation period, administering MMH, and determining subsequent latency to motor seizures in the chamber through continuous visual observation. Barbiturate was subsequently injected during the tonic phase of seizures, and all animals were allowed to recover.

The chamber measured 2 ft x 2 ft x 2.5 ft and provided sound attenuation, one-way viewing and positive ventilation. A microdot cable, suspended from a slip-ring and counterweight system, was attached to a receptacle on the heads of some animals as described below. The MMH, obtained from Matheson, Coleman and Bell (mol wt = 46.07, sp gr = 0.852 g/ml), was diluted to 20 mg/ml in normal saline solution. Latency to motor seizures was measured as the time, in minutes post-injection, to the onset of generalized tonic-clonic convulsions.

The No-Implant Group consisted of 10 healthy animals obtained from the UCLA Animal Care Facility. All of these cats had been processed through a standard 30-day period of isolation quarantine, and were delivered to our laboratory free of infection or disease. At the time of testing, these animals were merely removed from their home cage and evaluated in the test chamber as described above.

The Control-Implant Group was heterogeneous in makeup, consisting of 10 animals drawn from several other ongoing experiments in the laboratory. All of these animals had undergone surgery for placement of cortical and subcortical recording electrodes. All had indwelling stainless steel screw electrodes (1/8 in. tip diameter) threaded into the skull over sensorimotor cortex, at least some of which penetrated the dura. All had stereotaxically placed subcortical wire electrodes (stainless steel 26-gauge wire, insulated with epoxy except at the tips) in several structures including at least either thalamus, hypothalamus or both. Five of these animals had been utilized as noncontingent controls in EEG operant conditioning studies. The remaining 5 had participated in sleep-recording and evoked-potential studies. In all of these animals the implanted thalamic electrodes had been stimulated either at the time of surgery, to confirm proper localization, or postoperatively in somatosensory evoked response studies. All animals had extensive exposure to the recording chamber situation. On test days each animal was placed in the chamber for a 1-hour adaptation period and then injected with 10 mg/kg MMH intraperitoneally.

The SMR Implant Group consisted of 10 animals surgically prepared as above with cortical screw electrodes over sensorimotor cortex and subcortical wire electrodes placed stereotaxically into thalamus, hypothalamus or both. In most instances, these electrodes were situated in ventrobasal thalamus and posterolateral hypothalamus. This was the case also for at least half of the Control Implant Group animals.

EEG operant conditioning consisted of providing contingent food or positive brain stimulation reward following criterion production of sensorimotor rhythm activity. The required response was a 1-second train of 12-16 cps rhythmic activity from sensorimotor cortex at a voltage of at least 100% above baseline EEG amplitude. Six of the animals were rewarded with liquid food dispensed by a dip-cup mechanism in the chamber wall, and 4 were rewarded with 1-second trains delivered to posterolateral hypothalamus (the median forebrain bundle) at 4-8 volts, 0.6 msec duration, and a frequency of 250 Hz. The details of these procedures are described elsewhere (Wyrwicka and Sterman, 1968; Sterman et al., 1972b). These animals received 3 60-min training sessions per week over a total training period of 3 months. Four of these animals also participated in concurrent somatosensory evoked potential evaluation, in relation to a separate study.

RESULTS

The behavioral and physiological response patterns following exposure at convulsive doses to MMH have been described in detail previously (Sterman et al., 1969). Behaviorally, the pattern observed usually consisted of a characteristic sequence of emesis, vocalization, hyperventilation, salivation and hyperactivity, culminating in generalized tonic-clonic and myoclonic seizures. In the present study, a similar pattern was observed to precede seizures induced by exposure to 10 mg/kg doses of MMH. The mean latencies for each of these signs, excluding motor seizures, were essentially identical in all 3 groups.

Nine of the 10 cats in the No Implant Group showed this complete sequence. The remaining animal demonstrated the entire pattern, but did not experience a motor seizure during 6 hours of continuous observation. For the sake of statistical comparison, this animal was assigned a seizure latency of 290 min, which corresponded to the longest latency observed among the other animals in this group. All animals in the Control Implant Group had motor seizures, whereas only 9 of the 10 animals in the SMR Implant Group demonstrated motor seizures. A similar numerical adjustment was made in the latter group as in the No Implant Group.

The mean latency to motor seizures, together with standard deviations for each of the 3 experimental groups, is shown in Figure 1. The Control Implant Group had the shortest and most stable latency of the 3. In comparison to the mean value of 59.3 min for this group, both of the other groups showed significantly prolonged and more variable latencies. An analysis of variance test of these data indicated significant differences between means (f = 10.502, p < .001). Individual t-tests showed that the Control Implant Group had significantly shorter latencies than either the No Implant (t = 4.317, p < .01) or SMR Implant (t = 3.181, p < .01) animals. No significant difference was indicated between the No Implant and SMR Implant Groups (t = 1.709). Moreover, the various subgroups within the Control Implant Group (i.e., animals with noncontingent operant conditioning experience vs others) and SMR Implant Group (i.e., food vs brain stimulation reward) showed no marked differences from one another.

DISCUSSION

Hydrazine has proven to be an unusual convulsant by virtue of the characteristic long latency to seizures following exposure to its methylated derivatives. This feature, however, provides a useful experimental measure of seizure susceptibility, since it is related both to the dose and configuration of derivative compounds (Sterman et al., 1969; Back et al., 1970). Thus, the finding reported here that chronic implantation of intracranial electrodes reduced motor seizure latency leads to the conclusion that such experimental manipulations can significantly increase seizure susceptibility.

While it is impossible to determine any specific anatomical mediation of this effect, because of the heterogeneous makeup of the Control Implant Group, it appears that this may not be an important consideration by virtue of that very fact. This conclusion is supported further by the unique stability of seizure latencies in these animals relative to the other groups.

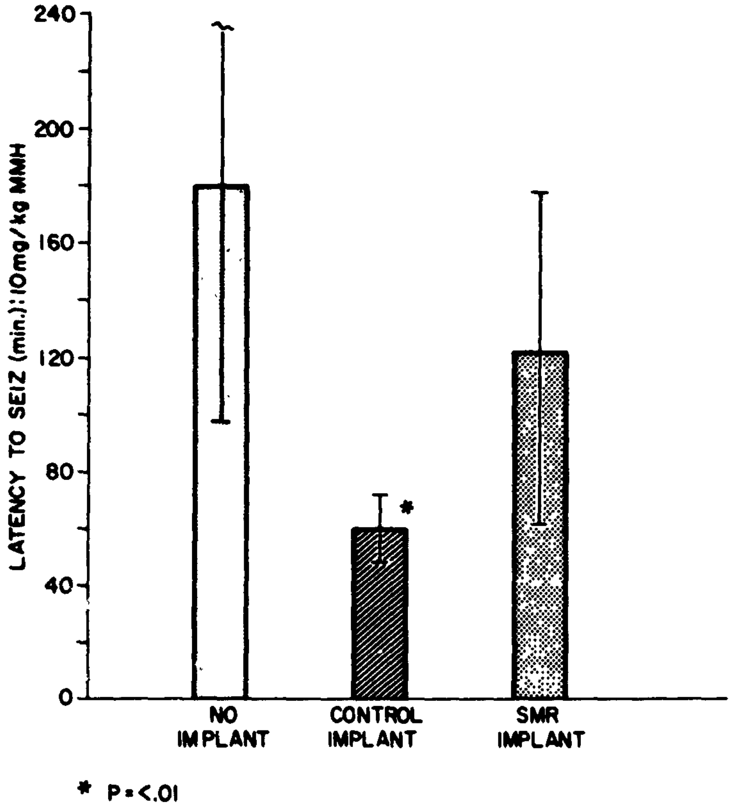

Figure 1. Graphic representation of the mean and standard deviation for latencies to the onset of motor seizures following 10 mg/kg dose (I.P.) of MMH is shown here for the 3 experimental groups defined in text. Note that the Control Implant Group showed a statistically significant reduction in latency when compared to the other two groups. The standard deviation of motor seizure latencies for this group was reduced markedly also.

It is interesting to note, further, that the mean latency value of 59.3 min found here was very similar to the mean value of 54.7 min reported from a smaller group of animals studied in 1969 (Sterman et al.). Electrodes had been placed in sensorimotor cortex and in thalamus and/or hypothalamus in all of these animals. This fact is consistent at least with the well-documented seizure inducing influence of damage to these structures resulting from traumatic, neoplastic or vascular lesions, or from chemical irritation. It is possible also that a subclinical inflammatory process or hypoxia resulting from exposure and interruption of the dura contributed to the increased susceptibility demonstrated by operated animals. It should be pointed out in this regard that these animals had been studied for periods ranging from several months to over 1 year and had shown no evidence of infection or overt cerebral abnormality.

Unoperated animals had the longest mean latency to motor seizures as a group, but also showed much greater variability than the Control Implant animals. This observation appears to reflect marked individual differences in susceptibility to MMH. It would be interesting to sort animals by reference to this parameter and to determine its reliability. Subsequent studies could examine possible genetic, biochemical or other physiological variables associated with this response dimension.

Finally, the significantly prolonged latency to motor seizures obtained in operated animals provided with SMR feedback training once again indicated a reduced seizure susceptibility resulting from this procedure. Interesting also is the fact that this group showed a variability in latency similar to the unoperated animals. Individual differences in response to training are suggested by this observation, a fact noted also in SMR studies with human epileptics (Sterman et al., 1974; Seifert and Lubar, 1975). If the operated animal represents an experimental model of increased susceptibility to MMH-induced seizures, then the results obtained indicate that therapeutic benefit is indeed derived by the method of EEG feedback training.