CENTRAL NERVOUS SYSTEM ACTIONS OF SOME SYNTHETIC TETRAHYDROCANNABINOL DERIVATIVES

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Recent advances in the chemistry of marihuana and the identification of its putative active ingredient Δ^9-tetrahydrocannabinol (Δ^9-THC) by Mechoulam and co-workers (23, 24) has renewed interest in synthetic tetrahydrocannabinol (THC) derivatives. The chemistry of marihuana elucidated by the early studies of Adams (1) and Todd (27) led to several synthetic compounds much more potent than naturally occurring THC derivatives. Among the synthetic THC derivatives synthesized by Adams and co-workers, the dimethylheptyl analogue (EA 1476, DMHP) was the most active (20–22). We were privileged to re-examine the pharmacology of these compounds between 1955 and 1959. An overview of their structure activity and general pharmacological actions including cardiovascular, respiratory, and hypothermic effects recently has been described by Hardman *et al.* (16–18). The data are further summarized in this symposium (19). An independent investigation of the two most potent compounds of this series was subsequently undertaken by Boyd and his colleagues (3–5, 8). These compounds are EA 1476 (DMHP), the dimethylheptyl and EA 1465 (MOP), the 1-methyloctyl substitution of Δ^{6A-10A}-THC. This manuscript describes further some of the central nervous system actions of DMHP in contrast to Δ^9-THC, when comparative data is available. A preliminary abstract of these findings has recently appeared (12).

EFFECTS ON CONDITIONED BEHAVIOR

Several investigators have described the effects of Δ^9-THC and related congeners on various operant behaviors in animals. Barry and Kubena (2) have shown that acclimation to the laboratory environment alters the response of rats to Δ^9-THC. Dose is an important factor. For example, doses of 4 mg/kg of Δ^9-THC produced initial excitation which could be enhanced in naive rats. Depression followed. Doses of 16 mg/kg produced mainly depression. Grunfeld and Edery (15) studied several active derivatives of hashish in a large variety of animal behaviors. The compound Δ^9-THC caused severe motor disturbances (swaying, dysbasia, adiadochokinesia), stupor and ptosis in dogs, and "tameness" with similar postural changes in monkeys. These behavioral effects were antagonized by d-amphetamine as was the cataleptoid reaction induced by Δ^9-THC in rats. There was a disruption of rat conditioned avoidance shuttle box behavior, but escape behavior to an electric shock was totally preserved. These studies are to be compared with the effects of DMHP in various animal avoidance behaviors. The results are very similar qualitatively but quantitatively the synthetic Δ^{6A-10A} analogue is more potent.

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Rat pole jump avoidance behavior. Pole jump avoidance behavior was used by Cook and Weidley (7) to differentiate the effects of barbiturates from chlorpromazine. It was shown that increasing doses of chlorpromazine produced more depression of avoidance than escape behavior. We used a similar procedure to study the effects of DMHP in 12 rats. A door buzzer stimulus of 15 sec duration was the conditioned stimulus (CS) and a 60 Hz electric shock of 1 ma was the unconditioned stimulus (US). The CS terminated at the onset of the US. The animals were trained to a behavioral criterion of at least 95 % avoidance behavior. Six trained rats given intraperitoneally a 95 % ethyl alcohol vehicle had 97 % avoidance responses before and 93 % avoidance responses after the vehicle. Escape behavior was also unaffected. In contrast 10 mg/kg of DMHP within 1 hr after intraperitoneal injection reduced avoidance behavior from a predrug state of 97 % to 47 % while escape behavior was reduced to 70 %. These data were highly statistically significant ($P < .01$). It was quite obvious that avoidance behavior was more selectively depressed than escape behavior by DMHP. This effect is grossly similar to that of narcotic analgesics such as morphine or neuroleptics like chlorpromazine in contrast to sedatives such as alcohol, barbiturates, meprobamate or benzodiazepines. The effects of some of the latter psychotropic agents on conditioned pole jump behavior have been described by Chodoff and Domino (6, 11).

Dog conditioned avoidance behavior. In view of the fact that the dog is especially useful for screening the pharmacological effects of marihuana extracts, a series of six adult dogs were trained to perform foreleg flexion to a 10,000 Hz auditory CS for 5 sec followed by a 0.7 ma pulsating d.c. (60 Hz) electric shock as the US. The CS overlapped the US. The comparative effects of various psychoactive drugs on this behavior have been previously published (13). Four dogs given DMHP intravenously in a dose of 0.25 mg/kg showed about a 75 % reduction in avoidance behavior. Mean escape behavior was much less reduced and the escape latency was prolonged from a control of 3.0 to 4.6 sec. Especially interesting was that when avoidance responses were made, they were markedly exaggerated in amplitude similar to a startle reaction.

Monkey two-way shuttle box avoidance behavior. In view of the "tranquilizing" effects of DMHP it was decided to study its effects on two-way shuttle box behavior with *Macaca mulatta* monkeys. The box was constructed as two separate chambers 2 by 2 by 4 ft with a small door in the middle wall to allow the animal to move freely from one compartment to the other. The floor of each compartment had an electric grid. Electric lights below each grid floor served as a conditioned stimulus. The duration of the conditioned stimulus was 10 sec. At the end of this time a 60 Hz electric shock of 4.5 ma was applied to the grid floor for 20 sec. A total of six monkeys were trained to avoid the electric shock. Three served as controls given the vehicle. Three others were given 0.5 mg/kg of DMHP intravenously and tested 1.5 hr later. The acquisition of this conditioned response was usually fairly rapid; it required about 50 trials in blocks of 5. An occasional monkey may exhibit great difficulty learning this response. Such animals were discarded. Intravenous doses of 1.0 mg/kg of DMHP completely incapacitated

the animals and it was impossible for them to perform in the shuttle box. Intravenous doses of 0.25 mg/kg of DMHP produced no noticeable effect on shuttle box performance. However, intravenous doses of 0.5 mg/kg of DMHP produced an interesting change in shuttle box behavior in two of the three monkeys studied. The third monkey showed no great effect, indicating a steep dose effect curve between 0.5 and 1.0 mg/kg, intravenously. The data of the two monkeys affected with 0.5 mg/kg of DMHP were pooled because they showed very similar changes. These consisted initially of 100 % blocked avoidance responses while about 50 % of the escape responses were intact. After repeated electric shocks an interesting behavioral reversal took place so that the animals' performances became almost normal for a period of time. A state of confusion appeared to develop after DMHP that resulted in the animals missing the escape door and bumping into walls in an attempt to escape the electric shock. After a few electric shocks were applied to the grid floor the animals temporarily became mentally aware and made the appropriate escape response. Additional trials (20 to 30) resulted in the return to relatively normal avoidance behavior. Three monkeys given alcohol vehicle controls without DMHP showed no such changes.

It is concluded that DMHP produces a "confusional" state in monkeys that can be temporarily reversed with strong afferent stimulation such as electric shock to the feet. These findings amplify and confirm previous data obtained with DMHP with trained rats and dogs. Avoidance behavior is more selectively depressed than escape behavior. The increased sensory input during the US has strong arousal producing effects which tend to restore behavior toward normal. A waxing and waning of the arousal level with a tendency for the US to cause a short lasting improvement seems a peculiar pharmacological attribute of certain doses of this drug.

ANALGESIC PROPERTIES

As described by Hardman et al. (16–19) DMHP in an intravenous dose of 1.0 mg/kg blocks the response of the dog to superficial pain (pinprick) within 1 to 2 min after injection. The response to deep pain (stepping on paw or tail of the dog) is maintained for 10 to 30 min after drug injection. Once analgesia to deep pain is obtained, the dogs are unable to be aroused from their depressed state.

Another method of determining analgesia (26) was employed in which the antinociceptive response of the rat tail to heat generated by a hot wire was evaluated. The rat would flick his tail off a holder when the heat of the hot wire located just below the tail holder became uncomfortable. A series of controls were established for each rat in groups of 5 to 16 and the procedure repeated at selected time intervals after receiving DMHP or morphine. The effect of the ethyl alcohol solvent was also assayed. It was found to lack analgesic activity in the high (95 %) concentrations employed. Only male rats weighing 50 to 100 g were utilized. These were young animals in which a minimum of cornified epithelium was present on their tails, thus reducing the variability of their control reaction times. None of the rat tails were exposed to the source of radiant heat for more than 15 sec on a given test in order to avoid tissue damage. If no reac-

tion occurred from the rat after 15 sec exposure, marked analgesia was considered evident. When nalorphine was used to antagonize the morphine or DMHP response, an arbitrary reduction of 50% in the reaction time was required to classify the nalorphine response as indicating true antagonism. Thus, a reaction time of less than 7.5 sec arbitrarily was required in an animal whose reaction time before nalorphine had been 15 sec as a result of giving either morphine or DMHP. The highest control reaction time recorded in this series of 290 animals was 7.5 sec. This meant that the drug under study had to increase the reaction time of the rat at least 100% to be classified as having produced marked analgesia.

It was found that DMHP produced a high mortality rate in the animals tested with doses of 10 to 100 mg/kg when administered intraperitoneally in a 95% ethyl alcohol vehicle. No fatalities were observed, however, when the drug was given orally in doses up to 150 mg/kg. Morphine was given orally in doses of 25 to 100 mg/kg. However, most of the research was done with a dose of 75 mg/kg. At this dose marked analgesia was observed in 90 to 100% of the rats tested. The results with 150 mg/kg of DMHP were not as uniform. Under these conditions marked analgesia was observed in 20 to 87% of the animals tested at any given time. The data are in agreement with the previous findings of Loewe and Nickerson (21) that the tetrahydrocannabinols are analgesic.

In morphine-treated animals (75 mg/kg orally), nalorphine, depending upon dose, was able to antagonize the prolonged reaction time in the majority of animals tested. A drug to nalorphine molar ratio (D/N) of 32 to 1 showed relatively ineffective antagonism but a ratio of 2/1 showed very clear-cut antagonism. Doses of DMHP of 150 and 200 mg/kg also prolonged the reaction time in roughly 50% of the animals. However, nalorphine was relatively ineffective in antagonizing this effect even when large doses were used. The effects of DMHP were antagonized by nalorphine (D/N ratio = 1) in less than 10% of the three groups of 12 animals tested at any given time; this suggests a non-specific effect. The wide variation in response may indicate erratic gastrointestinal absorption of morphine and DMHP. In fact, we now know that some of the synthetic THC derivatives such as DMHP are relatively ineffective when given per os or intraperitoneally in comparison to the intravenous route (25). The nature of the reponse of the rat to DMHP provides obstacles that will make evaluation of the analgesic activity of DMHP a difficult task in this test system. The data in the dog provide far more convincing evidence of analgesia than the rat tail test. Obviously, carefully run experiments in man can provide the only complete proof as to the presence or absence of true analgesic activity of DMHP.

ELECTROENCEPHALOGRAPHIC ALTERATIONS

#### Effects of DMHP on the cerebral electrical activity of chronic dogs

DMHP in doses of 0.25 mg/kg produced in the dog with chronic indwelling brain electrodes electroencephalographic (EEG) effects which paralleled gross behavior. Seven dogs were studied with conventional techniques. Within 20 min after the intravenous administration of DMHP the animals appeared drowsy and

at the same time showed high voltage, slow wave EEG activity in various cortical and subcortical areas. Any afferent stimulation quickly caused EEG and behavioral arousal. There was a tendency for the more anterior portions of the cerebral cortex of the dog to have greater high voltage, slow wave activity than the more posterior portions. However, these changes were not as clear-cut as they were in monkeys (see below). Following afferent stimuli the animals showed exaggerated startle responses. In a quiet environment they quickly slumped in the restraining apparatus and refused to stand, especially on their hindlegs. Frequently the animals alternated between periods of depression and activation. The intravenous administration of small doses (0.25 mg/kg) of d-amphetamine caused the high voltage, EEG slow waves to revert toward a low voltage, fast frequency pattern. The antagonism by d-amphetamine in this regard was not complete. After the administration of d-amphetamine the animals appeared to be more alert. However, they showed excessive startle responses. These effects were very similar to those observed in intact dogs as described by Hardman et al. (19). The partial antagonistic effects of d-amphetamine were relatively short lasting and the animals would revert to their previous stuporous state within an hour with concomitant EEG slowing. MOP showed similar EEG changes as DMHP but was slightly less potent.

Effects of DMHP on the cerebral electrical activity of chronic monkeys

A total of seven Macaca mulatta monkeys were prepared with chronic indwelling brain electrodes for recording EEG activity. In some monkeys alpha rhythm resembling the human alpha frequency was recorded from the parietal-occipital cortex. Figure 1A illustrates such activity in a monkey who was awake but quiet and relaxed. Loud noises quickly produced alpha blockade with subsequent low voltage, fast frequency waves. One hour after the intravenous administration of 0.5 mg/kg of DMHP in a peanut oil-lecithin, oil in water emulsion, the animal showed enhanced alpha waves. The more frontal areas of the brain also showed some slow waves. For example, the record in figure 1B shows that area 6 is much more affected than the arm motor cortex (area 4), the amygdala, and the posterior hypothalamus. Loud noises were still effective in producing blockade of the alpha rhythm and the subsequent low voltage-fast frequency EEG activity of an awake animal. There was a positive though incomplete correlation with the degree of cortical EEG slowing and gross behavior. After DMHP the animal was mildly sedated, and had a greater tendency to sleep. Loud noises easily aroused the animal behaviorally. At the same time a low voltage, fast frequency EEG was observed (see fig. 1B). The intravenous administration of 0.5 mg/kg of d-amphetamine caused the animal to become more alert. However, the EEG pattern was not completely normal (see fig. 1C).

There was some variation in the gross behavioral response of different monkeys to DMHP, especially with doses of 0.25 to 0.5 mg/kg, intravenously. Some animals showed primarily central nervous system depression with no exaggeration of motor movements (startle responses) upon arousal. Others showed marked motor movements upon arousal suggesting loss of inhibitory control. There was

A

B

C
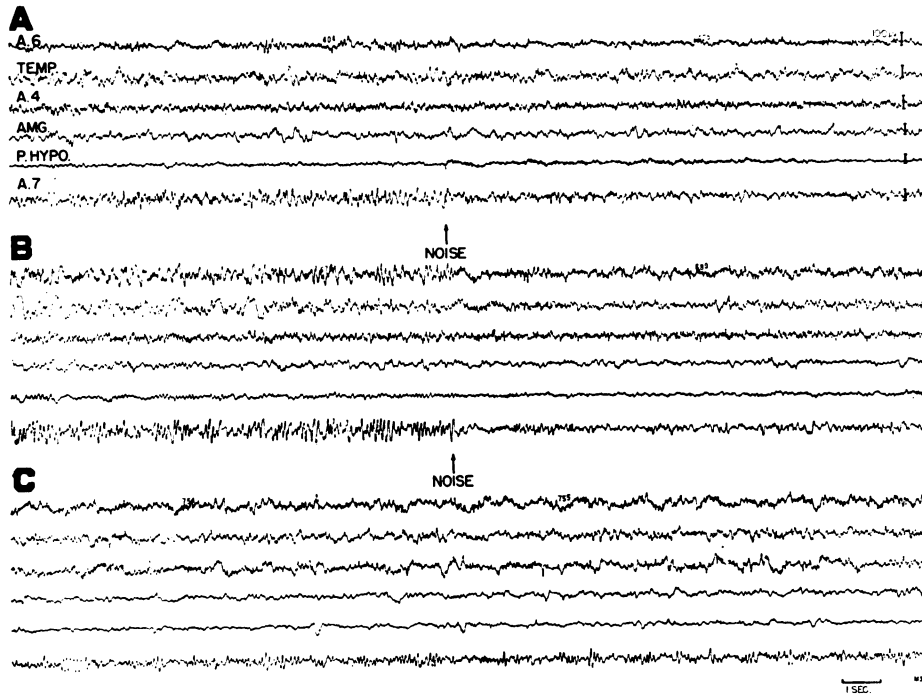
Fig. 1. Electroencephalographic effects of DMHP in a monkey with chronic indwelling brain electrodes. Panel A, control tracing. Panel B, 1 hr after 0.5 mg/kg of DMHP, intravenously. Panel C, 10 min after 0.5 mg/kg, intravenously, of d-amphetamine. Note that at the arrow (↑) loud noise induced electroencephalograph (EEG) activation before and after DMHP. The latter causes EEG slow waves and an enhanced amplitude of *alpha* waves. Symbols: A6, premotor cortex; Temp., temporal lobe; A4, motor cortex; Amg, medial amygdala; P. Hypo., posterior hypothalamus; A7, parietoccipital cortex. Paper speed as indicated. Calibration bars, 100 μv.

also some variation in the degree of central nervous system depression produced by single doses of DMHP (0.25 mg/kg, i.v.).

The EEG recordings from one animal showing a markedly exaggerated startle response to noise after DMHP is illustrated in figure 2. A small dose of DMHP (0.25 mg/kg, i.v.) produced some high voltage slow waves in the more frontal areas of the brain including area 6 and area 4 (leg). Some slowing was also seen in area 7 and in the caudate nucleus (see panel A, fig. 2). A loud noise produced short lasting behavioral arousal as well as a transient low voltage, fast frequency EEG pattern. One hour after an additional dose of 0.25 mg/kg of DMHP, the slow wave activity was more pronounced (see fig. 2B). A similar loud noise produced definite EEG and behavioral arousal, but also an exaggerated startle response (C) having a convulsive character. One hour after an additional 0.5 mg/kg of DMHP (total accumulative dose of 1 mg/kg) the spontaneous EEG and motor effects were even more pronounced. However, EEG activation was not as marked (see fig. 2C). The intravenous administration of 1 mg/kg of methyl-
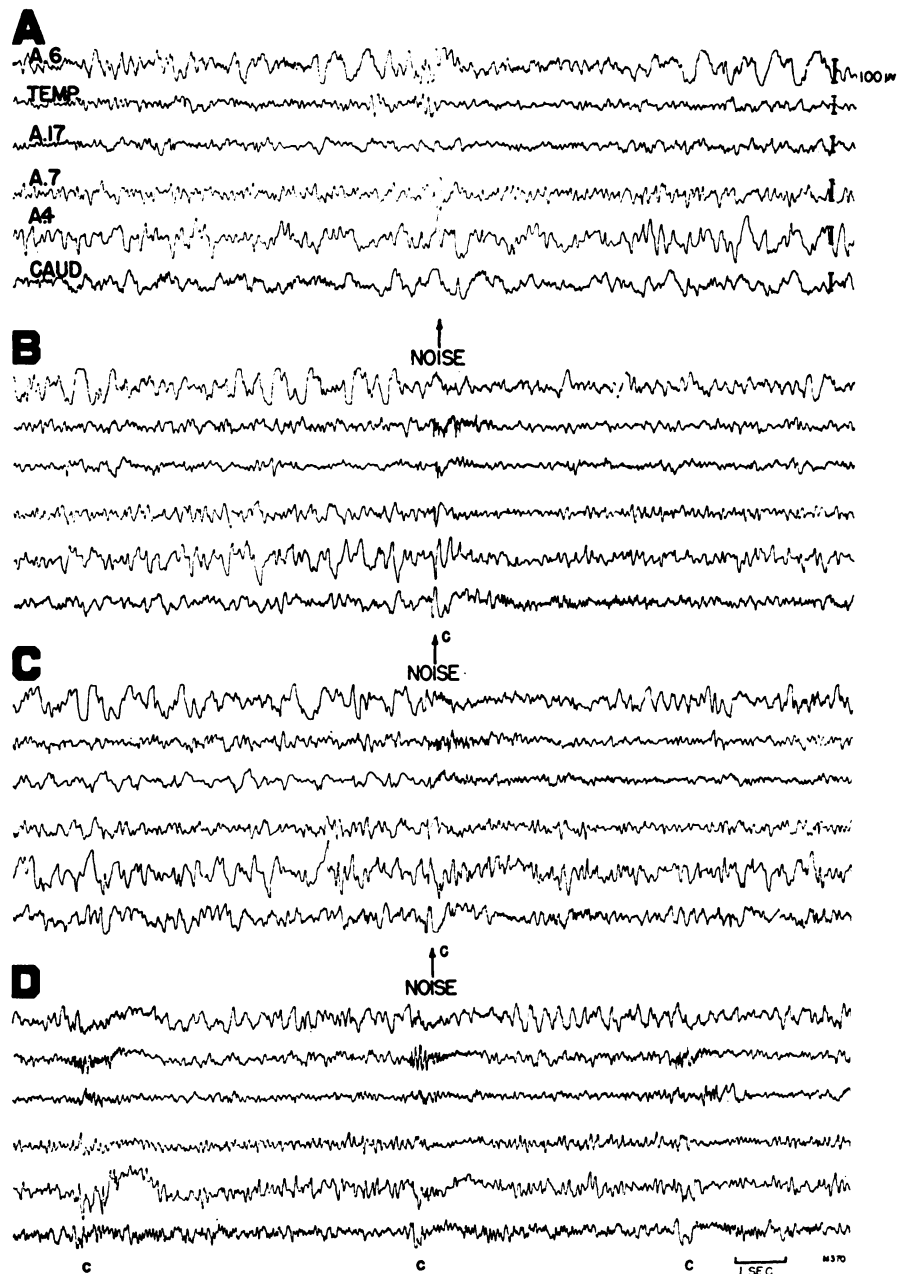
FIG. 2. Electroencephalographic effects of DMHP and partial antagonism by methylphenidate in a monkey with chronic indwelling brain electrodes. Panel A, 0.5 hr after 0.25 mg/kg of DMHP, intravenously. Panel B, 1 hr after an additional 0.25 mg/kg of DMHP, intravenously. Panel C, after an additional 0.5 mg/kg (accumulative dose 1.0 mg/kg). Panel D, 15 min after 1.0 mg/kg of methylphenidate. At each arrow (↑) loud noise induced a short lasting electroencephalograph (EEG) activation. After larger doses this was accompanied by an excessive startle response and convulsive (C) movements. Methylphenidate was a poor antagonist of the EEG effects. Spontaneous convulsive (C) movements were observed periodically. Symbols: As in fig. 1 plus Caud., caudate. Paper speed and calibration as noted.

phenidate caused a partial reversal of the EEG toward normal, but not in cortical area 6. For a short time the animal had frequent spontaneous exaggerated startle responses and convulsive movements (see fig. 2D). In general, methylphenidate appeared much less effective than d-amphetamine in antagonizing the EEG effects of DMHP.

The EEG effects in monkeys paralleled those obtained in dogs but certain species differences are worth noting. Although more sensitive to the administration of a given dose of DMHP, the dog tended to have less clear cut frontal-parietal high voltage slow waves than the monkey. Nevertheless, in both species the more frontal portions of the brain tended to show greater high voltage, slow waves than the posterior portions.

Effects of DMHP on the threshold to electrical stimulation in monkeys with chronic indwelling brain electrodes

Brainstem reticular formation. As described above, even after doses of 0.25 to 0.5 mg/kg of DMHP, EEG activation and behavioral arousal can be produced readily with various afferent stimuli. Therefore, in all probability DMHP in small doses causing ataxia and other behavioral phenomena does not have marked effects on the threshold to stimulation of the brainstem reticular formation. However, larger doses producing coma should elevate this threshold. This hypothesis was confirmed by actual experiments as shown by the data from three different monkeys in table 1. The parameters for electrical stimulation for the data in tables 1 and 2 were similar. Square wave pulses of 300 Hz, 0.5 msec duration for 5 sec were applied at different voltages as noted. Current strength of stimulation was monitored during only part of the study. The extent of correlation of voltage and current in five monkeys over a period of 6 months for three different brain areas varied from +0.89 to 0.99 with a mean of +0.97 which was highly statistically significant ($P > .001$). Inasmuch as voltage was measured in all animals, this index of threshold was therefore used.

The threshold of electrical stimulation of the brainstem reticular formation with 300 Hz square wave pulses in a typical monkey was 4 V. The responses obtained consisted of either looking around followed by moving in the restraining chair, or by increased attentiveness. Suprathreshold or maximal stimuli frequently produced complex motor movements. After the intravenous administration of 0.25 mg/kg of DMHP the threshold to reticular stimulation appeared to be slightly increased. For example, at the time of peak drug effect, in approximately 1 hr, stimulation of the brainstem reticular formation produced no detectable responses. However, stimulation at 6 V caused the animal to open his eyelids. The administration of a larger dose of DMHP in the order of a total of 0.5 mg/kg elevated the threshold still further. Similar effects were obtained at 1.0 mg/kg of DMHP. It can be noted in table 1 that at this dose doubling the voltage of stimulation to a total of 8 V still produced no detectable response. However, the administration of 0.5 mg/kg of d-amphetamine caused at this voltage a slight response that could be interpreted as arousal but it was not as clear-cut as the control. The data illustrated in table 1 represent an extreme case of an

TABLE 1

Some effects of DMHP and d-amphetamine or methylphenidate antagonism on the threshold to electrical stimulation of three Macaca mulatta monkeys with chronic indwelling brain electrodes

Brain Area	Control		0.25 mg/kg DMHP		0.5 mg/kg DMHP		1.0 mg/kg DMHP		0.5 mg/kg d-Amphetamine	
	Threshold stimulus	Response	Stimulus	Response	Stimulus	Response	Stimulus	Response	Stimulus	Response
Brainstem reticular formation	4 V	Looks around followed by moving around in chair	4 V	No response	4 V	No response	4 V	No response		
			6 V	Opened eyelids	6 V	No Response	6 V	No response	6 V	Very slight response
					8 V	Looks around aroused	8 V	No response	8 V	Slight response. Not as good as control
Medial amygdala	4 V	Licking fingers; stopped momentarily	4 V	No response	4 V	No response	4 V	No response		After 1.0 mg/kg methylphenidate
			6 V	No response	6 V	Delayed licking of chops			6 V	No response
							8 V	Slight stretching movements	8 V	Slight movement
Posterior hypothalamus	4 V	Sat erect; looked alert	4 V	Not as marked effects	4 V	No response	4 V	No response	4 V	No response
					6 V	No response	6 V	Opened eyes	6 V	Moved head
					8 V	Opened eyes	8 V	Opened eyes; looked up	8 V	Typical of control followed by startle response

TABLE 2

Some effects of DMHP and d-amphetamine antagonism on the threshold to electrical stimulation of a beagle dog with chronic indwelling brain electrodes

Brain Area	Control		0.25 mg/kg DMHP		0.5 mg/kg DMHP		0.5 mg/kg d-Amphetamine	
	Threshold stimulus	Response	Stimulus	Response	Stimulus	Response	Stimulus	Response
Brainstem reticular formation	5 V	Stopped moving	5 V	Awakens, more noisy after stimulation	5 V	No effect	5 V	Looks around; some motor movement
	6 V	Looked around			6 V	Startled with marked motor movements		
Medial amygdala	8 V	Stopped barking, licked chops, sniffing	8 V	No response	8 V	No response	8 V	No response
			9 V	Licked chops	9 V	No response	9 V	No response
					10 V	No response	10 V	No response
Posterior hypothalamus	4 V	Stiffened, looked up	4 V	Some response with jumpiness after stimulation	4 V	Jumpy after stimulation	3 V	Stiffened, looked up, jumpy
					5 V	Stiffened, looked up, very jumpy		

elevation in threshold due to stimulation of the brainstem reticular formation after administration of DMHP in an animal that showed marked CNS depression. Considerable individual animal variation was obtained. DMHP produced a state of mixed depression and stimulation. Some animals tended to show much more of the depressant effects of DMHP than others. Indeed, a few animals showed exaggerated startle responses that bordered on motor activity reminiscent of decerebrate rigidity. In such animals stimulation of the brainstem reticular formation produced typical exaggerated startle responses at near threshold voltage levels.

Amygdala. Electrical stimulation of the medial amygdala caused a variety of phenomena reminiscent of olfactory or taste sensations. Sometimes sniffing could be observed, frequently licking of the chops, chewing, licking of the fingers, or the cessation of this activity as well as very occasionally yawning. After the administration of DMHP the threshold for these responses was elevated. As indicated in table 1, the administration of 0.25 mg/kg of DMHP raised the threshold so that this activity could not be obtained at 4 or even 6 V. The addition of another 0.5 mg/kg of DMHP produced similar elevations in the threshold. Frequently at this dose exaggerated startle responses were observed and occasionally at a voltage of 6 V delayed licking of the chops. Larger doses of DMHP raised the threshold a great deal. The administration of either d-amphetamine or methylphenidate had little effect on the threshold of behavioral activity elicited from the amygdala. *Responses were not returned toward normal as in the case of stimulation of the brainstem reticular formation indicating that d-amphetamine had only partially antagonistic effects.*

Posterior hypothalamus. Threshold stimulation of the posterior hypothalamus frequently produced phenomena which could be interpreted as behavioral arousal. With larger intensities of stimulation piloerection, dilatation of the pupils, etc. were obtained. Increasing doses of DMHP tended to elevate the thresholds for these responses. The administration of d-amphetamine tended to revert this pattern toward normal. Thus, there was some antagonism insofar as responses from the posterior hypothalamus were concerned.

In addition to the areas studied above, the effects of DMHP were determined on the threshold to stimulation of the motor cortex of the monkey. The results obtained proved so variable that one could only conclude that they depended entirely upon the state of the animal. If the animal was severely depressed, the threshold for a Jacksonian motor seizure was elevated, but if the animal showed excessive startle responses the threshold was either not affected or lowered.

Some effects of DMHP on the threshold to electrical stimulation in dogs with chronic indwelling brain electrodes

Brainstem reticular formation. As in the monkey, there was considerable variation in the effects of DMHP in the dog that was dependent upon the degree of depression and excitement exhibited. Stimulation of the brainstem reticular formation frequently produced a cessation of movement if the animal happened to be moving or tended to cause the animal to look around. Excessive stimulation

at suprathreshold intensities produced bizarre motor movements. After the administration of 0.25 mg/kg of DMHP the threshold was not particularly altered and the animal was aroused to electrical stimulation as is indicated in table 2. Larger doses increased the threshold for arousal. Increasing the stimulus intensity to approximately 6 V produced startle responses with marked motor movement. The administration of equal amounts of d-amphetamine returned the threshold to stimulation of the brainstem reticular formation toward normal for a short period of time when gross behavioral antagonism was evident.

Amygdala. Stimulation of the medial amygdala produced somewhat similar responses to those obtained in the monkey. With threshold stimuli in the order of 4 to 8 V, the animals frequently stopped barking, licked their chops, sniffed, *etc.* Administration of DMHP elevated the threshold for these responses considerably. Larger doses in the order of 0.5 mg/kg elevated this threshold above 10 V. It is interesting to note that the administration o f d-amphetamine to such animals did not restore these thresholds toward normal.

Posterior hypothalamus. The threshold for stimulation of the posterior hypothalamus was usually in the order of 4 V. When stimulated, the animals appeared to look up, look around, and have some stiffening of their extremities. Occasionally piloerection was seen. After a small dose (0.25 mg/kg) of DMHP the threshold for this response was not greatly elevated. Frequently exaggerated jumpiness or startle responses were seen after electrical stimulation. With somewhat larger doses of DMHP the threshold for stimulation of the posterior hypothalamus was the same, although the response was considerably altered and accompanied by excessive jumpiness. Increasing intensities of stimulation produced effects similar to controls, in addition to excessive jumpiness and exaggerated startle responses. The administration of d-amphetamine frequently restored the threshold to normal or occasionally slightly below. Excessive jumpiness or startle responses were still observed.

It should be emphasized that both in the dog and monkey there is considerable variation in the effects of DMHP in modifying the threshold to stimulation of the brainstem reticular formation, medial amygdala, and the posterior hypothalamus. The effects obtained appeared to be dependent upon the condition of the animal and the degree of central nervous system depression and stimulation that was present. Thus, in animals that appeared to be grossly very depressed by large doses of DMHP and who did not show marked arousal responses to various afferent stimuli, the thresholds to stimulation of the various areas mentioned were frequently considerably elevated. The threshold to stimulation of the amygdala appeared to be much more elevated by DMHP than that of the other areas studied. Amphetamine frequently reversed the threshold toward normal, both in the brainstem reticular formation and the posterior hypothalamus but not in the amygdala. Animals who were excessively stimulated by DMHP, that is who showed excessive startle responses or almost frank convulsions of the decerebrate type, did not show marked elevations to the threshold of stimulation of the various areas studied and frequently at a threshold stimulus showed exaggerated convulsive-type activity.

Effects of DMHP on photic driving of the EEG of the dog

In view of the dramatic effects of LSD-25 and phencyclidine on photic driving of the EEG (10) it was of interest to determine the actions of DMHP. Dogs with chronic indwelling brain electrodes were used. As can be seen in Figure 3, photic driving at a frequency of 10 Hz produced complex EEG changes both in the visual cortex (area 17) and in the brainstem reticular formation (MR). Some change in the electrical activity is also seen in the prefrontal cortex (A8). However as expected, EEG driving in this area is very poor. The photic driving responses in the visual cortex and in the reticular formation were not simply one-to-one following. More typically, as is illustrated, after the flash of light, particularly at supramaximal intensities, multiple fast frequencies could be seen. Within half an hour after the administration of DMHP in an intravenous dose of 0.25 mg/kg, photic driving responses both in the visual cortex and in the reticular formation were altered. There appeared to be a decrease in the amount of interspersed fast frequency spike activity. Interestingly, in the parietal association cortex (A7) some photic driving responses were present that were not seen in the control (see fig. 3). After 0.5 mg/kg of DMHP photic driving responses were considerably
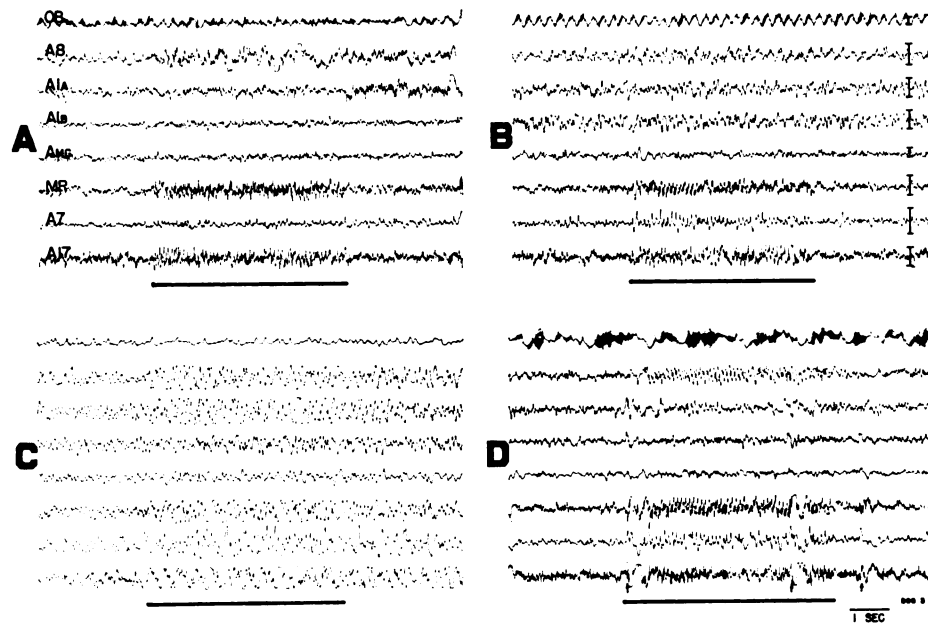
Fig. 3. Effects of DMHP on the electroencephalograph (EEG) and photic driving responses in a dog with chronic indwelling brain electrodes. Panel A, control. Panel B, approximately 1 hr after 0.25 mg/kg of DMHP, intravenously. Panel C, approximately 1 hr after 0.5 mg/kg of DMHP, intravenously. Panel D, approximately 5 min after 0.5 mg/kg of d-amphetamine, intravenously. The black bar below each panel indicates the time interval during photic driving at a frequency of 10 Hz. Symbols: O.B., olfactory bulb; A8, cortical area 8; Al$_A$, anterior portion of cortical area 1; Al$_B$, posterior portion of cortical area 1; AMG, medial amygdala; MR, brainstem reticular formation; A7, cortical area 7; A17, cortical area 17. All recording sites were confirmed histologically. Calibration height, 100 μv.

distorted as was the spontaneous EEG. It can be seen in figure 3 that almost all areas from which a record was taken showed high voltage, slow wave activity and that during photic driving this high voltage, slow wave activity was of high amplitude and somewhat greater frequency, but still grossly distorted. After either stimulation of the reticular formation or of the posterior hypothalamus, or intense peripheral afferent stimuli of various kinds when the animal was alerted and the EEG reverted toward normal, photic driving responses appeared again to be somewhat more normal. Similar effects were seen after the administration of 0.5 mg/kg of *d*-amphetamine. Exaggerated startle responses were seen with the subsequent artifacts in the EEG (see fig. 3 at the arrows). This phenomenon has been described above for the monkey. At this time the general pattern of the EEG reverted toward normal but definitely was not completely normal. Photic responses were somewhat similar to controls. It is interesting to note that at this time excellent approximately one-to-one photic driving was present. While *d*-amphetamine antagonized some effects of DMHP, it did not completely antagonize the effects of this agent on photic driving responses both in the normal visual projection areas and the enhanced photic driving in non-visual areas.

Effects of DMHP on the electrical correlates of a conditioned avoidance response in the dog

It has been described above that DMHP depressed (a) the conditioned avoidance pole jump response in the rat to an auditory stimulus, (b) the conditioned avoidance response in the monkey to continuous light, and (c) the conditioned avoidance response in the dog to a pure tone. It was of interest to determine whether the depression of the conditioned avoidance response was due to the lack of sensory input into specific sensory receiving areas. Because of the effects of DMHP on photic driving responses in the untrained dog, it was of interest to determine whether DMHP would have any effect on photic driving when this represented the conditioned stimulus, a procedure similar to that described above except a 10 Hz flashing strobe light was used as the CS. Three of the beagle dogs with chronic indwelling brain electrodes were trained to lift their foreleg in response to flickering light. Six channels were used for EEG recordings on a Grass model III EEG. One channel was used to record the leg lifting response of the dog, and the other to record the photic stimulus. Appropriate timers were used to program the sequence of the conditioned stimulus (CS) and the unconditioned stimulus (US) so that the intervals were automatically determined. The animal was trained so that lifting its foreleg whether for the CS or for the US automatically stopped the subsequent sequence. The CS was applied for 5 sec at a frequency of 10 Hz. At the end of 5 sec the US consisting of a 60 Hz a.c. electric shock of approximately 90 V was applied for at least 30 sec unless the animal made an appropriate withdrawal response. All animals were placed in a stockade to restrict their movements. For the first 60 trials three legs of each dog were tied in the restraining apparatus with only the foreleg with the applied electrode being moveable. After 60 trials the dogs were fairly well trained. Subsequently, it was only necessary to restrain the animals by means of the collar of the stockade. Rigid restraint of the

animals tended to induce anxiety and the animals learned with difficulty so this
was avoided. All trials were run in an electrically shielded box with a one-way
window. A source of compressed air was used in order to provide a distracting and
constant stimulus so that other extraneous noises would not interfere with the
performance of the animal. The experimental procedure consisted of removing
the animals from their cages and allowing them to roam freely in the dog room for
a period of 5 to 10 min during which time the investigator petted and played with
the animals. Subsequently, they were brought to the laboratory and placed in the
restraining apparatus. The leg was shaved with an electric clipper and saline
electrode paste applied to the leg with the stimulating electrode for the uncondi-
tioned stimulus. The left leg was used in two of the dogs and the right leg in
another. The electrode resistance usually was in the vicinity of 5000 ohms. The
experimental trials were given in groups of 10 per day. A random intertrial inter-
val of 1 to 3 min was used. There was a delay in this time if the dog was particu-
larly excited and doing a lot of spontaneous leg lifting. At the conclusion of 10
trials the dog was removed from the testing apparatus and the leg washed of
electrode paste. The animal was again played with for a few minutes, praised,
petted, etc. and then returned to the animal quarters. Before the drug studies
each animal had a total of 100 trials at which time all had learned to avoid to a
criteria of approximately 95 to 100 %, depending upon the dog. One week was
allowed between each dose of a given drug. With training, a naive dog with
chronic indwelling brain electrodes developed a clear-cut photic driving response
in various visual areas. This EEG response was very complex and difficult to
analyze. In three dogs which had almost 100 % avoidance responses, DMHP in an
intravenous dose of 0.25 mg/kg, markedly depressed the avoidance response to a
mean of about 5 % of control. With this dose the unconditioned response after an
electric shock applied to the foreleg was still present but was considerably de-
layed. During the unconditioned response the dogs continued to yell and cry out
but did not lift their legs as quickly as a normal animal. Therefore, it appeared
that the analgesic effects of DMHP under these circumstances was minimal.
Approximately 1 hr after DMHP photic driving responses were considerably
altered. Even though the animal did not respond with an avoidance response,
there was considerable whining and crying during presentation of the conditioned
stimulus, suggesting that the animal was aware of the significance of the condi-
tioned stimulus but for some reason, perhaps confusion, did not make the ap-
propriate avoidance response. Sensory information in the visual cortex, while
distorted as evidenced from the photic driving response, nevertheless was ap-
parently of such a type that the animal was aware of the significance of the condi-
tioning stimulus. Frequently, such a conditioning stimulus produced EEG activa-
tion even though the animal did not have an avoidance response. EEG activation
did not necessarily mean that the animal would make an avoidance response.
Interestingly, the administration of 0.25 mg/kg of d-amphetamine produced a ten-
dency for EEG activation as previously described. The conditioned photic driv-
ing responses became more prominent, perhaps due to the loss of the spindling and
slow waves. Nevertheless, photic driving was not as clear-cut as it was in the control.

Even though there was some tendency toward normalization of the electric record
and the animals looked more alert, they still did not show appropriate avoidance
responses. These actions of *d*-amphetamine on the electrical character of the
record as well as on the gross behavior of the animal tended to diminish within half
an hour. Therefore, one can conclude that the behavioral antagonistic effects of
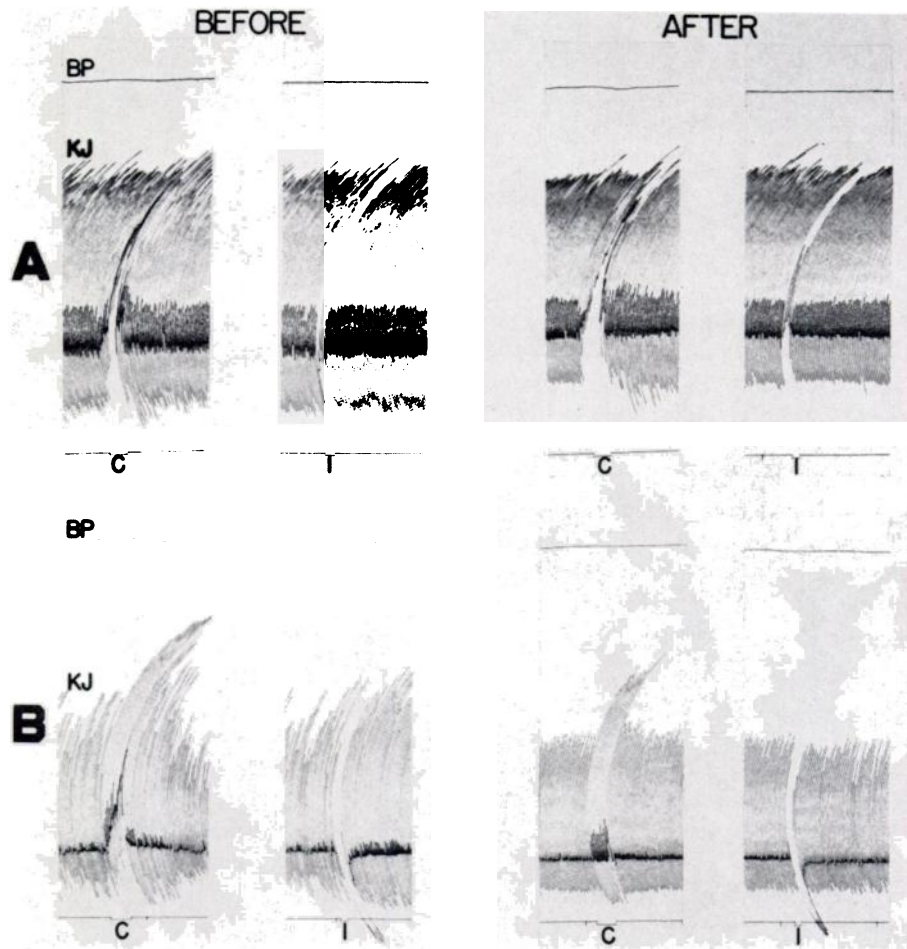amphetamine in the doses used were incomplete.

Fig. 4. Effects of DMHP on spinal cord reflexes in high spinal cats. The patellar reflex
(KJ) of two postether anesthetized cats, A and B, is recorded before and 1 hr after 1 mg/kg
of DMHP. Before, control; B.P., arterial blood pressure; K.J., knee jerk; C, stimulation of
contralateral sciatic nerve; I, stimulation of ipsilateral sciatic nerve. Parameters of elec-
trical stimulation 100 Hz square wave pulses of 1 msec, 0.5–10 V intensity. The patellar
reflex was elicited 1/sec with a mechanical hammer tapping the patellar tendon. Leg move-
ment was recorded by means of an isotonic muscle lever connected to an ink writing kymo-
graph. Note that these large doses of DMHP produced only very small depressant effects
in contrast to the effects in animals with an intact central nervous system.

EFFECTS ON SPINAL REFLEXES

Hardman *et al.* (16–19) have already described the effects of DMHP and related compounds on the spinal reflexes of intact animals. Especially dramatic was the depression of the flexor reflex and the hindlimb ataxia. These effects with DMHP are seen in intact animals in intravenous doses of 0.05 to 0.25 mg/kg.

High spinal cats

A series of four adult cats were anesthetized with diethyl ether-oxygen and high spinal transections performed at C-1. Spinal reflexes were then recorded as
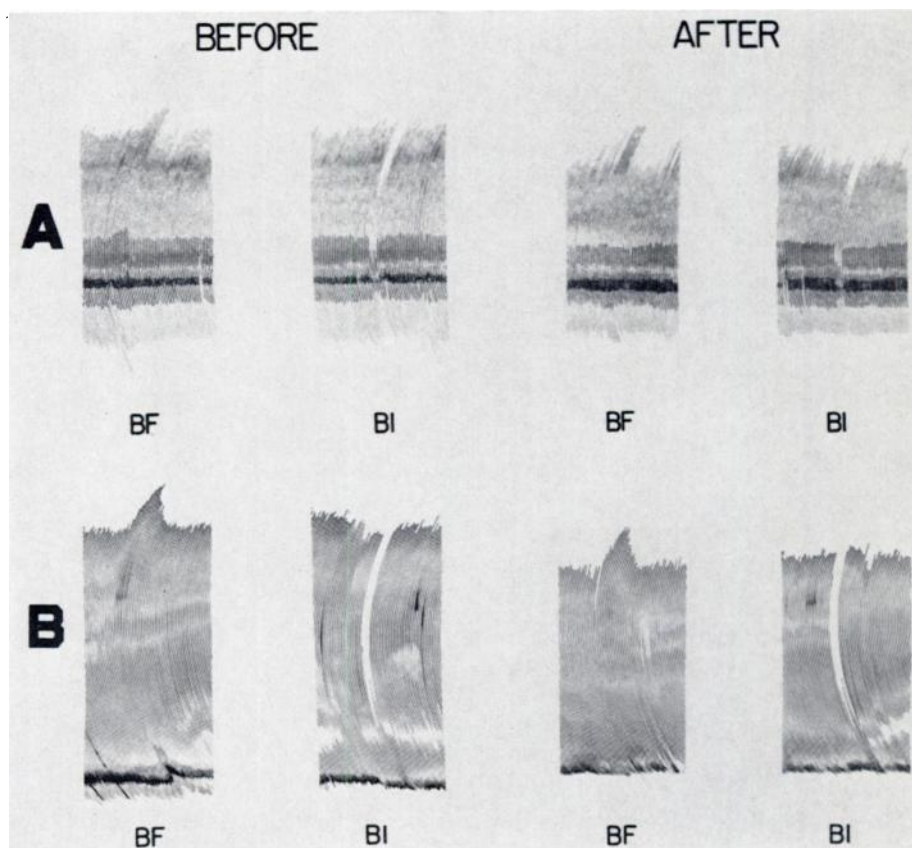

FIG. 5. Effects of DMHP on the modification of the patellar reflex by electrical stimulation of the bulbar reticular formation of chloralose anesthetized cats. Two cats, A and B, were anesthetized with *alpha*-chloralose and prepared for recording the patellar reflex as described in figure 4. In addition, bipolar stimulating electrodes were placed in the reticular formation for modifying the patellar reflex as shown. Before, Control; BF, bulbar facilitation; BI, bulbar inhibition; after, 1 hr after 1 mg/kg of DMHP, intravenously. Parameters of electrical stimulation were 3-4 V, 100 Hz square wave pulses of 0.1 msec. Note that DMHP depressed slightly the patellar reflex of these chloralose anesthetized animals. The percentage of depression of the effects of BI stimulation was greater than BF stimulation.

described previously (9) in postether anesthetized preparations. Only very large doses (1 mg/kg, i.v.) of DMHP were found to affect spinal cord reflexes. The changes produced were very slight and are questionably significant when the normal variability of these reflexes is considered. As illustrated in figure 4, electrical stimulation of the contralateral sciatic nerve produced crossed-extension (C) and electrical stimulation of the ipsilateral sciatic nerve produced inhibition of the patellar reflex (I). One hour after the administration of 1.0 mg/kg of DMHP the patellar reflex was slightly reduced. Crossed-extension was unaffected in cat A and slightly depressed in cat B. Inhibition of the patellar reflex did not appear to be significantly impaired. It was concluded that DMHP had very small effects on spinal cord reflexes in spinal cats in contrast to its dramatic effects in intact animals.

Modification of the effects of bulbar facilitation and inhibition of the patellar reflex

A series of four cats given *alpha* chloralose anesthesia (60-80 mg/kg, i.v.) were prepared for studying the effects of DMHP on bulbar reticular modulation of the patellar reflex with conventional techniques previously described (9).

The records of two different cats are illustrated in figure 5. Before DMHP electrical stimulation of the bulbar reticular formation (BF) produced facilitation of the patellar reflex and electrical stimulation of the medial bulbar reticular formation (BI) produced partial inhibition of the patellar reflex. In most cases DMHP in an intravenous dose of 1 mg/kg reduced very slightly bulbar facilitation and inhibition as well as the patellar reflex itself. Bulbar inhibition was occasionally more affected than bulbar facilitation. These findings suggest that DMHP may either potentiate chloralose anesthesia or depress in part bulbar facilitatory as well as inhibitory mechanisms or both. The phenomenon of exaggerated startle reflexes is a common pharmacological effect of DMHP. These data thus are consistent with the concept that a disinhibition of bulbar inhibitory pathways may occur after this drug.

These data are also consistent with the subsequent findings of Dagirmanjian and Boyd (8) that after low spinal transection the flexor reflex was not abolished by this compound. Only in midbrain transected cats were the responses to DMHP in their hands similar to animals with an intact central nervous system.

CONCLUSIONS

In the first edition of their textbook on neurochemistry published in 1955 Elliott *et al.* (14) referred briefly to the synthetic tetrahydrocannabinols. They remarked that, "Thus these . . . substances form a distinct and theoretically interesting group of central depressants." Between 1955 and 1959 we were involved in studying further the sites and mechanisms of actions of these compounds. We can heartily agree that these are indeed interesting agents whose central nervous system properties are unique. Because of the remarkable correlation of electrical brain activity and gross behavioral effects of these compounds, especially DMHP, they will probably become extremely useful in elaborating further the mechanisms of brain function. Blockade of conditioned avoidance responses in rats, dogs, and

monkeys does not appear to be the result of depression of afferent input primarily, although such input is distorted. Inasmuch as escape behavior is relatively normal, efferent motor output is likewise not greatly impaired unless large doses are given. The probable mechanism of action of the blockade of conditioned avoidance responses is on higher brain functions and may result from a confusional or depressed state.

On the basis of the neuropharmacological studies, the principal sites of action of DMHP appear to be in the cerebrum, especially in the secondary association cerebral cortices and related subcortical structures. DMHP has little effect on the brainstem activating system unless large doses are given in which case there is an elevation in threshold to arousal. The duration of arousal is characteristically reduced. The action of DMHP on the amygdala as one of the rhinencephalic structures appears to be primarily depressant. The depressant effects of DMHP on the spinal cord of high spinal transected cats are minor and cannot explain the marked swaying and depression of the flexor reflex seen after its administration to intact animals. The mechanism of the exaggerated startle responses including convulsive movements seen frequently after the administration of large doses of DMHP is unknown. However, it is of interest that although DMHP reduced slightly bulbar facilitation and inhibition as well as the patellar reflex itself, on a percentage basis bulbar inhibition occasionally was effected more than bulbar facilitation. This would indicate that DMHP has depressant effects on inhibitory brain systems as well.

It is suggested that many more synthetic THC compounds be made and become available to all interested scientists. There is much to be learned about nervous system function by studying their sites and mechanisms of action. Furthermore, some of these derivatives deserve clinical trials as potential therapeutic agents.

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