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BEHAVIORAL AND NEUROPHYSIOLOGICAL STUDIES OF UMDH IN THE CAT

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FOREWORD

This study was initiated by Project TORES (Toxicology Research) under the sponsorship of the Advanced Research Projects Agency, DOD, and procured by the U.S. Army Chemical Center, Maryland, as Research Grant Number DA-CML-18-108-C-51. Upon termination of Project TORES, procurement and administrative functions were transferred to the Aerospace Medical Division, Brooks Air Force Base, Texas. Technical monitorship was delegated to the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. The research was performed from May 1962 to July 1964, in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry." Drs. M. D. Fairchild and M. B. Sterman were the principal investigators for the Brain Research Institute, Center for the Health Sciences, University of California at Los Angeles, and Dr. K. C. Back was the contract monitor for the Aerospace Medical Research Laboratories.

The authors are grateful to Mr. H. Dubkin who was responsible for the construction of all apparatus described and for the data collection, and to Miss Joan Evans, Miss Edna Strom, Mrs. Maureen Carlisle and Miss Judy Parks for their able assistance.

This technical report has been reviewed and is approved.

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ABSTRACT

Studies concerning the behavioral and neurophysiological actions of UDMH in cats were carried out employing three separate experimental approaches: A) Collection of behavioral and electrophysiological data from animals prepared with indwelling recording electrodes and isolated in a controlled environment after various doses of UDMH, B) Determination of thresholds for seizure induction by electrical stimulation of the hippocampus in unanesthetized, but immobilized "acute" preparations, and C) Development of a unique behavioral apparatus which provides for the evaluation of subconvulsive doses of UDMH in relation to specific CNS regulatory mechanisms. The latter experiment has not yet reached the stage of UDMH administration and testing. The first set of experiments disclosed a direct relationship between dose and time of onset of characteristic behavioral and EEG signs preceding convulsions. The acute experiments indicate an initial increase in ventral hippocampal seizure threshold after administration of low doses of UDMH. This change is seen for the first two hours post-injection and may be related to the initial lethargy and delay in seizure occurrence characteristic of UDMH exposure.

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OF UDMH IN THE CAT

I. EEG and Behavioral Signs of UDMH Poisoning

A) Introduction

The objective of these experiments was to determine characteristic behavioral patterns, and their course of onset, following the intraperitoneal injection of convulsive and subconvulsive doses of UDMH. We then wished to relate associated electrical activity recorded from chronically indwelling electrodes placed on the cerebral cortex, as well as into various subcortical structures, to these behavioral manifestations. Some idea of the order and nature of the CNS changes produced by UDMH could, thus, be obtained.

B) Methods

Six male and female adult cats were surgically prepared for chronic CNS recording. The animals were deeply anesthetized with sodium pentobarbital and placed in a Kopf stereotaxic instrument. The calvarium was exposed, and small (PK-type A) stainless steel screws were placed into the calvarium over frontal, parietal, and occipital lobes of the cerebral cortex. Electrodes (bi-polar stainless steel) were then lowered stereotaxically into specific subcortical structures through small holes drilled into the overlying calvarium. Leads connected to both cortical and subcortical electrodes were brought to a Winchester plug, which was insulated and fixed to the skull with dental cement. The wound flaps were closed around the plug assembly and the animal was allowed to recover.

Several weeks later, the animal was placed in a large Lehigh Valley Electronics behavior observation chamber (Type 1317). This chamber was provided with internal lighting and ventilation. Its one-way mirror arrangement and sound attenuating properties allowed for the undisturbed observation of the experimental animal. A multistrand cable was attached to the Winchester plug mounting, and led into a six channel Grass III electroencephalograph.

Control observations were performed prior to all experimental injections. These observations consisted of continuous recording of EEG patterns with simultaneous notation of behavior. Control injections of normal saline solution were given to all animals during the course of this study. Experimental injections of 10, 20, 40, 60 and 120 mg/kg UDMH* were administered in a random order and observation continued until seizure and convulsion were observed, or until 4 hours of data had been obtained. Animals demonstrating seizure were immediately injected with 40 mg/kg sodium pentobarbital and placed in their home cage.

C) Results

1. Convulsive doses

If a given dose of UDMH was to lead subsequently to cortical seizure and convulsions a rather characteristic sequence of behavioral signs was observed. These included the onset of restlessness, retching and regurgitation, rapid respiration with panting, dilute and then viscous salivation, hyperkinesis, and finally grand mal seizure. Each of these patterns of behavior, some of them recurrent, was followed by a period of contrasting quiescence. This frequently took the form of sleep-like postures, particularly in the early stages, or quiet sitting and grooming. The postseizure state of high dose levels was characterized by status epilepticus several hours after the administration of barbiturate. The relationship between dose and time of onset of these various signs is shown in table 1. It can be seen from this data that the entire course of response is increasingly accelerated at higher dose levels.

The electrophysiologic correlates of these signs are shown in figure 1. Note the low frequency, high voltage pattern of the EEG during the pre-injection period and the presence of periodic 35-40 cps bursts in the basal forebrain (anterior preoptic area and basal telencephalon) and lenticular leads. This is characteristic of the normal semi-alert behavioral state (2, 6). The pattern observed after the onset of the various signs appeared to be consistent throughout these stages. The high frequency, low voltage picture in the cerebral cortex is typical of the highly

* The UDMH employed in these studies was provided by the U.S. Army through the University of Chicago. When opened, the material was found to be slightly yellow in color, and appeared to be reacting with the plastic container in which it was stored. The investigators, being unfamiliar with this substance, did not question this and proceeded to employ it in this state.

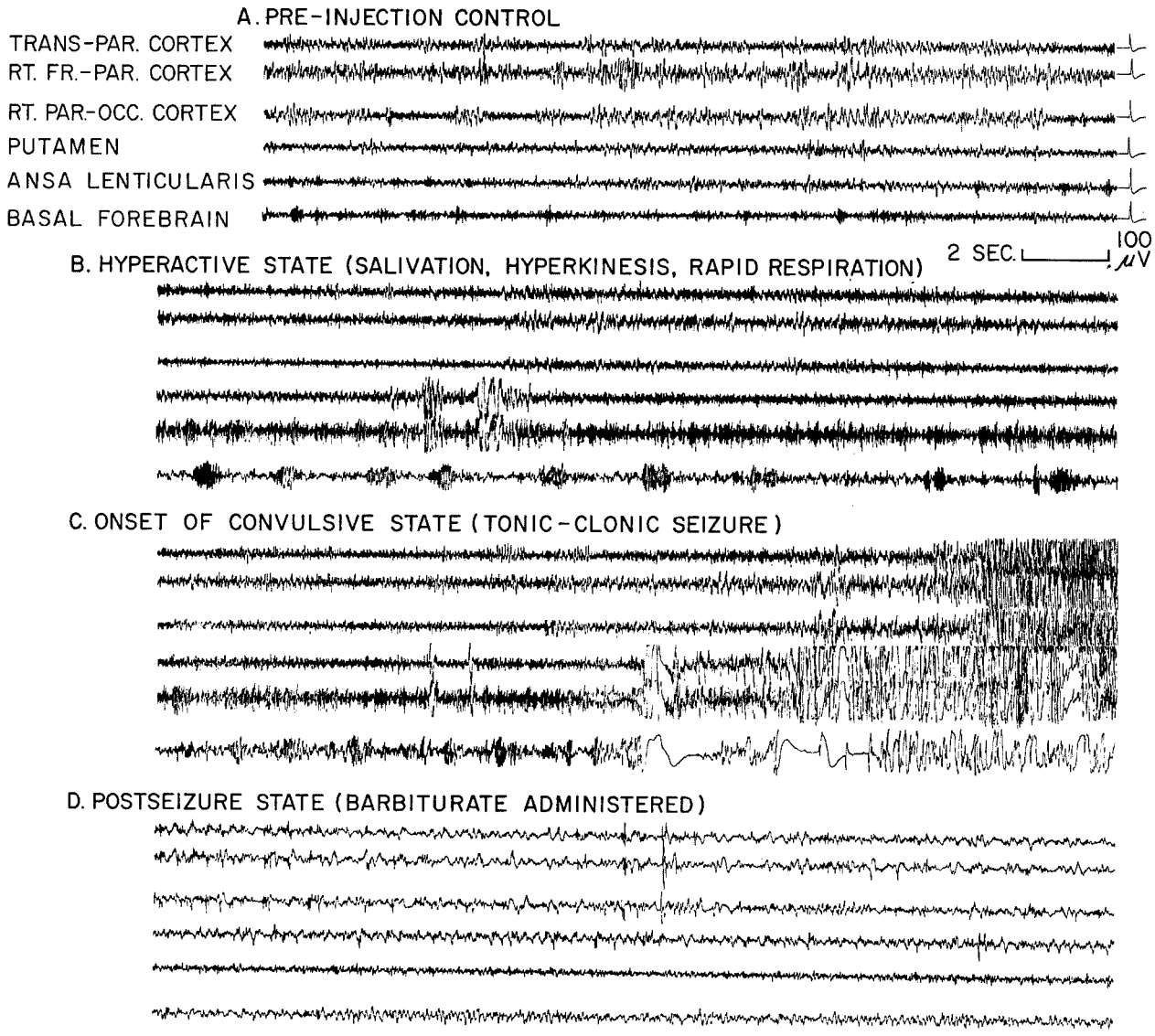

FIGURE 1. Characteristic changes in the electrical activity of the cerebral cortex and various subcortical structures following injection of a seizure inducing dose of UDMH.

TABLE 1: Course of onset of characteristic UDMH poisoning symptons as related to dose.

(Time in Minutes)

Dose mg/kg	Initial Restlessness	Retching	Panting	Salivate	Hyperexc.	Seizure
10	Behavior Remains Same	None	None	None	None	None
20	90	197	260	380	420	480
40	15	17	62	85	113	222
60	12	20	36	77	80	116
120	2	5	10	18	31	39

alerted animal. Petite mal seizure activity also was observed occasionally at this time. During the quiescent period following a given sign, the EEG becomes synchronized, presenting a pattern typical of drowsiness and light sleep. The enormous bursts from the basal forebrain region in figure 1 are clearly abnormal and present a picture which has never before been observed by these investigators. This area is generally considered to be involved in the mediation of high level inhibitory function. The bursting pattern observed in this region occurs exclusively during states of arousal and excitation, and is felt to be related to the interaction of arousal mechanisms and sensory input from the olfactory bulbs (2). The lenticular region, a part of the extrapyramidal motor system, also appeared to be hyperexcited, with many cells discharging rapidly. Toward the later portion of this stage, any strong sensory stimulus could induce EEG seizure and convulsion. The onset of EEG seizure discharge is preceded by spiking and seizure activity in the subcortical structures shown in figure 1.

2. Sub-convulsive Doses

From all outward appearances the EEG and behavior of an animal given subconvulsive doses (10 mg/kg or less) of UDMH does not differ appreciably from that seen after control injections of saline. There is a tendency for these animals to exhibit more quiescent or sleeping behavior; however, this observation is difficult to quantitate and, thus, evaluate in these experiments.

D) Conclusions

1. Convulsive doses of UDMH given intraperitoneally result in a characteristic sequence of behavioral signs, a definite change in the EEG, and a change in the electrical activity of certain subcortical structures. These pre-convulsive alterations do not appear in the animal receiving lower doses of this substance.

2. There was a direct relationship between the latency of onset of the behavioral signs and the amount of UDMH administered in these experiments.

3. Recordings from subcortical sites in the CNS after the administration of convulsive doses of UDMH indicate an increase in the cellular activity of extrapyramidal and basal forebrain structures. These sites temporally precede the cerebral cortex in the onset of seizure discharge, thus indicating a corticipetal progression of seizure activity which originates subcortically.

The electrophysiological findings reported here suggest that convulsive doses of UDMH result in a significant increase in the activity of the extrapyramidal motor system. They also suggest a marked amplification of sensory input to the CNS. Taken together, these factors could constitute a progressive, positive feedback situation which would lead, inevitably, to gross CNS seizure.

II. Effects of UDMH on Induced Hippocampal Seizures in the Acute Cat Preparation

A) Introduction

The hippocampus, by virtue of its configuration within the central nervous system, has been divided into two portions: the ventral hippocampus, which lies within the basal and medial aspect of the temporal lobe, and the dorsal hippocampus, situated caudal and dorsal to the former, at the posterior margin of the diencephalon. This limbic structure is distinguished from other CNS structures by its low threshold for seizure discharge following any form of trauma, including direct electrical stimulation. A functional distinction has been observed between the two anatomical portions of the hippocampus with regard to the induction of seizure activity by electrical stimulation. Seizure induced by stimulation of the ventral hippocampus is typically nonpropagated, or restricted only to the hippocampus. On the other hand, stimulation of the dorsal hippocampus results in a propagated seizure, which spreads to other subcortical structures and to the adjacent cerebral mantle. We wished to determine the effects of UDMH on the induction of these two types of seizure with the purpose of obtaining information about changes in the conduction pathways which mediate these differential patterns.

B) Methods

A tracheotomy and saphenous vein canulation was performed in adult cats under ether anesthesia. The scalp was opened and a portion of the calvarium and underlying dura removed. The animal was placed in a stereotaxic instrument, and all pressure points and wound margins anesthetized locally with procaine hydrochloride. The neuromuscular blocking agent, Flaxedil, was then administered intravenously, and the animal maintained on artificial respiration. Small stainless steel screws were placed into the calvarium over the various cerebral lobes, and bipolar electrodes lowered stereotaxically into the thalamus, hypothalamus and brainstem. All recording leads were connected to a Grass III electroencephalograph. Bipolar stainless steel electrodes were also placed stereotaxically into the dorsal and ventral hippocampus. These leads were attached, alternately, to the EEG machine or to a Grass S-4 stimulator, through an isolation unit.

The experimental animal was allowed to "blow off" the ether anesthetic prior to the beginning of stimulation. Either the dorsal or ventral hippocampus was stimulated, starting at low voltage levels, while recordings were taken from the non-stimulated hippocampal site.

It was, thus, possible to stimulate and record from the hippocampus simultaneously. Stimulation was always delivered at a frequency of 150 pps, and a 0.75 msec duration, with voltage varied for effect. After seizure had been induced, a period of recovery was provided before the next test, which involved the alternate hippocampal site. Threshold values were obtained in all animals prior to the injection of saline (control) or 15-30 mg/kg doses of UDMH intravenously. Threshold determinations were repeated every thirty minutes for five hours after injection. The animal was then killed with an overdose of barbiturate, and the brain removed for histological verification of electrode sites.

C) Results

The threshold values for saline and UDMH injections are shown in Table 2. It is apparent from these figures that the threshold for seizure induction by stimulation of the dorsal hippocampus remains very stable throughout the testing period. The control thresholds for ventral hippocampal seizure induction, however, show a gradual decrease after the first 60 to 90 minutes. This decrease amounts to some 30% of the original threshold, and remains consistent during the remaining tests. It would, therefore, appear to constitute a significant shift in seizure threshold. Thresholds after 15 mg/kg UDMH show a definite shift when compared to the control data. This dose is generally sub-convulsive in the behaving animal. There is an initial increase in ventral hippocampal threshold lasting 60 to 90 minutes. This contrasts with the period of stability seen in the control data. A gradual decrease in threshold follows. The dorsal hippocampus demonstrated a sharp decrease in threshold, which persisted throughout the testing session. This is a considerable alteration when compared with the stable data of control experiments. Animals receiving 30 mg/kg doses of UDMH usually began showing spontaneous cortical seizure patterns within one hour, thus preventing any further evaluation of seizure threshold.

D) Conclusions

1. The immobilized, unanesthetized animal not receiving UDMH demonstrated stable thresholds for seizure induced in the cerebral cortex by stimulation of the dorsal hippocampus. This stability is not observed in animals receiving subconvulsive doses of UDMH. Indeed, a sharp drop (approx. 30%) in threshold is seen. This suggests a possible increase in hippocampal-cortical conduction following the administration of such low doses.

2. The initial stability of threshold for nonpropagated seizure induced by stimulation of the ventral hippocampus in control animals is replaced by a definite increase in threshold after 15 mg/kg UDMH. This

TABLE 2: Effects of UDMH on seizure thresholds following electrical stimulation of the hippocampus. Stimulation at 150 pps; 0.75 msec; voltage thresholds as noted. NE indicates no seizure induced at 10 V or less.

Time (post-inj.)	CONTROL THRESHOLD VOLTAGE		THRESHOLD VOLTAGE FOLLOWING			
			15 mg/kg		30 mg/kg	
	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal
Pre Inject.	9	8	10	9	10	10
30 min.	9	8	NE	9	NE	10
60 min.	8	8	NE	6.5	Spontaneous	
90 min.	7	8	NE	6	Cortical	
120 min.	5	8	10	6	Seizures	
150 min.	5	8	7	5.5		
180 min.	6	9	8	5		
210 min.	6	8	7	7		
240 min.	7	7	5	5		
270 min.	7	8	6	5		

suggests an initial decrease in intra-hippocampal conduction after UDMH administration.

3. It was impossible to evaluate threshold shifts in animals receiving 30 mg/kg UDMH due to the occurrence of spontaneous cortical seizures.

4. A subcortical origin of UDMH induced seizures was implied in the previous section. The hippocampus may be an important pacemaker in this regard. If the initial increase in thresholds for intra-hippocampal conduction is a definite physiological response of this structure to UDMH, this fact may account for the consistently observed latency in the onset of seizure following critical exposure to this substance.

III. Running Velocity and Brain Stimulation in the Cat.

A) Introduction

The purpose of this investigation was to establish the interrelationships between electrical stimulation of the midbrain reticular activating system and the basal forebrain inhibitory system as measured by their effect on the running speed of cats negotiating a runway to obtain a food reward. Once established, this behavioral approach would also be employed to study various centrally active compounds in preparation for analyzing the effects of subconvulsive doses of UDMH on the central nervous system.

The first phase of this investigation has been completed. Electrical stimulation of the midbrain reticular activating system has been demonstrated to increase the velocity of cats in the runway; stimulation of the basal forebrain inhibitory system decreases velocity. When both brain areas are stimulated simultaneously, no significant change in velocity is observed.

B) Physical Equipment

1. The Runway. (Fig 2)

A runway is constructed of lumber, and measures four meters in length, one meter in width with sides one meter high. Boxes measuring one meter square are located at either end of the runway and are separated from it by fitted metal doors. Each box contains a liquid feeding device and a speaker, and serves as both a start and goal box. The run-path itself, which is located in the center of the runway, measures only 7.5 cm. in width and is supported approximately 15.0 cm. above metal troughs containing water. This relatively narrow pathway prevents the animal from varying his route from one box to the other and facilitates the detection of adverse locomotor effects from either brain stimulation or drug administration.

The cats' performance in the runway is timed to the nearest one-hundredth of a second by three electric clocks operated through photoelectric cells. These clocks are located on the runway side of each door, in the center of the run, and just in front of each feeding device. This arrangement permits the recording of times for negotiating the distance from: (1) Door to center of run (first-half time); (2) door to door (run path time); (3) Door to opposite feeder (full-run time). Subtraction of (1) from (2) yields "second-half time" and subtraction of (2) from (3) gives the time taken by the cat in approaching the feeder after entering the box.

Each door is operated by a specially modified 12 volt windshield

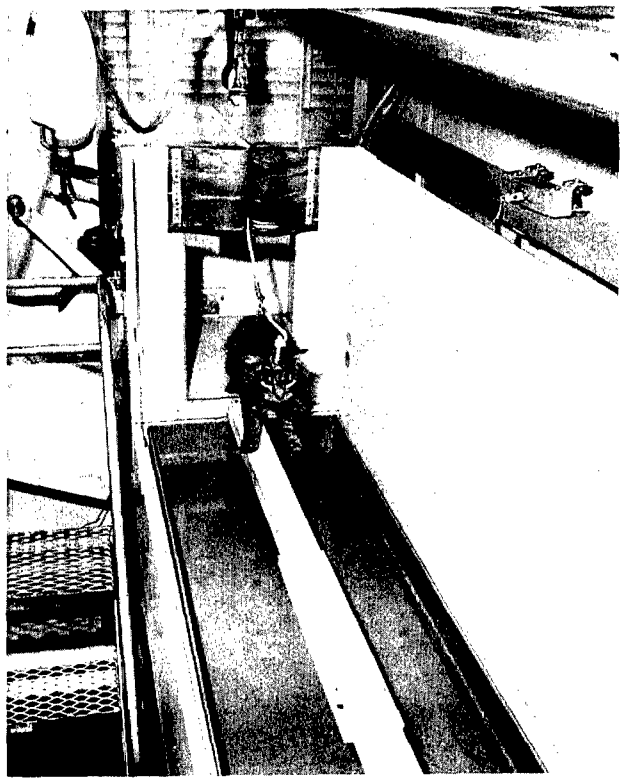

FIGURE 2. The Runway

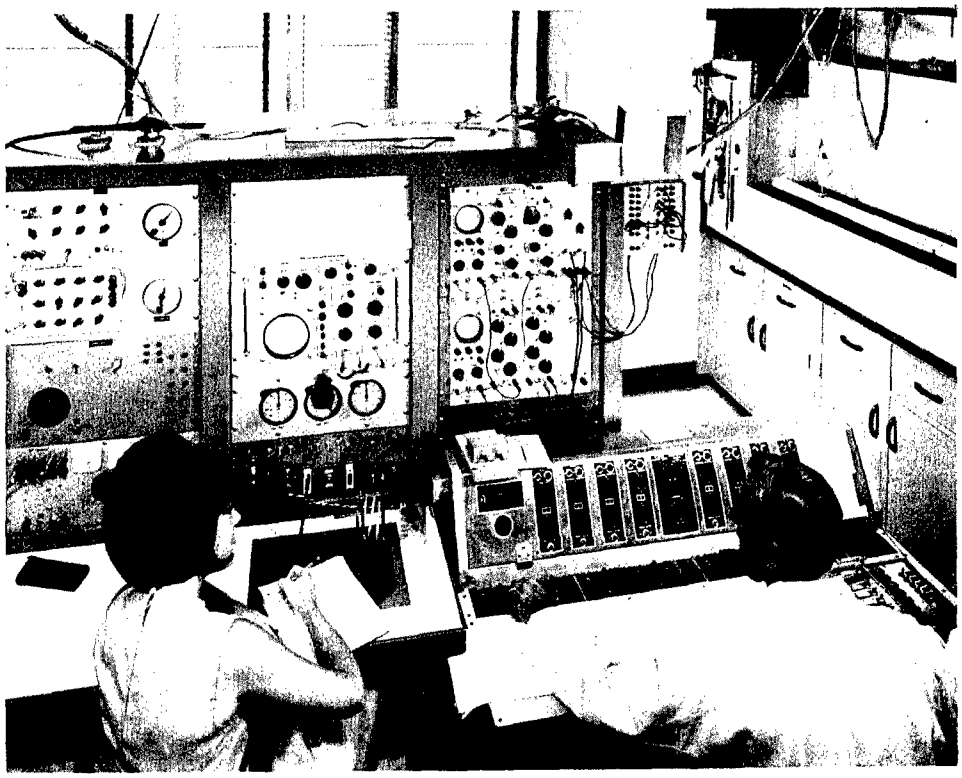

FIGURE 3. Stimulating and Recording Equipment

wiper motor. At the start of a run the motor pulls both doors down and away from the opening and then returns them smoothly to position after the animal has entered the opposite goal box. The doors are constructed of aluminum plate, and run in grooved tracks.

Once the cat is placed in either box at the start of a day's session, no further handling of the animal is necessary. Timing circuits automatically program a sequence of high frequency tone, brain stimulation, food delivery and delay time prior to the door opening. This sequence is repeated as the cat moves back and forth from one box to the other.

2. Stimulating and Recording Equipment. (Fig 3)

The experimental animals are prepared by the same procedures described in Section I. A cable connects the cat through the mounted plug to a Polyscientific brush-block assembly fixed on a trolley. This trolley runs on a track suspended over the entire length of the runway. The brush-block assembly is capable of turning freely while still maintaining good electrical contact, and thus prevents the animal from becoming entangled in the connecting cable. The brush-block also connects to a long, shielded, specially suspended cable leading to the recording and stimulating equipment.

Stimulation is accomplished by employing two independent Tektronic pulse and wave form generator systems which are connected to the animal through the secondary winding of a General Radio Corporation transformer. Stimulus current is monitored on the face of an oscilloscope as a voltage drop across either a 100 or 1000 Ohm resistor in series with the electrode impedance.

Recordings of CNS activity are visualized on a six channel grass model IIID electroencephalograph. Serious distortions arising from movement of the animal have largely been eliminated by employing "Microdot" cable.

C) Methods

1. Animal Preparations.

Cats of either sex weighing between 2.5 and 4.0 Kg. have had electrodes stereotaxically implanted in various brain areas. Electrodes implanted in subcortical sites are of the bipolar "Strut" type consisting of two 30 gauge stainless steel wires mounted on the side of 24 gauge hypodermic tubing and insulated except at the tip with an epoxylite resin. Cortical recordings are taken from small screws placed into, but not through, the skull. All electrodes are connected to a Winchester plug secured to the cat's skull by dental plastic.

2. Behavioral Procedures.

a) Conditioning

Following recovery from surgery the animals are placed on a deprivation diet in order to reduce their body weight to approximately 85% of normal. The cats are then preconditioned to the feeding devices in the runway and within 50 to 60 trials are usually alternating rapidly from one box to the other in order to obtain the several cubic centimeters of milk delivered from each feeding device.

The plug affixed to the animal's skull is then connected to the trolley. The connecting cable is attached to the leash ring of a standard small animal harness so that the load of the trolley is born by the shoulders rather than the head. A number of trials are necessary before the animal adapts to the unfamiliar drag of the trolley and cable. Since friction has been reduced to a minimum, most cats are not unduly disturbed by this situation.

The final step in the conditioning procedure is to introduce the cat to the opening and closing of the doors. Most animals become agitated by the door of a box swinging shut behind them, and at least five or six full training sessions have been found necessary before adaptation occurs.

Once fully conditioned, training sessions continue until running velocities become reasonably stable. Brain stimulation studies are then undertaken.

b) Stimulation Procedures.

The principal goal of these procedures was to determine stimulation parameters capable of measurably increasing running velocity with stimulation of the midbrain reticular activating system, and decreasing it with stimulation of the basal forebrain inhibitory system. In addition, we wished to interact these two systems so that their simultaneous stimulation would not significantly alter running velocity. All of this had to be accomplished utilizing current flows which had no other demonstrable effect on motor performance.

Each animal has electrodes placed bilaterally in both midbrain and forebrain structures. These sites were stimulated singly and in combination in order to determine the most effective sites for producing the desired alterations in running velocity. In addition, the most effective brain site stimulation parameters, in terms of frequency and pulse duration, were also investigated. This was accomplished by utilizing a three-way experimental design in four cats. The effects of 50, 150 and 300 cycles per second stimulation at pulse durations of 0.1, 0.5 and 1.0 millisecond were tested on left, right and bilateral electrode placements in both midbrain reticular and basal forebrain structures.

The following conclusions were drawn from the results of this investigation:

(1) Bilateral stimulation apparently offered no significant advantage over unilateral stimulation;

(2) In many cases one member of a bilateral electrode pair would be superior in effect to its opposite member. We assume minor variations in electrode placement to be responsible, but histological examination will be required to confirm this;

(3) A frequency of 300 cycles per second at 0.1 millisecond appeared to be most effective. Other combinations of frequency and pulse durations were also effective, but the above setting often gave the most consistent positive results.

c) Experimental Design.

Due to the inherent complexity and relative uniqueness of the experimental situation, a number of designs were attempted and subsequently abandoned during the initial phase of the work. A number of interesting and unexpected effects were encountered during this period. For instance, it was found that stimulation of one brain area for a number of consecutive trials could considerably alter its sensitivity. It was also noted that effectiveness of forebrain and reticular stimulation could vary in the same cat from day to day in a fashion which seemed to reflect some apparent "set" of the animal. Additionally, the effect of drive reduction due to gradual reduction in appetite during a session appeared to influence the effectiveness of brain stimulation in complex ways. A number of these observations would make interesting subjects for investigation in themselves but the major aim was to minimize intra-trial and intra-session variations. With this goal in mind the procedure outlined below was adopted.

The cat, with connecting cable attached, is placed in the left box of the runway where 25 seconds elapse prior to onset of a 2000 cycle per second tone. Two seconds following tone onset, the stimulators are switched on (during experimental trials) and 5 seconds later the door opens. Door opening trips a switch starting the first-half time and full-run time clocks and shutting off the tone. Just as the animal embarks on the run-path it breaks a photocell beam which starts the run-path time clock and when it reaches the middle of the run another photocell beam is interrupted stopping the first-half time clock and in addition pulsing a relay which delivers several cubic centimeters of milk to the feeder in the right hand box. Just prior to entering this box a third photocell is activated which stops the run-path time clock

and as the animal proceeds into the box and lowers its head to drink from the feeder, a final photocell is tripped which causes the door to close, stops the full-run time clock and shuts off the stimulators. This completes one trial. The timing and programming circuits then initiate exactly the same sequence of events and the animal returns to the left hand box where the cycle is again repeated.

Initial testing indicated that most cats will run at least 72 trials with reasonably stable velocity. When allowed to continue beyond this, they exhibit noticeable slowing and run times become erratic. Following each daily session the animals are fed 50 grams of solid food in their home cages. This supplement, in addition to approximately 150 cc. of milk ingested in the runway is sufficient to maintain consistent body weight in most cats, although they are weighed daily and dietary adjustments made when necessary.

Two nonstimulated cycles (a cycle is defined as one set of left to right and right to left trials) both precede and follow each cycle during which brain structures are stimulated. The cat's velocity during the cycle immediately preceding stimulation is taken as control while the cycle following stimulation is regarded as a recovery period. This sequence is repeated until each brain area has been stimulated singly and in combination a total of eight times.

D) Results.

To date, a total of seven cats have been tested in the runway. Two of these animals have been successful in that stimulation of the midbrain reticular formation has produced a significant increase in running velocity, stimulation of the basal forebrain has produced a decrease in velocity and simultaneous stimulation of these two areas has resulted in velocities similar to those observed in control trials. Partial success was realized in an additional two cats. In one case stimulation of the basal forebrain clearly slowed the cat, and in the other, reticular stimulation clearly accelerated running speed; however, in each animal the opposite effect was not clearly demonstratable. In both of these cats, however, a degree of interaction occurred when the two brain areas were stimulated simultaneously, in that reticular acceleration was decreased by basal forebrain stimulation and basal forebrain slowing was accelerated by reticular stimulation. The two remaining animals were unsuccessful. One of these has never adequately recovered from the effects of surgery, while the other did not overcome its fear of the operation of the doors in the runway. A seventh cat is currently in the final stages of the "conditioning" procedure and will soon be ready for stimulation testing.

Table 3 illustrates data obtained during a typical day's run in one of the cats which demonstrated the criteria for effect upon stimulation

TABLE 3: Design and data for typical runway trials. Cat #2, 3/6/64,
Stimulation: Left Basal Forebrain (LBF) 300 pps, 0.1 msec, 8.0 V. at 175 μA;
Right Reticular Activating System (RRAS) 300 pps, 0.1 msec, 0.75 V. at 25 μA.

Cycle No.	Brain Area Stimulated	Left to Right			Right to Left		
		First-Half	Full-Run	Run-Path	First-Half	Full-Run	Run-Path
2	(control)	2.02	5.27	4.07	2.23	5.75	4.35
3	LBF	3.17	6.83	4.83	2.43	6.01	4.59
5	(control)	1.74	4.84	3.60	1.96	5.04	3.72
6	RRAS	1.63	4.40	3.27	2.02	5.01	3.60
8	(control)	2.11	5.42	4.21	2.48	6.16	4.74
9	LBF + RRAS	2.04	5.61	4.30	2.18	5.47	4.05
11	(control)	1.59	4.64	3.42	1.88	4.67	3.25
12	RRAS	1.62	4.39	3.12	1.75	4.71	3.22
14	(control)	1.77	5.14	3.88	2.21	5.39	3.96
15	LBF	2.74	6.70	5.10	2.41	5.94	4.47
17	(control)	2.81	6.37	4.73	2.13	5.24	3.89
18	LBF + RRAS	1.88	5.08	3.59	1.87	4.85	3.46
20	(control)	2.08	5.35	3.94	2.06	5.15	3.73
21	LBF	2.57	6.52	5.94	2.37	5.85	4.29
23	(control)	2.29	5.66	4.24	1.95	5.13	3.73
24	RRAS	1.67	4.61	3.35	1.72	4.72	3.37
26	(control)	1.90	5.15	3.88	2.16	5.38	3.97
27	LBF + RRAS	2.08	5.73	4.23	2.69	6.51	5.00
29	(control)	2.25	6.02	4.58	2.18	5.53	4.12
30	RRAS	1.72	4.98	3.70	1.90	5.16	3.75
32	(control)	2.28	6.01	4.66	1.96	4.93	3.53
33	LBF	2.28	5.69	4.12	2.49	6.11	4.49
35	(control)	1.64	4.68	3.45	2.04	5.31	3.93
36	LBF + RRAS	1.75	4.70	3.32	2.12	5.38	4.04

of the reticular formation and basal forebrain.

Figure 4 presents a statistical analysis of the data shown in figure 1 and clearly demonstrates that a significant increase and decrease in velocity was produced by stimulation of midbrain reticular and basal forebrain structures respectively, while no significant difference from control times were observed during simultaneous stimulation of these brain areas.

E) Discussion.

Since elucidation of the midbrain reticular activating system by Magoun and his co-workers (3, 4) the concept of a brain mechanism capable of producing generalized central excitation and behavioral arousal has been generally accepted. In recent years the work of Sterman and Clemente (1, 5, 6) has provided evidence for an analogous central mechanism having representation in certain basal forebrain structures and subserving the function of inhibition and behavioral depression.

The experiments described in this section of the report have demonstrated, in a quantitative sense, that electrical stimulation of midbrain reticular and basal forebrain structures have opposite effects on the running speed of a cat negotiating a runway to obtain food reward. In addition they have shown that these two systems are capable of mutual antagonism within the central nervous system. This raises interesting speculations as to their role in the maintenance of central nervous system homeostasis and provides a model with which the activity of centrally active drugs can be investigated. It is hoped that insight can be gained into the central activity of subconvulsive doses of UDMH by assaying its effect on the dynamic balance of these two antagonistic brain mechanisms.

F) Suggestions for Future Research.

Future research efforts will be dedicated to studying the effects of centrally active compounds on the interaction of the midbrain reticular and basal forebrain systems. Classical stimulant and depressant drugs such as the amphetamines and barbiturates will be investigated from the standpoint of their effect on stimulus thresholds necessary to produce both increased and decreased running velocities and for their activity in altering the balance between these excitatory and inhibitory brain mechanisms. Various hallucinogenic and tranquillizing agents will be investigated in a similar manner.

Finally, the effect of subconvulsive doses of UDMH on these systems will be analyzed in the light of knowledge gained from studying the activity, in the same experimental situation, of drugs whose mechanism

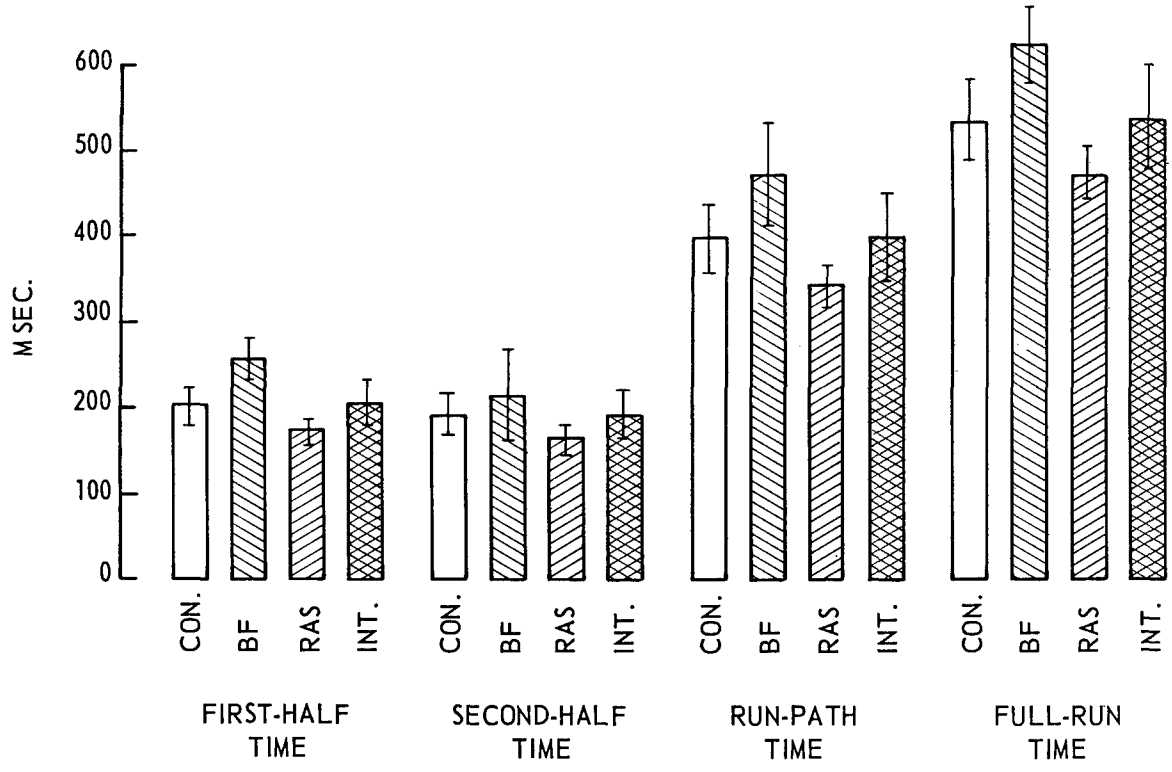

FIGURE 4. Histograms showing relative velocities in various segments of runway performance during control trials (cont.), basal forebrain (BF) and reticular formation (RAS) stimulation, and during simultaneous stimulation of both (INT). In every segment, with the exception of second-half times, statistical analysis indicated that BF decreased, RAS increased, and INT did not change velocity.

of action in the central nervous system is better known. Having defined changes produced by UDMH the antidotal properties of pyridoxine phosphate will be investigated. This procedure should yield information concerning whether the effects of subconvulsive doses are mediated by mechanisms similar to those responsible for the convulsive activity of the compound.

It is felt that this model offers considerable promise in uncovering some of the neurophysiological mechanisms involved in the production of generalized seizures by UDMH.

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Studies concerning the behavioral and neurophysiological actions of UDMH in cats were carried out employing three separate experimental approaches: A) Collection of behavioral and electrophysiological data from animals prepared with indwelling recording electrodes and isolated in a controlled environment after various doses of UDMH, B) Determination of thresholds for seizure induction by electrical stimulation of the hippocampus in unanesthetized, but immobilized "acute" preparations, and C) Development of a unique behavioral apparatus which provides for the evaluation of subconvulsive doses of UDMH in relation to specific CNS regulatory mechanisms. The latter experiment has not yet reached the stage of UDMH administration and testing. The first set of experiments disclosed a direct relationship between dose and time of onset of characteristic behavioral and EEG signs preceding convulsions. The acute experiments indicate an initial increase in ventral hippocampal seizure threshold after administration of low doses of UDMH. This change is seen for the first two hours post-injection and may be related to the initial lethargy and delay in seizure occurrence characteristic of UDMH exposure.

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